

Minireview

Genome degeneration affects both extracellular and intracellular bacterial endosymbionts

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Abstract

The obligate intracellular bacterial endosymbionts of insects are a paradigm for reductive genome evolution. A study published recently in *BMC Biology* demonstrates that similar evolutionary forces shaping genome structure may also apply to extracellular endosymbionts.

The expanding universe of bacterial insect symbionts

Insects are among the most successful animal groups in terrestrial ecosystems in terms of species richness and abundance. Symbiotic bacteria have a large part to play in this evolutionary success, often by contributing to host nutrition or defense against pathogens and predators. The bacterial companions may be facultative (secondary symbionts) or obligate (primary symbionts) for the host (Table 1). Symbionts can be found on the outer surface of the animals (ectosymbionts), as in leaf-cutter ants, which carry antibiotic-producing actinomycetes on the thorax that help to protect the cultivated fungus gardens [1]. Other symbionts live in various locations within the animals (endosymbionts), for example within the gut, such as the hindgut-inhabiting community required for wood digestion in termites [2], or the midgut endosymbionts of stinkbugs [3,4]. Moreover, endosymbionts can be found in various types of organs, such as the antennal glands of female bees (digger wasp), which harbor antibiotic-producing actinomycetes required to protect the eggs from fungal infestation [5] (Figure 1).

The most intimate bacteria-insect associations comprise obligate intracellular bacteria that reside in specialized host cells called bacteriocytes (Figure 1). The detailed molecular characterization of several such bacteriocyte-carrying animals, which include aphids, tsetse flies, psyllids, sharpshooters, cockroaches and ants, revealed a mainly nutritional basis to these associations, with the endosymbionts supplying important nutrients that were lacking in the host's food [6]. A striking hallmark of bacteriocyte symbioses is strictly vertical transmission of the symbiotic companions from the mother insect to her progeny, leading to frequent population bottlenecks in these bacteria that result in accelerated molecular evolution, for example, by fixation of even slightly deleterious mutations [7,8].

The complete isolation of these bacteria from other microbes as a result of their permanent intracellular lifestyle means a lack of horizontal gene transfer, resulting in a strict co-evolution of the symbionts with their hosts. In addition, a constant supply of metabolites from the host and a relatively stable environment relax selection pressure on the maintenance of many, mainly metabolic, genes [7,8]. This

Table 1**Comparison of basic features of endosymbiotic and free-living bacteria (ordered by genome size)**

	<i>Carsonella ruddii</i>	<i>Sulcia muelleri</i>	<i>Buchnera</i> spp.	<i>Mycoplasma genitalium</i>	<i>Blattabacterium</i> spp.	<i>Baumannia cicadellinicola</i>	<i>Wigglesworthia glossinidius</i>
Phylum	γ -Proteobacteria	Bacteroidetes	γ -Proteobacteria	Mollicutes	Bacteroidetes	γ -Proteobacteria	γ -Proteobacteria
Role as symbiont	Obligate, primary, mutualistic	Obligate, primary, mutualistic	Obligate, primary, mutualistic	Pathogen	Obligate, primary, mutualistic	Obligate, primary, mutualistic	Obligate, primary, mutualistic
Host	Psyllids	Sharpshooters	Aphids	Human	Cockroaches	Sharpshooters	Tsetse flies
Genome size (kbp)	160	245	422-686	580	Approximately 650	686	698
GC content (%)	16.5	22.4	20.1-26.2	31.7	Approximately 32	33.2	22.5
Biological function/disease	Nutrition	Nutrition	Nutrition	Genital infections	Nutrition	Nutrition	Nutrition
Location	Intracellular in bacteriocyte	Intracellular in bacteriocyte*	Intracellular in bacteriocyte	Cell associated	Intracellular in bacteriocyte	Intracellular in bacteriocyte*	Intracellular in bacteriocyte
Transmission	Vertical	Vertical	Vertical	Horizontal	Vertical	Vertical	Vertical
	<i>Blochmannia</i> spp.	<i>Candidatus Ishikawaella capsulata</i>	<i>Candidatus Rosenkranzia clausaccus</i>	<i>Chlamydia trachomatis</i>	<i>Sodalis glossinidius</i>	<i>Escherichia coli</i> K-12	<i>Sorangium cellulosum</i>
Phylum	γ -Proteobacteria	γ -Proteobacteria	γ -Proteobacteria	Chlamydiae	γ -Proteobacteria	γ -Proteobacteria	Myxobacteria
Role as symbiont	Obligate, primary, mutualistic	Obligate, primary, mutualistic	Obligate, primary, mutualistic	Pathogen	Facultative, secondary, commensal	Commensal	Environment
Host	Carpenter ants	Stinkbugs	Stinkbugs	Human	Tsetse flies	Mammalian intestine	Free-living
Genome size (kbp)	705-792	820-830	900-960	1,043	4,170 (972 pseudogenes) [†]	4,639	13,034
GC content (%)	27.4-29.6	38.9 (groEL) [§]	36-38 (groEL) [§]	41.3	54	51	71.4
Biological function/disease	Nutrition	Unknown	Unknown	Ocular, lung and genital infections	Influences parasite load (<i>Trypanosoma</i>) of host		
Location	Intracellular in bacteriocyte	Extracellular in midgut crypts	Extracellular in midgut crypts	Intracellular	Facultative intracellular	Extracellular	Extracellular
Transmission	Vertical	Vertical	Vertical	Horizontal	Horizontal/vertical	Horizontal	

**S. muelleri* lives with *B. cicadellinicola* in the same bacteriocyte. [†]The genome size of *S. glossinidius* is comparable to that of free-living Enterobacteriaceae, but it is in an early state of degeneration, as exemplified by the massive presence of pseudogenes and a coding capacity of only 51%. [§]The GC content of the *groEL* genes is presented for the stinkbug endosymbionts. In the other sequenced endosymbionts the *groEL* gene has the highest GC content, indicating that the overall GC content of the stinkbug endosymbionts is probably significantly lower.

has had dramatic consequences for the genome structure of the bacteriocyte endosymbionts. In general, these genomes are characterized by a strong AT bias (more than 70%), extremely reduced genome sizes of 160-800 kb, a complete stasis of genome structure, an extreme reduction in the

numbers of transcriptional regulators, and recombination and DNA repair factors, and high mutation rates [6-8]. Similar genomic features are also observed in pathogens, including *Mycoplasma* species and obligate intracellular chlamydiae and rickettsiae (Table 1). The strong AT bias

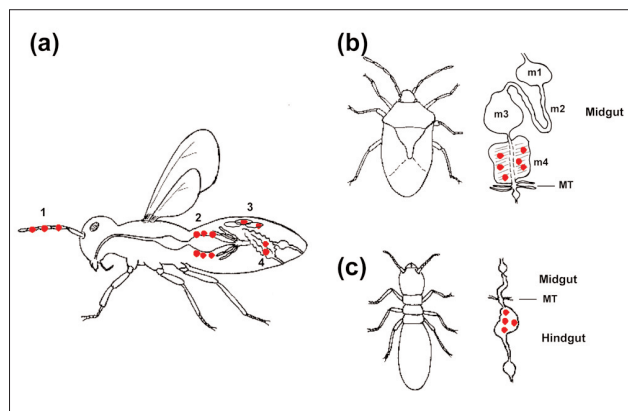


Figure 1

The diverse locations of endosymbionts in insects. The locations of the endosymbionts are shown in these schematic diagrams by red dots. (a) 1, The bee-wolf *Philanthus triangulum* harbors endosymbionts within the antennal segments [5]. 2, Bacteriocytes carrying primary endosymbionts can be localized within the midgut epithelium (carpenter ants) or in an organ-like structure called the bacteriome, which comprises a collection of bacteriocytes, located adjacent to the midgut (for example, in weevils, aphids and whiteflies) [6,7]. 3, Primary endosymbionts may also be present in the ovaries to ensure vertical transmission [6,7]. 4, Cockroaches and the termite *Mastotermes darwiniensis* harbor endosymbionts in a bacteriome within the fat body [13]. (b) Acanthosomatid stinkbugs harbor extracellular endosymbionts in crypts in a specialized part of the midgut (m4). The midgut is differentiated into four parts (m1 to m4) whereas the hindgut has a simple structure [4]. (c) Termites harbor a complex symbiotic community in their hindgut lumen [2]. In contrast to stinkbugs, the hindgut but not the midgut is differentiated into several parts with differing chemical milieux. MT, malpighian tubules.

leads to a significant increase in the number of basic amino acids in proteins, possibly resulting in alterations in their structure and function. It was proposed that, as a consequence, chaperonins such as GroEL, which might antagonize this possible deleterious effect by assisting such proteins to maintain their function, are constitutively over-expressed. This phenomenon has been observed in all endosymbionts examined so far [7].

An interesting difference between mutualists and pathogens is that in the beneficial bacteria genome degeneration preferentially tends to affect catabolic pathways, whereas in parasitic bacteria predominantly anabolic pathways are concerned, thus reflecting the different relationships of mutualists and pathogens with the host organism. The dramatic loss of genetic information and the concomitant reduction in the versatility necessary to thrive in changing environments inevitably causes an increased or absolute dependence of the bacteria on a few, or even a single, host species and, finally, an absolute connection to the host's evolutionary destiny. However, in the case of beneficial

symbioses obligate for both partners, the host itself becomes dependent on the endosymbiont and an increasing deterioration in the bacteria will be harmful for the host unless it is able to restore the essential functions provided by the bacteria in some way.

Stinkbugs and their endosymbionts

Stinkbugs have evolved fascinating strategies to permit colonization by beneficial bacteria and to guarantee their safe propagation to the progeny. In a recent article in *BMC Biology*, Takema Fukatsu and co-workers (Kikuchi *et al.* [4]) report on a novel aspect of the symbiotic relationship of stinkbugs with extracellular γ -proteobacteria [4], a continuation of their previous work on stinkbug endosymbionts [3]. A major conclusion of their investigations is that similar evolutionary forces are at work on obligate symbionts, whether they are extracellular or intracellular. It appears that the decisive evolutionary constraint is the spatial isolation of the bacteria, either by intracellular confinement in bacteriocytes or, as in the case of stinkbugs, by the development of specific host structures in the gut in which the extracellular symbionts are trapped as small populations that undergo frequent population bottlenecks.

Extracellular endosymbionts of the genus *Candidatus Ishikawaella*, which colonize stinkbugs of the family Plataspidae (Figure 2a), live in a well-separated section of the posterior midgut that harbors numerous crypts filled with the symbionts, thus forming an organ resembling the bacteriome (collection of bacteriocytes) of insects carrying intracellular symbionts [3]. Kikuchi *et al.* now find that in acanthosomatid stinkbugs (Figure 2b), symbionts of the novel genus *Candidatus Rosenkranzia* are located in specialized midgut crypts that are sealed off from the rest of the midgut, thereby leading to complete isolation of the bacteria (Figure 1) [4].

Although *Ishikawaella* and *Rosenkranzia* are extracellular, they have experienced changes in their genome structure similar to those seen in bacteriocyte symbionts - that is, a strong AT bias (greater than 62%) and a drastic reduction in genome size (genomes of 820-830 kb and 930-960 kb, respectively) (Table 1). Moreover, despite being extracellular, the endosymbionts show a quite strict pattern of co-evolution with their hosts. Although spatial isolation may lead to similar evolutionary trajectories in intra- and extracellular endosymbionts, future genome analysis of *Ishikawaella* and *Rosenkranzia* will reveal whether there are basic differences in the gene pools retained between extra- and intracellular symbionts as, for example, an extracellular location may expose bacteria to the host's immune system. The biological function of the stinkbug endosymbionts is

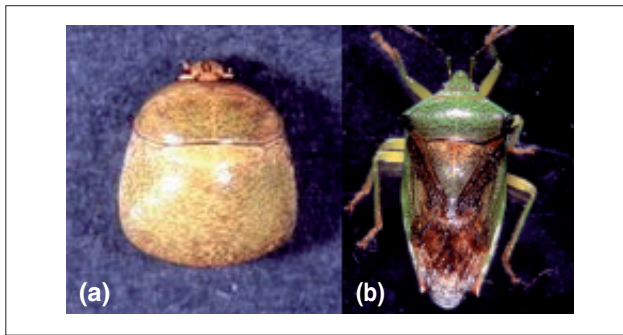


Figure 2
(a) *Megacopta cribraria*, a stinkbug of the family Plataspidae [3] **(b)** *Elasmostethus humeralis*, an acanthosomatid stinkbug [4].

still an open issue; the symbiosis is obligate, however, as elimination of the bacteria has severe consequences for the host insects, including increased mortality and sterility.

Symbiont transmission: 50 ways to leave your mother

The maternal transmission of mutualists to progeny and the manifold strategies that have evolved in insects to ensure safe propagation is a fascinating issue. In beewolf females the antenna-located symbionts are secreted into the brood chamber before oviposition and are then taken up by the larvae [5]. Obligate intracellular bacteriocyte endosymbionts can be transmitted via the presence of the bacteria in the reproductive tissue and invasion of the oocytes, as in the case of the endosymbiont *Blochmannia* of carpenter ants [6]. Alternatively, *Wigglesworthia*, the primary endosymbiont of the tsetse fly, is not only harbored within bacteriocytes but also within the lumen of the milk gland and is probably transmitted into the developing larvae via the milk secretions [9]. In the case of *Buchnera*, the primary endosymbiont of aphids, the bacteria are transmitted either to embryos in the viviparous morph or directly to eggs in the oviparous morph [10].

Because of the extracellular localization of the endosymbionts within the midgut, stinkbugs have developed very different transmission modes. In the plataspid stinkbugs, the posterior midgut of female, but not of male, adults is divided into distinct sections that are engaged in the production of complex structures containing *Ishikawaella* and called 'symbiont capsules', which are deposited together with the egg masses. These symbiont capsules are then ingested by newborn nymphs [3]. Vertical transmission in acanthosomatid stinkbugs is ensured by transfer to the egg surface via a specialized 'lubricating organ' in the abdomen, where endosymbionts are harbored in addition

to those in the sealed-off midgut crypts. When the eggs are deposited by the ovipositor, the closely associated lubricating organ harboring the endosymbionts transmits *Rosenkrantzia* by surface contamination of the eggs [4].

Are endosymbionts on the road to nowhere?

An open question is whether long-lasting obligate endosymbiosis (irrespective of location) might generally lead to a progressive degeneration of the bacterial partner due to increasing erosion of its genetic material, finally resulting in either a new type of intracellular organelle or in a useless bacterial remnant that might even become a burden to the host. In fact, *Carsonella ruddii*, the endosymbiont of psyllids, and *Buchnera aphidicola* BCc, the endosymbiont of the aphid *Cinara cedri*, may be examples of a possibly destructive end of the partnership (Table 1) [7]. In these primary endosymbionts, the genomes are reduced to dimensions approaching those of organelles (160 and 450 kb, respectively).

Gene loss in *B. aphidicola* BCc may be compensated for by incorporation of a secondary endosymbiont, *Candidatus Serratia symbiotica*, which is always present in addition to *B. aphidicola* and which may have taken over its symbiotic functions. However, in the case of *C. ruddii*, which has lost potential symbiotic functions in addition to vital cellular functions, no secondary replacement has been found so far. This might indicate that the host has acquired relevant genes from the bacterial partner, as has happened, for example, for the parasitic endosymbiont *Wolbachia* and several insect hosts [11,12]. Host genome sequencing is required to clarify this issue. If these considerations turn out to be a general rule for the evolutionary destiny of obligate and genetically isolated endosymbionts, then, independent of their cellular environment, these symbionts resemble exploited slaves rather than true mutualists.

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