AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology

Deterministic Assembly of Complex Bacterial Communities in Guts of Germ-Free Cockroaches

Aram Mikaelyan,[a,b] Claire L. Thompson,[a,b] Markus J. Hofer,[c] Andreas Brune[a,b]

Department of Biogeochemistry, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany[a]; LOEWE Center for Synthetic Microbiology (SYNMIKRO), Philipps-Universität Marburg, Marburg, Germany[b]; School of Molecular Bioscience, The University of Sydney, Sydney, NSW, Australia[c]

The gut microbiota of termites plays important roles in the symbiotic digestion of lignocellulose. However, the factors shaping the microbial community structure remain poorly understood. Because termites cannot be raised under axenic conditions, we established the closely related cockroach *Shelfordella lateralis* as a germ-free model to study microbial community assembly and host-microbe interactions. In this study, we determined the composition of the bacterial assemblages in cockroaches inoculated with the gut microbiota of termites and mice using pyrosequencing analysis of their 16S rRNA genes. Although the composition of the xenobiotic communities was influenced by the lineages present in the foreign inocula, their structure resembled that of conventional cockroaches. Bacterial taxa abundant in conventional cockroaches but rare in the foreign inocula, such as *Dysgonomonas* and *Parabacteroides* spp., were selectively enriched in the xenobiotic communities. Donor-specific taxa, such as endomicrobia or spirochete lineages restricted to the gut microbiota of termites, however, either were unable to colonize germ-free cockroaches or formed only small populations. The exposure of xenobiotic cockroaches to conventional adults restored their normal microbiota, which indicated that autochthonous lineages outcompete foreign ones. Our results provide experimental proof that the assembly of a complex gut microbiota in insects is deterministic.

Termites and cockroaches play important ecological roles in the turnover of lignocellulose in terrestrial ecosystems (1). These roles depend on the well-regulated nutritional relationships that they have evolved with their gut microbiota over millions of years (2). Compared to the gut microbiota associated with most other model insects, such as fruit flies (3) and bees (4), which are dominated by a few bacterial species, those of termites and cockroaches are considerably more diverse. Previous studies have shown that the composition of their gut microbiota reflects both the common evolutionary origin of and the dietary diversity within this group (5, 6), which makes them excellent models for investigating factors shaping complex gut communities in insects (7).

Comparative analyses of the bacterial gut microbiota of termites and cockroaches have revealed that the influence of host taxonomy in community composition is overshadowed by that of host diet (6). A more recent study on higher termites also identified diet as the primary determinant shaping bacterial community structure (8) and hypothesized that differences in diet impact the availability of microhabitats to particular bacterial lineages. These results strongly suggest that the intestinal environment is a strong driver of microbial community structure in termite guts, but the validity of this hypothesis still remains to be experimentally tested.

However, termites cannot be raised germ-free because of their obligate dependence on their gut microbiota. Also their elaborate social structure makes them intractable as gnotobiotic models because earlier instars must be nourished by nest mates. In comparison, their closest relatives, the noneusocial cockroaches, do not depend on colony members for nourishment and can be raised in isolation under axenic conditions (9). Moreover, the physicochemical conditions in cockroach guts (5, 10) are similar to those of many termites (11, 12), which would offer a surrogate environment at least for those core lineages of gut microbiota that are shared with termites (5, 6, 13). These characteristics make cockroaches excellent models to experimentally test theories on the selective role of the insect gut environment in the assembly of the intestinal microbial community.

We recently developed a gnotobiotic cockroach model based on *Shelfordella lateralis* (9), an omnivorous cockroach from the family Blattidae, the sister group of the termites (14). Using this model, we experimentally tested if the cockroach gut habitat could play an important role in determining gut community structure by inoculating germ-free individuals of *S. lateralis* with gut microbiotas from a set of donor organisms that include both close (termites) and distant (mice) relatives of cockroaches. The composition of the resulting foreign gut microbiota (xenomicrobiota) determined by pyrosequencing of amplified 16S rRNA genes was compared with that of the microbiota of cockroaches exposed to a conventional environment.

MATERIALS AND METHODS

Generation of germ-free cockroaches. *Shelfordella lateralis* was obtained from a commercial breeder and maintained as previously described (5). Germ-free cockroaches were obtained using the protocol described by Tegtmeier et al. (9). Briefly, mature oothecae were washed quickly in 0.1% sodium dodecylbenzenesulfonate and then sterilized in 2% peracetic acid solution for 5 min. Following surface sterilization, the oothecae were

Received 12 November 2015 **Accepted** 3 December 2015

Accepted manuscript posted online 11 December 2015

Citation Mikaelyan A, Thompson CL, Hofer MJ, Brune A. 2016. Deterministic assembly of complex bacterial communities in guts of germ-free cockroaches. Appl Environ Microbiol 82:1256–1263. doi:10.1128/AEM.03700-15.

Editor: H. L. Drake, University of Bayreuth

Address correspondence to Andreas Brune, brune@mpi-marburg.mpg.de.

A.M. and C.L.T. contributed equally to this work.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/AEM.03700-15.

rinsed in sterile water and transferred aseptically to sterile 50-ml polypropylene tubes and incubated at 25°C until hatching.

Germ-free cockroaches were screened for the absence of culturable microbes by macerating one hatchling from each ootheca and smearing it on the surface of a nutrient agar plate. Plates were incubated at 25°C for 1 month; in the rare cases where microbial growth was observed, the results obtained with the respective batch were discarded. Screening of germ-free hatchlings for unculturable contaminants by 16S rRNA gene amplification (9) yielded nothing but the sequences of a *Blattabacterium* sp., i.e., the maternally transmitted obligate endosymbiont found in the fat body of all cockroaches (15).

Inoculation with foreign gut microbiota. After germ-free hatchlings were transferred to fresh tubes in batches of five and starved for 1 week, live specimens of *Reticulitermes santonensis* (five individuals), *Zootermopsis nevadensis* (two individuals), or *Nasutitermes corniger* (five individuals) or mouse colon content (ca. 300 mg) was added to each tube. All termites were from laboratory colonies that were maintained on a diet of pine (*Reticulitermes santonensis* and *Zootermopsis nevadensis*) or birch wood (*Nasutitermes corniger* [6]). Colon content was from a C57BL/6 mouse dissected a few hours prior to inoculation.

Termites and colon content were typically consumed within 5 to 15 min. All cockroaches were aseptically transferred to 500-ml glass bottles containing autoclaved food, which consisted of wheat bran (36% fiber, 15% protein; Spielberger, Brackenheim, Germany) and microcrystalline cellulose powder (Sigma-Aldrich, Hamburg, Germany) mixed in a one-to-one ratio (by weight). Water was supplied via a 0.2-ml plastic tube containing a wet paper towel. Cockroaches were maintained for either 1 week or 4 weeks with water and food changed weekly.

Upon sampling, all cockroaches from each batch were dissected, and the guts of two identically treated batches were pooled and frozen at −20°C. DNA was extracted from each pool (10 guts per treatment) using a bead-beating protocol, as previously described (16). DNA was also extracted from the guts of 10 termites from the same colonies as those used for inoculation, from an aliquot of the mouse gut contents, from age-matched (1 week, 4 weeks) cockroaches that hatched germ-free but were raised in a conventional cockroach colony, and from adult cockroaches from the same colony. All samples were obtained in two replicates.

Conventionalization of xenobiotic cockroaches. Ten nymphs inoculated with the gut microbiota of *N. corniger* were transferred 1 week after inoculation to boxes containing 10 conventional adults of *S. lateralis* that were kept on the same bran-cellulose diet as the conventional cockroaches (see above). After 4 weeks, both the nymphs and the adults were dissected, their guts were pooled and frozen, and DNA was extracted as described above. The experiment was conducted in two replicates.

Pyrosequencing of 16S rRNA genes. DNA from all samples was amplified using primers 343Fmod (TAC GGG WGG CWG CA) and 784Rmod (GGG TMT CTA ATC CBK TT) targeting the V3-V4 region of the bacterial 16S rRNA gene (12). Both primers had an additional, sample-specific 6-bp barcode at the 5′ end, as described by Köhler et al. (12). Adaptor ligation, subsequent amplification, and pyrosequencing (454 GS FLX with Titanium technology; Roche) were done commercially (GATC Biotech, Constance, Germany).

Pyrotag libraries were processed for quality using the standard operating procedure described by Schloss et al. (17), except that only reads with a minimum length of 250 bp and an average phred quality score of 35 were selected (using a 5-base sliding window). Quality-checked reads were sorted into different multifasta files using the sample-specific barcodes contained in the sequences. Following the removal of the barcodes and primers, the reads from each sample were clustered into operational taxonomic units (OTUs; 99% sequence identity) using USEARCH (18). The entire data set was submitted to the NCBI Short Read Archive (see below).

Analysis of community structure. For the analysis of taxonomic composition, a representative phylotype from each of the OTUs in a sample was classified with the RDP classifier (19) implemented in the mothur software suite (20) at a confidence cutoff of 80%, using DictDb v. 3.0 as reference database (13). Genus level lineages were ranked by determining their cumulative contribution to the principal component analysis of the gut communities as previously described (6, 21). Additionally, Morisita-Horn distances were calculated for the communities at the genus level and visualized using nonmetric multidimensional scaling (NMDS) with the vegan package (22) in the R statistical software suite (23).

Similarities in the phylogenetic structure of the communities were calculated using the taxonomy-independent weighted UniFrac metric (24) as implemented in mothur. Pairwise UniFrac distances were calculated for all 34 samples based on a maximum likelihood tree calculated using FastTree (1,000 sequences per sample [25]) and ordinated by NMDS.

Phylogenetic analysis of short reads. The OTU representatives in replicate libraries from each treatment were merged to produce one fasta file per treatment and imported into the ARB (26) implementation of DictDb v. 3.0 (13), aligned, and added to the guide tree using the add-by-parsimony tool in ARB. OTUs that formed more than 0.5% of the reads in each of the merged data sets were selected and subjected to phylogenetic analysis with closely related full-length reference sequences in their respective phylogenetic neighborhoods (maximum likelihood criteria; the general time-reversible model) using FastTree (25). The relative abundances of each OTU in the merged data sets were annotated as circles using the APE package (27) written for the R software suite (23).

Accession numbers. The data set for the entire project was submitted to the NCBI Sequence Read Archive (SRP063974). Accession numbers for the individual samples are given in Table 1.

RESULTS

Pyrosequencing of amplified 16S rRNA genes of the gut microbiota. We inoculated groups of germ-free cockroaches with gut microbiota from different donors and characterized the resulting xenomicrobiota using pyrosequencing of amplified 16S rRNA genes (Fig. 1A). Per library, 1,504 to 13,376 quality-checked sequence reads were obtained. Classification using DictDb (13) successfully assigned 99% of the reads to defined phyla, but the assignment success decreased with taxonomic depth. Nevertheless, the classification success typically remained well above 70% down to the genus level for termites, mice, and both conventional and xenobiotic cockroaches (for details, see Table S1 in the supplemental material).

Comparison of natural and xenobiotic communities. Distance-based ordinations using Morisita-Horn and weighted UniFrac metrics showed that conventionally raised cockroaches had considerable similarity in bacterial community structure, irrespective of their age (Fig. 1B and C). The same was true for xenobiotic communities derived from the same donor. However, community structure in xenobiotic cockroaches differed considerably from that of their respective donors.

The distribution and abundance of bacterial genera in the different samples (Fig. 2) showed several common patterns that explain the overall clustering observed in the ordination analyses. The normal gut microbiota of conventional cockroaches was dominated by genus level groups affiliated with the families *Lachnospiraceae*, *Porphyromonadaceae*, and *Ruminococcaceae*. The most prevalent of these groups was *Dysgonomonas*, which dominated the communities in all conventionally raised cockroaches. Genus level lineages in the *Alistipes* complex (*Rikenellaceae*) and *Fusobacterium* (*Fusobacteriaceae*), which were reproducibly detected in the gut microbiota of 1-week-old conventional nymphs, became scarce at 4 weeks after inoculation, which suggested that

TABLE 1 Gut microbiota samples obtained from donors and treated germ-free cockroaches at different times postinoculation and accession number for each sample in the NCBI Sequence Read Archive

ID	Species	No. of wks postinoculation	Accession no. (a, b)[b]
Donors			
1	*Zootermopsis nevadensis* (lower termite)	—[a]	SAMN04095828, SAMN02228084
2	*Reticulitermes santonensis* (lower termite)	—	SAMN04095823, SAMN02228089
3	*Nasutitermes corniger* (higher termite)	—	SAMN04095818, SAMN02228099
4	*Mus musculus* (mouse)	—	SAMN04095810, SAMN04095811
Conventional cockroaches			
5	Nymphs	1	SAMN04095802, SAMN04095803
6	Nymphs	4	SAMN04095800, SAMN04095801
7	Young adults	12	SAMN04095804, SAMN04095805
Xenobiotic cockroaches inoculated with gut microbiota from:			
Z. nevadensis			
8	Nymphs	1	SAMN04095826, SAMN04095827
9	Nymphs	4	SAMN04095824, SAMN04095825
R. santonensis			
10	Nymphs	1	SAMN04095821, SAMN04095822
11	Nymphs	4	SAMN04095819, SAMN04095820
N. corniger			
12	Nymphs	1	SAMN04095814, SAMN04095815
13	Nymphs	4	SAMN04095812, SAMN04095813
Mouse			
14	Nymphs	1	SAMN04095808, SAMN04095809
15	Nymphs	4	SAMN04095806, SAMN04095807
Challenge expt			
16	Nymphs from ID 12 exposed to ID 17	4	SAMN04095816, SAMN04095817
17	Conventional adults	—	SAMN04095798, SAMN04095799

[a] —, not applicable because the age of the individuals was not determined.
[b] Each inoculation was conducted in replicate (a, b). The entire data set is available also under accession number SRP063974.

these lineages are early colonizers of the gut. However, their relative abundance increased again in conventional adults.

The strongest differences in the distribution of genus level bacterial lineages were observed between xenobiotic cockroaches and the corresponding donors (Fig. 2). Many donor-specific lineages that dominated the respective inocula were either absent or scarce in the corresponding xenomicrobiota (Fig. 2). For example, members of the genus *Endomicrobium*, which occur as endosymbionts of gut flagellates in lower termites (28, 29), were abundant in *Zootermopsis nevadensis* and *Reticulitermes santonensis* but not detected in cockroaches inoculated with these donors (Fig. 2). The same situation was observed with members of *Fibrobacteres*, which colonize wood fibers in higher termites of the genus *Nasutitermes* (30); they were abundant in *Nasutitermes corniger* but not detected in the xenobiotic cockroaches inoculated with this donor. Similarly, members of the S24-7 cluster of *Bacteroidales*, which predominated in the mouse gut, were not detected in the mouse-derived xenomicrobiota.

Enrichment of donor-specific bacterial lineages. One of the most striking features was the preferential enrichment of members of the family *Porphyromonadaceae* in xenobiotic cockroaches inoculated with the gut microbiota of lower termites (Fig. 2; see also Table S2 in the supplemental material). *Dysgonomonas* (family *Porphyromonadaceae*) dominated the xenomicrobiota derived from *Z. nevadensis* and *R. santonensis*, both 1 week (43% and 45% of the respective communities) and 4 weeks (29% and 65% of the respective communities) after inoculation.

However, phylogenetic analysis of the short reads classified as *Dysgonomonas* in the context of a comprehensive set of full-length 16S rRNA gene sequences previously obtained from the intestinal tracts of insects and mammals (DictDb v. 3.0 [13]) revealed that conventional and xenobiotic cockroaches each harbored distinct operational taxonomic units (OTUs) (Fig. 3A). The major phylotypes in the termite-derived xenomicrobiota clustered with clones from termites, whereas those from conventionally raised cockroaches clustered with clones from *S. lateralis*. However, a few OTUs from conventional cockroaches were found also in the termite-derived xenomicrobiota (Fig. 3A), which suggested the presence of closely related lineages of *Dysgonomonas* in phylogenetically distant donors that cannot be resolved using short reads. This is in agreement with the presence of almost identical *Dysgonomonas* phylotypes even in more distantly related hosts (i.e., in the termite *Coptotermes formosanus* and in the beetle *Pachnoda ephippiata*).

While the xenomicrobiota derived from *N. corniger* gut microbiota was dominated by members of *Streptococcaceae*, the xenomicrobiota derived from the mouse gut inoculum showed a predominance of *Parabacteroides* (*Porphyromonadaceae*), which accounted for 61% and 11% of the respective communities 1 week and 4 weeks after inoculation (Fig. 2). Although *Parabacteroides* lineages were present also in conventionally raised cockroaches, phylogenetic analysis of the short reads again indicated the presence of distinct phylotypes in mouse and cockroach guts (Fig. 3B). Those from the mouse gut were most closely related to *Parabac-*

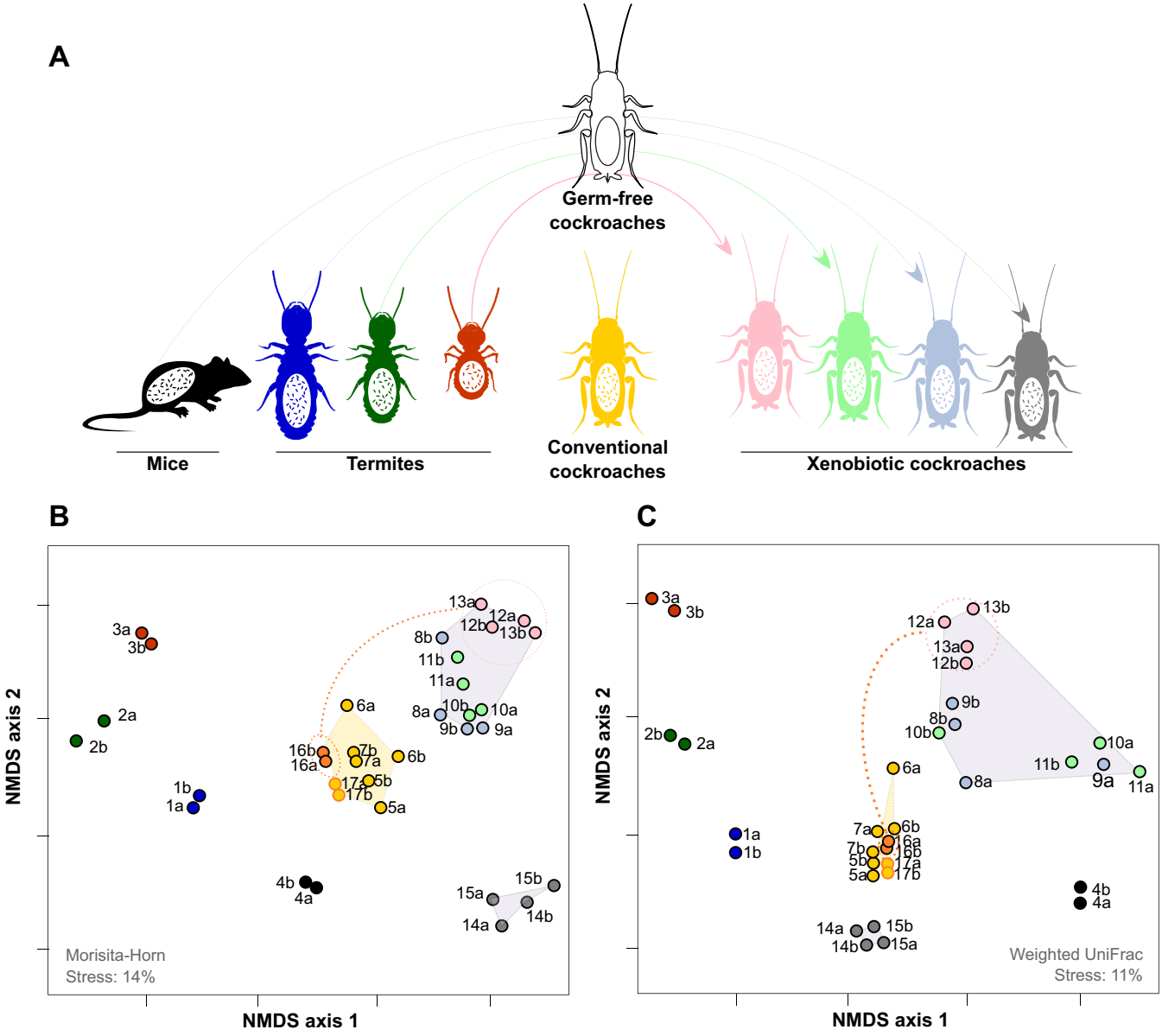

FIG 1 Inoculation of germ-free cockroaches with foreign gut microbiota (A) and nonmetric multidimensional scaling analysis of community structure using Morisita-Horn (B) and Weighted UniFrac (C) metrics. The color code indicates the communities of donors (saturated colors) and xenobiotic cockroaches (lighter colors); orange dots with black borders in panels B and C represent xenobiotic cockroaches inoculated with *Nasutitermes corniger* gut microbiota (pink dots) and then 1 week later exposed to conventional adults (yellow dots with orange borders) for 4 weeks (connected by a dotted line). For additional sample information, see Table 1.

teroides johnsonii and clustered with clones from mammalian guts, while those from the cockroach gut clustered with clones from *S. lateralis*.

Conventionalization of xenobiotic cockroaches. When xenobiotic cockroaches inoculated with the gut microbiota of *N. corniger* were removed from their axenic conditions and challenged by exposure to conventional adults for 6 weeks, they acquired a gut microbiota that clustered tightly with that of conventional cockroaches in both ordination analyses (Fig. 1). The taxonomic classification indicated that this shift was caused mostly by the loss of *Streptococcaceae* and the gain of several bacterial lineages that were typical of conventional cockroaches, such as members of the genera *Bacteroides*, *Parabacteroides*, *Dysgonomonas*, and *Fusobacterium* and those of the *Alistipes* complex. Notably, the uptake of cockroach-specific phylotypes was not limited to bacterial taxa that are characteristically abundant in cockroaches but also included rare members of the community, such as the members of the "insect cluster" of *Fibrobacteres* (Fig. 2). Although the sequences representing this cluster obtained from the conventionalized roaches could not be classified beyond the class level because of the confidence threshold employed by the RDP classifier, the phylogenetic neighborhood of the phylotypes confirmed them to be members of cockroach cluster IIb (Fig. 3C).

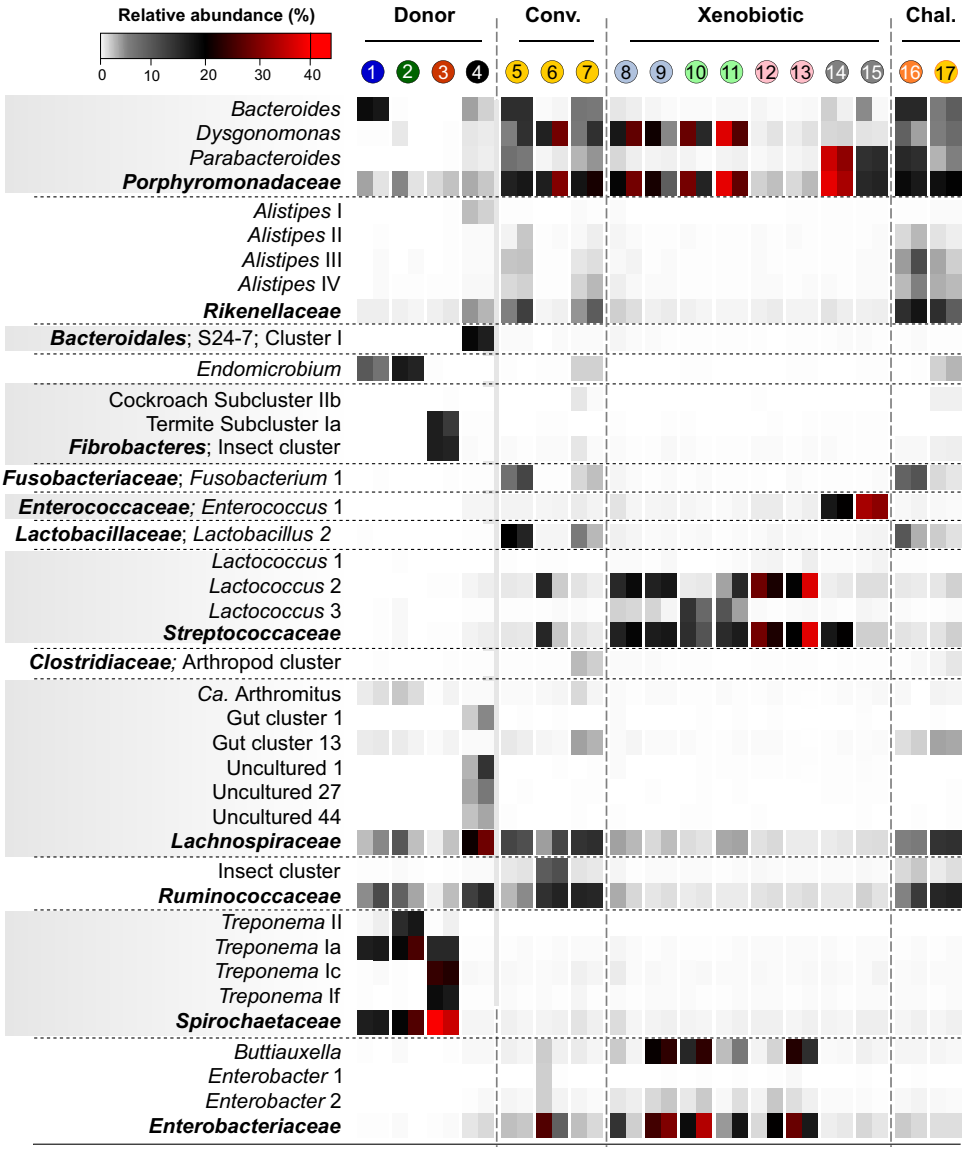

FIG 2 Distribution and relative abundance of important genus level and family level taxa in the gut microbiota of donor organisms and cockroaches (Conv., conventional; Chal., xenobiotic nymphs challenged with conventional adults). The numbers indicated above the heatmap correspond to the samples in Table 1; results of replicate samples (a, b) are always next to each other. For additional sample information, see Table 1.

DISCUSSION

Our study demonstrates that the cockroach gut habitat preferentially selects particular bacterial lineages from the gut microbiota of a phylogenetically diverse range of hosts. The assembly of these xenobiotic communities is deterministic, and certain bacterial lineages, irrespective of their origin, colonize the gut habitat more successfully than others. These results extend the findings of Seedorf and colleagues (31), who observed a similar influence of the gut environment on the selection of bacterial lineages in mice, and show that the guts of insects can be as selective as those of mammals and that community assembly is governed by similar "rules."

Fundamental and realized niches. Although the bacterial lineages in each inoculum in principle have equal opportunities to colonize the germ-free gut, our results show that only a subset of the available lineages successfully establish in this environment. Closely related bacterial lineages that colonize the guts of both cockroaches and termites (5, 6) can do so because they have the similar fundamental niches, i.e., similar ranges of environmental conditions conducive to their colonization without the influence of interspecific interactions (32). However, the relative abundance of these "core" lineages would depend on its "realized" niche (32), i.e., the fraction of the fundamental niche that it occupies in the presence of interactions with other organisms.

The best example illustrating the concept of the realized niche are members of the genus *Dysgonomonas*, which are able to colonize the guts of both termites and cockroaches, which in turn indicates that the fundamental niches of these opportunistic bacteria must be similar. However, members of this genus are considerably more abundant among the normal gut microbiota of *S.*

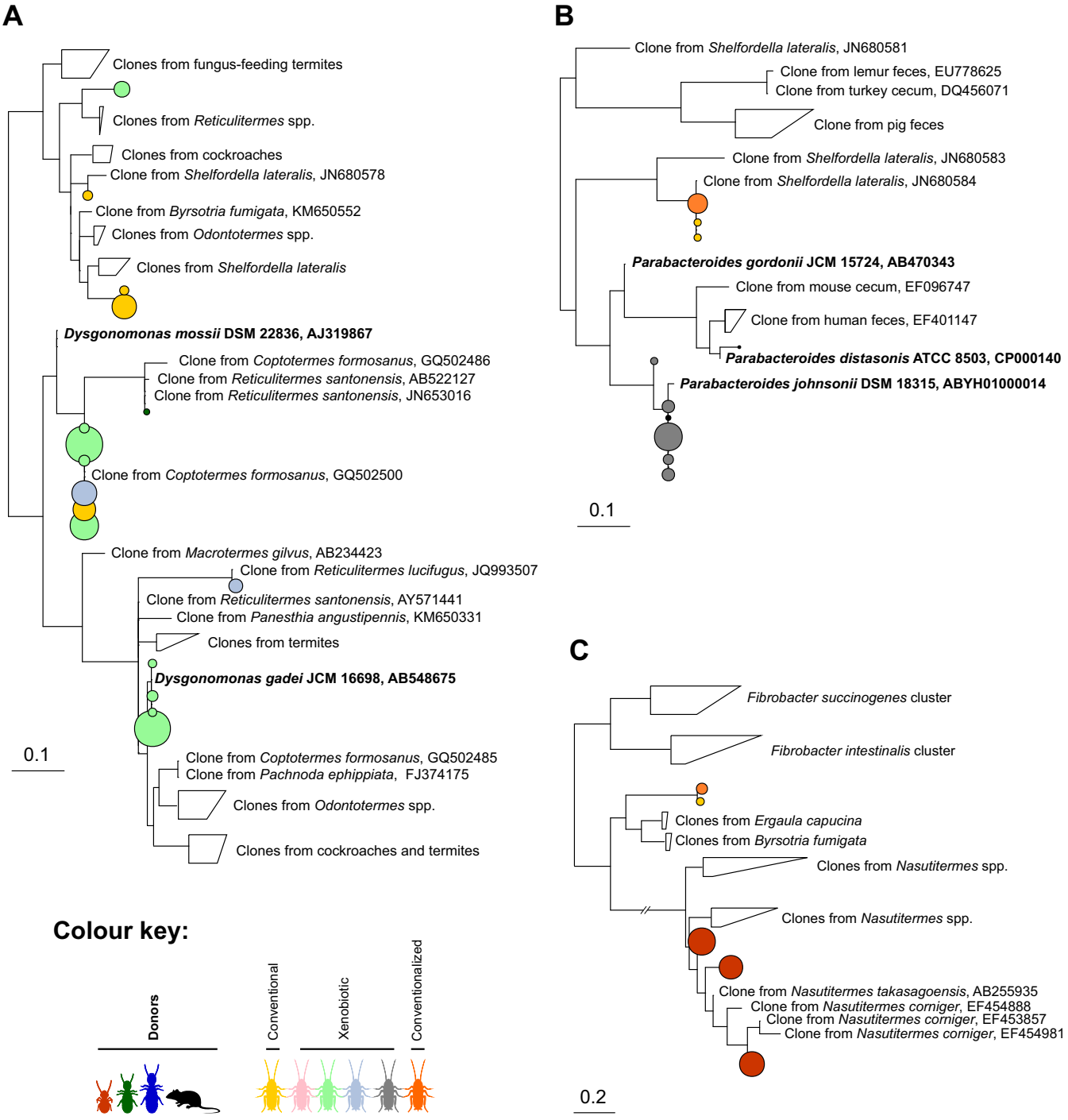

FIG 3 Phylogenetic analysis of representative sequences of the genera *Dysgonomonas* (A), *Parabacteroides* (B), and *Fibrobacteres* (C) obtained from xenobiotic cockroaches and the respective donors. The circle size indicates the relative abundance of each OTU in the respective library; the color code is the same as described for Fig. 1.

lateralis and other cockroaches than in most termites (5, 6, 33; this study), and they are preferentially enriched only in the xenomicrobiota of all cockroaches inoculated with the gut microbiota of lower termites but not with that of the higher termite *N. corniger*, although they are present (but not abundant) in the inoculum. Apparently, the realized niche of *Dysgonomonas* species in the guts of termites and xenobiotic cockroaches is modulated by the interaction with other populations, which can exclude poor competitors even if their fundamental niches overlap. The same argument would explain the dominance of *Parabacteroides* species in mouse-derived xenobiotic cockroaches; these species are extremely rare members of the gut microbiota of the donor.

Habitat specificity. It is important to consider that the niche, whether fundamental or realized, is a property of the organism

and not of the environment. Nevertheless, it is intimately linked with the latter through the habitat, which is the physical environment of an organism, including both biotic and abiotic factors. This interplay between niche and habitat is best illustrated by the example of *Fibrobacteres*, which are considered to play an important role in fiber digestion and are physically associated with the surface of wood particles in the guts of *Nasutitermes* spp. (30). A cockroach-specific clade of *Fibrobacteres* was recently recovered as an abundant member of the gut microbiota in litter-feeding cockroaches (13). Interestingly, despite their low abundance in our data sets, host-specific phylotypes from *S. lateralis* (this study) were found to cluster with these clones, which suggested that even omnivorous cockroaches fed with a fiber-rich diet are colonized by members of *Fibrobacteres*.

Despite the abundance of *Fibrobacteres* in the *N. corniger* inoculum, termite-specific phylotypes of *Fibrobacteres* were unable to colonize the cockroach gut, which indicated that there must be differences in the properties of the lineages colonizing the respective host and the environmental factors in the respective habitats. First, the ability of the termite-specific lineages to realize a niche in the guts of cockroaches probably depends on the fundamental differences in the quality of fiber (wood versus bran). Second, wood-feeding termites show several adaptations in their digestive system that are specifically geared toward making wood particles more accessible to digestion, including an extreme comminution of wood in the foregut and an alkaline pretreatment of the particles in the anterior hindgut; these adaptations are absent from omnivorous cockroaches and may affect bacterial colonization the ability of the cockroaches to digest wood (for a review, see reference 1).

In contrast to *Fibrobacteres*, little is known about the fundamental niche of *Dysgonomonas* species or the influence of environmental factors on their realized niches in cockroach and termite guts. Most cultured representatives of the genus are apparently facultative anaerobes (34–36), and one of the possible factors contributing to their colonization success (over obligate anaerobes) could be the degree of oxygen penetration into the gut. The oxygen partial pressures in the hindgut of young nymphs of *S. lateralis* (9) are higher than in adult cockroaches (5) and termites (e.g., references 12 and 37). Also the *Parabacteroides* lineages that dominated the guts of xenobiotic cockroaches inoculated with mouse gut microbiota may be better adapted to an exposure to oxygen during colonization, which is in agreement with the observation that *Parabacteroides* spp. are the most consistent early colonizers of the gut of germ-free piglets (38).

Competition between allochthonous and autochthonous bacterial lineages. Previous studies with *Drosophila* spp. have shown that alterations to the diet can substantially impact the composition of the gut microbiota (39, 40). In *S. lateralis*, however, even fundamental shifts in the diet resulted in a gut microbiota that—also highly dynamic among different individuals—is quite stable at the family level (33). These contrasting results suggest that in cockroaches, which have a much more complex gut microbiota than *Drosophila* spp., the gut habitat might be playing a stronger role in selecting a taxonomically (and possibly functionally) restricted community. The results of the present study strongly support the important role of the gut habitat in the selection of bacterial lineages in *S. lateralis*, where the allochthonous bacterial lineages in xenobiotic cockroaches were from the same core families or even core genera represented in the microbiota of normal cockroaches (5, 6, 33).

In order to test if autochthonous bacterial lineages encountered in the natural gut microbiota preferentially colonize the gut of *S. lateralis*, we exposed cockroaches harboring a xenomicrobiota to cockroaches with a normal gut microbiota. The results clearly show that cockroach-specific lineages are more competitive in their natural habitat than their foreign relatives, which suggests that autochthonous bacterial lineages have an advantage in colonizing the cockroach gut. A similar phenomenon has been reported also for the bee gut, where a host-specific strain of *Snodgrassella alvi* proved to be more competitive than foreign ones (41). In addition to having a competitive advantage as individual bacterial lineages, the autochthonous microbiota may have a combined competitive advantage together over the foreign microbiota.

Conclusion. This study uses germ-free cockroaches to clearly demonstrate that the assembly of complex gut microbiota in insects can be both predictable and deterministic. While previous analyses of the normal gut microbiota of cockroaches already suggested that the cockroach gut habitat preferentially selects specific bacterial lineages from the environment (10, 33), our study shows, however, that if a preferred lineage is absent from the inoculum, the gut habitat selects other lineages with similar fundamental niches.

In contrast to what occurs in the eusocial termites, where the entire gut community may be transmitted through the exchange of droplets of hindgut fluid between colony mates (proctodeal trophallaxis), the gut microbiota of cockroaches is most likely assembled through environmental inoculation (see reference 7). However, our results clearly demonstrated that this process is not entirely stochastic and that the cockroach gut is far from being an environment that indiscriminately accepts bacterial lineages from the environment. Rather, the gut environment preferentially selects lineages that are specifically adapted to this habitat. The germ-free cockroach model will help provide a better mechanistic understanding of the role played by the cockroach host in determining the structure of its complex gut communities.

ACKNOWLEDGMENTS

This work was supported within the LOEWE program of the State of Hesse (Center for Synthetic Microbiology) and by the Max Planck Society. A.M. received a fellowship from the SYNMIKRO Post-Doc program. C.L.T. received a postdoctoral fellowship of the Alexander von Humboldt Foundation.

We thank Katja Meuser for the excellent technical assistance and Karen A. Brune for linguistic comments on the manuscript.

REFERENCES

1. **Brune A.** 2014. Symbiotic digestion of lignocellulose in termite guts. Nat Rev Microbiol **12:**168–180. http://dx.doi.org/10.1038/nrmicro3182.
2. **Bourguignon T, Lo N, Cameron SL, Šobotník J, Hayashi Y, Shigenobu S, Watanabe D, Roisin Y, Miura T, Evans TA.** 2015. The evolutionary history of termites as inferred from 66 mitochondrial genomes. Mol Biol Evol **32:**406–421. http://dx.doi.org/10.1093/molbev/msu308.
3. **Wong AC-N, Chaston JM, Douglas AE.** 2013. The inconstant gut microbiota of *Drosophila* species revealed by 16S rRNA gene analysis. ISME J **7:**1922–1932. http://dx.doi.org/10.1038/ismej.2013.86.
4. **Martinson VG, Danforth BN, Minckley RL, Rueppell O, Tingek S, Moran NA.** 2011. A simple and distinctive microbiota associated with honey bees and bumble bees. Mol Ecol **20:**619–628. http://dx.doi.org/10.1111/j.1365-294X.2010.04959.x.
5. **Schauer C, Thompson CL, Brune A.** 2012. The bacterial community in the gut of the cockroach *Shelfordella lateralis* reflects the close evolutionary relatedness of cockroaches and termites. Appl Environ Microbiol **78:**2758–2767. http://dx.doi.org/10.1128/AEM.07788-11.

6. **Dietrich C, Köhler T, Brune A.** 2014. The cockroach origin of the termite gut microbiota: patterns in bacterial community structure reflect major evolutionary events. Appl Environ Microbiol **80:**2261–2269. http://dx.doi.org/10.1128/AEM.04206-13.
7. **Brune A, Dietrich C.** 2015. The gut microbiota of termites: digesting the diversity in the light of ecology and evolution. Annu Rev Microbiol **69:**145–166. http://dx.doi.org/10.1146/annurev-micro-092412-155715.
8. **Mikaelyan A, Dietrich C, Köhler T, Poulsen M, Sillam-Dussès D, Brune A.** 2015. Diet is the primary determinant of bacterial community structure in the guts of higher termites. Mol Ecol **24:**5284–5295. http://dx.doi.org/10.1111/mec.13376.
9. **Tegtmeier D, Thompson CL, Schauer C, Brune A.** 4 December 2015. Oxygen affects colonization and metabolic activities of gut bacteria in a gnotobiotic cockroach model. Appl Environ Microbiol http://dx.doi.org/10.1128/AEM.03130-13.
10. **Bauer E, Lampert N, Mikaelyan A, Köhler T, Maekawa K, Brune A.** 2015. Physicochemical conditions, metabolites, and community structure of the bacterial microbiota in the gut of wood-feeding cockroaches (Blaberidae: Panesthiinae). FEMS Microbiol Ecol **91:**1–14. http://dx.doi.org/10.1093/femsec/fiu028.
11. **Li H, Sun J, Zhao J, Deng T, Lu J, Dong Y, Deng W, Mo J.** 2012. Physicochemical conditions and metal ion profiles in the gut of the fungus-growing termite *Odontotermes formosanus*. J Insect Physiol **58:**1368–1375. http://dx.doi.org/10.1016/j.jinsphys.2012.07.012.
12. **Köhler T, Dietrich C, Scheffrahn RH, Brune A.** 2012. High-resolution analysis of gut environment and bacterial microbiota reveals functional compartmentation of the gut in wood-feeding higher termites (*Nasutitermes* spp.). Appl Environ Microbiol **78:**4691–4701. http://dx.doi.org/10.1128/AEM.00683-12.
13. **Mikaelyan A, Köhler T, Lampert N, Rohland J, Boga H, Meuser K, Brune A.** 2015. Classifying the bacterial gut microbiota of termites and cockroaches: a curated phylogenetic reference database (DictDb). Syst Appl Microbiol **38:**472–482. http://dx.doi.org/10.1016/j.syapm.2015.07.004.
14. **Inward DJG, Vogler AP, Eggleton P.** 2007. A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. Mol Phylogenet Evol **44:**953–967. http://dx.doi.org/10.1016/j.ympev.2007.05.014.
15. **Lo N, Bandi C, Watanabe H, Nalepa C, Beninati T.** 2003. Evidence for cocladogenesis between diverse dictyopteran lineages and their intracellular endosymbionts. Mol Biol Evol **20:**907–913. http://dx.doi.org/10.1093/molbev/msg097.
16. **Paul K, Nonoh JO, Mikulski L, Brune A.** 2012. "Methanoplasmatales," Thermoplasmatales-related archaea in termite guts and other environments, are the seventh order of methanogens. Appl Environ Microbiol **78:**8245–8253. http://dx.doi.org/10.1128/AEM.02193-12.
17. **Schloss PD, Gevers D, Westcott SL.** 2011. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. PLoS One **6:**e27310. http://dx.doi.org/10.1371/journal.pone.0027310.
18. **Edgar RC.** 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics **26:**2460–2461. http://dx.doi.org/10.1093/bioinformatics/btq461.
19. **Wang Q, Garrity GM, Tiedje JM, Cole JR.** 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol **73:**5261–5267. http://dx.doi.org/10.1128/AEM.00062-07.
20. **Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF.** 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol **75:**7537–7541. http://dx.doi.org/10.1128/AEM.01541-09.
21. **Otani S, Mikaelyan A, Nobre T, Hansen LH, Koné NA, Sørensen SJ, Aanen DK, Boomsma JJ, Brune A, Poulsen M.** 2014. Identifying the core microbial community in the gut of fungus-growing termites. Mol Ecol **23:**4631–4644. http://dx.doi.org/10.1111/mec.12874.
22. **Oksanen JF, Blanchet G, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson G, Solymos P, Stevens MHH, Wagner H.** 2015. vegan: community ecology package. 2.0-0. R package version 2.3-0. http://CRANR-project.org/package=vegan.
23. **R Core Team.** 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computation, Vienna, Austria. http://www.R-project.org/.
24. **Lozupone CA, Hamady M, Kelley ST, Knight R.** 2007. Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. Appl Environ Microbiol **73:**1576–1585. http://dx.doi.org/10.1128/AEM.01996-06.
25. **Price MN, Dehal PS, Arkin AP.** 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol **26:**1641–1650. http://dx.doi.org/10.1093/molbev/msp077.
26. **Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar Buchner A, Lai T, Steppi S, Jobb G, Förster W, Brettske I, Gerber S, Ginhart AW, Gross O, Grumann S, Hermann S, Jost R, König A, Liss T, Lüßmann R, May M, Nonhoff B, Reichel B, Strehlow R, Stamatakis A, Stuckmann N, Vilbig A, Lenke M, Ludwig T, Bode A, Schleifer K-H.** 2004. ARB: a software environment for sequence data. Nucleic Acids Res **32:**1363–1371. http://dx.doi.org/10.1093/nar/gkh293.
27. **Paradis E, Claude J, Strimmer K.** 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics **20:**289–290. http://dx.doi.org/10.1093/bioinformatics/btg412.
28. **Stingl U, Radek R, Yang H, Brune A.** 2005. "Endomicrobia": cytoplasmic symbionts of termite gut protozoa form a separate phylum of prokaryotes. Appl Environ Microbiol **71:**1473–1479. http://dx.doi.org/10.1128/AEM.71.3.1473-1479.2005.
29. **Ohkuma M, Sato T, Noda S, Ui S, Kudo T, Hongoh Y.** 2007. The candidate phylum "Termite Group 1" of bacteria: phylogenetic diversity, distribution, and endosymbiont members of various gut flagellated protists. FEMS Microbiol Ecol **60:**467–476. http://dx.doi.org/10.1111/j.1574-6941.2007.00311.x.
30. **Mikaelyan A, Strassert JFH, Tokuda G, Brune A.** 2014. The fibre-associated cellulolytic bacterial community in the hindgut of wood-feeding higher termites (*Nasutitermes* spp.). Environ Microbiol **16:**2711–2722. http://dx.doi.org/10.1111/1462-2920.12425.
31. **Seedorf H, Griffin NW, Ridaura VK, Reyes A, Cheng J, Rey FE, Smith MI, Simon GM, Scheffrahn RH, Woebken D, Spormann AM, Van Treuren W, Ursell LK, Pirrung M, Robbins-Pianka A, Cantarel BL, Lombard V, Henrissat B, Knight R, Gordon JI.** 2014. Bacteria from diverse habitats colonize and compete in the mouse gut. Cell **159:**253–266. http://dx.doi.org/10.1016/j.cell.2014.09.008.
32. **Hutchinson GE.** 1957. Concluding remarks. Cold Spring Harbor Symp Quant Biol **22:**415–427. http://dx.doi.org/10.1101/SQB.1957.022.01.039.
33. **Schauer C, Thompson C, Brune A.** 2014. Pyrotag sequencing of the gut microbiota of the cockroach *Shelfordella lateralis* reveals a highly dynamic core but only limited effects of diet on community structure. PLoS One **9:**e85861. http://dx.doi.org/10.1371/journal.pone.0085861.
34. **Hofstad T, Olsen I, Eribe ER, Falsen E, Collins MD, Lawson PA.** 2000. *Dysgonomonas* gen. nov. to accommodate *Dysgonomonas gadei* sp. nov., an organism isolated from a human gall bladder, and *Dysgonomonas capnocytophagoides* (formerly CDC group DF-3). Int J Syst Evol Microbiol **50:**2189–2195. http://dx.doi.org/10.1099/00207713-50-6-2189.
35. **Pramono AK, Sakamoto M, Iino T, Hongoh Y, Ohkuma M.** 2015. *Dysgonomonas termitidis* sp. nov., isolated from the gut of the subterranean termite *Reticulitermes speratus*. Int J Syst Evol Microbiol **65:**681–685. http://dx.doi.org/10.1099/ijs.0.070391-0.
36. **Yang Y, Zhang N, Ji S, Lan X, Zhang K, Shen Y, Li F, Ni J.** 2014. Dysgonomonas macrotermitis sp. nov, isolated from the hindgut of a fungus-growing termite. Int J Syst Evol Microbiol **64:**2956–2961. http://dx.doi.org/10.1099/ijs.0.061739-0.
37. **Brune A, Emerson D, Breznak JA.** 1995. The termite gut microflora as an oxygen sink: microelectrode determination of oxygen and pH gradients in guts of lower and higher termites. Appl Environ Microbiol **61:**2681–2687.
38. **Laycock G, Sait L, Inman C, Lewis M, Smidt H, van Diemen P, Jorgensen F, Stevens M, Bailey M.** 2012. A defined intestinal colonization microbiota for gnotobiotic pigs. Vet Immunol Immunopathol **149:**216–224. http://dx.doi.org/10.1016/j.vetimm.2012.07.004.
39. **Staubach F, Baines JF, Künzel S, Bik EM, Petrov DA.** 2013. Host species and environmental effects on bacterial communities associated with *Drosophila* in the laboratory and in the natural environment. PLoS One **8:**e70749. http://dx.doi.org/10.1371/journal.pone.0070749.
40. **Chandler JA, Morgan Lang J, Bhatnagar S, Eisen JA, Kopp A.** 2011. Bacterial communities of diverse *Drosophila* species: ecological context of a host-microbe model system. PLoS Genet **7:**e1002272. http://dx.doi.org/10.1371/journal.pgen.1002272.
41. **Kwong WK, Engel P, Koch H, Moran NA.** 2014. Genomics and host specialization of honey bee and bumble bee gut symbionts. Proc Natl Acad Sci U S A **111:**11509–11514. http://dx.doi.org/10.1073/pnas.1405838111.