

The Gut Microbiota of Termites: Digesting the Diversity in the Light of Ecology and Evolution

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Abstract

Termite guts harbor a dense and diverse microbiota that is essential for symbiotic digestion. The major players in lower termites are unique lineages of cellulolytic flagellates, whereas higher termites harbor only bacteria and archaea. The functions of the mostly uncultivated lineages and their distribution in different diet groups are slowly emerging. Patterns in community structure match changes in the biology of different host groups and reflect the availability of microbial habitats provided by flagellates, wood fibers, and the increasing differentiation of the intestinal tract, which also creates new niches for microbial symbionts. Whereas the intestinal communities in the closely related cockroaches seem to be shaped primarily by the selective forces of microhabitat and functional niche, the social behavior of termites reduces the stochastic element of community assembly, which facilitates coevolution and may ultimately result in cospeciation.

Contents

INTRODUCTION	146
EVOLUTION OF SYMBIOTIC DIGESTION	147
THE TERMITE GUT HABITAT	148
Gut Structure	148
Microhabitats	148
Environmental Factors	150
DIVERSITY OF THE GUT MICROBIOTA	150
Termite Gut Flagellates	151
Bacteria	151
Archaea	153
FUNCTIONAL NICHES	153
Polymer Degradation	153
Hydrogen Metabolism	155
Oxygen Consumption	155
Nitrogen Metabolism	155
COEVOLUTIONARY PATTERNS	156
Evidence for Cospeciation	156
Habitat-Specific Lineages	156
Host Patterns and Core Microbiota	157
DRIVERS OF COMMUNITY STRUCTURE	158

INTRODUCTION

Among all organisms that degrade lignocellulose, termites are the most efficient. They combine high assimilation rates with a speed of cellulose digestion that surpasses that of other invertebrates and match wood-rotting fungi in their ecological impact (4, 118). Like other animals thriving on a fiber-rich diet, termites cooperate with a dense assemblage of microorganisms that contribute functions that are lacking in the host.

Our understanding of symbiotic digestion in termite guts has substantially increased during the past two decades (15). This includes the important role of the host in comminution and enzymatic pretreatment of the digesta; only the processes unlocking lignified plant fibers remain enigmatic (72, 120). The diversity of the gut microbiota and its role in the digestive processes have been reviewed in detail (e.g., 16, 37, 81). However, the forces shaping community structure remain contentious, and general reviews on the gut microbiota of insects have discussed host phylogeny, gut environment, and dietary preferences as potential drivers (25, 28).

Recent progress in the field increased the resolution of diversity studies and allowed a broader taxon sampling, which allowed the reliable detection of less abundant symbionts and provided sufficient coverage to detect patterns in community structure across the entire termite host range. In this review, we critically analyze the diversity of the termite gut microbiota in the light of ecology and evolution. We will briefly outline the current knowledge on the diverse aspects of symbiotic digestion, but we will focus on the importance of microhabitat, functional niche, and possible host factors in shaping the intestinal communities. We have tried our best to connect readers with all relevant literature, but because of space restrictions, many references to the original work had to be replaced by review articles.

Microbiota: the microbial component of an ecosystem, here used for the sum of the microbial communities associated with a specific host

Microhabitat: the small-scale environment of a species or community, comprising both abiotic and biotic factors

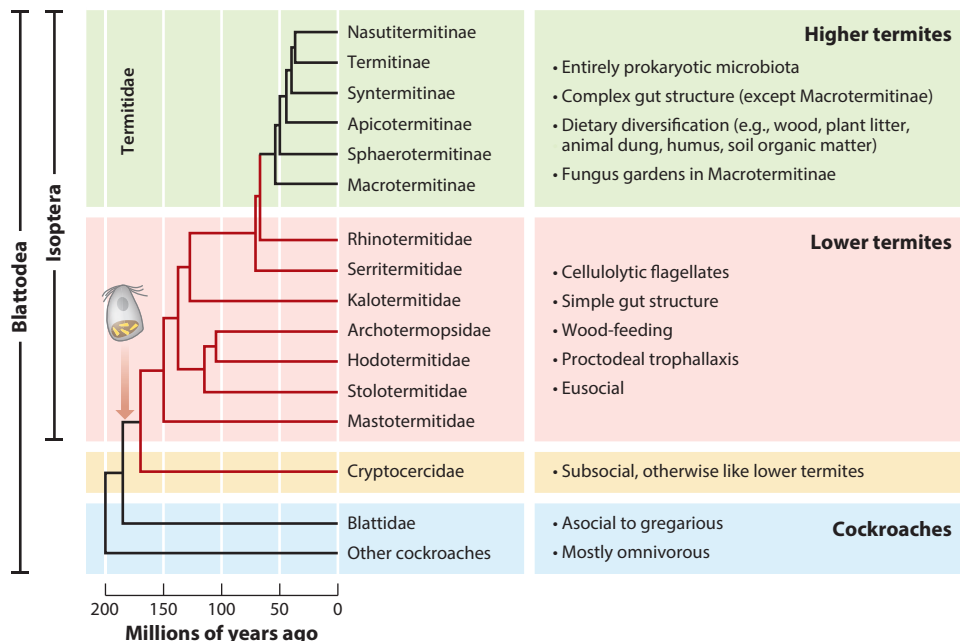


Figure 1

Phylogeny of termites (Isoptera), illustrating their origin within the radiation of cockroaches (together forming the Blattodea), with the omnivorous Blattidae as sister group, and important events in the evolution of the digestive symbiosis. Red branches indicate the presence of cellulolytic flagellates that were acquired by a common ancestor of lower termites and Cryptocercidae, which gave rise to their wood-feeding lifestyle. The loss of flagellates in the higher termites gave rise to an enormous dietary diversification (chronogram based on data from 8).

EVOLUTION OF SYMBIOTIC DIGESTION

It is now generally accepted that termites originated within the radiation of cockroaches from a presumably wood-feeding, subsocial ancestor (8, 61, 69, 70). The most recent molecular phylogenies revealed that the split between the termite line and their sister group, the blattid cockroaches, occurred during the Middle Jurassic (at least 150–170 Mya) (**Figure 1**). A key event in the evolution of termites was the acquisition of cellulolytic flagellates by a common ancestor of termites (Isoptera) and their sister group, the Cryptocercidae (hereafter included in all statements concerning lower termites), which must have provided a strong boost of their capacity for lignocellulose digestion. The symbiosis between termites and flagellates was stabilized by the development of proctodeal trophallaxis; this trait ensures the reliable transfer of flagellates among nestmates and across generations and is part of their complex social behavior, which started with long-lasting biparental care in the subsocial Cryptocercidae and culminated in an elaborate caste system and the sharing of labor in termites (see 53, 61, 70). Together with the cellulolytic activities of the host, which are present in the entire blattodean lineage, the flagellates form the dual cellulolytic system of lower termites (16, 120)—a far more efficient means for symbiotic digestion of lignocellulose than those of other detritivorous and xylophagous cockroaches (53).

It is therefore surprising that the youngest of all termite families, the Termitidae, which arose about 50 Mya (8), is no longer associated with cellulolytic protists (81). The loss of flagellates in the higher termites resulted in an entirely prokaryotic gut microbiota and was accompanied by

Proctodeal trophallaxis: social behavior in which nestmates imbibe droplets of hindgut contents; serves both in nutrition and in the transfer of symbionts

numerous symbiotic innovations and an enormous dietary diversification, which in turn brought about enormous ecological and evolutionary success (4).

Ecosystem:

a community of organisms and the abiotic components of their environment, connected by a network of interactions

THE TERMITE GUT HABITAT

The intestinal tracts of termites are small ecosystems with a wide range of microhabitats that strongly differ in their abiotic and biotic environment. Many of the environmental features are intrinsic properties of the gut, whereas others result from physiological activities of the host or the microbial residents in the respective location. In addition, the types of habitats available for microbial colonization have changed during the evolutionary history of the host.

Gut Structure

Termites share the basic gut structure of cockroaches (**Figure 2**). The foregut transports food from the mouthparts into a spacious crop, where it is incubated with secretions from the salivary glands. After further comminution by the gizzard, food passes into the midgut, where it is digested by enzymes secreted by the midgut. The digestion products are resorbed by the midgut epithelium and gastric ceca. The remaining material is transported into the hindgut (proctodaeum), which consists of a short ileum (P1) followed by an enteric valve (P2), colon (P3 and P4), and rectum (P5) and harbors the bulk of the gut microbiota. After the removal of water and ions, the residues of digestion are released as feces (17).

The crop of termites is much smaller than that of cockroaches; the midgut is shortened, and the ceca are reduced or entirely absent. In all lower termites, the anterior colon (P3), which may be somewhat expanded already in cockroaches, is strongly dilated into a single, voluminous paunch (78). In higher termites (with the exception of Macrotermitinae), the hindgut is further elongated and differentiated into several proctodeal compartments (79).

Cockroaches and termites harbor bacteria in all gut regions. Although the foregut and midgut are loaded with digestive enzymes (proteases, lysozymes, chitinases) secreted by the salivary glands and epithelia and the passage of food through the midgut is relatively rapid (27), there seem to be sites of microbial fermentations, as indicated by the accumulation of lactate and short-chain fatty acids (e.g., 2, 54, 104). The hindgut is the major site of microbial colonization, which is reflected also in the high concentrations of short-chain fatty acids in most hindgut compartments (e.g., 54, 104). An exception is the alkaline P1 compartment of wood- and soil-feeding higher termites, which is populated only in relatively low numbers (54, 106).

Microhabitats

The rapid passage of the digesta requires that to prevent washout, a microorganism must either swim fast enough or associate with particles that are retained in the gut longer than the liquid fraction. The gut flagellates of lower termites either are highly motile and able to actively maintain their position in the gut, or possess organelles for attachment to the cuticle of the gut wall (e.g., 91). Given that flagellates make up the bulk of the hindgut volume, it is not surprising that the majority of prokaryotes in the hindgut of lower termites colonize the surface, cytoplasm, and even nucleus of these protists (15). The transfer of gut contents among nestmates extends the life span of the flagellate habitats beyond that of an individual termite.

The lumen itself is not a favorable microhabitat for bacterial cells, except for the large spirochetal forms, which swim fast enough to actively maintain their position. In wood-feeding higher termites, the retention time of wood particles is longer than that of the gut fluid. Similar

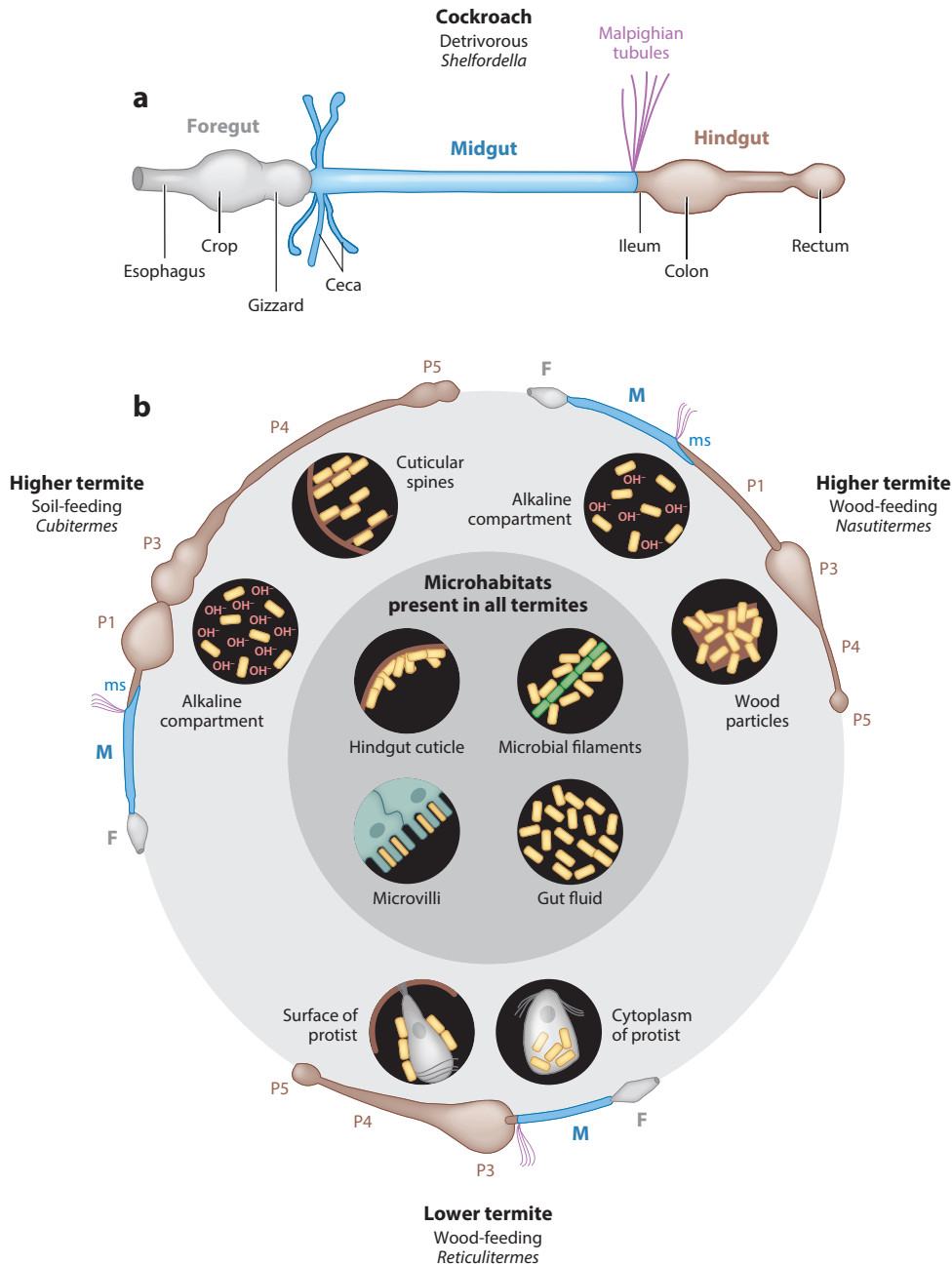


Figure 2

Anatomy of termite guts and important microbial habitats in different host groups. (a) The basic plan of the undifferentiated cockroach gut. (b) Termite guts are derived from the same basic plan, but while the foregut (F) and midgut (M) are reduced relative to those of cockroaches, the hindgut is increasingly elongated and may be differentiated into a mixed segment (ms) and several proctodeal compartments (P1–P5), which provide additional microhabitats for microbial colonization (nomenclature after 79).

mechanisms seem to exist in soil feeders, where small clay particles (rich in organic matter) are retained longer than large sand grains (27). It has been estimated (based on DNA content) that almost one-third of the microbial biomass in the luminal fluid of the hindgut paunch (P3) of a wood-feeding *Nasutitermes* species is firmly associated with the fiber fraction (65).

In insect guts, only the midgut is endodermal and allows direct access of microorganisms to the epithelial surfaces, which are protected by the peritrophic membrane. Associations of bacterial cells with the microvilli of the epithelial brush border or the ectoperitrophic space of the mixed segment have been observed (6, 10, 116). The foregut and hindgut are of ectodermal origin and are always lined with a cuticle. However, in the hindgut region, the cuticle has characteristic pores or pits (10, 19), which may increase the permeability for acetate and other short-chain fatty acids. The cuticle offers plenty of surfaces and is usually covered by a dense microbial biofilm. Cuticular spines in the P4 compartment of certain higher termites provide additional attachment sites for the microbiota (5). However, during ecdysis insects replace their entire cuticle, and the hindgut has to be recolonized after each molt.

Environmental Factors

The physicochemical conditions in the different gut compartments are affected by both the biotic and the abiotic environment. Although oxygen continuously enters the gut via the host epithelia, its efficient removal by the gut microbiota renders the center of all dilated hindgut compartments anoxic. It is important to realize that because termite guts are small, diffusive transport of metabolites along their steep radial concentration gradients is much more important than axial transport and convective mixing by peristalsis and also unaffected by the activity of the flagellates (see 16).

Compared with the larger guts of most vertebrates, insect guts have an enormous surface-to-volume ratio (11), which increases the relative importance of aerobic processes but also facilitates the exchange of both gaseous and dissolved products of microbial metabolism at the epithelial surfaces. The redox potential of the different microhabitats is modulated by their oxygen status, the production of redox-active compounds like hydrogen or ferrous iron (in soil-feeding termites), or differences in intestinal pH (see 16).

Also, host secretions should have a strong effect on the microbiota in different gut compartments. In the anterior gut, digestive enzymes in saliva and in midgut secretions provide sugars or amino acids as substrates for the resident microbiota, but they also digest microbial biomass (30, 117, 120). Passage through the anterior gut may represent a barrier to colonization by foreign microorganisms or pathogens, and the mechanisms by which hindgut microbiota transferred to nestmates via proctodeal trophallaxis evades digestion (and in the case of flagellates, mechanical disruption by the gizzard) remain unknown. Also, the extreme alkalinity in the anterior hindgut of soil-feeding termites should affect the viability of transient microbiota and select for lineages adapted to this habitat.

DIVERSITY OF THE GUT MICROBIOTA

The termite gut microbiota comprises all three domains of life: Bacteria, Archaea, and Eukarya (flagellate protists occur in lower termites) (15, 81). Most of the flagellates are easily identified on the basis of their morphological features, whereas characterization of the bacterial and archaeal communities requires molecular tools. The isolates obtained from termite guts are typically not very abundant (12); in particular, the termite-specific lineages remain mostly uncultured.

Termite Gut Flagellates

Most termite gut flagellates belong to the phylum Parabasalia (81). Three of the six classes of parabasalids (i.e., the traditional hypermastigids) are composed of species that are unique to the guts of lower termites (77). They are large enough to phagocytize wood particles, and their great motility due to multiple flagella prevents washout—probably adaptations to the termite diet and the microbial habitat in the termite gut. The ancestral Trichomonadea, which are also found in other habitats, are generally much smaller and feed on bacteria or dissolved nutrients. The larger cell size in some cellulolytic lineages is probably a response to the same evolutionary pressure (77). Many, but not all, species of lower termites harbor flagellates of the order *Oxymonadida* (phylum *Preaxostyla*). Some lineages developed special holdfasts that attach to the hindgut cuticle, and the cells can be so small that they disappear within the bacterial biofilm (112).

Molecular studies revealed that the diversity of termite gut flagellates is greater than expected (e.g., 31, 32, 109), and many species await detailed phylogenetic and ultrastructural characterization. New lineages are still being discovered (50, 91), and even seemingly identical morphospecies turn out to comprise different phylotypes (e.g., *Trichonympha* species: 51, 111, 130), adding to the notion that each termite species has unique symbionts. First attempts to assess the diversity and community structure of termite gut flagellates by amplicon sequencing indicated the need for an improved phylogenetic framework and universal primer sets (92, 110).

Bacteria

The bacterial gut microbiota of termites comprises only a few dominant phyla, with distinct differences between the major host groups (**Figure 3a**). Over the last two decades, clone libraries of 16S rRNA genes have provided a wealth of information on bacterial diversity in a variety of termite guts (36, 81), and more recent studies have added information for termite genera that were so far not represented (e.g., 33, 63). Many of the libraries, particularly from older studies, were relatively small, and the diversity of the gut communities was severely undersampled. The application of next-generation sequencing technologies resolved these issues and also allowed the study of differences in community structure across a wide range of termite species (22, 83, 92, 100), among individuals of the same species obtained from geographically separated colonies or subjected to different dietary regimens (7, 95), or between different gut compartments or luminal fractions (23, 54, 65).

Spirochaetes are characteristic members of all termite gut communities (9, 37). They are phylogenetically highly diverse and comprise various monophyletic groups of termite-specific lineages (e.g., 39, 82). Individual lineages differ in abundance between host groups (22, 92), either occur as free-swimming cells or are associated with the surface of flagellates (9, 47) or the fiber fraction (65), and may comprise different functional guilds (see below). The highest proportion of spirochetes is found in wood-feeding termites, whereas their numbers among fungus-cultivating and humus-feeding termites are typically rather low (22, 63, 83).

Bacteroidetes are highly abundant in fungus-cultivating termites (e.g., 36, 63) and contribute to the similarity of gut microbiota between termites and cockroaches (22, 83). Many of the predominant taxa (e.g., *Alistipes*, *Dysgonomonas*, *Paludibacter*, and *Parabacteroides*) readily isolated from termite guts (90, 101, 125) possess a general preference for intestinal habitats: They are encountered in the guts of mammals as well. However, there are also many family-level clades (e.g., Bacteroidales cluster V) that consist exclusively of representatives encountered in termites and cockroaches (74, 104). Similarly, *Firmicutes* are represented by common gut bacteria (mostly

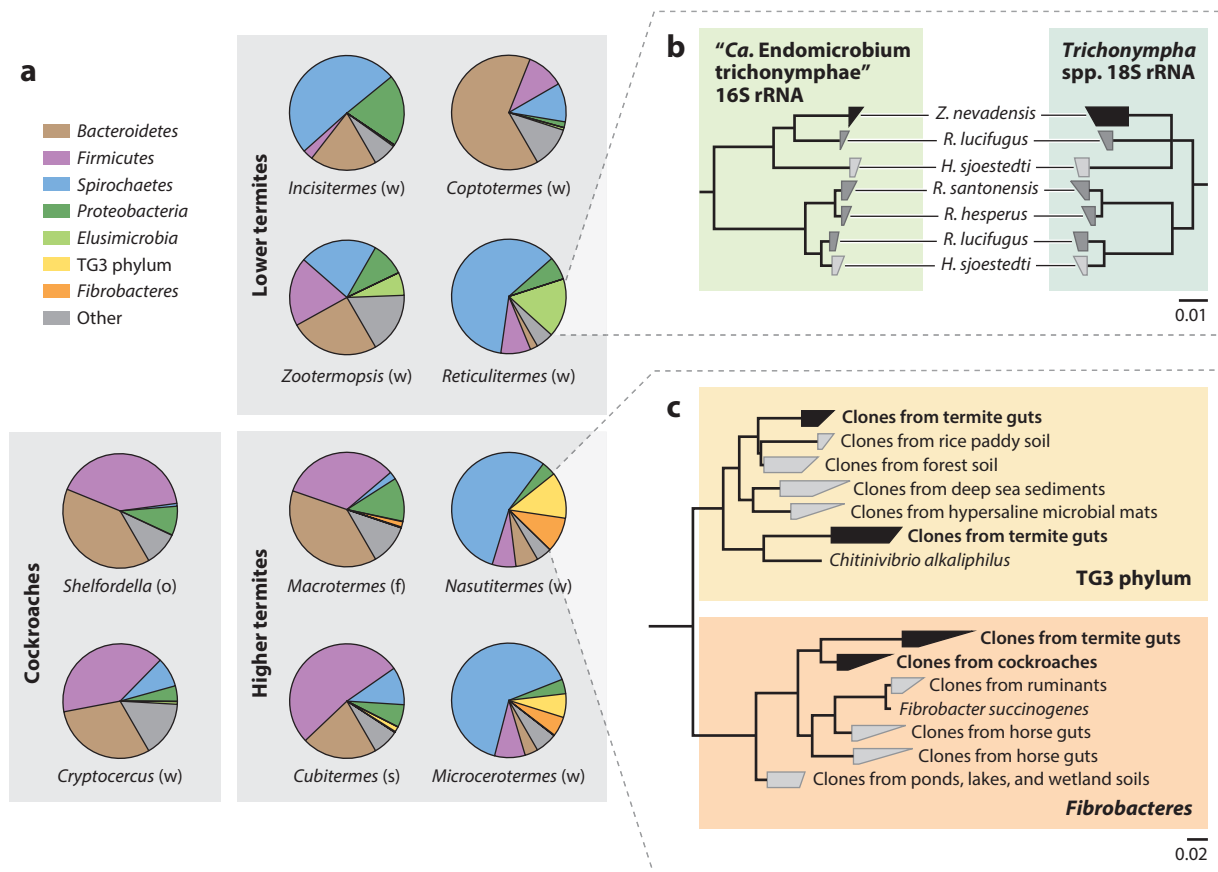


Figure 3

Diversity of the gut microbiota of termites and cockroaches. (a) Phylum-level differences between representatives of major host groups (data from 22). (b) Comparison of the phylogenetic trees (small-subunit rRNA) of "*Candidatus Endomicrobium trichonymphae*" and their *Trichonympha* hosts from various lower termites (*Hodotermopsis sjoestedti*, *Reticulitermes hesperus*, *Reticulitermes lucifugus*, *Reticulitermes santonensis*, and *Zootermopsis nevadensis*), illustrating that the strict cospeciation between the flagellates and their symbionts does not extend to the termite host (data from 45). (c) Phylogenetic tree (16S rRNA) of the *Fibrobacteres* and the candidate phylum TG3, illustrating the presence of termite-specific clusters and their relationship to clones from other environments (A. Mikaelyan, N. Lampert, & A. Brune, unpublished data). Scale bars in panels b and c denote substitutions per site. Abbreviations: f, fungus-feeding; o, omnivorous; s, soil-feeding; w, wood-feeding.

Lachnospiraceae and *Ruminococcaceae*) and highly specific lineages associated with the hindgut cuticle of arthropods (e.g., "*Candidatus Arthromitus*"; 114) or the alkaline gut compartments of higher termites (106, 115). *Proteobacteria* are also more abundant in cockroaches and Macrotermitinae than in other termite groups. Among *Deltaproteobacteria*, various *Desulfovibrio*-related lineages and a deep-branching clade (Rs-K70 cluster) are encountered in all host groups; both comprise strains associated with flagellates (98, 102, 108).

Whereas the above-mentioned phyla are represented in all termites, others may be absent or of low abundance in some groups. Members of the *Elusimicrobia* make up a large proportion of the bacterial community in many lower termites (22) and have been identified as endosymbionts of certain flagellates ("*Candidatus Endomicrobium*"; 14). The fiber-associated members of *Fibrobacteres* and the candidate phylum TG3 are abundant in wood-feeding higher termites but

have been detected also in other lineages (22, 38, 92). *Planctomycetes* form large populations only in the posterior hindgut compartments of soil-feeding Termitinae (55), but they also occur in low abundance in other groups (92). *Verrucomicrobia* related to “*Candidatus* Nucleococcus,” an intranuclear symbiont of termite gut flagellates (103), are abundant in several lower termites but found also in hosts that lack flagellates (22).

Archaea

There are four major lineages of *Euryarchaeota* in termite guts: *Methanosarcinales*, *Methanomicrobiales*, *Methanobacteriales*, and a deep-branching clade distantly related to the nonmethanogenic *Thermoplasmatales* (see 13, 40). The latter were identified as a new order of methanogens that was initially referred to as *Methanoplasmatales* but is now called *Methanomassiliicoccales*, after the first isolate of the order. Comparative genome analysis of “*Candidatus* Methanoplasma termitum,” a highly enriched culture from the gut of a higher termite (85), with strains from the human gut indicated a new mode of energy metabolism in all members of this lineage (56).

The greatest diversity of archaea is found in higher termites of the subfamily Termitinae, particularly the soil-feeding lineages, which harbor distinct archaeal communities of presumably hydrogenotrophic and methylotrophic populations in each hindgut compartment (see 13, 85). Archaeal communities in lower termites are dominated by *Methanobrevibacter* species (40), but a recent survey using pyrotag libraries revealed that their diversity is greater than indicated by the earlier, clone-based studies (92). This includes an uncultured lineage of *Thaumarchaeota* that had been detected in soil-feeding termites (29).

FUNCTIONAL NICHES

The major products of symbiotic digestion of lignocellulose in the termite hindgut are short-chain fatty acids and microbial biomass. Whereas the fermentation products are resorbed by the hindgut epithelium and drive the energy metabolism of the termite, the microbial biomass has an important nutritive value for the host (15). The functional roles of individual microbial populations are not always clear, but after decades of research, it is possible to sketch major niches of the hindgut ecosystem that are relevant for termites of all feeding guilds (**Figure 4a**).

Polymer Degradation

The primary function of the hindgut microbiota is to depolymerize recalcitrant plant fiber (16, 37). In the hindgut of lower termites, the flagellates produce a broad suite of glycoside hydrolases for the efficient breakdown of phagocytized wood, including various cellulases (exoglucanases and endoglucanases) and diverse hemicellulases (e.g., xylanases, arabinosidases, mannosidases) (see 72). Recently, the protist community has been identified also as the major source of chitinase activity in the hindgut of *Zootermopsis angusticollis* (96). It is important to note that hydrolysis takes place in the digestive vacuoles of flagellates, which not only sequesters the wood particles from the hindgut fluid but should also prevent access of luminal bacteria to the sugars produced by the depolymerization process. However, a recent metabolomics study indicates a major contribution of hindgut bacteria in lower termites to the degradation of cellodextrins (117).

The scenario differs in higher termites, where the absence of flagellates requires new modes of fiber digestion. The oldest strategy is that of the Macrotermitinae, which digest wood or lignocellulosic plant litter with the help of a lignin-degrading basidiomycete fungus (*Termitomyces* spp.). This fungus is not part of the gut microbiota but is cultivated in fungus gardens (combs) in the

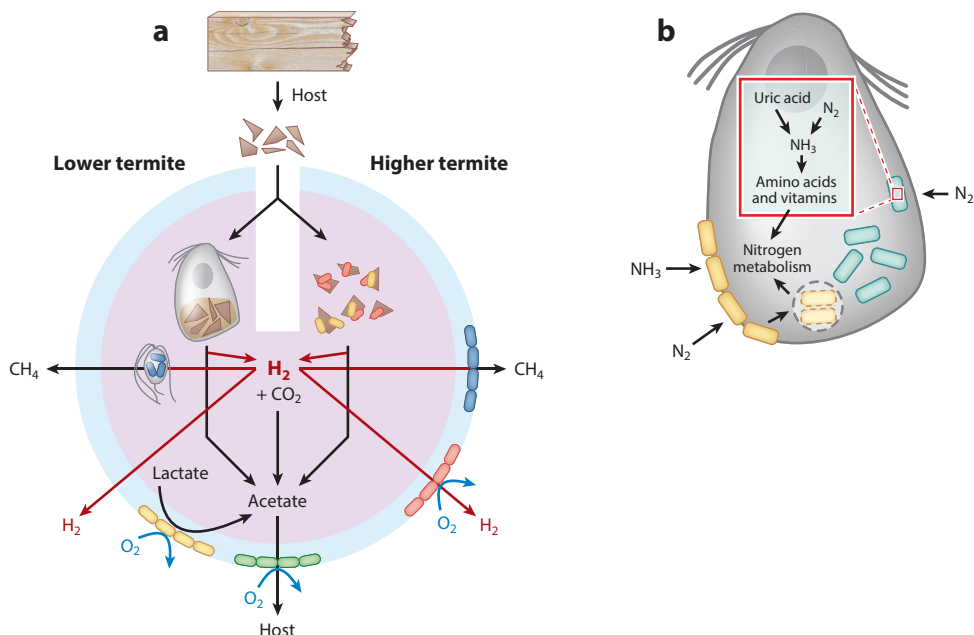


Figure 4

Major functional niches of the hindgut ecosystem. (a) The fermentative breakdown of wood polysaccharides to acetate and CO_2 differs between lower and higher termites (flagellates versus fiber-associated bacteria). Hydrogen is a major intermediate, giving rise to methanogenesis and reductive acetogenesis. Microorganisms located in the microoxic gut periphery catalyze oxygen-dependent processes (15). Arrows indicate fluxes of carbon (black) and hydrogen (red). (b) Contribution of symbionts to the nitrogen metabolism of their flagellate host. Ectosymbionts fix nitrogen and/or assimilate ammonia; they are phagocytized and subsequently digested. Endosymbionts fix nitrogen and recycle uric acid (15, 37).

nests (73, 88). The termites harvest older combs, which consist of partially digested lignocellulose and fungal biomass. The composition of the ingested material varies among the genera of fungus-cultivating termites, and this may be the reason for differences in the composition of their bacterial microbiota (22, 83). Fiber digestion in the fungus comb is incomplete and continues in the gut, as indicated by metagenomic analyses of several species of fungus-cultivating termites (60, 89, 127). The gut bacteria, specifically members of the *Bacteroidetes*, encode large numbers of glycosyl hydrolases implicated in the breakdown of polysaccharides of plant and fungal cell walls.

The other subfamilies of higher termites evolved strategies to unlock partially humified lignocellulose as a new dietary resource. During humification, which may start with the dung of herbivorous mammals or decaying wood and plant litter, there is a continuous decrease in cellulose content and a relative increase in the residual complex polysaccharides and nitrogenous products derived from microbial biomass. The community structure and abundance of different glycoside hydrolase families clearly differ between wood- and dung-feeding termites (35). Peptides derived from microbial biomass are an important component of soil organic matter and probably a major dietary resource of true soil feeders (16), which may explain the abundance of *Firmicutes* (22, 92).

Several lineages of higher termites apparently returned to a wood-feeding lifestyle (8). In the absence of flagellates, the wood particles entering the hindgut are available for bacterial colonization. A recent study of *Nasutitermes* spp. linked the cellulolytic activities in the fiber fraction to

fiber-associated members of *Fibrobacteres*, *Spirochaetes*, and the TG3 phylum (65), which explains the abundance of cellulase genes in hindgut metagenomes that were assigned to *Fibrobacteres* and *Spirochaetes* (35, 119). The earlier literature has been reviewed in detail (16, 37, 72).

Hydrogen Metabolism

Hydrogen is a central intermediate in fermentative processes and often accumulates to high concentrations (**Figure 4a**). In lower termites, it is released during the oxidation of polysaccharides to acetate and CO₂ by the cellulolytic flagellates (87); in higher termites (54, 59), the identity of the primary fermenters is not clear. Low hydrogen concentrations observed in some termite species (20, 87) are probably caused not by a lack of production but by a close coupling of hydrogen-producing and hydrogen-consuming processes (15).

In wood-feeding termites, most of the hydrogen produced in the gut is converted to additional acetate via reductive acetogenesis from CO₂ (16). The capacity of several isolates for reductive acetogenesis (9) and inventories of functional genes (*fhs*, *coo*) involved in the Wood-Ljungdahl pathway (e.g., 64, 84, 98) indicate that both lower and higher termite species possess diverse populations of homoacetogenic spirochetes. However, the particular role of the diverse FeFe hydrogenases (*hyd*) present in the gut microbiota (e.g., 1, 129) in the production and consumption of hydrogen is not clear. Even the closely related isolates from *Treponema* cluster Ia may differ in their capacity for reductive acetogenesis (9, 26), underscoring that not all of the diverse lineages of termite gut treponemes are necessarily homoacetogenic.

Hydrogenotrophic methanogenesis is a characteristic process of the termite gut microbiota, but it is typically of minor importance in wood-feeding species, most likely because of the hydrogen limitation of methanogens, which is due to their locations in the hindgut periphery (13). The reasons for the much higher methane emissions of fungus-cultivating and soil-feeding termites are not clear but may be rooted either in the structure of the methanogenic communities, which include large populations of lineages with obligately methylophilic representatives (see above), or in their spatial organization, which allows the transfer of reducing equivalents between gut compartments (13). In lower termites with unusually high methane emission, the methanogens are associated with flagellates located in the hydrogen-rich gut center (see 40).

Oxygen Consumption

Because termite guts are so small, the influx of oxygen across the hindgut wall is enormous (11). Therefore, the isolation of facultative and strictly aerobic bacteria from termite guts is not surprising. Particularly, the acetate-oxidizing microaerophiles colonizing the hindgut wall appear to be well adapted to the hypoxic conditions in the periphery of the hindgut (49, 122, 123). Oxygen is used as an electron sink also by fermenting bacteria, as indicated by the shift from propionate to acetate during the metabolism of lactate (16). Even strict anaerobes, such as methanogens colonizing the hindgut wall, can remove oxygen from their environment using hydrogen as a reductant (13). The removal of oxygen with hydrogen, which is worthless for the termite, increases the yield of acetate and other useful fermentation products. Oxygen is also an essential cosubstrate for the oxidative metabolism of aromatic compounds; genome sequences revealed cryptic capacities for oxygenase activities in anaerobic termite gut spirochetes (62).

Nitrogen Metabolism

The low nitrogen content of lignocellulose is a serious constraint to the growth of wood-feeding termites. The hindgut microbiota plays an important role in the fixation, recycling, and upgrading

Coevolution:

evolutionary process caused by reciprocal adaptations between two species; does not necessarily lead to cospeciation

Cospeciation:

synchronized speciation of coevolving partners; leads to cocoladogenesis

Cocladogenesis:

simultaneous evolutionary splitting of two species, resulting in congruent topologies of their phylogenetic trees; usually indicates cospeciation

of nitrogen (15, 37). The microbial fermentation products formed in the hindgut are directly absorbed by the host epithelia, whereas the biomass can be accessed by the digestive enzymes in the midgut only after proctodeal trophallaxis. Even in dung-feeding and humivorous species, where the mineralization of nitrogenous food constituents leads to a net formation of ammonia in the digestive process (71), the insect's need for essential amino acids and vitamins can be met only by digesting microbial biomass in the midgut.

The diversity of homologs of the *nifH* gene, a functional marker for nitrogen fixation, indicates broad diazotrophic potential in termite gut communities (e.g., 48, 124). In lower termites, symbionts of flagellates seem to play an important role in the fixation and/or upgrading of nitrogen (20, 42, 43) (**Figure 4b**). The identity of the microorganisms responsible for the high rates of nitrogen fixation in wood-feeding higher termites is unclear (124).

COEVOLUTIONARY PATTERNS

Coevolution between termites and members of their gut microbiota had already been postulated in the early studies of bacterial diversity in termite guts, which had observed clusters of phylogenetically related bacteria in closely related but geographically isolated hosts (e.g., 39, 74). The most recent high-throughput sequencing approaches, which allow a much larger sampling of taxa, confirmed the presence of distinct coevolutionary patterns in microbiota across the entire host range (22, 92, 110). Although the distribution of certain microbial lineages is in agreement with cospeciation of symbiont and host, the distribution of others seems to reflect changes in microhabitats and functional niches that occurred during host evolution.

Evidence for Cospeciation

The great similarity of the gut microbiota within and among colonies of the same termite species (e.g., 44, 92, 95) suggests that proctodeal trophallaxis stabilizes the microbial community structure within a colony and ensures the faithful transfer of symbionts across generations, which should ultimately lead to cospeciation. However, cocoladogenesis with the termite host has so far been firmly established only in the case of "*Candidatus* Azobacteroides pseudotrichonymphae," an endosymbiont of *Pseudotrichonympha* flagellates, and termites of the family Rhinotermitidae, which cospeciate with these flagellates, giving rise to a termite-specific clade of bacterial symbionts (76). In the cases of the endosymbiotic "*Candidatus* Endomicrobium trichonymphae" and the ectosymbiotic "*Candidatus* Armantifilum devescovinae" (21), the trees of the entire bacterial clades are perfectly congruent only with that of their flagellate hosts (**Figure 3b**); their cocoladogenesis with termites, however, is disturbed, possibly by lateral transfers of flagellates between different termite lineages (52).

Habitat-Specific Lineages

However, not all symbionts of flagellates are cospeciating with their respective host. An example is "*Candidatus* Desulfovibrio trichonymphae," which is found in many but not all species of *Trichonympha* and seems to be acquired in a nonhereditary manner (102, 108). There are numerous other cases among *Elusimicrobia*, *Spirochaetes*, and *Bacteroidetes* (e.g., 21, 47, 75), where closely related bacteria colonize the surface or cytoplasm of distantly related flagellates. By contrast, the same habitats may be colonized also by entirely unrelated symbionts of presumably similar function—such as nitrogen fixation (20, 43), provision of amino acids and vitamins (42, 108), and propelling

of the host cell (41, 121)—which strongly suggests a selective effect of microenvironment and/or functional niche.

There seems to be a predisposition in certain bacterial lineages to colonize a particular habitat whenever the opportunity arises. Lineages of recognized symbionts are usually part of termite-specific clusters (46, 74), which suggests that the gut serves as a reservoir of bacteria that are capable of colonizing flagellates or other suitable microhabitats. Members of the *Bacteroidales* are regularly encountered at the gut wall (e.g., 54, 68), whereas certain lineages of *Clostridiales* show a clear preference for alkaline gut compartments (54, 106, 115). Many of the taxa among *Bacteroidetes* and *Firmicutes* that are encountered also in the guts of other insects and mammals seem to have a general preference for intestinal habitats.

Microhabitats and niches are not constant factors but change during host evolution. The loss of flagellates in higher termites and the new opportunities arising for cellulolytic bacteria explain the disappearance of flagellate-specific symbionts and the eventual appearance of presumably fiber-associated lineages in the wood-feeding groups (22, 65, 92). The termite-specific clades in the *Fibrobacteres* and the TG3 phylum (**Figure 3c**) are composed of lineages that are apparently specific for certain termite genera but are not necessarily cospeciating across the entire host range (38). The sister-group position of clones from leaf-feeding cockroaches suggests niche selection as a major determinant.

Host Patterns and Core Microbiota

There is a strong phylogenetic signal in the overall structure of the termite gut microbiota (**Figure 5a**). Changes in the abundance of particular lineages coincide with major events in host evolution (22, 92, 110) and are reflected also in inventories of functional genes (124, 128). However, the overall similarity of the microbiota of congeneric termites (54, 92) contrasts with the simultaneous presence of phylotypes that are in obvious contradiction to cospeciation across the entire host range (22). Also, in comparative analyses of the gut microbiota of other insects (18, 126) and mammals (58, 80) where a relationship between host phylogeny and community structure has been observed, the importance of dietary factors as determinants of community structure seems to increase with phylogenetic distance.

The phylogenetic analysis of an entire community will always result in a mixed signal of both hereditary and environmentally acquired lineages, some of which may be cospeciating with certain host clades whereas others are only occasionally encountered. These problems are at least partially resolved if the analysis is restricted to a core microbiota of similar phylotypes that are represented in the majority of the host species and are selected using a tree-based definition—i.e., classification against a curated reference database (e.g., 22, 83). The size of the core is scale dependent because the chance that a lineage is represented across the entire host range decreases with increasing evolutionary distance (22, 83, 92).

A cluster analysis of the bacterial core microbiota across a wide range of termites and cockroaches resolved the general phylogenetic relationship among the major host groups (**Figure 5b**). Within the host groups, however, a strong phylogenetic signal was present only among the higher termites (family Termitidae), where the trees of host and microbiota were almost congruent. Among the lower termites, which were represented mostly by members of different families, the trees were highly divergent and the sister-group position of *Cryptocercus* was lost, indicating that signals of cospeciation are weak or entirely absent at least at the family level. The entire absence of any phylogenetic signal among the cockroaches underscores that the strong similarity in community structure between the phylogenetically highly divergent host lineages is not a product of coevolution.

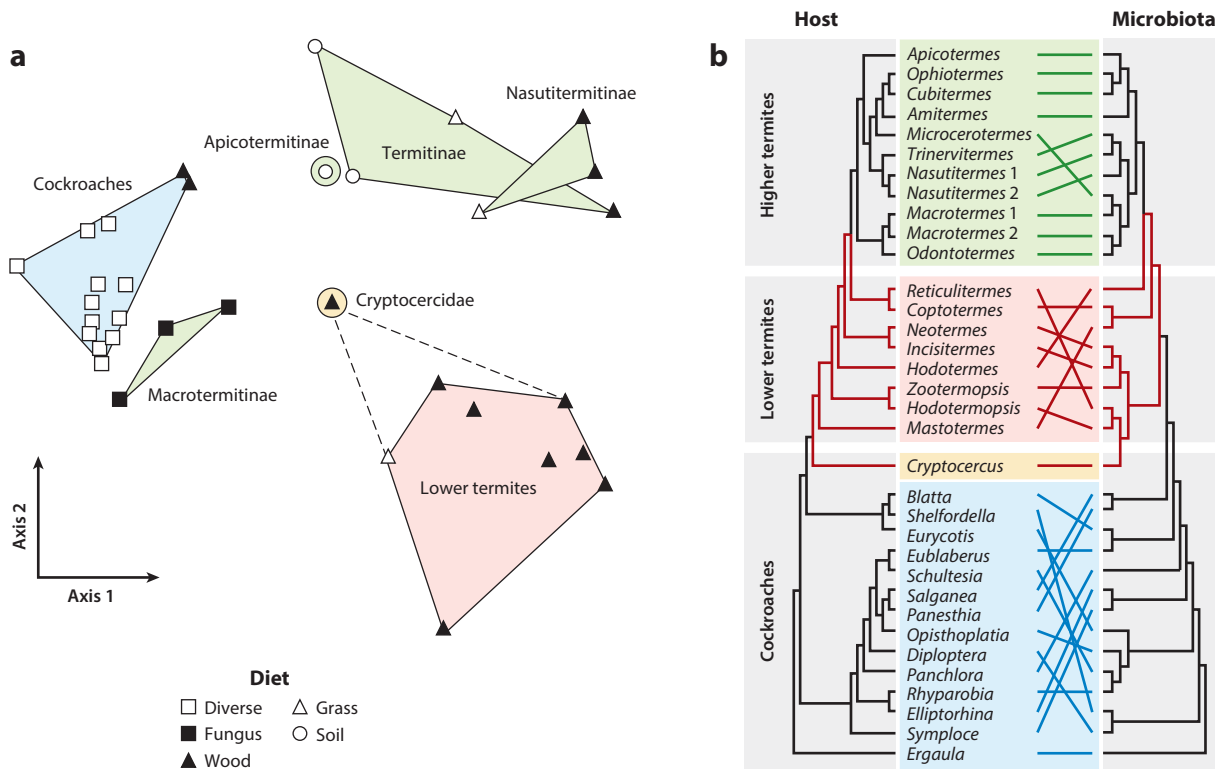


Figure 5

Phylogenetic patterns in the bacterial communities in the guts of termites and cockroaches. (a) Similarity-based ordination of community structure shows a strong clustering according to host groups but also weak dietary signals (modified from 22 with permission). (b) Comparison of host phylogeny (data from 8) and cumulative phylogeny of the core microbiota (data from 22), showing a clear separation of the gut microbiota according to the major host groups but evidence for coevolution only in higher termites.

DRIVERS OF COMMUNITY STRUCTURE

In contrast to the hereditary symbioses of insects and their intracellular symbionts, a digestive symbiosis is an open association, and both deterministic and stochastic processes should contribute to the assembly of the gut microbial community (25). The guts of cockroaches are colonized by ingestion of bacteria acquired from the environment, either together with the food source or by coprophagy (53, 69, 70), which introduces a strong stochastic element into habitat selection and would explain the highly similar yet individually variable community structure of cockroaches (2, 99, 105). In termites, however, proctodeal trophallaxis adds another deterministic component that attenuates the stochastic element by ensuring a reliable transfer of symbionts across generations and allows reciprocal adaptations that create host specificity and may eventually lead to cospeciation.

The obvious drivers of community structure in the termite gut ecosystem are differences in the microhabitats and functional niches, which are often difficult to distinguish. Examples are the microorganisms colonizing the gut wall, which contribute to oxygen reduction but at the same time must be equipped to deal with the toxic effects of reactive oxygen species, and the bacterial lineages colonizing the wood particles in higher termites, which must be able to attach to the fiber but also fulfill an important function in digestion (see above). The resulting patterns of

Coprophagy:

consumption of feces of individuals from the same or other species

biodiversity depend on the level of resolution (i.e., they differ between compartments, luminal fluid, gut wall, and other surfaces; 54, 65, 68). Because a habitat always includes biotic factors, cooperative metabolic interactions between species have to be considered (97).

Presently, direct experimental evidence for habitat selection is available only for vertebrate guts, where the inoculation of germfree hosts with the microbiota of unrelated donors results in communities that closely resemble that of conventional individuals (93, 107). Also, changes in community structure provoked by artificial diet shifts may help to identify cases of niche selection (66, 67). However, the strong discrepancies between the results of similar studies indicate that conclusions have to be regarded with caution, particularly if the underlying mechanisms are not investigated. In lower termites, changes in bacterial community structure or function (7, 44, 94) may merely reflect shifts in the flagellate community in response to diet (46, 113). The strong stochastic element in the gut microbiota of cockroaches may mask a diet response (3, 86, 105).

It is reasonable to expect the presence of molecular mechanisms that allow the host to actively control the composition of its gut microbiota, either by favoring beneficial bacteria or by excluding undesired competitors and harmful pathogens (25, 28). Antimicrobial peptides and reactive oxygen species are important components of the innate immune system of insects and seem to regulate the abundance and composition of the microbiota in *Drosophila* (24, 57). As in other insects, the saliva of termites contains small antifungal peptides and gram-negative-bacteria-binding proteins with chitinase activity (e.g., 34). It is possible that host-specific members of the gut microbiota have developed mechanisms to evade such host defenses.

SUMMARY POINTS

1. Termite guts harbor a dense and diverse microbiota that is essential for symbiotic digestion.
2. The social behavior of termites ensures a reliable transfer of microbiota across generations.
3. The strategy of symbiotic digestion changed during the evolutionary history of the host.
4. The microbiota comprises many termite-specific lineages whose abundance differs among host groups.
5. The availability of microhabitats and the availability of functional niches are major drivers of community structure.
6. Coevolution is strongly enhanced by the vertical transmission of symbionts, which facilitates reciprocal adaptations.
7. Specific lineages may develop obligate host dependence that ultimately results in cospeciation.

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