Methanogens in the Gastro-Intestinal Tract of Animals

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Contents

Abstract Nearly all vertebrates host methanogens in their gastro-intestinal tracts. However, a great fraction of vertebrates emits only traces of methane from their faeces (~1 nmol/g faeces/h) and has no significant amounts of methane in their breath. In contrast, many animals host some 100 times more methanogens in their gastro-intestinal tract and emit methane in their breath. These substantial differences are not caused by different feeding habits; rather a genetic factor controls the presence of large amounts of methanogens. The attribute "methane production" is evolutionarily stable, and the loss of this character obeys Dollo's law: once lost in the course of evolution, this character cannot be acquired another time.

Also invertebrates can host methanogens in their gastro-intestinal tract. In contrast to the vertebrates, only a few taxa of arthropods emit methane: millipedes, termites, cockroaches and scarab beetles. All other arthropods in our study did not emit methane and did not host even traces of methanogens. As in vertebrates, the diet of the animals is not crucial for the presence of methanogens. Again, a genetic factor seems to control the presence or absence of methanogens. Methanogenesis is also a prerequisite for the presence of intestinal anaerobic protozoa with endosymbiotic methanogens, but not for the presence of impressive structural

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differentiations of the hindgut epithelium, which – in methanogenic taxa – host enormous amounts of methanogens.

1 Introduction

Methanogens are the dominating archaeal organisms and they possess a great phylogenetic and ecological diversity (Woese et al. 1990; Liu and Whitman 2008). They occupy a broad spectrum of ecological niches, including the cytoplasm of unicellular anaerobic eukaryotes and the gastro-intestinal tract of various animals (Lange et al. 2005). Most methanogens have not been cultured yet, and their diversity can only be deduced from the analysis of their 16S rRNA genes. Methanogens in complex anaerobic environments are frequently involved in interspecies hydrogen transfer thereby improving fermentations and electron transfer in syntrophic communities of bacteria and archaea (Schink 1997; Stams and Plugge 2009; Worm et al. 2010). Earlier studies have suggested that the endosymbiotic methanogens of protozoa occur only in protists with hydrogenosomes, i.e. hydrogen-producing organelles of mitochondrial descent (Fenchel and Finlay 1995; Hackstein et al. 2006a; Hackstein and Tielens 2010). On the other hand, hydrogenosomes are not the same, and methanogens are not always present in protists with hydrogenosomes; sometimes, endosymbiotic methanogens are present in protists without hydrogenosomes (Fenchel and Finlay 2010; Hackstein and Tielens 2010). It has been shown that methanogens in protists exhibit a certain host specificity (Fenchel and Finlay 1995), but on the other hand, endosymbiont replacements seem to be possible, especially in evolutionary timescales (van Hoek et al. 2000). Notably, methanogens in the gastro-intestinal tracts of animals are not found everywhere; in some animals methanogens are abundant, in others they are only of very low abundance or even completely absent. In this chapter, we will discuss the elusive distribution of methanogens in the gastro-intestinal tract of vertebrates and arthropods.

2 Vertebrates

Vertebrates are born (or hatch from the egg) with a sterile gastro-intestinal (GI) tract. Soon after birth or hatching the GI tract becomes colonized by bacteria and archaea. Eventually, after reaching adulthood, vertebrates, and especially mammals, host a very complex and numerous microbiota in their guts (Zoetendal et al. 2006; Liu and Whitman 2008). These microbiota are host-specific and clearly different from free-living bacterial communities (Ley et al. 2008a, b). Virtually all these microbiota include methanogens (Miller and Wolin 1986; Hackstein and van Alen 1996; Hackstein et al. 1996). However, the number of methanogens varies at least by two orders of magnitude between species, with the consequence that the faeces of certain species emit less than 1 nmol/g/h of methane while the faeces of

other species produce much more than 100 nmol/g/h. A systematic analysis of more than 250 species of vertebrates reveals a bimodal distribution of the methane emissions from faeces with about 85 species producing less than 5 nmol/g/h and about 123 producing more than 50 nmol/g/h (Fig. 1; Hackstein and van Alen 1996). Only a few species produce intermediate amounts of methane. If one assumes that one methanogenic archaeon produces approximately 1 fmol methane per hour (10^{-15} mol/h) then one gram of faeces of the low producers could host not more than 10^6 methanogens. Accordingly, high methane producers could host more than 10^8 methanogens in one gram of faeces. This range has been confirmed by enumberation (Miller and Wolin 1982; Doré et al. 1995; El Oufir et al. 1996; Liu and Whitman 2008). These differences have physiological consequences: while the high methane producers emit significant amounts of methane with their breath, the concentrations of methane in the breath of low producers do not exceed the atmospheric background concentration (Hackstein and van Alen 1996). Therefore, we will name the species with faecal emissions of less than 3 nmol/g/h "non-producers" and those with emissions above 20 nmol/g/h "producers".

Methane producers and non-producers are not randomly distributed (Table 1). With a few exceptions, individuals of the same species share their methane status, as well as representatives of closely related species. This raises the question as to whether it is possible to identify the reasons for the presence or absence of high amounts of methanogens in the GI tract. It had been assumed that a plant-based diet rich in fibres provides the basis for the presence of high numbers of methanogens (Miller and Wolin 1986). This is clearly the case in ruminants such as cattle, sheep and goats. These animals, but also hindgut-fermenters such as horse and elephant produce high amounts of methane (Table 1). On the other hand, bamboo-eating pandas do emit only traces of methane, but vegetarian chiropters do not produce methane. In contrast, carnivorous crocodiles, giant snakes and ant-eating species such as the great ant-eater, the tamandua and the aardvark release large amounts of

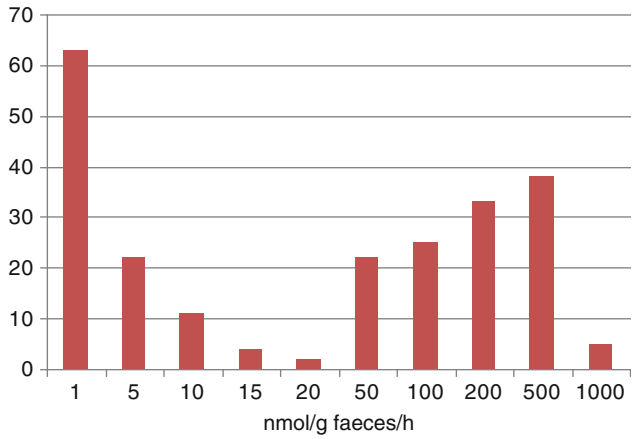

Fig. 1 Histogram of the mean methane emission rates (nmol/g faeces/h, abscissa) by the faeces of 225 amniotes. Ordinate: number of species

Table 1 Methane production in vertebrates

	No. of species	Methane producer	Non-producer	Mean methane production producer/non-producer[a]
Xenopus laevis	1	1	–	Methane in breath
Emydidae	1	1		Methane in breath
Testudinae	4	4	–	30–109/–
Caiman crocodilus	1	1	–	181/–
Iguanidae	3	2	1	275–322/5
Pythonidae	3	3	–	59/–
Ratites	8	4	4	93–414/0.2–0.7
Anser anser	1	1	–	79/–
Anas platyrhynch.	1	1	–	70/–
Galli	2	2	–	102–142/–
Columba livia	1	–	1	–/0.3
Passer domesticus	1	–	1	–/0.7
Strigiformes	3	–	3	–/0.2–0.5
Tachyglossus	1	1	–	44/–
Marsupialia	9	8	1	6–499/0.03
Choleopinae	2	2	–	7–290/–
Myrmecophagidae	2	2	–	134–208
Tenrecidae	2	–	2	–/0.1–0.4
Erinaceinae	2	–	2	–/0.2–0.8
Sorex spec.	1	–	1	No methane in breath
Talpa europaea	1	–	1	No methane in breath
Tupaia bergeri	1	–	1	–/1
Chiroptera	4	–	4	–/1–2
Macroscrelides	1	–	1	–/0.2
Cheirogaleus med.	1	1	–	5[b]/–
Lemuridae	10	10	–	7–505/–
Loridae	3	3	–	11–79/–
Galagonidae	2	1	1	4/3
Alouatta caraya	1	1	–	73/–
Aotinae	3	3	–	113–373/–
Atelinae	3	3	–	155–347/–
Cebinae	2	–	2	–/0.2–0.4
Callimico goeldii	1	–	1	–/1
Pitheciinae	2	2	–	129–433/–
Callithricidae	10	4	6	7–77/0.5–3
Cercopithecinae	11	11	–	31–530/–
Colobinae	6	6	–	219–459/–
Hylobatidae	4	4	–	234–433/–
Hominidae	4	4	–	135–417/–
Canidae	4	–	4	–/0.1–4
Felix silvestris	1	–	1	–/0.1
Procyon lotor	1	–	1	–/0.3
Ailurinae	4	–	4	–/0.2–2
Ursinae	6	–	6	–/0.3–3
Viveridae	4	–	4	–/0.1–1
Delphinapterus l.	1	–	1	No methane in breath
Tursiops truncatus	1	–	1	No methane in breath
Trichecus manatus	1	1	–	51/–

(*continued*)

Table 1 (continued)

	No. of species	Methane producer	Non-producer	Mean methane production producer/non-producer[a]
Proboscidea	2	2	–	9–41/–
Equidae	2	2	–	30–118/–
Tapiridae	2	2	–	66–311/–
Rhinoceros unicor.	1	1	–	8/–
Procavia capensis	1	1	–	257/–
Orycteropus afer	1	1	–	15/–
Suidae	3	3	–	30–68/–
Tayassu tajacu	1	1	–	329/–
Choeropsis liber.	1	1	–	76/–
Camelidae	3	3	–	73–121/–
Giraffidae	2	2	–	21–41
Ovibos moschatus	1	1	–	72/–
Cervinae	5	5	–	69–423/–
Odocoileinae	3	3	–	53–138
Alces alces	1	1	–	110/–
Cephalophus mon.	1	1	–	29/–
Tragelaphinae	3	3	–	66–435/–
Bovinae	2	2	–	116–226/–
Caprinae	6	6	–	21–4,230/–
Reduncinae	2	2	–	14–59/–
Manis tricuspis	1	–	1	–/0.2
Sciuridae	17	7	10	8–142/0.01–4
Castor fiber	1	–	1	–/1
Jaculus jaculus	1	–	1	–/0.3
Cricetinae	8	2	6	9/0.3–2
Cricetomys gamb.	1	1	–	100/–
Gerbellinae	2	–	2	–/0.1–0.4
Murinae	7	1	6	26/0.1–2
Myoxidae	2	–	2	–/0.1–2
Graphiurus murin.	1	-	1	–/0.6
Hystricidae	3	3	–	29–108/–
Thryonomys swin.	1	1	–	3[b]/–
Erethizontidae	2	2	–	90–583/–
Chinchilla laniger	1	1	–	128/–
Caviinae	4	4	–	7–237/–
Dolichotis patago.	1	1	–	25/–
Hydrochorus hyd.	1	1	–	311/–
Dasyproctidae	3	3	–	87–176/–
Octodontidae	2	2	–	2[b]–8/–
Capromys pilorid.	1	1	–	28/–
Myocastor coypus	1	1	–	440/–
Lagomorpha	3	3	–	4[b]–42/–

For a more extended version of this Table see Hackstein and van Alen (1996)

[a]Range of the mean emissions of producers/mean emissions of non-producers as nmol methane/g faeces/h

[b]Classified as methane producer on the basis of their maximal emissions

methane from their faeces; armadillos emit low, but still significant concentrations of methane. Only one species of ant-eating animals, the pangolin, is a non-producer (Table 1). Therefore, a plant-based, fibre-rich diet cannot be the primary reason for the presence of large numbers of methanogens. Also the presence of a highly differentiated GI tract does not necessarily predispose for methane production: while African and South American ostriches do produce methane, their Australian/New Zealandian relatives emu and cassowary, which possess a GI tract of similar complexity and use a comparable diet, are non-producers (Table 1; Fig. 2). Also dolphins and whales, which possess complex foregut differentiations, do not produce methane (Table 1). Thus, neither the presence of a highly differentiated GI tract nor a fibre-rich diet predispose automatically for the presence of high numbers of methanogens.

The just-mentioned example of the methane-producing and non-producing ostriches provides evidence for the intrinsic reasons for the presence/absence of methanogens. A phylogenetic tree of the ostriches based on the mitochondrial 12S rRNA genes (Fig. 2) reveals that these birds are monophyletic. The methane-emitting African/South American ostriches occupy a basal position in the phylogenetic tree, while the non-producing Australian/New Zealandian ostriches and ratites are found in the top of the tree. The latter ostriches and ratites share a recent common ancestor, and this ancestor obviously lost the property to host methanogens, a property that is shared by all its descendents. This argues that a heritable, genetic property provides the basis for the presence of large numbers of methanogens in the GI tract. Also the study of South American apes supports this interpretation. While all old-world apes and monkeys are methane producers, the methane status of the New World apes can differ by species, even by subspecies. For example, the marmoset *Leontopithecus rosalia rosalia* produces only 1 nmol/g/h methane, whereas the closely related *Leontopithecus rosalia chrysomelas* is a producer of some 70–500 nmol/g/h faecal methane. Among the Callitricidae (marmosets and tamarins), which include the two *Leontopithecus* species, four species

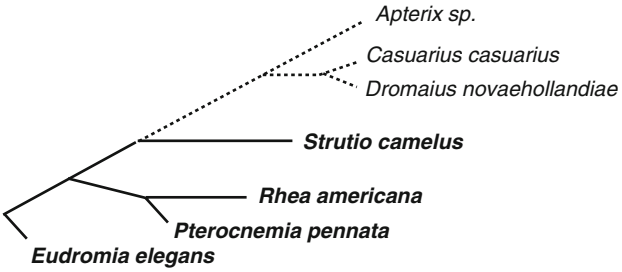

Fig. 2 Evolution of ratites: Faeces of emu (*Dromaius novaehollandiae*), cassowary (*Casuarius casuarius*), and kiwi (*Apteryx* sp.) do not emit significant amounts of methane (max. 2 nmol/g/h). Faeces of ostrich (*Strutio camelus*), nandu (*Rhea americana*), Darwin nandu (*Pterocnemia pennata*), and tinamou (*Eudromia elegans*) produce between 137 and 414 nmol/g/h methane. The tree is based on mitochondrial 12S rDNA sequence data of Cooper et al. (1992). Redrawn after Hackstein and van Alen 1996

are producers and six species are non-producers in our screen (Table 1; Hackstein and van Alen 1996). Moreover, seven species of monkeys belonging to the Cebidae produce large amounts of methane through their faeces, while two *Cebus* species emit only traces. This means that the property "methane production" can be lost at the species level in the absence of any significant differences in diet or other physiological parameters.

Also, among the Rodentia the character "methane production" can be lost at the species, subspecies, or even at the population level. For example, 7 of the 17 species of sciurids studied produced methane while ten did not (Table 1). The species *Sciurus vulgaris* was identified as methane producer, but a highly inbred population of ten individuals did not produce methane. Since differences in the diet can be excluded, a dietary basis for the character "methane production" can be excluded.

This holds also true for the Muridae, where non-producers predominate. From the nine species of Critecinae and Cricetomyinae studied, only three species (golden hamster, muskrat and giant pouched rat) emitted methane. From the seven species belonging to the Murinae, only one, *Leopoldamys sabanus*, produced methane (Table 1). Recently, it has been shown that also certain strains of laboratory rat are methanogenic while other strains are non-methanogenic (Florin et al. 2000). In clear contrast, all species of carnivores, chiropters and insectivores did not produce methane irrespective of their diet, whereas all artiodactyls and perissodactyls studied produced methane. Also all caviomorphs, hystricomorphs and lagomorphs tested produced methane (Table 1).

Thus, a direct correlation between diet and methane production can be excluded, and an alternative explanation for the significant differences found among the species examined seems difficult. Notably, a lack of infection by methanogens in "non-producers" can also be excluded since virtually all species tested produce at least traces of methane indicating the presence of methanogens (Table 1). However, if we incorporate the property "methane producer"/"non-producer" in a phylogenetic tree that is based on the analysis of mammalian protein sequences (Miyamoto and Goodman 1986), a rationale for the phenomenon "methane production" becomes evident (Fig. 3). Non-producers cluster as whole branches, or they are found in terminal positions of the tree. In other words, methane production is a primitive-shared (plesiomorphic) character, while the loss of methane production is a shared-derived (synapomorphic) character. The loss of methane production clearly obeys Dollo's rule: once lost in the course of evolution, the competence for methanogenesis cannot be restored. This holds also true for those losses at lower taxonomic levels (e.g. species level) that could not be included into the phylogenetic tree. The integration of the methane data into other phylogenetic trees, for example the "classical" tree of Novacek (1992) or the molecular trees of Li et al. (1990) or of Janke et al. (1994) does not lead to a different interpretation. Consequently, the loss of the competence to host large numbers of methanogens is an evolutionary stable character that must have a heritable, genetic basis. This can also explain the loss of methanogenesis in populations where the Hardy-Weinberg equation describes the distribution of genetic characters. Evidence for the presence of producers and non-producers in a species have been described here for the species *S. vulgaris* (Table 1),

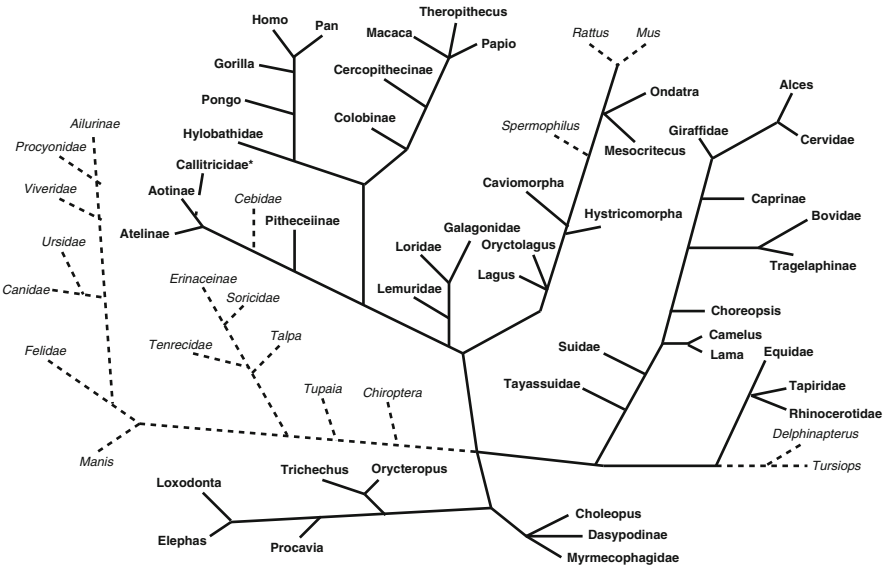

Fig. 3 Integration of information about methane production into the protein sequence based phylogenetic tree of Miyamoto and Goodman (1986). Producers: *roman*, non-producers: *italics*; *asterisk* only four out of the ten species are methane producers. Redrawn after Hackstein and van Alen (1996)

and are also well known for strains of laboratory rats and notably, human populations (Miller and Wolin 1982; Segal et al. 1988; Brusa et al. 1993; Hudson et al. 1993; Doré et al. 1995; Florin et al. 2000; Levitt et al. 2006).

At the moment, one can only speculate about the physiological or biochemical basis for the presence of large amounts of methanogens in the guts and faeces of many animals and their significantly lower number in certain other species. Different levels of bile acids have been assumed as modulators of the methane production (Florin and Jabbar 1994), but a general function of bile acids as a physiological control of methanogenesis seems unlikely. The presence of a receptor for methanogenic archaea or some other adherence mechanism could potentially explain the prolonged persistence of high numbers of methanogens in the gut. In the absence of the receptor-mediated adhesion to the gut wall, methanogens are easily removed from the G-I tract in the course of digestion, compensated only by high division rates of the methanogens. This might allow maintaining only titres of methanogens that are 30–100 times lowerthan in species with an adhesion mechanism for methanogens.

It is surprising that so many animals host high numbers of methanogens that cause a significant emission of methane. Most of these methanogenic animals possess “alloenzymatic” intestines that depend in their function on the presence of complex symbiotic microbial associations (Langer 1988, 1991, 1994; Langer and Snipes 1991). Alloenzymatic intestines are characterized by well developed hindguts and caeca and also by the evolution of rumina and other forestomach fermenting organs without rumination. Recently, the analysis of the microbiomes of the

various animals has provided evidence for the existence of characteristic "foregut" and "hindgut" microbiota (Ley et al. 2008b) supporting the anatomical studies. Notably, there is evidence that the postnatal development of these differentiations depends on the presence of effective microbial fermentations, in particular the presence of certain fermentation products such as propionate and butyrate (Jesse et al. 1994). In contrast, "autoenzymatic" intestines do not have the need for microbial symbionts for their digestions, and consequently, they do not possess the differentiations that are characteristic for the alloenzymatic guts. However, they host specific microbial communities that are characteristic for "simple" GI tracts (Ley et al. 2008b). Most of these intestines are found among the animals that belong to the category of "non-producers" of methane. This does not mean that monogastric, autoenzymatic intestines are devoid of any significant microbiota. Our measurements have shown that virtually all of the non-producers of methane emit significant amounts of hydrogen (Hackstein and van Alen 1996), which is indicative of intensive microbial fermentations. But as mentioned above, these fermentations do not contribute to the digestion of resistant biopolymers, and they do not induce any of the intestinal differentiations that are found in the methanogenic animals with alloenzymatic digestion. Even a fibre-rich diet of a non-methanogenic animal does not correlate with any alloenzymatic differentiation of the G-I tract. The best example is the giant panda, which relies completely on a bamboo diet but does not possess any fermentative intestinal differentiation. His gut microbiome classifies his GI tract as belonging to the "simple" type (Ley et al. 2008b). Notably, evolution allowed the development of panda's famous additional thumb, but not of a well-developed caecum or colon. On the other hand, the leaf-eating, methanogenic colobid monkeys evolved a foregut fermenting structure similar to the rumen of the ruminants with a microbiome that resembles that of ruminants (Ley et al. 2008a, b).

A secondary loss of methanogenesis is possible in alloenzymatic animals with foregut differentiations, e.g. dolphins and whales (Fig. 3), but also in animals with hindgut differentiations, e.g. many murids and certain New World monkeys and apes. Monogastric, autoenzymatic animals are primarily non-methanogenic (Fig. 3). The correlation between the presence of high numbers of methanogens and the presence of intestinal differentiations is striking and for sure not accidental. Obviously, methanogens fulfil a crucial role in intestinal fermentations that allow the digestion of plant polymers (Schink 1997; Stams and Plugge 2009). It is likely that this role cannot be taken over by other hydrogen-consuming bacteria. Notably, the presence of high numbers of methanogens must be controlled by one or several genetic factors, since the evolutionary loss of the capacity to host high numbers of methanogens cannot be restored. Also the population-specific distributions of methane producers and non-producers in human populations are indicative of a genetic basis (Segal et al. 1988; Hudson et al. 1993; Brusa et al. 1993; Levitt et al. 2006). Furthermore, the analysis of the trait "methane production" in pedigrees reveals inheritance patterns that are compatible with the interpretation of an autosomal dominant inheritance (Hackstein et al. 1995). Twin studies that rejected a genetic influence on the methane status of humans might be erroneous due to a

statistical analysis that seems not suitable for the detection of different classes of methane producers/non-producers (Florin et al. 2000). It has also been discussed above that a lack of proper infection with methanogens can be excluded for the explanation of the bimodal distribution of producers and non-producers, since also non-producers exhibit a low level of methanogenesis in their faeces. Thus, the presence of high numbers of methanogens in the G-I tract of vertebrates is still elusive, but obviously under the control of one or several genetic factors. It is for sure not the consequence of particular dietary habits or the presence of intestinal differentiations.

3 Arthropods

Arthropods represent by far the largest global biodiversity of all multicellular animals. Despite their small size and the tiny volumes of their intestinal tracts many arthropods host a complex microbiota in their guts (Bayon 1980; Hackstein and Stumm 1994; Cazemier et al. 1997; Hackstein 1997; Egert et al. 2003; Brune 2006; Hackstein et al. 2006b; Warnecke et al. 2007). Already in 1953, Paul Buchner in his seminal monograph (Buchner 1953) described the fascinating world of symbiotic associations between arthropods and bacteria. He emphasized not only the enormous diversity of differentiations of the intestinal tract, but also the more direct associations between bacteria and their hosts involving specialized tissues and organs (e.g. "bacteriomes"). There were a lot of speculations about the contribution of the symbionts to the host's nutrition, but only recently the progress in molecular biological techniques and bioinformatics allowed unravelling of the molecular basis of some of these symbiotic associations (Moran 2003, 2007; Moran and Baumann 2000; Hoffmeister and Martin 2003; Canback et al. 2004; Dillon and Dillon 2004; Dale and Moran 2006; Moya et al. 2008; Ruby 2008). In remarkable contrast to the situation in vertebrates, the role of methanogenic archaea is very limited in the arthropod world. While nearly all vertebrates host at least traces of methanogens, the vast majority of the arthropod taxa is completely devoid of methanogens. In principle, the detection of methanogens in the GI tract of arthropods is easy, since arthropods exhale intestine-born methane with their breath (Bijnen et al. 1996). Due to their small size, the methane production of arthropods can be measured non-invasively by incubating the intact specimen in stoppered glass vials. With a standard gas chromatograph, it is possible to detect sub-nanomolar concentrations of methane after the prolonged incubation of individual or several specimens. In this way, the presence of less than 10^6 methanogens in the GI tract of a single arthropod can be detected.

In a first experiment, we screened more than 110 representatives of 35 higher taxa of terrestrial arthropods for methane and hydrogen emissions (Hackstein and Stumm 1994; Table 2). In a second experiment, we analysed some 70 strains of cockroaches representing 44 different species (Hackstein 1997; Hackstein et al. 2006b; Table 3). To confirm the presence or absence of methanogens in the GI

Table 2 Methane and hydrogen production in invertebrates

	Common name	Methane	Hydrogen	Protists
Araneae	Spiders			
Araneus diadematus[a] (A)		−	+	−
Acari	Mites and ticks			
Boophilus microplus		−	−	−
Isopoda	Sawbugs			
Oniscus asellus[a] (A)		−	−	−
Porcellio scaber (A)		−	−	−
Chilopoda	Centipedes			
Lithobius forficatus (A)		−	−	−
Diplopoda	Millipedes			
Chicobolus sp. (J)		+	+	−
Mestosoma hylaeicum (A)		−	−	−
Orthoporus sp. (J)		+	+	C
Pycnotropis acuticollis (A)		+	+	nd
Rhapidostreptus virgator (A)		+	+	C
Unidentified A (J)		+	+	−
Unidentified B (J)		+	+	−
Unidentified D (J)		+	+	C
Unidentified K (J)		+	+	C
Glomeris sp.[a] (A)		−	+	−
Julus sp.[a] (A)		−	−	−
Polydesmus sp.[a] (A)		−	−	−
Tachypodojulus niger[a] (A)		−	−	−
Thysanura	Bristletails			
Lepisma saccharina (A)		−	−	−
Collembola	Springtails			
Folsmia candida (J, A)		−	−	−
Acrididae	Short-horned grashopper			
Locusta migratoria (A)		−	−	−
Schistocerca gregaria (A)		−	−	−
Unidentified[a] (A)		−	−	−
Gryllidae	Crickets			
Achaeta domesticus (A)		−	−	−
Decticus sp.[a] (A)		−	−	−
Gryllus bimaculatus (A)		−	−	−
Ventralla quadrata (A)		−	−	−
Phasmidae	Stick and leaf insects			
Eurycantha calcerata (A)		−	−	−
Pharnacia acanthopus (A)		−	−	−
Sipyloidea sipylus (A)		−	−	−
Mantidae	Mantids			
Hierodula membranacea (A)		−	−	−
Blattidae	Cockroaches			
Blaberus craniifer (A)		+	+	C
Blaberus fuscus (L, A)		+	−	C
Blaberus giganteus (L)		+	+	C
Blatta orientalis (A)		+	+	−
Blatella germanica (A)		+	+	−
Ectobius sp.[a] (A)		−	−	−
Gromphodorhina port. (L; A)		+	+	C
Leucophaea sp. (A)		+	+	−

(*continued*)

Table 2 (continued)

	Common name	Methane	Hydrogen	Protists
Panchlora nivea (A)		−	−	−
Periplaneta americana (L, A)		+	−	C
Periplaneta australasia (L, A)		+	+	C
Pycnoscelus suriname. (L, A)		+	+	C
Supella supellectilium (L, A)		+	−	F
Isoptera	Termites			
Cryptotermes brevis (A)		+	−	nd
Heterotermes indicola (A)		+	−	F
Mastotermes darwiniensis (A)		+	−	F
Reticulotermes santonen. (A)		+	−	F
Dermaptera	Earwigs			
Forficula auricularia[a] (A)		−	−	−
Heteroptera	Bugs			
Dysdercus intermedius (L, A)		−	+	−
Oncopeltus fasciatus (L, A)		−	−	−
Platymerus biguttata (A)		−	−	−
Pyrrhocoris apterus (L, A)		−	−	−
Cicadoidea	Cicadas			
Nephotettis cincticeps (A)		−	−	−
Aphididae				
Aphis fabae (L, A)		−	−	−
Apidae				
Apis mellifera (A)		−	−	−
Carabidae	Ground beetles			
Carabus sp.[a] (A)		−	−	nd
Pterostichus niger[a] (A)		−	−	nd
Silphidae	Carrion beetles			
Necrophorus vespillo[a] (A)		−	−	nd
Dermestidae	Dermestid beetles			
Dermestes frischi (A)		−	−	nd
Tenebrionidae	Darkling beetles			
Oryzaephilus sp. (L, A)		−	−	nd
Scarus tristis (L)		−	−	nd
Tenebrio sp. (L)		−	−	nd
Tribolium confusum (L, A)		−	−	nd
Zophobas morio (L, A)		−	−	nd
Cryptophagidae	Silken fungus beetles			
Alphitobius diapecur. (L, A)		−	−	nd
Bostrychidae	Branch and twig borers			
Acanthocelides panac. (L, A)		−	−	nd
Rhizopertha dominica (L, A)		−	−	nd
Sitophilus graminarius (L, A)		−	−	nd
Anobiidea	Death-watch beetles			
Anobium punctatum (L)		−	−	nd
Oligomerus ptilinoides (L)		−	−	nd
Ptilinus pectinicorni (L)		−	−	nd
Stegobium panaceum (A)		−	−	nd
Xestobium rufovillosum (L)		−	−	nd
Lyctidae	Powder-post beetles			
Luctus africanus (L)		−	−	nd
Luctus brunneus (L)		−	−	nd

(*continued*)

Table 2 (continued)

	Common name	Methane	Hydrogen	Protists
Minthea rugicollis (L)		−	−	nd
Dynastinae	Rhinocer beetles			
Dynastes hercules (L)		+	+	F
Cetoniidae	Rose chafers			
Cetonia aurata (L)		+	+	F
Dicronorrhina micans (L)		+	+	F
Eudicella gralli (L, A)		+	+	F
Eudicella smittii (L)		+	+	F
Pachnoda bhutana (L, A)		+	+	F
Pachnoda ephippuata (L)		+	+	F
Pachnoda marginata (L)		+	−	F
Pachnoda nachtigalli (L, A)		+	−	F
Pachnoda savignyi (L, A)		+	−	F
Potassia cuprea (L, A)		+	+	F
Phyllopertha horticola[a] (A)		−	−	−
Geotrupinae	Dung beetles			
Geotrupes sp.[a] (A)		−	+	nd
Geotrupes sp[a] (A)		−	−	nd
Cerambycidae	Longhorn beetles			
Hylotrupes bajulus (L)		−	−	−
Chrysomelidae	Leaf beetles			
Crioceris asparag[a] (A)		−	−	nd
Diabrotica baltea (A)		−	−	nd
Leptinotarsa decemlinea. (A)		−	+	nd
Phaedon cochleariae (L, A)		−	+	nd
Cucurlionidae	Weevils			
Otiorrhynchus sulcatus (A)		−	+	nd
Lepidoptera	Butterflies and moths			
Aphomia sociella[a] (L)		−	+	−
Bombyx mori (L)		−	−	nd
Caligo memnon (L)		−	−	nd
Danaus plexippus (L)		−	+	nd
Ephestia kühniella (L)		−	−	nd
Galleria mellonella[a] (L, A)		−	−	−
Heliotis virescens		−	−	nd
Pieris brassicae[a] (L)		−	−	nd
Plutella xylostella (L)		−	−	nd
Spodoptera frugiperda (L)		−	−	nd
Trabala vishnou (L)		−	−	nd
Diptera	Flies			
Hylemyia antiqua (L)		−	−	−
Musca domestica (P, A)		−	−	−
Tipula sp.[a] (L)		−	+	−
Siphonaptera	Fleas			
Ctenocephalides felis (L).		−	−	nd

nd not determined, *C* ciliates, *F* flagellates, *L* larva, *A* adult
[a]Endemic European species from the field

Table 3 Methane emission in cockroaches

Species	Methane emissions	Hindgut differentiation	Protists in hindgut
Blattoidea			
Blattinae			
Blatta orientalis	+	+	C
Deropeltis sp.	+	+	C
Periplaneta americana	+	+	C
Periplaneta australasiae	+	+	C
Periplaneta brunnea	+	+	C
Periplaneta fulginosa	+	+	C
Polyzosteriinae			
Eurycotis floridana	+	+	C
Blaberoidea			
Polyphagidae			
Polyphaginae			
Polyphaga aegyptiaca	+	+	C
Blattellidae			
Plectopterinae			
Eudromiella sp. (Costa Rica)	−	−	−
Lupparia sp. (Luzon, Philippines)	−	−	−
Supella longipalpa	−	−	−
Supella supellectilium	+	+	F
Blattellinae			
Blattella germanica	+ and −	+/−	−
Ischnoptera sp.	nd	−	−
Loboptera decipiens	nd	−	−
Parcoblatta lata	+	nd	nd
Shawella couloniana	−	−	−
Symplece pallens	−	nd	nd
Ectobiinae			
Ectobius sylvestris	−	−	−
Ectobius sp.	−	−	−
Nyctiborinae			
Nyctibora sp. *(Costa Rica)*	+	+	−
Blaberidae			
Blaberoid complex			
Zetoborinae			
Schultesia lampyridiformis	+	+	−
Blaberinae			
Archimandrita sp.	+	+	−
Blaberus craniifer	+	nd	nd
Blaberus fuscus	+	+	C
Blaberus discoidalis	+	+	C
Blaberus giganteus	+	+	C
Blaberus sp. *CR*	+	nd	nd
Byrsotria fumigata	+	+	C
Eublaberus distanti	+	+	−

(*continued*)

Table 3 (continued)

Species	Methane emissions	Hindgut differentiation	Protists in hindgut
Eublaberus posticus	+	+	−
Blaptica sp.	+	+	C + F
Panchloroid complex Pycnoscelinae			
Pycnoscelus surinamensis	+	+	C + F
Diplopterinae			
Diploptera punctata	+	+	C
Panchlorinae			
Panchlora nivea	−	−	−
Oxyhaloinae			
Gromphodorhina chopardi	+	+	C
Gromphodorhina portentosa	+	+	C
Leucophaea maderae	+	+	−
Nauphoeta cinerea	+	+	F
Gen. near Griffiniella	+	+	C
Epilamproid complex Epilamprinae			
Rhabdoblatta sp.	+	+	−

nd not determined, *C* ciliates, *F* flagellates, *hindgut differentiations* presence of an enlarged, well differentiated hindgut

tracts, individual arthropods were dissected and subjected to an analysis with the aid of epifluorescence microscopy (Hackstein and Stumm 1994). Epifluorescence microscopy allows the detection of individual methanogenic archaea due to their blue F_{420} autofluorescence that is characteristic for methanogens (Doddema and Vogels 1978; Figs. 4 and 5).

Our analysis revealed that only representatives of four out of the 35 taxa studied emitted methane: millipedes, cockroaches, termites, and scarab beetles. All other species did not produce methane, but sometimes hydrogen instead (Table 2). Also the microscopical inspection did not provide evidence for the presence of any methanogen in the arthropods belonging to a non-methanogenic taxon (Hackstein and Stumm 1994). A correlation with the diets of various arthropods was not evident since even species with a strong plant- and fibre-rich diet were completely amethanogenic (e.g. crickets, locusts, stick insects). A size factor could also be excluded since even tiny larvae of methanogenic species with a gut volume of about 1 μL emitted methane. Thus, as in vertebrates, methane production is characteristic for certain taxa, and therefore, controlled by an intrinsic, hereditary, genetic property of the host. This assumption is supported by the observation that certain species of cockroaches (which belong to a methanogenic taxon) have lost the capacity to host methanogens. These species belong predominantly to the Blattellinae and Plectopterinae (Table 3). Even amethanogenic strains of *Blattella germanica* and *Periplaneta americana* were found. These amethanogenic strains

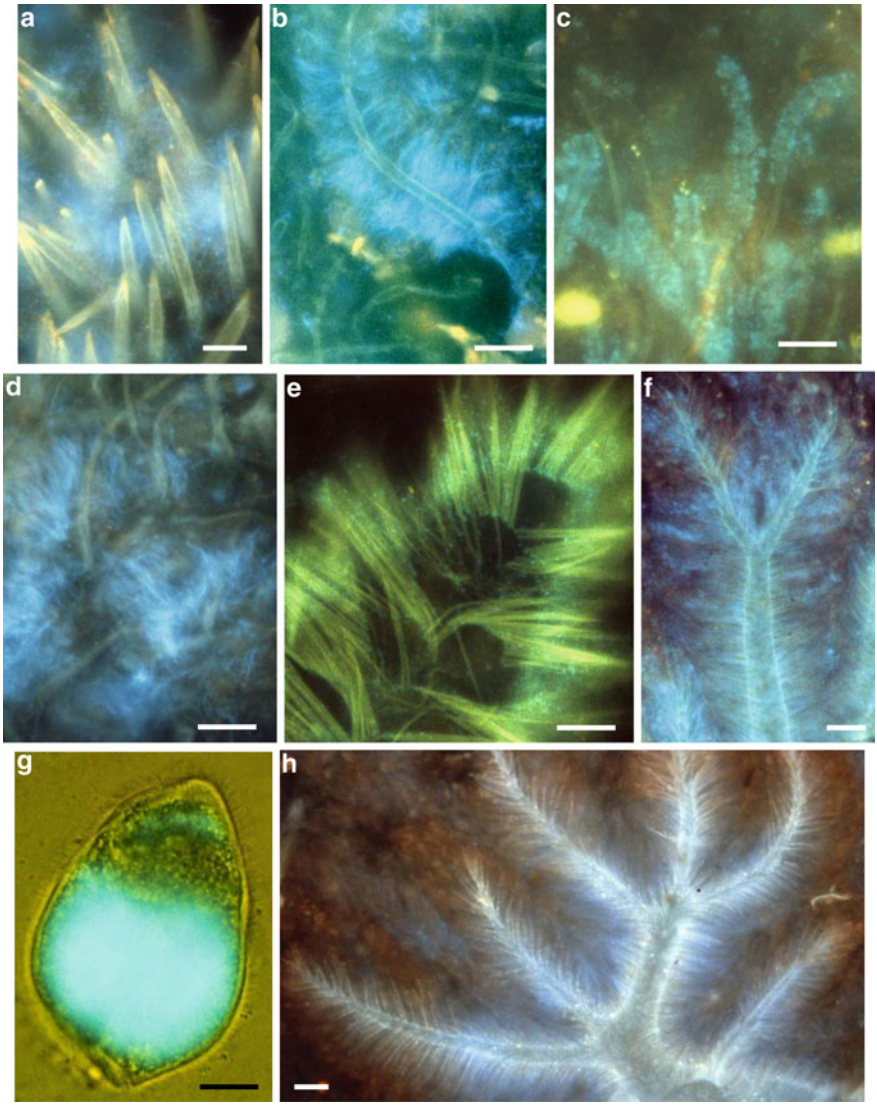

Fig. 4 Methanogenic archaea in the hindguts of the various arthropods, detected by fluorescence microscopy. The *blue* autofluorescence caused by the cofactor F_{420} indicates the presence of methanogens. The *yellowish*/*greenish* fluorescence originates from the chitinous cuticular structures of the arthropod hosts. (**a**) Filamentous methanogens loosely associated with cuticular hairs in the anterior part of the hindgut of the cockroach *Diploptera punctata*. Bar: 10 µm. (**b**) Filamentous methanogens adhering with their tips to cuticular hairs of the hindgut of the cockroach *Nauphoeta cinerea*. Bar: 10 µm. (**c**) Coccoid methanogens closely associated with cuticular hairs of the hindgut of the cockroach *Leucophaea maderae*. Bar: 10 µm. (**d**) Filamentous methanogens in the posterior hindgut of *Diploptera punctata*. There is no evidence for a close association with cuticular hindgut structures. Bar: 10 µm. (**e**) Small, coccoid methanogens between cuticular hairs (*yellowish* autofluorescence) covering the hindgut of *Nyctibora* sp. Note that many

could be transiently infected with methanogens by co-culture with methanogenic species. However, soon after the removal of the donor insects, the methanogens in the recipients were lost. This means that these amethanogenic strains had definitively lost the capacity to maintain permanently methanogens in their GI tract. Trials to infect amethanogenic species that belong to an amethanogenic taxon were unsuccessful: saw bugs and crickets could not be infected with methanogens – not even transiently (Hackstein and Stumm 1994; Hackstein et al. 1996).

Therefore, we analysed the GI tract and the intestinal surfaces in more detail. The complete GI tracts of the arthropods were dissected and studied by epifluorescence, phase contrast, and differential interference contrast (DIC) microscopy. Arthropod guts are clearly compartmentalized in the anterior–posterior direction. In general, it is possible to identify an oesophagus, crop, midgut and hindgut (Dettner and Peters 2003). The intestinal tract of millipedes and cockroaches is structured relatively simple (Fig. 6a), while the GI tract of cetonid and scarabeid larvae is dominated by a voluminous midgut and hindgut (Fig. 6c). The GI tract of termites, especially of humivorous species is highly structured and consists of compartments with a very variable pH value (Fig. 6b). Notably, in all methanogenic arthropods studied so far, the methanogens are restricted to the hindgut (Hackstein and Stumm 1994), also the methanogens, which are associated with anaerobic, gut-dwelling protozoa. Only parasites, such as gregarines (lacking methanogenic endosymbionts) can be found in the midgut, which, however, in the methanogenic arthropods hosts a complex and numerous microbiota of (facultatively) anaerobic bacteria. The strongly alkaline pH in the midgut of humivorous insects could explain the absence of methanogens and symbiotic protozoa, which depend on habitats with a moderate, near neutral pH as found in the hindguts. However, the midguts of cockroaches possess a more or less neutral pH. Nevertheless, methanogens are completely absent from this compartment. Whether the peritrophic membrane (Dettner and Peters 2003), which wraps the gut contents during their passage through the anterior parts of the GI tract, prohibits the colonization by methanogens, remains unclear. However, it is noteworthy to mention that the peritrophic membrane becomes disintegrated in the hindgut.

The methanogens occur free-floating in the hindgut lumen, attached to food particles, adhering to the gut wall, or as endosymbionts of protists. In certain insects, cuticular differentiations such as trichomes or complex epithelial differentiation of the gut wall (e.g. “pseudosetae”, Figs. 4f, h, 7 and 8) enlarge the inner

Fig. 4 (continued) methanogens are found at the basis of the hairs, adhering to the cuticle of the hindgut, at a distance of only a few micrometers to the tracheoles, which support aerobic mitochondrial metabolism in the hindgut epithelium. Bar: 10 μm. (**f**) Coccoid methanogens closely associated with a “pseudoseta” from the hindgut of a larva of the scarab beetle *Pachnoda marginata*. Bar: 10 μm. (**g**) An anaerobic nyctotheroid ciliate from the hindgut of the cockroach *Byrsotria fumigata*. Note the intensive autofluorescence of F_{420} originating from endosymbiotic methanogens. Bar: 10 μm. (**h**) Filamentous methanogens closely associated with a pseudoseta from the hindgut of a larva from the scarab beetle *Pachnoda bhutana*. Bar: 10 μm. Reproduced with permission from Hackstein et al. (2006b)

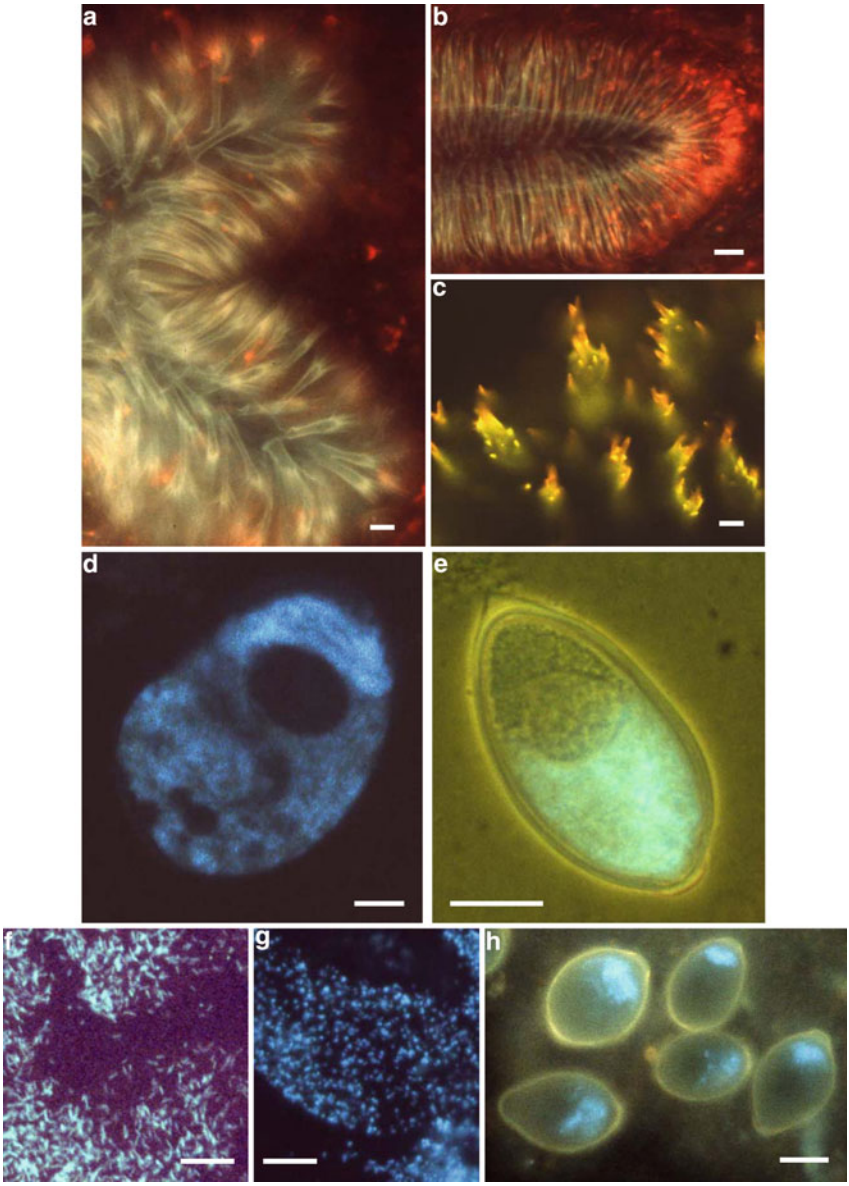

Fig. 5 (**a**, **b**) Differentiations of the hindgut epithelium of short-horned grasshoppers and crickets. Note the complete absence of blue-fluorescing methanogens. Both taxa do not produce methane. (**a**) *Phaeophylacris bedoides*, a cave-dwelling cricket (Bar 10 μm). (**b**) Unidentified, European short-horned grasshopper (Bar: 10 μm). (**c**) Cuticular structures at the junction between midgut and hindgut of the cockroach *Rhabdoblatta* sp. These chitinous bracts are likely to have a function in disrupting the peritrophic membrane before the gut contents enter the hindgut (Bar: 10 μm). (**d**) An anaerobic nyctotheroid ciliate from the hindgut of the cockroach sp. The blue autofluorescence stems from numerous endosymbiotic methanogens. The *dark spot* identifies the location of the

surface of the hindgut by several orders of magnitude. These structures provide attachment sites for a complex microbiota, which includes methanogens as a dominant component (Figs. 4f, h and 7). However, the presence of such elaborated differentiations of the gut wall does not per se enable the colonization by methanogens. Also non-methanogenic insects possess such structures – without any trace of a methanogenic archaeon (Fig. 5a, b). Notably anaerobic protozoa with endosymbiotic methanogens were also found exclusively in the hindgut of many (but not all) methanogenic arthropods (Figs. 4g and 5d–h). Such protozoa were never found in the GI tract of non-methanogenic animals.

The morphology of both the intestinal and the endosymbiotic methanogens is rather variable suggesting the presence of various species of methanogens. Only three species from termite guts and one from a cockroach gut have been isolated and cultured in vitro (Leadbetter and Breznak 1996; Leadbetter et al. 1998; Sprenger et al. 2000). All four species of methanogens adhere to the internal surface of the hindgut. PCR – and T-RFLP guided profiling studies in termites and cetonids confirmed the anticipated diversity of intestinal archaea, which are clearly different from non-gut communities (Ohkuma et al. 1995, 1999; Shinzato et al. 1999, Tokura et al. 2000; Brauman et al. 2001; Friedrich et al. 2001; Egert et al. 2003; Donovan et al. 2004; Miyata et al. 2007). Also the *Nyctotherus*-like ciliates from the hindgut of methanogenic cockroaches and millipedes and their methanogenic endosymbionts are different at the 16S rDNA level from each other and from free-living gut methanogens (van Hoek et al. 1998, 2000). The endosymbiotic methanogens are similar to, but distinct from gut-dwelling *Methanobrevibacter* species. The free-living relatives of *Nyctotherus* host different methanogens belonging to the Methanomicrobiales (van Hoek et al. 2000). The ciliates and their endosymbionts predominantly co-speciate, suggesting a vertical inheritance of the endosymbionts. The exceptions from the co-speciation argue for infrequent endosymbiont replacements (van Hoek et al. 2000; Hackstein et al. 2002).

The adherence of the methanogens to the internal surfaces and the supporting structures of the hindgut might explain the persistence of methanogens in the arthropod guts. It is conceivable that their adherence is under genetic control explaining the occurrence of methanogens in certain taxa, their absence in other taxa and the amethanogenic strains of otherwise methanogenic arthropods.

Fig. 5 (continued) macronucleus, which does not contain methanogens (Bar: 10 μm). (**e**) Cyst (resting stage) of the ciliate shown in Fig. 4g (i.e. from the hindgut of the cockroach *Byrsotria fumigata*). The blue autofluorescence discloses the presence of methanogens also in cysts (Bar: 10 μm). (**f**, **g**) Endosymbiotic methanogens from ciliates thriving in the hindgut of the cockroach *Periplaneta americana* (strain Amsterdam) (**f**), and the cockroach *Blaberus* sp. (strain Amsterdam) (**g**). The methanogens were released from the ciliates by gentle squashing. Note the different shapes of the methanogens (Bar: 5 μm) (**h**) Cysts of ciliates from the hindgut of a cockroach belonging to the Oxyhaloinae (Genus near Griffiniella) containing endosymbiotic methanogens (blue autofluorescence) (Bar: 10 μm). Reproduced with permission from Hackstein et al. (2006b)

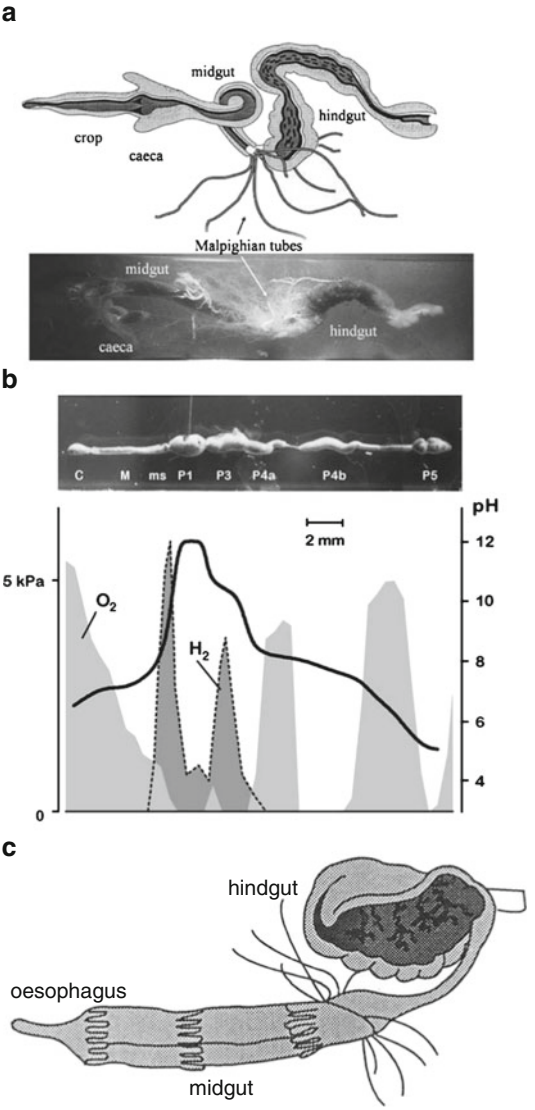

Fig. 6 Macroscopical views of the intestinal tract of cockroaches (**a**), termites (**b**) and larvae of scarab beetles (**c**). (**a**) *Above*: cartoon of the intestinal tract of a cockroach (*Periplaneta americana*). *Below*: a picture of a gut of *Periplaneta americana*, which has been embedded into agarose for microsensor measurements (after removal of the crop). (**b**) The unravelled intestinal tract of a termite (*Cubitermes* sp.) to demonstrate the complex longitudinal compartmentalisation of the termite gut. A microsensor is inserted into compartment P1. The plot below displays the longitudinal variations in pH (*solid line*) and the partial pressures of O_2 and H_2. *C* Crop, *M* midgut, *ms* mixed segment, P1-5: proctodeal regions. (**b**) Reproduced with permission from Brune and Friedrich (2000). (**c**) A cartoon demonstrating the gross organisation of the intestinal tract of the larva of the scarab beetle *Pachnoda* spec. The midgut is highly alkaline. The interior of the hindgut is shown to indicate the location of the pseudosetae (*black* structures; c.f. Figs. 4f, h and 7). Reproduced with permission from Hackstein et al. (2006b)

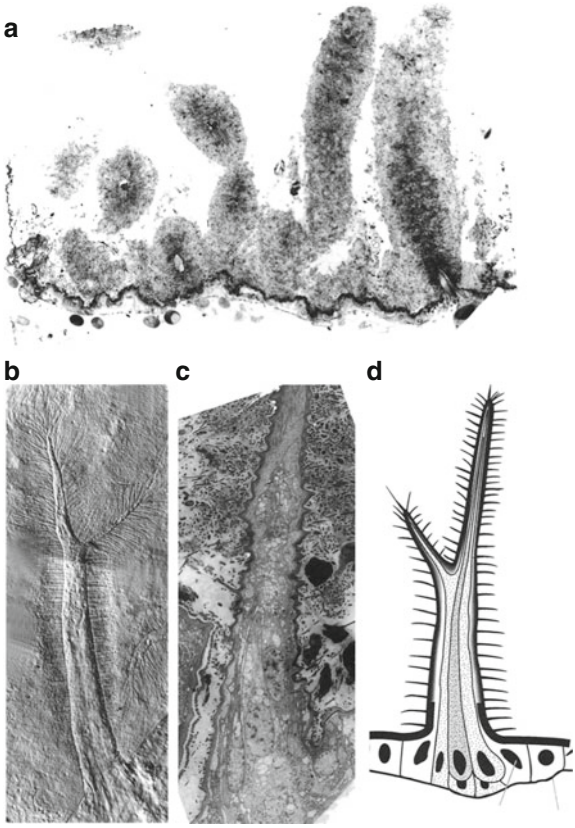

Fig. 7 (**a**) Light micrograph (semi-thin section) of the hindgut epithelium of a larva of *Dynastes hercules* (Scarabaeidae). Villus-like structures, measuring between 200 and 500 μm protrude into the lumen of the hindgut. These structures, which we have named "pseudosetae", are composed of several, elongated cells of the hindgut epithelium and covered by a complex prokaryotic microbiota, including methanogens (c.f. Fig. 4f, h). (**b**) Light micrograph (differential interference contrast) of a single pseudoseta from the hindgut of a *Pachnoda marginata* (Scarabaeidae) larva after the removal of the bacteria adhering to this structure. The surface of the pseudoseta is covered with a cuticle, which carries numerous hairs (trichomes) enhancing the surface by about two orders of magnitude. Sizes 100–300 μm. (**c**, **d**): Electron micrograph (**c**) and cartoon (**d**) of a single pseudoseta. Note that tracheae and tracheoles as well as mitochondria are lacking in the distal parts of the pseudosetae. The vacuoles are most likely involved in the transport of fermentation products (mainly short chain fatty acids) generated in the lumen of the hindgut to the hindgut epithelium, and eventually to the hemolymph. *Black* ovals in (**d**) indicate the nuclei of the hindgut epithelium and the pseudoseta. Reproduced with permission from Hackstein et al. (2006b)

The adherence of the methanogens to the gut wall and the small size of the guts create a problem for the survival of the methanogens. Methanogens are strictly anaerobic (Liu and Whitman 2008), but at the gut wall they experience a continuous influx of oxygen. Due to their small size, arthropod guts possess a large surface to volume ratio (Brune and Friedrich 2000, Hackstein et al. 2006b) that makes it

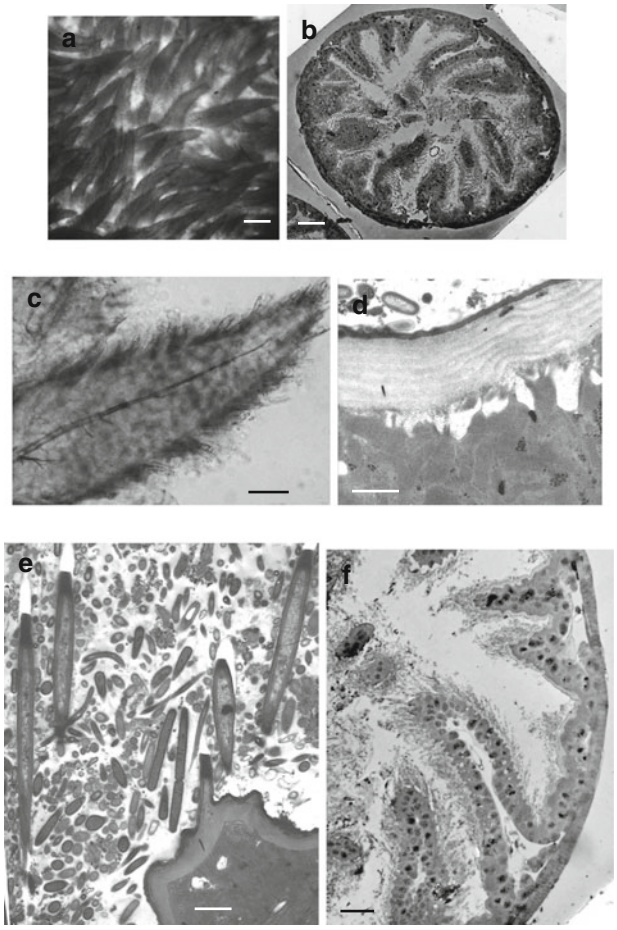

Fig. 8 (**a–f**) Various aspects of the hindgut epithelium of the cockroach *Nyctibora* sp. (**a**) Low magnification light microscopy reveals that the inner surface of the hindgut is covered by villus-like protrusions of the hindgut epithelium (Bar 200 μm). (**b**) A cross-section of the hindgut shows that these villi fill nearly the whole volume of the gut (Bar 200 μm). (**f**) The same aspect at higher magnification (Bar: 100 μm) reveals the presence of tracheae inside of these villi. (**c**) A light micrograph at higher magnification (Bar: 100 μm), which shows that tracheae and tracheoles are present in each of the villi. (**d**) Mitochondria with many cristae are found just below the cuticle, which covers the epithelial cells at the luminal side (Bar: 1 μm). (**e**) Electron micrograph of a villus, which is associated with numerous bacteria forming a complex microbiota strongly adhering to the villus with its trichomes (several of which are cut). Note the trachea inside the epithelial cell (Bar: 2 μm). Reproduced with permission from Hackstein et al. (2006b)

difficult to maintain anaerobic conditions inside the gut. However, aerobic and facultatively anaerobic bacteria located close to the gut wall sequester the oxygen and generate a steep oxygen gradient across the gut wall with the consequence that the lumen, but not the wall of the gut becomes completely anaerobic. In lower termites, the centre of the gut is populated by flagellates that generate hydrogen,

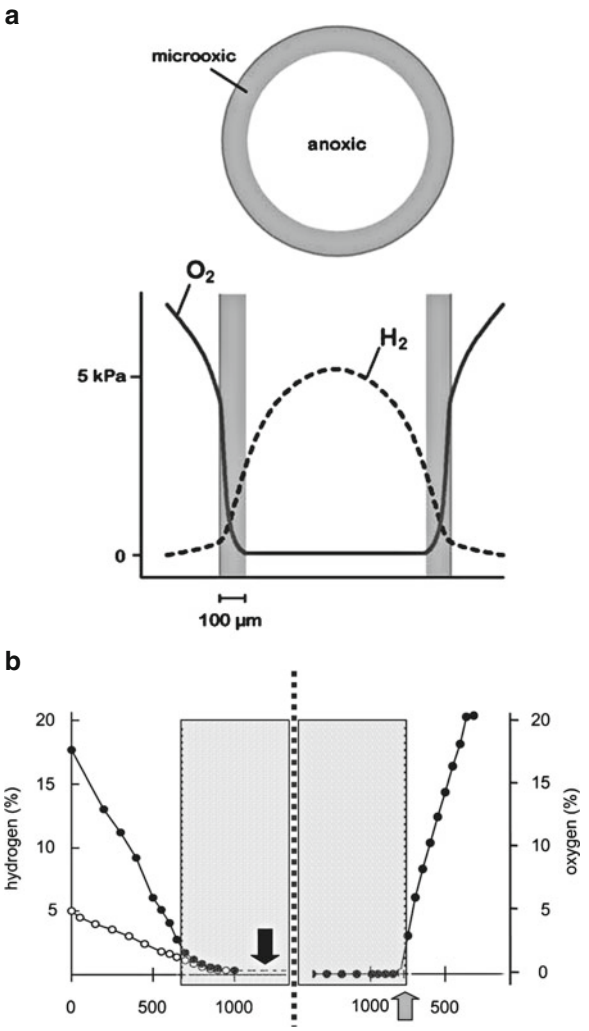

Fig. 9 Cartoons illustrating the radial profiles of H_2 and O_2 partial pressures in termite (**a**) and cockroach guts (**b**), respectively, as measured with the aid of microsensors at explanted guts, which had been embedded into agarose (Brune et al. 1995; for such a set-up, see Fig. 6a, b). Very steep O_2 gradients (**a**, and right part of **b**) are caused by the respiration of facultatively aerobic microbiota with the consequence of small microoxic zones at the periphery of the hindgut lumen (cross-section in **a**, and *grey arrow* in **b**, *right panel*). The H_2 peak in the termite hindgut is caused by hydrogen-producing protozoa. The hydrogen diffuses out of the gut, being partially consumed by methanogens colonizing the hindgut wall. In (**b**), hydrogen is generated throughout the hindgut lumen, but the presence of methanogens throughout the lumen keeps the partial pressure of hydrogen low (*black arrow* at the *left panel*). The hydrogen-consuming communities are not saturated, since even the application of external hydrogen at a partial pressure of 18% does not cause higher than background levels of hydrogen in the gut lumen [*left panel*, *open circles* (5% H_2) and *black circles* (18% H_2)]. The *shaded areas* indicate the location of the left and right halves of the hindgut, respectively. Abscissa: distance to the surface of the agarose in micrometers. (**a**) Reproduced with permission from Brune and Friedrich (2000). Reproduced with permission from Hackstein et al. (2006b)

which accumulates in the centre of the gut and diffuses outwards through the gut wall, where methanogens and other bacteria create a hydrogen sink (Fig. 9a). Thus, in lower termites the methanogens at the gut wall occupy a position between an inside-directed oxygen gradient and an outside-directed hydrogen gradient. In cockroaches, there is no outside directed hydrogen gradient, since the gut ciliates possess endosymbiotic methanogens and since the free-living methanogens are more evenly distributed throughout the hindgut (Fig. 9b). Nevertheless, as also in cockroaches the hindgut microbiota generate a steep oxygen gradient and produce hydrogen in the lumen of the gut that is consumed in situ by interspecies hydrogen transfer. The lack of accumulation of hydrogen even after incubation of hindguts in a hydrogen atmosphere indicates that hydrogen is limiting methanogenesis in the hindgut (Fig. 9b).

Interestingly, we detected hydrogen emissions in many of the methanogenic species in our screen (Table 2; Hackstein and Stumm 1994). The explanation for this paradox lies in the fact that dense populations of (facultatively) anaerobic bacteria in the midgut generate substantial amounts of hydrogen that – in the absence of methanogens – diffuses out of the midgut. Part of this hydrogen is exhaled with the breath, while another substantial part is transferred to the methanogenic hindgut by intercompartment hydrogen transfer (Lemke et al. 2001). The anatomy of the intestinal tract of termites, cockroaches, and scarab beetle larvae favours such an intercompartment hydrogen transfer (Lemke et al. 2001). We estimated that the hydrogen, which is transferred to the hindgut, contributes to some 25–30% of the methane production in the hindgut (Lemke et al. 2001; Hackstein et al. 2006b).

4 Conclusions

Methanogenic archaea in the intestinal tract of vertebrates and arthropods fulfil an important role in interspecies hydrogen transfer (Schink 1997; Stams and Plugge 2009). Remarkably, significant amounts of methanogens are only present in part of the vertebrate taxa and in four of the many orders of arthropods. Obviously, neither diet nor structure of the GI tract can explain the presence of significant amounts of methanogens in certain taxa and their absence in others. Notably, the taxonomic position of the host or population constraints is crucial for the methane status. This means that hereditary, genetic factors of the host control the presence of symbiotic methanogens in the GI tract of animals.

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