Title

Grandeur Alliances: Symbiont Metabolic Integration and Obligate Arthropod Hematophagy.

Journal

Trends in Parasitology, 32(9)

Authors

Rio, Rita
Weiss, Brian
Attardo, Geoffrey

Publication Date

2016-09-01

DOI

10.1016/j.pt.2016.05.002

Peer reviewed

Published in final edited form as:

Trends Parasitol. 2016 September ; 32(9): 739–749. doi:10.1016/j.pt.2016.05.002.

Grandeur alliances: Symbiont metabolic integration and obligate arthropod hematophagy

Rita V.M. Rio[1,*], **Geoffrey M. Attardo**[2], and **Brian L. Weiss**[2]

[1]53 Campus Drive, Department of Biology, West Virginia University, Morgantown, WV 26506

[2]Yale School of Public Health, Department of Epidemiology of Microbial Diseases, New Haven, CT 06520

Abstract

Several arthropod taxa live exclusively on vertebrate blood. This food source lacks essential metabolites required for the maintenance of metabolic homeostasis, and as such, these arthropods have formed symbioses with nutrient-supplementing microbes that facilitate their host's 'hematophagous' feeding ecology. Herein we highlight metabolic contributions of bacterial symbionts that reside within tsetse flies, bed bugs, lice, reduviid bugs and ticks, with specific emphasis on B vitamin and cofactor biosynthesis. Importantly, these arthropods can transmit pathogens of medical and veterinary relevance and/or cause infestations that induce psychological and dermatological distress. Microbial metabolites, and the biochemical pathways that generate them, can serve as specific targets of novel control mechanisms aimed at disrupting the metabolism of hematophagous arthropods, thus combatting pest invasion and vector-borne pathogen transmission.

Keywords

hematophagy; blood; microbiota; vector; B vitamins; symbiont

Microbiota play significant roles towards host biology

Microbial symbiosis, once regarded as an ecological anomaly, is now recognized as a major driver of metazoan evolution. Microbial symbionts impact all aspects of their host's biology, including growth [1, 2], behavior (reviewed in [3, 4]), immunological priming [5-7] and ecological plasticity, such as thermal tolerance [8], resistance against natural enemies [9-11], detoxification of pesticides [12, 13] and body coloration [14]. These crucial functions provide fascinating examples of how microbial symbionts facilitate the phenotypic complexity exhibited by their animal hosts [15, 16]. Alliances with bacteria, regarded as repositories of high metabolic diversity [17], also drive host ecological expansion by

*Correspondence: rita.rio@mail.wvu.edu.

enabling the occupation of specialized and often resource-restricted niches. For example, bacterial symbionts provision nutrients and catabolize recalcitrant biomass [18-21], thus allowing their hosts to thrive on highly restricted, nutrient poor, diets.

In this review, we highlight examples of how evolution driven host-symbiont metabolic integration has enabled two obligate hematophagous insects, the tsetse fly (*Glossina* spp.) and the bed bug (*Cimex* spp.), to flourish on nutritionally restricted vertebrate blood. Additionally, we briefly discuss the nutrient provisioning roles of lice, reduviid bugs and tick microbiota, which serve to illustrate the parallels in endosymbiont evolution and metabolism. Notably, analogous patterns of evolution have also occurred in the rich array of microbial partnerships of insects feeding on other types of restricted diets, such as phloem and xylem sap (reviewed in [19]). The geographic distribution of these and other blood-feeding arthropods is spreading at a historically alarming rate due to a variety of factors including environmental changes, pesticide resistance, globalization and the rise in urban landscapes [22-26]. These insects, as well as other blood-feeding arthropods, pose significant public health challenges because of the pathogens they transmit, the dermatological pathologies caused by bites (including allergic reactions and potential secondary infections with skin-associated pathogens), and the detrimental psychological ramifications associated with infections and/or infestations. Thus, understanding the molecular mechanisms that underlie microbiota-facilitated hematophagy is of vital importance, as detailed knowledge of these interactions can lead to the development of novel targets and control mechanisms for disrupting pest biology and pathogen transmission.

Tsetse fly

Tsetse flies (Diptera: Glossinidae), localized exclusively to sub-Saharan Africa, are of medical significance as the cyclical and obligate vector of African trypanosomes (*Trypanosoma spp.*). These flagellate protozoa are the causative agents of human and animal African trypanosomiases, which are neglected diseases that result in significant morbidity and mortality across much of Africa [27-29]. In addition to potentially harboring trypanosomes, tsetse flies are associated with a consistent and restricted (i.e. low taxonomic richness) intestinal microbiota [30]. The simplicity of the microbiota, in stark contrast to those of many other animals, likely arises from two unique facets of tsetse's biology. First, both male and female tsetse feed exclusively on sterile vertebrate blood. Second, tsetse employ a unique mode of reproduction known as adenotrophic viviparity during which all of embryogenesis, and the majority of larval development, occur within the sterile maternal uterus [31]. These biological traits significantly curtail the fly's exposure to microbes during most life stages.

Tsetse's enteric microbiota consists primarily of two Gammaproteobacteria, the ancient obligate mutualist *Wigglesworthia* spp. [32] and the more recently acquired commensal, *Sodalis* spp. [33]. Both 16S rRNA clone libraries [34, 35] and Illumina deep sequencing of the V4 hypervariable region of the eubacterial 16S rRNA gene [36] confirm the simplicity of the microbiota and the numerical dominance of *Wigglesworthia* over *Sodalis*. These two symbionts are vertically transmitted when developing intrauterine larvae imbibe milk, produced by a maternal milk gland, that contains the two bacteria [37, 38]. Additionally,

Sodalis present in the male spermatophore can be transferred to females during copulation and then passed on to the offspring, thus demonstrating evidence of paternal transmission [39]. Lastly, tsetse flies can also harbor *Wolbachia* infections, primarily belonging to the A supergroup [40, 41], which are mostly confined to reproductive tissue [42]. Similar to *Wigglesworthia* and *Sodalis*, *Wolbachia* infections are also transmitted through the matriline, albeit via infected ovaries [43], and may result in a cytoplasmic incompatibility phenotype during embryogenesis [44]. Multiple transfers of massive segments of the *Wolbachia* genome into the *G. morsitans morsitans* genome has occurred [45], although the impact of these lateral transfer events still remain to be determined. Environmentally acquired microbes are also present in the gut of adult field flies [36, 46-48]. However, this population is transient, comprises only a small percentage of the total bacteria present, and likely lacks a functional role with respect to tsetse's biology.

Wigglesworthia

The *Wigglesworthia*-tsetse symbiosis extends back 50-80 million years [49]. This relationship has persisted through tsetse species radiation, and has driven stringent co-evolution between the two partners. In both male and female tsetse, *Wigglesworthia* are located (Fig. 1) within specialized cells (bacteriocytes) that collectively comprise a bacteriome organ located at the anterior end of the fly midgut. An additional extracellular population is located within the female-specific milk gland [37]. These two *Wigglesworthia* populations likely perform distinct functional roles; the bacteriome-associated cells supplement nutrients lacking in vertebrate blood [50-53], while milk gland associated cells prime development of their host's immune system [7, 35] and contribute to evolutionary persistence of the symbiosis via transmission to developing intrauterine larvae [38, 54].

Wigglesworthia-produced metabolites

Antibiotic-mediated elimination of *Wigglesworthia* results in the loss of tsetse fecundity via abortion of early stage larval progeny [55]. Supplementation of the blood meal with nutrient rich yeast extract [44], a cocktail of B vitamins [56] or homogenates of bacteriome tissue from *Wigglesworthia*-harboring wild-type flies [52] partially restores fecundity in *Wigglesworthia*-deficient females. These findings support the fact that vertebrate blood lacks sufficient B vitamins [57] necessary to sustain tsetse reproductive processes, and that these nutritional requisites are provisioned by the obligate mutualist *Wigglesworthia*. In support of this theory, *Wigglesworthia's* highly streamlined genome (~700 kb) has retained loci that encode pathways capable of synthesizing several B vitamins, including thiamine (vitamin B1), pyridoxine (vitamin B6) and folate (vitamin B9)[54, 58]. Below we highlight how these vitamins contribute to the physiological homeostasis of tsetse and its limited microbiota.

Thiamine (B1)—*Wigglesworthia's* ability to synthesize thiamine likely reflects the bacterium's co-evolution with its tsetse host. This feature may also represent an early signature of co-evolution between the obligate mutualist and other members of fly's microbiota, as a means of evading antagonism within the tsetse holobiont (the host and associated microbiota) [59]. One of the few distinctions in gene retention between the *Wigglesworthia* and *Sodalis* genomes lies in thiamine synthesis and transport. These symbionts are metabolically intertwined with respect to their biological need for thiamine.

More specifically, *Wigglesworthia* is capable of *de novo* thiamine monophosphate (TMP) production, while *Sodalis* has apparently lost this capability, a fact supported by the presence of pseudogenes and missing loci in the commensal microbe's TMP biosynthetic pathway [60]. Despite its inability to produce this vitamin, *Sodalis* requires thiamine to maintain metabolic homeostasis and is thus dependent on exogenous sources. Consequently, this bacterium retains a concentration-dependent thiamine ABC transporter (TbpAThiPQ) that enables salvage from the environment. *Wigglesworthia* transcription of the thiamine biosynthetic locus, *thiC*, varies throughout tsetse development, likely reflecting differences in demand throughout the fly's life cycle [51]. Moreover, transcription of the biosynthetic pathway and *Wigglesworthia* population density may be regulated to accommodate exogenous thiamine supplementation of the blood meal [51], although whether this is bacterium or host-mediated remains to be determined. The *Wigglesworthia-Sodalis* interdependency may be used to exemplify the Black Queen Hypothesis (BQH) [61], which highlights requisites for the evolution of cooperation between species. The BQH states that the evolution of a cooperative community may involve the production of a leaky product by one species, inadvertently providing a public resource, followed by relaxed selection on these biosynthetic pathways within the genome of a beneficiary. Together these processes drive interspecies dependency. Microbiota genome complementation, not only towards the host but also towards other members of the community, has been described in several insect systems [62-65]. This complementation may drive synergistic equilibrium and accelerate further interdependence [66], indicative of selection at the host rather than the individual symbiont population level.

Pyridoxine (B6)—Tsetse flies differ from most other insects in the use of proline as a precursor for ATP production (reviewed in [67]) through the tricarboxylic acid (TCA) cycle, rather than carbohydrates, such as trehalose or glucose. Proline fuels various tsetse activities including flight [68] and lactation during intrauterine larval development [52]. Notably, *Wigglesworthia* also plays a role in host proline homeostasis through the production of the essential cofactor pyridoxal phosphate (the active form of vitamin B6). Vitamin B6 is essential for the enzymatic function of alanine-glyoxylate aminotransferase (AGAT), the first step in proline regeneration from alanine within tsetse's fat body tissue [52]. In support of symbiont B6 provisioning, tsetse lacking *Wigglesworthia* have significantly lower levels of circulating B6, and correspondingly lower proline levels, within their hemolymph. This phenotype results in elevated larval abortion by pregnant females [52]. Interestingly, trypanosomes also utilize proline as a carbon source during their development within the tsetse host [69-71], and proline levels within the fly's hemolymph are significantly reduced when they harbor late trypanosome infections [52]. This suggests B6 reserves may affect maturation of infections and/or be necessary for the induction of tsetse immune responses that combat infection with parasitic trypanosomes [72, 73]. Additionally, competition for proline between tsetse and trypanosomes may account for why parasitized females exhibit significantly reduced fecundity in comparison to their uninfected counterparts [69].

Folate (B9)—The genomes of *Wigglesworthia* from divergent tsetse species (*G. morsitans* and *G. brevipalpis*) exhibit extraordinary chromosomal synteny and gene retention [54, 58]. However, one of the few distinctions between the genomes of these bacteria is that

Wigglesworthia glossinidia morsitans (*Wgm; Wigglesworthia spp*. within the *G. morsitans* host) retains a complete pathway that converts phosphoenolpyruvate (PEP) and erythrose 4-phosphate into chorismate, which is a precursor for aromatic amino acid and vitamin production [74, 75]. Moreover, *Wgm* is then able to incorporate chorismate into the *p*-aminobenzoate (PABA) biosynthesis branch for downstream folate (vitamin B9) production. The higher transcriptional activity of representative loci within the *Wgm* folate/chorismate biosynthesis pathways, coupled with the greater folate abundance within female *G. morsitans* bacteriomes, particularly during gestation, supports the significance of B9 availability towards early female sexual development and reproduction [53]. Inhibition of the *Wgm* folate biosynthesis pathway within maternal bacteriomes results in an increase in the time required for larvigenesis and the production of smaller larvae [53]. The relationship between the capacity of different *Wigglesworthia* to synthesize vitamin B9 and differential vector competency across associated tsetse species [76-81] remains unknown. However, because trypanosomes are folate auxotrophs [82, 83], variations in the production of this vitamin may contribute towards the developmental progression of this parasite in different tsetse hosts. It is tempting to speculate that distinctions in the biosynthetic capabilities of *Wigglesworthia spp*. within different tsetse species may impact tsetse ecological variation, for example by influencing preference for vertebrate blood meals of varying nutritional composition [84, 85]. Conversely the reciprocal situation, in which tsetse nutritional ecology may have shaped *Wigglesworthia* genome content, thus selecting for the presence or absence of certain loci, may have also occurred.

Sodalis

Compared to that of obligate *Wigglesworthia*, commensal *Sodalis* has a relatively recent evolutionary association with tsetse [86, 87]. In fact, this bacterium's presence within natural tsetse populations is stochastic [46, 48, 88]. *Sodalis* exhibits a broad tissue tropism within the fly (Fig. 1), but is predominantly found in the midgut [89]. The function of *Sodalis* in tsetse is poorly understood, and the degree to which and how *Sodalis* contributes to tsetse vector competency remain a contentious topic [90]. One theory proposes that the chitinolytic activity of *Sodalis* within tsetse's midgut may potentiate trypanosome infection susceptibility in teneral (newly eclosed) flies through the release of N-acetyl-d-glucosamine (GlcNAc) and its inhibition of anti-trypanosomal lectins [91, 92] found naturally in low abundance in young adult flies. The extent of *Sodalis* integration with the *Wigglesworthia* symbiont likely extends beyond B1 metabolism, and is currently being investigated. Interestingly, the absence of *Wigglesworthia* within tsetse results in the loss of *Sodalis* in subsequent generations [93]. Lastly, the identification of *Sodalis*-like bacteria have been described in numerous insect species including various Hemiptera, Diptera, Coleoptera and Phithiraptera [94-101], although little is known concerning genome modifications following establishment within these very different hosts [102, 103].

Bed bug

The common bed bug, *Cimex lectularius*, is an obligatory blood feeder throughout all mobile life stages [104, 105]. Bed bugs may serve to facilitate the transmission of various pathogens, including American trypanosomes and harmful bacteria and arboviruses

[106-108], although the magnitude of disease transmission in the field, and consequent relevance to public health, remains to be determined. Furthermore, whether bed bugs serve as true vectors, or simply facilitate mechanical transmission, is also unknown. Bed bugs harbor *Wolbachia* (*w*Cle) that belong to the F supergroup and are thus phylogenetically similar to beneficial *Wolbachia* strains harbored by filarial nematodes [109]. The bed bug-*Wolbachia* symbiosis is mutualistic, and as such, this bacterium does not negatively impact bed bug fitness as do different *Wolbachia* strains found in other insect hosts (reviewed in [110]). *w*Cle are found within bacteriocytes that cumulatively comprise a pair of bacteriomes located adjacent to the bedbug gonads (Fig. 1), which facilitates vertical transmission [111]. A yet unidentified gammaproteobacterium may also be found co-occurring within bed bug bacteriocytes and sporadically throughout other tissues, including Malpighian tubules and ovariole pedicels [111]. The function of this bacterium remains unknown, but its localization suggests a role in waste recycling.

Biotin and Riboflavin

*w*Cle provisions nutrients that facilitate the bed bug hematophagous life style, and in the absence of this symbiont, the host exhibits impaired development and reproductive sterility [111]. Symbiont elimination and vitamin supplementation experiments indicate that *w*Cle provisions biotin (vitamin B7) and riboflavin (vitamin B2) to its bed bug host [112, 113]. Interestingly, the biotin biosynthetic pathway was acquired by an ancestor of *wCle* through a lateral gene transfer event from an unrelated bacterium [112]. In contrast to biotin biosynthesis, all available insect-associated *Wolbachia* genomes retain a complete riboflavin biosynthesis pathway [112]. Weak or conditional fitness benefits, such as riboflavin provisioning or other advantages conferred by *Wolbachia* [114] within other insects, may facilitate the invasion and spread of *Wolbachia* in host populations while lessening the burden of parasitism associated with these infections.

Symbiont nutrient contributions to lice, tick and reduviid bug hosts

Lice

The human body louse, and several genera of hard and soft ticks, house endogenous symbiotic bacteria that likely also supply essential metabolites to their host. The human body louse, *Pediculus humanus humanus*, feeds exclusively on blood from the human body and can vector several pathogens, including *Rickettsia prowazekii, Borrelia recurrentis* and *Bartonella quintana* (the causative agents of epidemic typhus, relapsing fever and trench fever, respectively; [115]). This human ectoparasite also harbors a primary endosymbiont, provisionally designated '*Candidatus* Riesia pediculicola' [116], a member of the Enterobacteriaceae family within the Gammaproteobacteria [117]. In nymphs and adult males, *R. pediculicola* is localized to bacteriocytes that collectively form a 'stomach disc' within the midgut, while in adult females this microbe is housed within the lateral oviducts and posterior oocyte poles, thus suggestive of the route of maternal transmission via extracellular symbiont migration [116, 118] This bacterium has co-evolved extensively with its louse host, and as such, has a highly streamlined genome consisting of a 574,526 bp linear chromosome and a 7,628 bp extra-chromosomal plasmid [119]. In the absence of *R. pediculicola*, louse nymphs perish during their first molt [120]. This outcome is likely

reflective of the fact that this symbiont is required for *P. h. humanus* to produce pantothenic acid (vitamin B5). Interestingly, three genes (*panB*, *panC* and *panE*) that encode enzymes necessary for pantothenic acid synthesis are located on *R. pediculicola*'s multi-copy plasmid [119, 121]. This arrangement may reflect an evolution-driven mechanism that reduces the risk of gene loss during symbiont genome degeneration while ensuring adequate levels of gene expression [119]. Interestingly, the genome of *R. pediculicola* lacks antibiotic resistance genes, suggesting that targeting this symbiont through antibiotic administration may be a novel method of controlling *P. h. humanus* infestations [120, 122].

Ticks

Ticks are also obligate blood feeders (and prolific disease vectors; [123]) and thus house endosymbiotic bacteria for the likely purpose of acquiring nutrients absent from their exclusive food source. These microbes include members of the genera *Coxiella*, *Rickettsia* and *Francisella*, some of which are closely related to vertebrate pathogens [124-127]. These bacteria are trans-stadially and trans-generationally transmitted in several tick hosts [128-134] and present genomic features characteristic of extensive co-evolution with their tick hosts, as exemplified through co-cladogenesis. For example, the genomes of the *Rickettsia* endosymbionts (*R. buchneri*) from *Ixodes pacificus* and *I. scapularis* encode the full set of gene orthologues (*folA*, *folC*, *folE*, *folKP* and *ptpS*) required for *de novo* folate biosynthesis [133, 135]. Similarly, the chromosome of the *Coxiella*-like symbiont from the lone star tick, *Amblyomma americanum* is highly reduced (657 kb; [126]), and like that of its counterpart found in *Rhipicephalus turanicus*, encodes major vitamin and cofactor biosynthesis pathways, including those that produce folic acid, pyridoxine, lipoic acid, thiamine phosphate, biotin and riboflavin [126, 136]. Antibiotic treatment of *A. americanum* reduces symbiont density and results in a significant increase in time to oviposition and a significant reduction in the number of larvae that hatch and that are subsequently viable [137]. These reduced fitness parameters appeared to result specifically from the loss of *Coxiella* and not *Rickettsia* [137, 138], suggesting that the former bacterium is a primary endosymbiont of *A. americanum* (and likely other tick species). Little is known concerning the functional roles that *Francisella* may play relative to tick biology, with greater research emphasis on discriminating between the highly virulent human pathogen, *F. tularensis*, and *Francisella*-like endosymbionts (FLEs) to inform epidemiological studies [139, 140].

Reduviid bugs

The reduviid bug, *Rhodnius prolixus*, is the principle vector of *Trypanosoma cruzii*, the causative agent of Chagas disease (also referred to as New World, or American trypanosomiasis) [141]. This insect houses an obligate symbiont from the genus *Rhodococcus*, which is vertically transmitted to coprophagic (i.e. ingesting feces) first instar nymphs [142]. The genome of *Rhodococcus* (*R. rhodnii*) housed in *R. prolixus* encodes several B vitamin biosynthesis genes, including those involved in the production of thiamine (B1), riboflavin (B2), niacin (B3), pantothenate (B5), pyridoxal (B6), biotin (B7), tetrahydrofolate (B9) and cobalamin (B12) [143]. *R. prolixus* that lack *R. rhodnii* die prematurely during early nymphal development [142] and the obligate nature of this symbiosis is likely either a function of bacterial vitamin supplementation or being digested by the host, with various cellular components providing deficient nutrients [142, 144, 145] .

Concluding remarks

In this paper we summarize the current state of knowledge regarding symbiont nutrient contributions to arthropod hosts that feed exclusively on vertebrate blood throughout their lifespan. The defining themes in the relevant literature represent hallmarks of obligate symbioses that have emerged after extensive periods of host-bacteria coevolution. These themes also serve as examples of convergent evolution on the part of distant bacteria that retain B vitamin and cofactor biosynthesis pathways despite selection towards reduced genomes within their strictly blood feeding hosts. Prominently, nutrient provisioning bacterial symbionts often reside within cells (bacteriocytes) that together form specialized bacteriome organs that may afford immunological protection and/or increase the efficiency of provisioning roles and the accumulation of produced metabolites. Next-generation sequencing of bacterial genomes has allowed us to acquire putative insights, and thus develop hypotheses, about the metabolic relationship between hematophagous arthropods and their obligate symbionts (see Outstanding questions). In order to more rigorously address these theories through functional experimentation, we must develop techniques for culturing obligate symbionts outside of their hosts, either in cell lines [135, 146, 147] or (better yet) in cell-free media. We must also improve upon proteomics-related technologies (such as mass-spectrometry) used to confirm symbiont metabolite production and distribution. ‘Starving’ hematophagous arthropods by obstructing symbiont nutrient provisioning processes may serve as a novel strategy for reducing infestations and the spread of vector-borne diseases.

Acknowledgements

We are grateful to members of our laboratories, notably Dr. Serap Aksoy, for their contributions to the work presented on the tsetse model system. We thank Drs. Tim Driscoll and Victoria Verhoeve for insightful discussions on tick endosymbionts. We also thank the NIH/NIAID for their generous funding that has supported some of the work described herein.

References

1. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004; 101:15718–15723. [PubMed: 15505215]
2. Turnbaugh PJ, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444(7122):1027–31. [PubMed: 17183312]
3. Hughes, DP. Host manipulation by parasites. 1st ed. Vol. xiii. Oxford University Press; Oxford: 2012. p. 2248 p. of col. plates
4. Diaz Heijtz R, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A. 2011; 108(7):3047–52. [PubMed: 21282636]
5. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO Rep. 2006; 7(7):688–93. [PubMed: 16819463]
6. Wang J, Wu Y, Yang G, Aksoy S. Interactions between mutualist *Wigglesworthia* and tsetse peptidoglycan recognition protein (PGRP-LB) influence trypansome transmission. Proc. Natl. Acad. Sci. U S A. 2009; 106:12134–12138.
7. Weiss BL, Maltz M, Aksoy S. Obligate symbionts activate immune system development in the tsetse fly. Journal of immunology. 2012; 188(7):3395–403.
8. Dunbar HE, et al. Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. PLoS Biol. 2007; 5(5):e96. [PubMed: 17425405]

9. Moreira LA, et al. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and Plasmodium. Cell. 2009; 139(7):1268–78. [PubMed: 20064373]
10. Oliver KM, Russell JA, Moran NA, Hunter MS. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proc. Natl. Acad. Sci. USA. 2003; 100(4):1803–1807. [PubMed: 12563031]
11. Koch H, Schmid-Hempel P. Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. Proc Natl Acad Sci U S A. 2011; 108(48):19288–92. [PubMed: 22084077]
12. Kikuchi Y, et al. Symbiont-mediated insecticide resistance. Proc Natl Acad Sci U S A. 2012; 109(22):8618–22. [PubMed: 22529384]
13. Broderick NA, Raffa KF, Handelsman J. Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. Proc Natl Acad Sci U S A. 2006; 103(41):15196–9. [PubMed: 17005725]
14. Tsuchida T, et al. Symbiotic bacterium modifies aphid body color. Science. 2010; 330(6007):1102–4. [PubMed: 21097935]
15. Dawkins, R. The extended phenotype : the gene as the unit of selection. Vol. viii. Freeman; Oxford Oxfordshire ; San Francisco: 1982. p. 307
16. Moran NA. Symbiosis as an adaptive process and source of phenotypic complexity. Proc Natl Acad Sci U S A. 2007; 104(Suppl 1):8627–33. [PubMed: 17494762]
17. Margulis, L.a.D.S. Acquiring Genomes a Theory of the Origin of Species. Basic Books; New York, NY: 2002.
18. Flint HJ, et al. Microbial degradation of complex carbohydrates in the gut. Gut Microbes. 2012; 3(4):289–306. [PubMed: 22572875]
19. Hansen AK, Moran NA. The impact of microbial symbionts on host plant utilization by herbivorous insects. Molecular ecology. 2014; 23(6):1473–96. [PubMed: 23952067]
20. Flint HJ. Polysaccharide breakdown by anaerobic microorganisms inhabiting the Mammalian gut. Adv Appl Microbiol. 2004; 56:89–120. [PubMed: 15566977]
21. Flint HJ, et al. Interactions and competition within the microbial community of the human colon: links between diet and health. Environmental microbiology. 2007; 9(5):1101–11. [PubMed: 17472627]
22. Acheson ES, Kerr JT. Looking forward by looking back: using historical calibration to improve forecasts of human disease vector distributions. Vector Borne Zoonotic Dis. 2015; 15(3):173–83. [PubMed: 25793472]
23. Messina JP, et al. Climate Change and Risk Projection: Dynamic Spatial Models of Tsetse and African Trypanosomiasis in Kenya. Ann Assoc Am Geogr. 2012; 102(2):1038–1048. [PubMed: 26316656]
24. Moore S, et al. Predicting the effect of climate change on African trypanosomiasis: integrating epidemiology with parasite and vector biology. J R Soc Interface. 2012; 9(70):817–30. [PubMed: 22072451]
25. Doggett SL, et al. Bed bugs: clinical relevance and control options. Clin Microbiol Rev. 2012; 25(1):164–92. [PubMed: 22232375]
26. Potter M, Rosenberg B, Henriksen M. Bugs without borders: defining the global bed bug resurgence. 2010; 18:8–20.
27. Webster JP, et al. One health - an ecological and evolutionary framework for tackling Neglected Zoonotic Diseases. Evol Appl. 2016; 9(2):313–33. [PubMed: 26834828]
28. Connor RJ. The impact of nagana. The Onderstepoort journal of veterinary research. 1994; 61(4): 379–83. [PubMed: 7501369]
29. Simarro PP, et al. Estimating and mapping the population at risk of sleeping sickness. PLoS Negl Trop Dis. 2012; 6(10):e1859. [PubMed: 23145192]
30. Aksoy S. Tsetse - A haven for microorganisms. Parasitol. Today. 2000; 16:114–118. [PubMed: 10689331]
31. Benoit JB, et al. Adenotrophic viviparity in tsetse flies: potential for population control and as an insect model for lactation. Annu Rev Entomol. 2015; 60:351–71. [PubMed: 25341093]

32. Aksoy S. *Wigglesworthia* gen nov. and *Wigglesworthia glossinidia* sp. nov., taxa consisting of the mycetocyte-associated, primary endosymbiont of tsetse flies. Int. J. Syst. Bacteriol. 1995; 45(4): 848–851. [PubMed: 7547309]
33. Dale, C.a.I.M. *Sodalis* gen. nov. and *Sodalis glossinidius* sp. nov., a microaerophilic secondary endosymbiont of the tsetse fly *Glossina morsitans morsitans*. Int. J. Syst. Bacteriol. 1999; 49:267–275. [PubMed: 10028272]
34. Maltz MA, et al. OmpA-mediated biofilm formation is essential for the commensal bacterium *Sodalis glossinidius* to colonize the tsetse fly gut. Appl Environ Microbiol. 2012; 78(21):7760–8. [PubMed: 22941073]
35. Weiss BL, Wang J, Aksoy S. Tsetse immune system maturation requires the presence of obligate symbionts in larvae. PLoS Biology. 2011; 9(5):e1000619. [PubMed: 21655301]
36. Aksoy E, et al. Analysis of multiple tsetse fly populations in Uganda reveals limited diversity and species-specific gut microbiota. Appl Environ Microbiol. 2014; 80(14):4301–12. [PubMed: 24814785]
37. Attardo GM, Lohs C, Heddi A, Alam UH, Yildirim S, Aksoy S. Analysis of milk gland structure and function in Glossina morsitans: Milk protein production, symbiont populations and fecundity. J. Insect Physiol. 2008; 51:1236–1442. [PubMed: 18647605]
38. Ma WC, Denlinger DL. Secretory discharge and microflora of milk gland in tsetse flies. Nature. 1974; 247:301–303.
39. De Vooght L, et al. Paternal Transmission of a Secondary Symbiont during Mating in the Viviparous Tsetse Fly. Mol Biol Evol. 2015; 32(8):1977–80. [PubMed: 25851957]
40. Zhou W, Rousset F, O'Neill SL. Phylogeny and PCR-based classification of *Wolbachia* strains using wsp gene sequences. Proc. R. Soc. Lond. B. 1998; 265:509–515.
41. Doudoumis V, et al. Detection and characterization of *Wolbachia* infections in laboratory and natural populations of different species of tsetse flies (genus *Glossina*). BMC microbiology. 2012; 12(Suppl 1):S3. [PubMed: 22376025]
42. Cheng Q, Ruel T, Zhou W, Moloo S, Majiwa P, O'Neill S, Aksoy S. Tissue distribution and prevalence of *Wolbachia* infections in tsetse flies, *Glossina* spp. Med. Vet. Entomol. 2000; 14(1): 44–50. [PubMed: 10759311]
43. O'Neill SL, Gooding RH, Aksoy S. Phylogenetically distant symbiotic microorganisms reside in *Glossina* midgut and ovary tissues. Med. Vet. Entomol. 1993; 7:377–383. [PubMed: 8268495]
44. Alam U, et al. *Wolbachia* symbiont infections induce strong cytoplasmic incompatibility in the tsetse fly *Glossina morsitans*. PLoS Pathog. 2011; 7(12):e1002415. [PubMed: 22174680]
45. Brelsfoard C, et al. Presence of extensive *Wolbachia* symbiont insertions discovered in the genome of its host *Glossina morsitans morsitans*. PLoS Negl Trop Dis. 2014; 8(4):e2728. [PubMed: 24763283]
46. Geiger A, et al. First isolation of *Enterobacter*, *Enterococcus*, and *Acinetobacter* spp. as inhabitants of the tsetse fly (*Glossina palpalis palpalis*) midgut. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases. 2009; 9(6):1364–70.
47. Geiger A, et al. *Serratia glossinae* sp. nov., isolated from the midgut of the tsetse fly *Glossina palpalis gambiensis*. Int J Syst Evol Microbiol. 2010; 60(Pt 6):1261–5. [PubMed: 19667382]
48. Lindh JM, Lehane MJ. The tsetse fly *Glossina fuscipes fuscipes* (Diptera: Glossina) harbours a surprising diversity of bacteria other than symbionts. Antonie van Leeuwenhoek. 2011; 99(3):711–20. [PubMed: 21203841]
49. Chen XA, Song L, Aksoy S. Concordant evolution of a symbiont with its host insect species: Molecular phylogeny of genus *Glossina* and its bacteriome-asssociated endosymbiont Wigglesworthia glossinidia. J. Mol. Evol. 1999; 48(1):49–58. [PubMed: 9873076]
50. Snyder AK, et al. Nutrient provisioning facilitates homeostasis between tsetse fly (Diptera: Glossinidae) symbionts. Proc Biol Sci. 2010; 277(1692):2389–97. [PubMed: 20356887]
51. Snyder AK, McLain C, Rio RV. The tsetse fly obligate mutualist *Wigglesworthia morsitans* alters gene expression and population density via exogenous nutrient provisioning. Appl Environ Microbiol. 2012; 78(21):7792–7. [PubMed: 22904061]

52. Michalkova V, et al. Vitamin B6 generated by obligate symbionts is critical for maintaining proline homeostasis and fecundity in tsetse flies. Appl Environ Microbiol. 2014; 80(18):5844–53. [PubMed: 25038091]
53. Snyder AK, Rio RV. "*Wigglesworthia morsitans*" Folate (Vitamin B9) Biosynthesis Contributes to Tsetse Host Fitness. Appl Environ Microbiol. 2015; 81(16):5375–86. [PubMed: 26025907]
54. Rio RV, et al. Insight into the transmission biology and species-specific functional capabilities of tsetse (Diptera: glossinidae) obligate symbiont *Wigglesworthia*. MBio. 2012; 3(1)
55. Nogge G. Sterility in tsetse flies (*Glossina morsitans* Westwood) caused by loss of symbionts. Experentia. 1976; 32:995–996.
56. Nogge G. Significance of symbionts for the maintenance of the optimal nutritional state for successful reproduction in hematophagous arthropods. Parasitol. 1981; 82:101–104.
57. Edwards MA, Kaufman ML, Storvick CA. Microbiologic assay for the thiamine content of blood of various species of animals and man. Am. J. Clin. Nutr. 1957; 5:51–55. [PubMed: 13394540]
58. Akman L, Yamashita A, Watanabe H, Oshima K, Shiba T, Hattori M, Aksoy S. Genome sequence of the endocellular obligate symbiont of tsetse, *Wigglesworthia glossinidia*. Nat. Gen. 2002; 32(2): 402–407.
59. Snyder AK, Rio RVM. Interwoven Biology of the Tsetse Holobiont. Journal of Bacteriology. 2013; 195(19):4322–4330. [PubMed: 23836873]
60. Toh H, et al. Massive genome erosion and functional adaptations provide insights into the symbiotic lifestyle of *Sodalis glossinidius* in the tsetse host. Genome Res. 2006; 16(2):149–56. [PubMed: 16365377]
61. Morris JJ, Lenski RE, Zinser ER. The Black Queen Hypothesis: evolution of dependencies through adaptive gene loss. MBio. 2012; 3(2)
62. McCutcheon JP, von Dohlen CD. An interdependent metabolic patchwork in the nested symbiosis of mealybugs. Curr Biol. 2011; 21(16):1366–72. [PubMed: 21835622]
63. McCutcheon JP, Moran NA. Parallel genomic evolution and metabolic interdependence in an ancient symbiosis. Proc Natl Acad Sci U S A. 2007; 104(49):19392–7. [PubMed: 18048332]
64. Kwong WK, et al. Genomics and host specialization of honey bee and bumble bee gut symbionts. Proc Natl Acad Sci U S A. 2014; 111(31):11509–14. [PubMed: 25053814]
65. Van Leuven JT, et al. Sympatric speciation in a bacterial endosymbiont results in two genomes with the functionality of one. Cell. 2014; 158(6):1270–80. [PubMed: 25175626]
66. Wu D, et al. Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. PLoS Biol. 2006; 4(6):e188. [PubMed: 16729848]
67. Leak, SGA. Tsetse biology and ecology, their role in the epidemiology and control of trypanosomes. CABI publishing; New York, NY: 1999.
68. Bursell E. Aspects of the Metabolism of Amino Acids in the Tsetse Fly, Glossina (Diptera). J Insect Physiol. 1963; 9(4):439–452.
69. Hu C, et al. Infections with immunogenic trypanosomes reduce tsetse reproductive fitness: potential impact of different parasite strains on vector population structure. PLoS Negl Trop Dis. 2008; 2(3):e192. [PubMed: 18335067]
70. Hurd H. Manipulation of medically important insect vectors by their parasites. Annu Rev Entomol. 2003; 48:141–61. [PubMed: 12414739]
71. Hurd H. Host fecundity reduction: a strategy for damage limitation? Trends in parasitology. 2001; 17(8):363–8. [PubMed: 11685895]
72. Hao Z, Kasumba I, Lehane MJ, Gibson WC, Kwon J, Aksoy S. Tsetse immune responses and trypanosome transmission: Implications for the development of tsetse-based strategies to reduce trypanosomiasis. Proc. Natl. Acad. Sci. U S A. 2001; 98(22):12648–12653. [PubMed: 11592981]
73. Nayduch D, Aksoy S. Refractoriness in tsetse flies (Diptera: Glossinidae) may be a matter of timing. Journal of Medical Entomology. 2007; 44(4):660–5. [PubMed: 17695022]
74. Herrmann KM, Weaver LM. The Shikimate Pathway. Annu Rev Plant Physiol Plant Mol Biol. 1999; 50:473–503. [PubMed: 15012217]
75. Dosselaere F, Vanderleyden J. A metabolic node in action: chorismate-utilizing enzymes in microorganisms. Crit Rev Microbiol. 2001; 27(2):75–131. [PubMed: 11450855]

76. Peacock L, et al. The influence of sex and fly species on the development of trypanosomes in tsetse flies. PLoS Negl Trop Dis. 2012; 6(2):e1515. [PubMed: 22348165]
77. Harley JM. Comparison of the susceptibility of infection with *Trypanosoma rhodesiense* of *Glossina pallidipes*, *G. morsitans*, *G. fuscipes* and *G. brevipalpis*. Ann Trop Med Parasitol. 1971; 65(2):185–9. [PubMed: 5090259]
78. Moloo SK, Kutuza SB. Comparative-Study on the Susceptibility of Different *Glossina* Species to *Trypanosoma Brucei-Brucei* Infection. Tropical Medicine and Parasitology. 1988; 39(3):211–213. [PubMed: 3194664]
79. Moloo SK, et al. Study on the sequential tsetse-transmitted *Trypanosoma congolense*, *T-brucei brucei* and *T-vivax* infections to African buffalo, eland, waterbuck, N'Dama and Boran cattle. Veterinary Parasitology. 1999; 80(3):197–213. [PubMed: 9950344]
80. Moloo SK, Kabata JM, Sabwa CL. A Study on the Maturation of Procyclic *Trypanosoma-Brucei-Brucei* in *Glossina-Morsitans Centralis* and *G-Brevipalpis*. Medical and veterinary entomology. 1994; 8(4):369–374. [PubMed: 7841491]
81. Moloo SK, Okumu IO, Kuria NM. Comparative susceptibility of *Glossina longipennis* and *G-brevipalpis* to pathogenic species of *Trypanosoma*. Medical and veterinary entomology. 1998; 12(2):211–214. [PubMed: 9622377]
82. Berriman M, et al. The genome of the African trypanosome *Trypanosoma brucei*. Science. 2005; 309(5733):416–22. [PubMed: 16020726]
83. Jackson AP, et al. The genome sequence of *Trypanosoma brucei gambiense*, causative agent of chronic human african trypanosomiasis. PLoS Negl Trop Dis. 2010; 4(4):e658. [PubMed: 20404998]
84. Schweigert BS, Pearson PB. The folic acid content of blood from various species. Am J Physiol. 1947; 148(2):319–22. [PubMed: 20284550]
85. Leamon CP, et al. Impact of high and low folate diets on tissue folate receptor levels and antitumor responses toward folate-drug conjugates. J Pharmacol Exp Ther. 2008; 327(3):918–25. [PubMed: 18791065]
86. Aksoy S, Chen X, Hyspa V. Phylogeny and potential transmission routes of midgut associated endosymbionts of tsetse (Diptera: Glossinidae). Insect Mol. Biol. 1997; 6(2):183–190. [PubMed: 9099582]
87. Weiss BL, Mouchotte RM, Rio RVM, Wu Y, Wu Z, Heddi A, Aksoy S. Interspecific transfer of bacterial endosymbionts beween tsetse fly species: infection establishment and effect on host fitness. Appl Environ Microbiol. 2006; 72:7013–7021. [PubMed: 16950907]
88. Alam U, et al. Implications of microfauna-host interactions for trypanosome transmission dynamics in *Glossina fuscipes fuscipes* in Uganda. Appl Environ Microbiol. 2012; 78(13):4627–37. [PubMed: 22544247]
89. Cheng Q, Aksoy S. Tissue tropism, transmission, and expression of foreign genes in vivo in midgut symbionts of tsetse flies. Insect Mol. Biol. 1999; 8(1):125–132. [PubMed: 9927181]
90. Soumana IH, et al. The bacterial flora of tsetse fly midgut and its effect on trypanosome transmission. J Invertebr Pathol. 2013; 112(Suppl):S89–93. [PubMed: 22841948]
91. Maudlin I, Welburn SC. Lectin mediated establishment of midgut infections of *Trypanosoma congolense* and *Trypanosoma brucei* in *Glossina morsitans*. Trop. Med. Parasitol. 1987; 38:167–170. [PubMed: 3432950]
92. Welburn SC, Arnold K, Maudlin I, Goday GW. *Rickettsia*-like organisms and chitinase production in relation to transmission of trypanosomes by tsetse flies. Parasitology. 1993; 107:141–145. [PubMed: 8414668]
93. Wang J, et al. Intercommunity effects on microbiome and GpSGHV density regulation in tsetse flies. J Invertebr Pathol. 2013; 112(Suppl):S32–9. [PubMed: 22874746]
94. Boyd BM, et al. Two bacteria, *Sodalis* and *Rickettsia*, associated with the seal louse *Proechinophthirus fluctus* (Phthiraptera: Anoplura). Appl Environ Microbiol. 2016
95. Kono M, et al. Infection dynamics of coexisting beta- and gammaproteobacteria in the nested endosymbiotic system of mealybugs. Appl Environ Microbiol. 2008; 74(13):4175–84. [PubMed: 18469124]

96. Kaiwa N, et al. Primary gut symbiont and secondary, *Sodalis*-allied symbiont of the Scutellerid stinkbug *Cantao ocellatus*. Appl Environ Microbiol. 2010; 76(11):3486–94. [PubMed: 20400564]

97. Grunwald S, Pilhofer M, Holl W. Microbial associations in guts systems of wood- and bark-inhabiting longhorned beetles [Coleoptera: Cerambyicidae]. Syst. Appl. Microbiol. 2010; 33:25–34. [PubMed: 19962263]

98. Toju HTH, Koga R, Nikoh N, Meng XY, Kimura N, Fukatsu T. "*Candidatus* Curculioniphilus buchneri" a novel clade of bacterial endocellular symbionts from weevels of the genus Curculio. Appl Environ Microbiol. 2010; 76:275–282. [PubMed: 19880647]

99. Fukatsu T, Koga R, Smith WA, Tanaka K, Nikoh N, Sasaki-Fakatus K, Yoshizawa K, Dale C, Clayton DH. Bacterial endosymbiont of the slender pigeon louse, Columbicola columbae, allied to endosymbionts fo grain weevils and tsetse flies. Appl Environ Microbiol. 2007; 73:6660–6668. [PubMed: 17766458]

100. Novakova E, Hyspa V. A new *Sodalis* lineage from the bloodsucking fly *Craterina melbae* (Diptera: Hippoboscoidea) originated independently of the tsetse flies symbiont *Sodalis glossinidius*. FEMS Microbiol. Lett. 2007; 269:131–135. [PubMed: 17227456]

101. Kaiwa N, et al. Bacterial symbionts of the giant jewel stinkbug *Eucorysses grandis* (Hemiptera: Scutelleridae). Zoological science. 2011; 28(3):169–74. [PubMed: 21385056]

102. Oakeson KF, et al. Genome degeneration and adaptation in a nascent stage of symbiosis. Genome Biol Evol. 2014; 6(1):76–93. [PubMed: 24407854]

103. Clayton AL, et al. A novel human-infection-derived bacterium provides insights into the evolutionary origins of mutualistic insect-bacterial symbioses. PLoS Genet. 2012; 8(11):e1002990. [PubMed: 23166503]

104. Reinhardt K, Siva-Jothy MT. Biology of the bed bugs (Cimicidae). Annu Rev Entomol. 2007; 52:351–74. [PubMed: 16968204]

105. Benoit JB, et al. Unique features of a global human ectoparasite identified through sequencing of the bed bug genome. Nat Commun. 2016; 7:10165. [PubMed: 26836814]

106. Salazar R, et al. Bed bugs (*Cimex lectularius*) as vectors of *Trypanosoma cruzi*. Am J Trop Med Hyg. 2015; 92(2):331–5. [PubMed: 25404068]

107. Adelman ZN, Miller DM, Myles KM. Bed bugs and infectious disease: a case for the arboviruses. PLoS Pathog. 2013; 9(8):e1003462. [PubMed: 23966852]

108. Meriweather M, et al. A 454 survey reveals the community composition and core microbiome of the common bed bug (*Cimex lectularius*) across an Urban Landscape. PloS one. 2013; 8(4):e61465. [PubMed: 23585900]

109. Baldo L, et al. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. Appl Environ Microbiol. 2006; 72(11):7098–110. [PubMed: 16936055]

110. Werren JH, Baldo L, Clark ME. *Wolbachia*: master manipulators of invertebrate biology. Nat Rev Microbiol. 2008; 6(10):741–51. [PubMed: 18794912]

111. Hosokawa T, et al. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. Proc Natl Acad Sci U S A. 2010; 107(2):769–74. [PubMed: 20080750]

112. Nikoh N, et al. Evolutionary origin of insect-*Wolbachia* nutritional mutualism. Proc Natl Acad Sci U S A. 2014; 111(28):10257–62. [PubMed: 24982177]

113. Moriyama M, et al. Riboflavin Provisioning Underlies *Wolbachia's* Fitness Contribution to Its Insect Host. MBio. 2015; 6(6):e01732–15. [PubMed: 26556278]

114. Zug R, Hammerstein P. Bad guys turned nice? A critical assessment of *Wolbachia* mutualisms in arthropod hosts. Biol Rev Camb Philos Soc. 2015; 90(1):89–111. [PubMed: 24618033]

115. Badiaga S, Brouqui P. Human louse-transmitted infectious diseases. Clin Microbiol Infect. 2012; 18(4):332–7. [PubMed: 22360386]

116. Sasaki-Fukatsu K, et al. Symbiotic bacteria associated with stomach discs of human lice. Appl Environ Microbiol. 2006; 72(11):7349–52. [PubMed: 16950915]

117. Allen JM, et al. Evolutionary relationships of "*Candidatus* Riesia spp.," endosymbiotic enterobacteriaceae living within hematophagous primate lice. Appl Environ Microbiol. 2007; 73(5):1659–64. [PubMed: 17220259]

118. Perotti MA, et al. Host-symbiont interactions of the primary endosymbiont of human head and body lice. Faseb J. 2007; 21(4):1058–66. [PubMed: 17227954]
119. Kirkness EF, et al. Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. Proc Natl Acad Sci U S A. 2010; 107(27): 12168–73. [PubMed: 20566863]
120. Perotti, MA.; Kirkness, EF.; Reed, DL.; Braig, HR. Endosymbionts of lice, in Insect Symbiosis. 3 ed. Bourtzis, K., editor. Taylor & Francis; Boca Raton, FL: 2009. p. 205-220.
121. Boyd BM, et al. Genome sequence of *Candidatus* Riesia pediculischaeffi, endosymbiont of chimpanzee lice, and genomic comparison of recently acquired endosymbionts from human and chimpanzee lice. G3 (Bethesda). 2014; 4(11):2189–95. [PubMed: 25213693]
122. Sassera D, et al. Microbial symbiosis and the control of vector-borne pathogens in tsetse flies, human lice, and triatomine bugs. Pathog Glob Health. 2013; 107(6):285–92. [PubMed: 24188239]
123. de la Fuente J, et al. Overview: Ticks as vectors of pathogens that cause disease in humans and animals. Front Biosci. 2008; 13:6938–46. [PubMed: 18508706]
124. Noda H, Munderloh UG, Kurtti TJ. Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals. Appl Environ Microbiol. 1997; 63(10): 3926–32. [PubMed: 9327557]
125. Rounds MA, et al. Identification of endosymbionts in ticks by broad-range polymerase chain reaction and electrospray ionization mass spectrometry. Journal of Medical Entomology. 2012; 49(4):843–50. [PubMed: 22897044]
126. Smith TA, et al. A *Coxiella*-like endosymbiont is a potential vitamin source for the Lone Star tick. Genome Biol Evol. 2015; 7(3):831–8. [PubMed: 25618142]
127. Baldridge GD, et al. Transovarial transmission of *Francisella*-like endosymbionts and *Anaplasma phagocytophilum* variants in *Dermacentor albipictus* (Acari: Ixodidae). Journal of Medical Entomology. 2009; 46(3):625–32. [PubMed: 19496436]
128. Phan JN, et al. Molecular detection and identification of *Rickettsia* species in *Ixodes pacificus* in California. Vector Borne Zoonotic Dis. 2011; 11(7):957–61. [PubMed: 21413886]
129. Cheng D, et al. Prevalence and burden of two rickettsial phylotypes (G021 and G022) in *Ixodes pacificus* from California by real-time quantitative PCR. Ticks Tick Borne Dis. 2013; 4(4):280–287. [PubMed: 23522936]
130. Clay K, et al. Microbial communities and interactions in the lone star tick, *Amblyomma americanum*. Molecular ecology. 2008; 17(19):4371–81. [PubMed: 19378409]
131. Lalzar I, Friedmann Y, Gottlieb Y. Tissue tropism and vertical transmission of *Coxiella* in *Rhipicephalus sanguineus* and *Rhipicephalus turanicus* ticks. Environmental microbiology. 2014; 16(12):3657–68. [PubMed: 24650112]
132. Machado-Ferreira E, et al. *Coxiella* symbionts in the Cayenne tick *Amblyomma cajennense*. Microb Ecol. 2011; 62(1):134–42. [PubMed: 21611689]
133. Hunter DJ, et al. The *Rickettsia* Endosymbiont of *Ixodes pacificus* Contains All the Genes of De Novo Folate Biosynthesis. PloS one. 2015; 10(12):e0144552. [PubMed: 26650541]
134. Cheng D, et al. Host blood meal-dependent growth ensures transovarial transmission and transstadial passage of *Rickettsia* sp. phylotype G021 in the western black-legged tick (*Ixodes pacificus*). Ticks Tick Borne Dis. 2013; 4(5):421–6. [PubMed: 23876278]
135. Kurtti TJ, et al. *Rickettsia buchneri* sp. nov., a rickettsial endosymbiont of the blacklegged tick *Ixodes scapularis*. Int J Syst Evol Microbiol. 2015; 65(Pt 3):965–70. [PubMed: 25563918]
136. Gottlieb Y, Lalzar I, Klasson L. Distinctive Genome Reduction Rates Revealed by Genomic Analyses of Two *Coxiella*-Like Endosymbionts in Ticks. Genome Biol Evol. 2015; 7(6):1779–96. [PubMed: 26025560]
137. Zhong J, Jasinskas A, Barbour AG. Antibiotic treatment of the tick vector *Amblyomma americanum* reduced reproductive fitness. PloS one. 2007; 2(5):e405. [PubMed: 17476327]
138. Kurlovs AH, et al. *Ixodes pacificus* ticks maintain embryogenesis and egg hatching after antibiotic treatment of *Rickettsia* endosymbiont. PloS one. 2014; 9(8):e104815. [PubMed: 25105893]

139. Versage JL, et al. Development of a multitarget real-time TaqMan PCR assay for enhanced detection of *Francisella tularensis* in complex specimens. Journal of clinical microbiology. 2003; 41(12):5492–9. [PubMed: 14662930]

140. Michelet L, et al. Discriminating *Francisella tularensis* and *Francisella*-like endosymbionts in *Dermacentor reticulatus* ticks: evaluation of current molecular techniques. Vet Microbiol. 2013; 163(3-4):399–403. [PubMed: 23415475]

141. Liu Q, Zhou XN. Preventing the transmission of American trypanosomiasis and its spread into non-endemic countries. Infect Dis Poverty. 2015; 4:60. [PubMed: 26715535]

142. Beard CB, Cordon-Rosales C, Durvasula RV. Bacterial symbionts of the triatominae and their potential use in control of Chagas disease transmission. Annu Rev Entomol. 2002; 47:123–41. [PubMed: 11729071]

143. Pachebat JA, et al. Draft Genome Sequence of *Rhodococcus rhodnii* Strain LMG5362, a Symbiont of *Rhodnius prolixus* (Hemiptera, Reduviidae, Triatominae), the Principle Vector of *Trypanosoma cruzi*. Genome Announc. 2013; 1(3)

144. Harrington J. Studies on *Rhodnius prolixus*: growth and development of normal and sterile bugs, and the symbiotic relations. Parasitology. 1960; 50:279–286. [PubMed: 14399809]

145. Hill P, Campbell JA, Petrie IA. *Rhodnius prolixus* and its symbiotic actinomycete: a microbiological, physiological and behavioural study. Proc R Soc Lond B Biol Sci. 1976; 194(1117):501–25. [PubMed: 12514]

146. Mattila JT, et al. Isolation of cell lines and a rickettsial endosymbiont from the soft tick *Carios capensis* (Acari: Argasidae: Ornithodorinae). Journal of Medical Entomology. 2007; 44(6):1091–101. [PubMed: 18047211]

147. Duh D, et al. *Rickettsia hoogstraalii* sp. nov., isolated from hard- and soft-bodied ticks. Int J Syst Evol Microbiol. 2010; 60(Pt 4):977–84. [PubMed: 19666817]

Outstanding questions

1. What are the cues that initiate the establishment of nutritional symbioses and how do bacterial symbionts adapt to their host's background (i.e. physiological, metabolic, genomic) upon and during establishment? *Sodalis* and *Sodalis*-like symbionts are in the process of establishing symbiotic relationships with a broad range of insects that employ very different nutritional ecologies. These systems may provide a wealth of information on adaptive processes that underlie nascent symbiotic associations.
2. What are the transporters and signaling mechanisms that regulate dialog in nutrient provisioning between partners? How are symbiont-provisioned nutrients distributed to and processed within different host tissues? Do the signals that regulate these processes originate from the host, the symbiont(s) or both?
3. Does the constitution of host-associated microbiotas change in response to environmental alterations? May microbiotas contribute to a host's ability to rapidly adapt to ecological novelties?
4. How frequently and to what extent do different microbial symbionts interact within a host? Are symbionts metabolically integrated with each other, as well as their host?
5. Studies that investigate the relationship between animals and their microbiotas are largely performed using model systems that have been colonized for extensive periods of time. Do these colonized host-microbe systems accurately reflect, from a biological perspective, their counterparts that occur in native habitats?
6. Can we identify symbiont-derived molecules that may be targeted for the purpose of controlling pests and the pathogens they transmit?
7. Bacterial symbionts are present in medically significant arthropod vectors. Are (and if so how) these symbionts contributing metabolically and/or immunologically to their host's vector competency? Similarly, are these symbionts contributing metabolites that are required by pathogens in order to successfully cycle through their arthropod host?
8. Deficiencies in the blood meal have driven the co-evolution of symbionts with their blood feeding hosts. Have (and if so how) components of blood driven selection for bacteria capable of utilizing these for fitness?

TRENDS BOX

- Invertebrate-microbe symbioses, often of limited taxonomic richness, provide a foundation towards understanding the structure and function of more complex microbiotas. Basic concepts gained from these invertebrate model systems may be scalable to the complex microbiota of other animals, including humans.
- The distantly related bacterial symbionts of strictly hematophagous arthropods exemplify convergent evolution in that they have undergone genome reduction following host establishment but still maintain the capability to synthesize essential nutrients, mainly B-group vitamins.
- These symbionts are critical towards the basic physiology and maintenance of metabolic homeostasis of their animal hosts, and as such, may provide novel avenues for pursuit in pest management strategies.

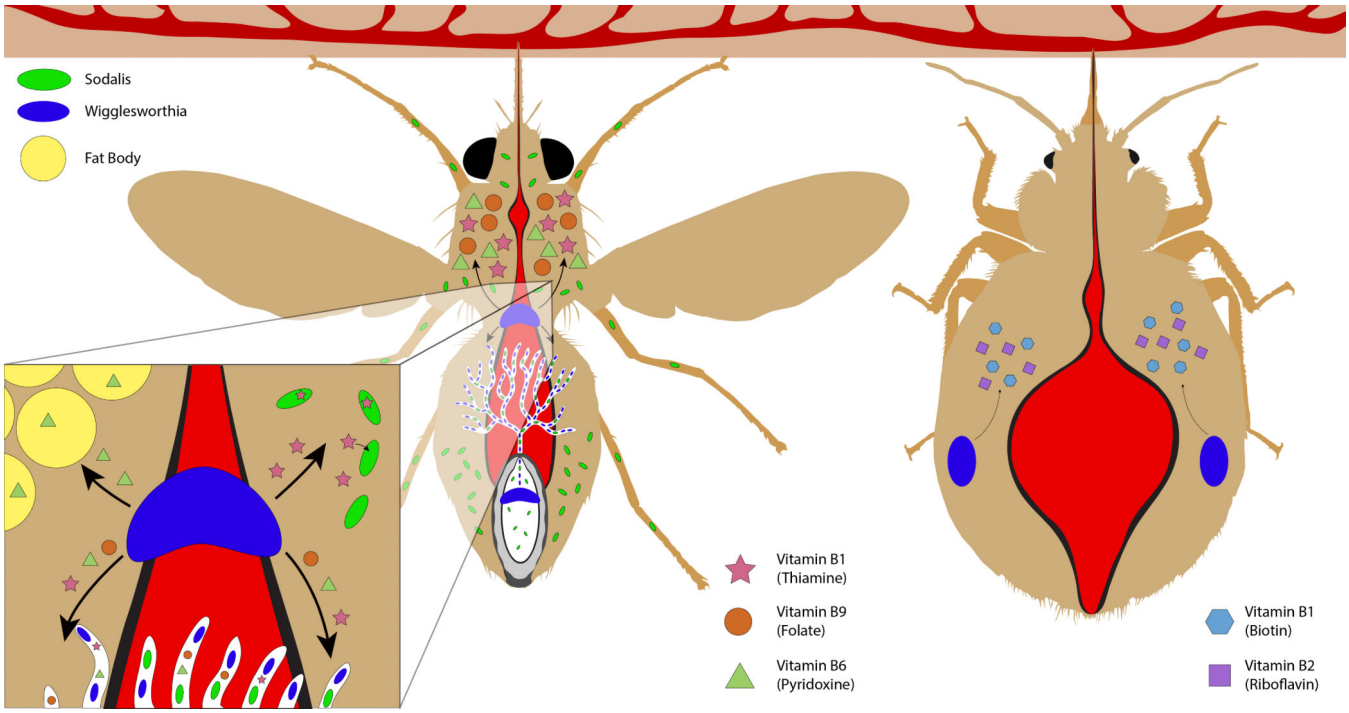

Figure 1. Symbiont nutrient complementation in tsetse flies and bed bugs

Tsetse's obligate symbiont, *Wigglesworthia*, is found within bacteriocytes in adult and larval stage flies. *Sodalis*, tsetse's commensal symbiont, exhibits a broad tissue tropism and is found intra- and extracellularly in many host tissues. Extracellular populations of *Wigglesworthia* and *Sodalis* are transmitted to developing intrauterine larvae via maternal milk gland secretions. *Wigglesworthia*-provisioned metabolites are secreted from tsetse's bacteriome into the hemolymph, from which they are taken up by the female's fat body and milk gland for energy production and larval uptake. *Sodalis* also scavenges thiamine produced by *Wigglesworthia*. Bed bugs have entered into an obligate symbiosis with the bacterium *Wolbachia* (strain *w*Cle). *wCle* resides within a pair of bacteriomes immediately adjacent to the bed bug's gonads, thus facilitating vertical transmission. When treated with antibiotics to eliminate their obligate symbionts, both tsetse flies and bed bugs exhibit impaired development and reproductive sterility.

Table 1

Bacterial symbionts of obligate hematophagous arthropods involved in metabolic homeostasis.

Arthropod host	Symbiont	Symbiont location	Nutrient provisioning	Means of transmission
Tsetse flies (Insecta: Diptera)	*Wigglesworthia*	Bacteriocytes at anterior midgut	Folate (B9) Pyridoxine (B6) Thiamine (B1)	Vertical, milk gland route
	Sodalis	Wide tissue tropism Intra- and extracellular	Unknown	Vertical, milk gland route
Bed bugs (Insecta: Hemiptera)	*Wolbachia (w*Cle)	Bacteriocytes adjacent to gonads	Biotin (B7) Riboflavin (B2)	Vertical, ovarian
	Unidentified gammaproteobacterium	Adjacent to w*Cle*, Malpighian tubules, ovary	Unknown	Unknown
Lice (Insecta: Phthiraptera)	*Candidatus* Riesia pediculicola	Bacteriocytes, stomach discs	Pantothenic acid (B5)[a]	Vertical, ovarian
Reduviid bugs (Insecta: Hemiptera)	*Rhodococcus*	Hindgut Extracellular	Biotin (B7)[a] Cobalamin (B12)[a] Niacin (B3)[a] Pantothenate (B5)[a] Pyridoxine (B6)[a] Riboflavin (B2)[a] Tetrahydrofolate (B9)[a] Thiamine (B1)[a]	Mixed, coprophagy
Ticks (Arachnida: Parasitiformes)	*Coxiella*	Wide tissue tropism	Biotin (B7)[a] Folic acid (B9)[a] Pyridoxine (B6)[a] Riboflavin (B2)[a] Thiamine phosphate (B1)[a]	Vertical, ovarian
	Rickettsia	Ovaries	Folate (B9)[a]	Vertical, ovarian
	Francisella	Hemolymph	Unknown	Vertical, ovarian

[a]Putatively assigned, based on symbiont genome capabilities.