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Nutritional symbiosis and ecology of host-gut microbe systems in the Blattodea

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Cockroaches and termites (Order: Blattodea) have been the subject of substantial research attention for over a century due, in part, to a subset of them having a strong propensity to cohabitate with humans and their structures. Recent research has led to numerous insights into their behavior, physiology, and ecology, as well as their ability to harbor taxonomically diverse microbial communities within their digestive systems, which include taxa that contribute to host growth and development. Further, recent investigations into the physiological and behavioral adaptations that enable recalcitrant polysaccharide digestion and the maintenance of microbial symbionts in cockroaches and termites suggests that symbionts contribute significantly to nutrient provisioning and processing.

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Gut microbial diversity

Cockroaches and termites exhibit a wide range of dietary strategies that include omnivory, xylophagy, detritovory, humivory and coprophagy, which are influenced by the diverse, largely uncharacterized microbes inhabiting their digestive tracts. Recent experimental work suggests that host taxonomy [1,2•,3], gut compartmentalization [4•,5] and diet [6,7] significantly impact gut bacterial community composition. Blattodea species host stable core microbiota whose relative abundances respond to different diets and they are consistently dominated by members of the Firmicutes, Bacteroidetes and Proteobacteria, with Spirochetes being distinctively abundant in some termites [7–9,10•]. Micro-eukaryotes (i.e. protists) are more diverse and abundant in 'lower termites' than in cockroaches [11•,12], yet they are prevalent in wood-feeding cockroaches [13•]. Wood-feeding 'lower termites' (e.g. Mastotermitidae, Termopsidae, and Rhinotermitidae) maintain microbial communities with increased protozoan diversity relative to cockroaches and 'higher termites', and they are enriched with Spirochetes and Bacteroidetes that live within or on the surfaces of endemic protists [1,2•,14,15]. Bacterial diversity increases in 'higher termites' (e.g. Macrotermitinae, Termitinae, Apicotermitinae and Nasutitermitinae) that feed on diverse diets and are characterized by a notable abundance of Spirochetes and other poorly defined taxa [1,2•,6]. Diet strongly correlates with gut microbiota composition within the 'higher' termites, where fungus-cultivating Macrotermitinae resemble that of omnivorous cockroaches, dominated by Firmicutes, Bacteroides and Proteobacteria, and relatively few Spirochetes. The inverse is observed in wood-feeding Nasutitermitinae and Termitinae gut microbiota [1,6]. Interestingly, gut microbiota composition in litter-feeding, soil-feeding and humus-feeding Nasutitermitinae and Termitinae are more similar to omnivorous cockroaches, except for the increased abundance of Spirochetes, which further illustrates the relationship between diet and gut microbial diversity [1,6].

Comprehensive surveys of viruses, Amoebozoa, fungi, protozoans, and helminths within Blattodea gut microbial communities using nucleic acid sequencing approaches are sparse. Metagenomic profiling of the *Coptotermes formosanus* gut bacteriophage community identified ~566 phage phylotypes/colony with 960 unique phylotypes across three colonies [16•], which hints at considerable bacteriophage diversity within *C. formosanus* and perhaps the Blattodea in general. A survey of *Entamoeba* from *Periplaneta americana*, *Blaptica dubia*, and *Gromphadorhina oblongonota* cockroaches identified 134 unique rDNA sequences from nine new *Entamoeba* clades, which greatly expands the known diversity of cockroach-dwelling *Entamoeba* [17•], yet little is known about their biology and ecological roles. 18S rDNA metagenomic profiling of *Blattella germanica* hindguts detected a diverse array of fungi, nematodes, *Candidia*, flagellates and ciliates across both lab-reared and wild-caught individuals [18••]. While fungal and nematode sequences were ubiquitous, flagellates and ciliates were restricted to insects from a few locations and were low to absent in lab-reared insects [18••]. Oxymonad flagellates are abundant in the gut microbiota of wood-feeding

cockroaches and termites [19,20] and several new species of *Monocercomonoides* and *Blattamonas* were cultivated from several omnivorous cockroaches [21•]. Thelastomatid nematodes (Nematoda: Oxyurida: Thelastomatidae) are common inhabitants of the cockroach hindgut where they graze on gut microbiota, and their feeding impacts both host growth and development and hindgut bacterial community diversity [22,23]. In contrast, few gut-dwelling nematodes have been identified in termites, with isolated reports of Thelastomatid nematodes [24] and the species description of *Stomachorhabditis fastidiosa* [25].

Host insect/microbe interactions

The expanded hindgut paunch of Blattodea [26,27] and specific subcompartmentalization of termite hindguts [28] reflect dramatic adaptations to their abundant gut microbiota. Distinct bacterial communities have been demonstrated within subcompartments of the termite hindgut, with greater similarity between homologous subcompartments of different taxa of soil and humus feeding higher termites than among different subcompartments of an individual taxon [4•]. The recent availability of several Blattodea genomes and transcriptomes reveal additional possible adaptations to harboring diverse gut microbiota. In particular, gene families associated with the innate immune system, including PGRP and GNBP pattern recognition molecules within IMD and Toll pathways [29,30•] and antimicrobial peptide genes, have expanded through multiple duplication events [31•,32]. These data suggest that they may employ a high-resolution taxon recognition system to manage their diverse gut microbiota.

Early studies eliminating or suppressing gut bacteria using germ-free methods and antibiotics demonstrated positive effects of bacterial colonization on cockroach and termite growth and development [33–35,36••]. Furthermore, drastic reduction of fat body endosymbionts (*Blattabacterium* spp.) of cockroaches using antibiotics led to high mortality, slow growth, and severely reduced reproductivity [37]. Depletion of gut bacteria and *Blattabacterium* spp. using antibiotics decreased exogenous glucose-U-^{14}C or $Na_2{}^{35}SO_4$ incorporation into insect-extracted amino acids, [38,39] and increased urate accumulation in fat bodies [40], suggesting a microbial participation in amino acid provisioning and nitrogen cycling. Recent ^{13}C stable isotope analysis in *Periplaneta americana* and *Reticulitermes flavipes* supported this by demonstrating that insect-assimilated essential amino acids were likely of bacterial origin [41,42]. Additionally, antibiotic suppression of gut microbiota decreased the standard metabolic rate in *P. americana*, suggesting that gut microbiota contribute significantly to energy expenditure of the cockroach [43•].

Omnivorous cockroach gut bacteria can degrade dietary polysaccharides like cellulose [44,45], and cellulolytic symbionts are important for termite and wood-feeding cockroach diet assimilation. While wood-feeding cockroaches and lower termites rely upon cellulolytic protists (and their bacterial mutualists) for lignocellulose feeding [46], polysaccharide-degrading bacteria are important cellulolytic components of the higher termite gut microbiota [47•]. Beyond contributing to dietary digestion, gut microbiota can also degrade and detoxify xenobiotics. Antibiotic depletion of gut microbiota in insecticide-resistant *Blattella germanica* cockroaches increased indoxacarb insecticide sensitivity and partial resistance was conferred upon indoxacarb-susceptible strains following coprophagy of feces, and the microbes within, from insecticide-resistant *B. germanica* [48•]. A complementary study reported that several endosulfan-degrading bacteria were isolated from *Blatta orientalis* digestive tracts, yet host protection against this insecticide was not examined [49]. Finally, antibiotic-treated *B. germanica* that were infected with the entomopathogenic fungus *Metarhizium anisopliae* exhibited 50% greater mortality than untreated, infected insects, which suggests that gut bacteria reduced entomopathogen susceptibility [50•].

In addition to providing host protection, gut bacteria and their products can impact host behavior. Volatile carboxylic acids (VCA) present in frass (feces) are among fecal aggregation agents used by *B. germanica* to attract nestmates, facilitating several beneficial outcomes (i.e. predator avoidance, mate location, access to nutrient-rich feces). Choice-based assays revealed that frass from wild-type *B. germanica* was more attractive than frass from antibiotic-treated insects to 1st-instar nymphs [51]. Several VCAs were absent in frass from antibiotic-treated insects and inoculation of frass with aerobic bacteria cultivated from untreated insects rescued the aggregation response, strongly suggesting bacterial origins of aggregation agents.

Microbe/microbe interactions

Diverse interspecies symbioses exist in Blattodea digestive tracts that include protist-bacterial endo-symbiosis and ecto-symbiosis, nematode-bacterial ectosymbioses, and nematode and protist predatory behaviors that too can shape the gut microbial community.

Protist-Endosymbiont Interactions. Methanogenic archaea-bacteria, delta-protobacterial sulfate-reducers and spirochete acetogens are common endosymbionts of cellulolytic ciliates and flagellates native to cockroach and termite guts [52••,53,54•]. Recent genomic analyses have confirmed the potential for these organisms to act as H_2 sinks within their hosts and hint at the importance of syntrophy in promoting endosymbiont acquisition [52••,53,54•]. *Trichonympha* spp. protists are abundant in several termite lineages and they harbor bacterial endosymbionts that belong to several distinct bacterial phyla. Members of the *Elusimicrobia* phylum, namely the 'Endomicrobia' spp., reside within *Trichonympha* spp. and have been detected in the gut lumens of termites,

cockroaches and other animals [55]. Genome annotations of five *Candidatus* 'Endomicrobium trichonymphae' phylotype Rs-D17 genomovars revealed highly similar genome content and structure, including amino acid and vitamin provisioning capabilities and intact CRISPR/Cas systems, suggesting a persistent defense against foreign DNA [56,57]. Given the nitrogen demands of amino acid provisioning, it is notable that these endosymbionts lacked diazotrophic genes observed in the cultivated *Endomicrobium proavitum*, which also inhabits *Reticulitermes* hindguts, but do not always physically associate with *Trichonympha* protists [58•]. Extensive genome sequence sampling of the *Elusimicrobia* is underway, with >130 projects (as of October 2019) registered with GenBank, which can reveal how members of this taxon have evolved across diverse hosts. The trichonymphid endosymbiont *Candidatus* 'Ancillula trichonymphae' (Actinobacteria:Bifidobacteriales) lacks nitrogen-fixing genes yet can potentially provision amino acids and vitamins using ammonia assimilation and completely fermentative energy metabolic pathways [59]. The Bacteroidetes trichonymphid endosymbiont *Candidatus* 'Azobacteroides pseudotrichonymphae' resides in Rhinotermitidae and has nitrogen-fixation genes that likely support the mutualism through metabolite provisioning [60]. The detection of a circularly permuted bacteriophage genome encoding a glutamine-tRNA absent in the bacterial chromosome in the related *Can.* 'A. pseudotrichonymphae' phylotype ProJPt-1 adds an intriguing additional layer to symbioses in these organisms [61•].

Ectosymbionts of Gut Microbes and Microbial Grazing. Protist ectosymbionts are abundant in termite guts, with Spirochetes and 'Synergistes' species associated with and propelling *Mixotricha paradoxa* [62] and *Caduceia versatilis* [63], respectively, through coordinated movements. TG2 'Margulisbacteria' attach to *Treponema* spirochetes who are themselves attached to termite flagellates, exhibiting a multilevel symbiosis [64•]. Hydrogenesis via cellulosic fermentation by 'Margulisbacteria' is thought to benefit their acetogenic spirochete partner. The Bacteroidales ectosymbiont of *Barbulanympha* protists within the cockroach *C. punctulatus* can fix ^{15}N, which the protist also assimilates [65], while, in contrast, the *Candidatus* 'Symbiothrix dinenymphae' (order: Bacteroidales) ectosymbiont of *Dinenympha* spp. protists from the lower termite *Reticulitermes speratus* lacks nitrogen fixation genes, yet it encodes lignocellulose degradation enzymes [66]. Bacterial ectosymbionts of nematodes belonging to the *Bacteroidales* termite cluster V, *Rikennellaceae* and *Ruminococcaceae* groups have been observed in two thelastomatid species residing in *Panesthia angustipennis* woodroach digestive tracts [67•]. Microbe grazing by *Leidynema appendiculatum* nematodes within *P. americana* and *P. fuliginosa* hindguts altered gut community composition, increasing overall taxonomic diversity and enriching the community with Proteobacteria [22]. Finally, sympatric bacterivorous ciliates in *Panesthia* cockroaches exhibited distinct grazing preferences amongst available bacterial species, suggesting long-term ciliate-bacterial food web associations that likely impact gut community composition [13•].

Microbial community assembly

Coprophagy, trophallaxis, exuvium consumption, cannibalism, and necrophagy are common behaviors observed across Blattodea and hypothesized to aid in the transfer and retention of gut symbionts [20,68–70], including protozoa [71], nematodes [72] and methanogens [12]. Recent 16S rRNA gene sequencing studies have demonstrated how gut bacterial communities are acquired [73•], change over time [74] and across different gut environments [4•,5,75]. Gut and frass microbial diversity in *B. germanica* significantly overlap, but 13–20% of gut taxa were absent from frass, suggesting that coprophagy is not the only means for gut microbial community acquisition [18••]. Rifampicin treatment of *B. germanica* gut communities over two generations depleted gut community diversity to nearly 10% of untreated cockroaches, and coprophagy of frass from untreated insects resulted in the gut community rebounding to ~50% of the untreated species diversity [73•]. Single exposure conventionalization of germ-free *P. americana* cockroaches resulted in intermediate growth and morphological phenotypes between germ-free and wild-type cockroaches, suggesting that multiple exposures to frass and its associated microbiota are necessary for normal growth and development [36••]. Inoculations of germ-free *Shelfordella lateralis* cockroaches with gut microbiota from termite and mouse guts resulted in colonization of bacteria congeneric to those of a *S. lateralis*-nativegut bacterial community, suggesting that the cockroach gut could select for specific taxa [76]. Work with germ-free cockroaches has also demonstrated how culturable bacteria that are native to the gut can impact gut oxygen levels, which in turn can affect gut community membership that includes taxa that are oxygen-sensitive (i.e. Bacteroidetes) [77].

Conclusions and future perspectives

Our expanding recognition of the diversity of life found within Blattodea digestive tracts will provide the basis for extended study of more specific interactions within this environment. Gut microbes participate in polysaccharide digestion, xenobiotic degradation, and amino acid and vitamin provisioning, and further detailing of the functional contributions of individual taxa to symbiotic digestion warrants attention. Likewise, newly discovered protist-bacterial symbioses provide opportunities to dissect interspecies interactions. Finally, the Blattodea, especially cockroaches, are amenable to cultivation under both conventional and germ-free conditions, which highlights them as an excellent platform for future efforts to identify the mechanisms underlying host-microbe and microbe-microbe interactions.

Conflict of interest statement

Nothing declared.

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The diversity of termite and cockroach dwelling candidate phylum 'Margulisbacteria' was surveyed by 16S rDNA sequencing revealing a class-level clade of phylotypes originating from termites. Single cell genomes from fluorescence-assisted cell sorted termite gut filtrates were sequenced, yielding a fragmented genome of 'Margulisbacteria' from a lower termite and metagenomic sequencing of capillary picked protists yielded three genomes from two species of lower termite. FISH microscopy localized all phylotypes on *Treponema* spirochetes, motivating the further recovery of the genome of one 'Margulisbacteria' associated spirochete from the metagenome. Analysis of the genomes indicates a likely syntrophic relationship, with the 'Margulisbacteria' degrading cellulose and evolving H2 and the spirochete utilizing H2 in acetogenesis.

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Blattella germanica cockroaches were continuously fed rifampicin antibiotic over 30 days upon reaching adulthood and hindgut bacterial communities were characterized by 16S rDNA profiling in comparison to hindgut communities from non-antibiotic fed cockroaches. A second generation of insects was produced by the antibiotic treated cockroaches and maintained on antibiotic, removed from antibiotic, or removed from antibiotic and provided with feces of the control colony of cockroaches. Gut microbial community diversity was reduced in first generation antibiotic treated insects compared to untreated insects and greatly reduced in second generation insects maintained on antibiotics. Diversity of gut microbial communities rebounded in second generation insects that were removed from antibiotic and increased further in insects that were both

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