Host–symbiont–pathogen interactions in blood-feeding parasites: nutrition, immune cross-talk and gene exchange

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Abstract

Animals are common hosts of mutualistic, commensal and pathogenic microorganisms. Blood-feeding parasites feed on a diet that is nutritionally unbalanced and thus often rely on symbionts to supplement essential nutrients. However, they are also of medical importance as they can be infected by pathogens such as bacteria, protists or viruses that take advantage of the blood-feeding nutritional strategy for own transmission. Since blood-feeding evolved multiple times independently in diverse animals, it showcases a gradient of host–microbe interactions. While some parasitic lineages are possibly asymmetric and manage to supplement their diet from other food sources, other lineages are either loosely associated with extracellular gut symbionts or harbour intracellular obligate symbionts that are essential for the host development and reproduction. What is perhaps even more diverse are the pathogenic lineages that infect blood-feeding parasites. This microbial diversity not only puts the host into a complicated situation – distinguishing between microorganisms that can greatly decrease or increase its fitness – but also increases opportunity for horizontal gene transfer to occur in this environment. In this review, I first introduce this diversity of mutualistic and pathogenic microorganisms associated with blood-feeding animals and then focus on patterns in their interactions, particularly nutrition, immune cross-talk and gene exchange.

Multiparticle interactions in microbiomes of blood-feeding parasites

Due to their specialized diet and dependence on vertebrate hosts, blood-feeding animals serve as diverse ecological niches for beneficial, commensal and pathogenic microorganisms (Lehane, 2005; Rio et al. 2016). In different blood-feeding lineages, distinct phylogenetic origins, feeding strategy and preference for vertebrate hosts have led to differences in microbiome composition and to the origin of species-specific symbioses adapted to particular hosts. Since blood-feeding arthropods are also the most prominent vectors of causative agents of diseases such as malaria, sleeping sickness, filariasis, dengue, typhus, and plague, their microbiome interactions are of great importance. For some blood-feeding lineages, stable beneficial endosymbioses are either hypothesized to be absent such as in some hard ticks (Ross et al. 2017) or the host is known to be relying on only a few symbionts such as in tsetse flies (Rio et al. 2012; Bing et al. 2017). Host–symbiont–pathogen interactions in these parasitic lineages are thus relatively simple to study. On the contrary, numerous blood-feeding lineages such as mosquitoes rely on loosely associated gut symbionts, and fragmentary data on host–symbiont–pathogen interactions are available only for a handful of these species (Damiani et al. 2010; Capone et al. 2013; Minard et al. 2013; Coon et al. 2014; Wang et al. 2017).

Several decades of research on individual microorganisms of blood-feeding parasites has provided us with a wealth of species-specific experimental data (Ribeiro and Francischetti, 2003; Graça-Souza et al. 2006), and recent developments in microbiome characterization methods will hopefully allow comprehensive comparative analyses proposed by the Parasite Microbiome Project (Dheilly et al. 2017). First, the long history of experimental work shows that majority of blood-feeding parasites depend on beneficial symbionts for nutrition, particularly provision of B-vitamins or cofactors missing from the blood diet (Wigglesworth, 1929, 1936; Aschler, 1932; Brecher and Wigglesworth, 1944; Puchtla, 1954, 1955; Michalkova et al. 2014; Nikoh et al. 2014; Manzano-Marin et al. 2015; Douglas, 2017), and some of these symbionts perhaps also contribute to blood digestion (Indergand and Graf, 2000; Pas et al. 2008). Second, immature immune system of animal blood-feeding lineages such as larvae of tsetse flies was shown to be dependent on beneficial bacteria for maturation (Weiss et al. 2011, 2012) and the innate immune system is highly modified for harbouring beneficial bacteria (Kim et al. 2011; Wang and Aksoy, 2012; Bing et al. 2017). Microbiome composition also plays a clear role in vector competence (Weiss and Aksoy, 2011; Weiss et al. 2013) and many of microbiome interactions occurring in blood-feeding parasites seem to be antagonistic. Last for this review, but definitely not least, microbiome interactions in blood-feeding animals often result in all possible directions of gene exchange: (i) between two microorganisms coexisting in the same host (Richmond and Smith, 2007; Nikoh et al. 2014), (ii) from a microorganism to its
animal host (Brelsfoard et al. 2014) or (iii) from an animal host to its microorganism (Klasson et al. 2009; Woolfit et al. 2009).

All of these interactions outlined above and discussed throughout this review are of medical and veterinary importance since they can be potentially leveraged for the elimination of diseases transmitted by blood-feeding vectors (reviewed by Berasategui et al. 2015). A fascinating aspect in the biology of blood-feeding parasites is also the interactions with the vertebrate host the haematophagous parasite feeds on. However, these interactions are out of scope of this review and were already thoroughly discussed elsewhere (Schoeler and Wikel, 2001; Fontaine et al. 2011). Here, I focus on nutrition, immune cross-talk and gene exchange and review these interactions for microbiome members of blood-feeding parasites with particular attention being paid to the interactions among the parasitic host, its obligate symbionts and other facultative/pathogenic bacteria and eukaryotes in the microbiome.

**Multiple independent origins of blood-feeding in animals**

Blood-feeding has originated multiple times independently as a feeding strategy in animals as diverse as arthropods, nematodes, platyhelminths, annelids and vertebrates (Table 1). Vertebrates that at least partially feed on blood include parasitic lampreys and other fishes (Tetlock et al. 2012), some bird species such as vampire ground finches (Schluter and Grant, 1984) and mammals such as vampire bats (Carrillo-Araujo et al. 2015). Haematophagy is, however, mostly a domain of arthropods (insects, ticks and mites) and other invertebrates (e.g. leeches, nematodes and Schistosoma spp.; Table 1). The most species-rich blood-feeding animals are insects with estimated 14 000 blood-feeding species (Adams, 1999) of mosquitoes, black flies, sand flies, biting midges, tabanids, tssetse flies, bat flies, louse flies, lice, fleas, kissing bugs and bed bugs (Table 1). Consequently, different animal lineages greatly differ in the level of dependence on blood (Mans and Neitz, 2004; Lehane, 2005) – either being their main (obligatory haematophagy) or partial food source (facultative haematophagy) (Fig. 1). Facultative haematophages feed also on other alternative diets and they are thus in most cases not fully dependent on microorganisms to provide them with nutrients such as B-vitamins and cofactors. Facultative haematophagy is, for example, known from the vampire ground finch Geospiza sepentriionalis (Schluter and Grant, 1984) or males of vampire moths Calyptra spp. (Bänziger, 1975). What is the effect of this episodic blood-feeding on microbiome composition was never studied in detail.

In other blood-feeding parasites such as mosquitoes, blood-feeding is only used by adults. Both sexes feed on plant juices and nectar, but only adult females feed on blood (Takken and Verhulst, 2013). Interestingly, a gradient of dependence on a blood meal occurs in mosquitoes. It can be either not required for successful reproduction (autogenous species), required only for the second clutch of eggs (partially anautogenous), or absolutely crucial for reproduction (anautogenous species) (Lehane, 2005). Pre-existing energy/nutrient reserves play an important role during the first genotypic cycle of female mosquitoes (Zhou et al. 2004) and larval microbiome composition can be responsible for either providing these reserves or initiating other processes essential for mosquito development. Recently, aerobic respiration by bacteria in larvae was identified as a crucial factor that triggers growth and ecdysone-induced molting of mosquitoes (Coon et al. 2017). In contrast to facultative haematophages, obligate haematophages such as lice, bed bugs or kissing bugs cannot survive on other diets than blood and their blood dependence (Table 1) is usually reflected by obligate nutritional bacteria (Beard et al. 2002; Kirkness et al. 2010; Nikoh et al. 2014).

Most blood-feeding insects undergo complete metamorphosis (i.e. are holometabolous such as fleas and all dipterans). Hemimetabolous parasites comprise only true bugs (bed bugs and kissing bugs) and lice. Interestingly, the only strictly haematophagous holometabolous insects that also house obligate intracellular bacteria are Hippoboscoidea flies (tssetse flies, louse flies and bat flies). These dipterans develop by the so-called adenotrophic viviparity – larvae are retained within the female’s body, nourished through secretions of ‘milk glands’ (also used for symbiont transfer), and pupate immediately after birth (Lehane, 2005).

**Remarkable diversity of mutualistic, commensal and pathogenic microorganisms in parasites feeding on blood**

Similarly to beneficial symbioses of other animals, symbioses of blood-feeding invertebrates can be roughly divided into two groups based on their cellular localization: extracellular and intracellular (Moran et al. 2008; Engel and Moran, 2013). Numerous blood-feeding animals only house extracellularly localized gut symbionts that have to be acquired de novo every generation from the environment. Such extracellular symbioses seem to be more common in facultatively blood-feeding dipterans, but they are also found in some obligatory blood-feeding arthropods, for instance kissing bugs (Heteroptera: Reduviidae: Triatominae). Unlike to social insects, stinkbugs or some beetles (Kikuchi et al. 2009; Kwong and Moran, 2016; Salem et al. 2017), none of the gut symbionts reported from blood-feeding arthropods have been convincingly shown to have relatively direct translational transmission (e.g. by egg smearing or individual-to-individual transfer) and have to be acquired every generation from their environment, for example, by coprophagy of actinomyces Rhodococcus rhodnii by Rhodnius prolixus kissing bugs (Beard et al. 2002; Eicher and Schaub, 2002). This acquisition of microbiota from the environment inevitably leads to much higher dynamicity in microbiome composition (e.g. symbiont losses, multiple origins and replacements) and in some lineages, such as in Ixodes scapularis ticks, a stable microbiome is probably absent and the importance of microbiota for the host reproduction and development should be thoroughly tested (Ross et al. 2017).

The second group of blood-feeding animals, exemplified by lice or bed bugs, houses intracellular bacteria in specialized cells (bacteriocytes) sometimes even forming organs (bacteriomes) and these bacteria are heritable through oocyte transfer or in a unique case of viviparous Hippoboscoidea (tssetse flies, louse flies and bat flies) through secretions of ‘milk glands’ from the mother to larvae (Hosokawa et al. 2012; Balmad et al. 2013; Novakova et al. 2015). In a similar manner to other heritable symbiotic bacteria, genomes of these symbionts undergo genome reduction (Table 2) and many other changes well known for intracellular symbioses (McCutcheon and Moran, 2011). Enlarged host bacteriocytes housing symbionts are in many cases somehow connected to the gut, either being a direct portion of midgut in tssetse flies and louse flies (Balmad et al. 2013; Novakova et al. 2015) or localized in proximity of the digestive tract and reproductive tissues in many lice species, bat flies or bed bugs (Ries, 1931; Buchner, 1965; Sasaki-Fukatsu et al. 2006; Hosokawa et al. 2010, 2012). Surprisingly, intracellular symbionts of blood-feeding animals are localized freely in the cytoplasm and retain at least some components of bacterial cell envelope, namely peptidoglycan matrix and outer membrane proteins (Akman et al. 2002; Kirkness et al. 2010). The intracytoplasmic localization is in stark contrast to symbionts of plant-feeding insects that are surrounded by a host-derived symbiosomal membrane (McCutcheon and Moran, 2011). These cellular features are likely responsible for less severe genome reduction (>500 kbp) of symbionts in
blood-feeding animals when compared with symbionts of plant-sap-feeding insects that are more integrated in the host cell (Morse et al., 2013; Duron et al., 2017; Sochová et al., 2017). One question has been pervasive in the literature about blood-feeding parasites for decades. What were ‘free-living’ ancestors of obligate symbionts in these parasites? Research progress of the last few years seems to have answered this question. Majority of obligate symbionts in blood-feeding parasites originate from facultative and pathogenic ancestors such as Wolbachia wCle in bed bugs, Arsenophonus/Riesia in louse flies and lice, Legionella polyplacis and Sodalis-allied symbionts in lice, Coxiella and Francisella-allied symbionts in ticks, and *Providencia siddallii* in leeches (Table 2).

### Table 1. Selected blood-feeding parasites and their microbiomes

<table>
<thead>
<tr>
<th>Host lineage</th>
<th>Nutritional mutualists</th>
<th>Facultative and pathogenic bacteria</th>
<th>Facultative and pathogenic eukaryotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filarial nematodes (Nematoda: Filarioidea)</td>
<td>Wolbachia sp.</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vampire bats (Chordata: Desmodontiformes)</td>
<td>Diverse gut bacteria</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lampreys (Chordata: Petromyzontiformes)</td>
<td>Aeromonas spp.</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lice (Insecta: Phthiraptera)</td>
<td>Sodalis-allied, Arsenophonus-allied, Legionella polyplacis</td>
<td>Rickettsia prowazekii, Bartonella quintana, Borrelia recurrentis, Wolbachia spp.</td>
<td>–</td>
</tr>
<tr>
<td>Bed bugs (Insecta: Heteroptera)</td>
<td>Wolbachia sp. Wcle</td>
<td>Unidentified-Enterobacteriales sp.</td>
<td>–</td>
</tr>
</tbody>
</table>

Viruses are not shown here since most of the arthropod species can transmit a diversity of arboviruses. I note that the table is not exhaustive and only shows major microbiome members reported to date. Blood-feeding lineages with no bacterial symbionts detected so far such as hookworms (Nematoda: Strongylida), barber’s pole worms (Nematoda: Filarioidea) and Schistosoma blood flukes (Platyhelminthes: Trematoda) were omitted from this table for simplicity.
Diversity of facultative bacteria in blood-feeding parasites is still relatively under-explored, although common facultative bacteria from several genera (Wolbachia, Cardinium, Rickettsia, Arsenophonus and Sodalis) were found in a number of hosts (Table 2) (Palavesam et al. 2012; Lawrence et al. 2015; Kelly et al. 2017). Even less explored is the diversity of unicellular eukaryotes. This is particularly striking because many insect pathogens and commensals, such as apicomplexans, trypanosomatids, amoebae, ciliates and microsporidia (Becnel et al. 2005; Morrison, 2009; Maslov et al. 2013; Vávra and Lukeš, 2013; Geiger et al. 2016), are due to their life cycle present in the gut lumen, along gut microvilli, in salivary glands, near to bacteriocytes, or even inside oocytes of blood-feeding animals. Such co-occurrences likely result in more interactions with beneficial symbionts than currently anticipated. Possible interactions could include scavenging of nutrients synthesized by obligate bacteria or hiding from the host immune system in the symbiotic tissue.

**Nutritional interactions between blood-sucking parasites and their obligate symbionts**

Genome and transcriptome sequencing has revolutionized the study of interactions between symbiotic bacteria and their animal hosts (McCutcheon and Moran, 2011). It is now rarely questioned that obligate and co-obligate symbionts provide B-vitamins and co-factors to blood-feeding hosts (Douglas, 2017). Interestingly, there are at least two groups of obligately blood-sucking arthropods, kissing bugs and some tick lineages, that do not depend to obligate intracellular symbionts for acquisition of B-vitamins (da Mota et al. 2012; Ross et al. 2017). Therefore, these compounds remain to be either acquired from blood or provided by environmentally acquired extracellular gut symbionts. What is generally not clear is which particular B-vitamins and co-factors are truly needed by different blood-feeding species and which are needed only by their symbiotic bacteria. Additional nutritional co-operations between blood-feeding hosts could likely also involve amino acid and nitrogen metabolism or participation on blood digestion.

So far, there are paired host–symbiont genomes available from only three obligately blood-sucking arthropods – Wigglesworthia glossinidia from tsetse flies, Riesia pediculicola from human lice and Wolbachia sp. Cle from bed bugs (Akman et al. 2002; Kirkness et al. 2010; International Glossina Genome Initiative, 2014; Nikoh et al. 2014; Benoit et al. 2016; Rosenfeld et al. 2016). This lack of data hinders drawing any strong conclusions about nutritional interactions in the blood-sucking systems because it is not certain which co-factors are needed by host-encoded enzymes. Based only on genomic data, Wigglesworthia, Riesia and Wolbachia sp. Cle should be capable of synthesizing biotin, riboflavin, folate and pyridoxine (Fig. 2). Obligate symbionts in other blood-feeding systems appear to be also capable of providing nicotinamide, pantothenate/coenzyme A and thiamine (Fig. 2). Thiamine provision is perhaps the most controversial since this cofactor is clearly acquired from the blood diet and imported into bacterial cells by a thiamine ABC transporter (Fig. 2) in hominid lice, tsetse flies and louse flies (Kirkness et al. 2010; Rio et al. 2012; Nováková et al. 2015).

Contrary to plant-feeding insects where the host cell expression complements amino acid biosynthesis carried out by symbionts (Hansen and Moran, 2011), the host role in biosynthesis of symbiont-provided B-vitamins is basically absent in blood-feeding arthropods. For example, it is in tsetse flies limited only to the expression of a multi-vitamin transporter to distribute B-vitamins from bacteriocytes to other tissues (Bing et al. 2017). However, RNA-seq (or quantitative proteomics) studies inspecting blood-feeding parasites are rarely including data for both the host and its microbiome, so further research is needed to inspect possible roles of bacterial symbionts in other key physiological processes such as blood digestion and haeme detoxification (Williamson et al. 2003; Sojka et al. 2013).

The importance of symbiotic bacteria for amino acid and nitrogen metabolism in blood-sucking animals is usually considered to be of lower importance than co-factor provision, although several pathways producing amino acids are sometimes retained (Rio et al. 2012; Pachebat et al. 2013; Nováková et al. 2015; Boyd et al. 2016). These pathways can be of biological importance, for example, the shikimate pathway is retained in the
The genome of *W. glossinidia* from *Glossina morsitans* but absent in the genome of *Glossina brevipalpis* (Rio et al. 2012). Chorismate, a shikimate pathway product, can be used for the synthesis of phenylalanine and folate, and might thus increase vector competency of *G. morsitans* for African trypanosomes (*Trypanosoma brucei brucei*). Trypanosomes cannot synthesize these compounds but are known to encode transporters to scavenge them from the environment (Rio et al. 2012).

### Immune cross-talk: distinguishing between pathogenic and beneficial microorganisms

Host control and immunity maintenance of vertically transmitted obligate symbionts have been mainly studied in symbiotic animals that feed on other diets than blood, for example, in *Sitophilus* weevils (Login et al. 2011). Several ancient and well-established hereditary symbionts in Hemiptera have been shown to be missing bacterial cell envelope structures recognized by the insect immune system – peptidoglycan and lipopolysaccharides (McCutcheon and Moran, 2011). However, as discussed above, even the most extremely reduced symbiont genomes from blood-sucking parasites still retain some of the structures recognized as of bacterial origin by the host peptidoglycan-recognition proteins (PGRPs) or Gram-negative binding proteins.

Interestingly, two insect groups with complete genomes for both the host and its obligate symbiont available (aphids and lice) have jettisoned PGRPs, genes from the immunodeficiency signalling (IMD) pathway and many antimicrobial peptides (Gerardo et al. 2010; Kirkness et al. 2010). Additional genome data imply that if the PGRPs are present, as shown, for example, in *Rhodnius prolixus* (Pachebat et al. 2013), they might be turned off.

<table>
<thead>
<tr>
<th>Blood-feeding animal</th>
<th>Obligate intracellular endosymbiont</th>
<th>Genome size (Mbp)</th>
<th>GC (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leeches</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haementeria officinalis</td>
<td><em>Providencia siddallii</em> γ-proteobacteria (Enterobacteriales)</td>
<td>0.84</td>
<td>23.9</td>
<td>Manzano-Marin et al. (2015)</td>
</tr>
<tr>
<td>Ticks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amblyomma americanum</td>
<td><em>Coxiella</em>-like endosymbiont γ-proteobacteria (Legionellales)</td>
<td>0.66</td>
<td>34.6</td>
<td>Smith et al. (2015)</td>
</tr>
<tr>
<td>Rhipicephalus turanicus</td>
<td><em>Coxiella</em> rudoviae γ-proteobacteria (Legionellales)</td>
<td>1.7</td>
<td>38.2</td>
<td>Gottlieb et al. (2015)</td>
</tr>
<tr>
<td>Amblyomma maculatum</td>
<td><em>Francisella</em>-like endosymbiont γ-proteobacteria (Thiotrichales)</td>
<td>1.56</td>
<td>31.8</td>
<td>Gerhart et al. (2016)</td>
</tr>
<tr>
<td>Lice</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pedicinus badii</td>
<td><em>Puchettia</em> sp. PRUG γ-proteobacteria (Enterobacteriales)</td>
<td>0.53</td>
<td>24.2</td>
<td>Boyd et al. (2017)</td>
</tr>
<tr>
<td>Pediculus humanus</td>
<td><em>Riesio pediculicola</em> γ-proteobacteria (Enterobacteriales)</td>
<td>0.58</td>
<td>28.5</td>
<td>Kirkness et al. (2010)</td>
</tr>
<tr>
<td>Pediculus schoeffi</td>
<td><em>Riesio pediculicola</em> γ-proteobacteria (Enterobacteriales)</td>
<td>0.57</td>
<td>31.8</td>
<td>Boyd et al. (2014)</td>
</tr>
<tr>
<td>Pthirus gorillei</td>
<td><em>Riesio</em> sp. γ-proteobacteria (Enterobacteriales)</td>
<td>0.53</td>
<td>25.0</td>
<td>Boyd et al. (2017)</td>
</tr>
<tr>
<td>Proechinophthirus fluctus</td>
<td><em>Sodolis</em> sp. γ-proteobacteria (Enterobacteriales)</td>
<td>2.18</td>
<td>50</td>
<td>Boyd et al. (2016)</td>
</tr>
<tr>
<td>Polyplax serrata</td>
<td><em>Legionella polyplacis</em> γ-proteobacteria (Legionellales)</td>
<td>0.53</td>
<td>23.0</td>
<td>Říhová et al. (2017)</td>
</tr>
<tr>
<td>Kissing bugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhodnius prolixus</td>
<td><em>Rhodococcus rhodnii</em> Actinobacteria (Actinomycetales)</td>
<td>4.38</td>
<td>69.7</td>
<td>Pachebat et al. (2013)</td>
</tr>
<tr>
<td>Bed bugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cimex lectularius</td>
<td><em>Wolbachia pipiens</em> str. wCle α-proteobacteria (Rickettsiales)</td>
<td>1.25</td>
<td>36.3</td>
<td>Nikoh et al. (2014)</td>
</tr>
<tr>
<td>Tsetse flies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glossina brevipalpis</td>
<td><em>Wigglesworthia glossinidia brevipalpis</em> γ-proteobacteria (Enterobacteriales)</td>
<td>0.68</td>
<td>22.5</td>
<td>Akman et al. (2002)</td>
</tr>
<tr>
<td>Glossina morsitans</td>
<td><em>Wigglesworthia glossinidia morsitans</em> γ-proteobacteria (Enterobacteriales)</td>
<td>0.72</td>
<td>25.2</td>
<td>Rio et al. (2012)</td>
</tr>
<tr>
<td>Louse flies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipoptena cervi</td>
<td><em>Arasphorus lipoptenarum</em> γ-proteobacteria (Enterobacteriales)</td>
<td>0.84</td>
<td>24.8</td>
<td>Nováková et al. (2016)</td>
</tr>
<tr>
<td>Melophagus ovinus</td>
<td><em>Arasphorus melophagi</em> γ-proteobacteria (Enterobacteriales)</td>
<td>1.16</td>
<td>32.2</td>
<td>Nováková et al. (2015)</td>
</tr>
</tbody>
</table>

Table 2. Genome properties of obligate nutritional symbionts of blood-feeding parasites
in tsetse flies, one of the PGRPs retains an amidase activity. By recycling peptidoglycan in bacteriocytes and milk glands of female tsetse flies, the activity shields symbionts from recognition by other PGRPs and expression of lineage-specific antimicrobial peptides mediated by the IMD pathway (Wang et al. 2009).

Living both extracellularly and intracellularly in different insect tissues (Fig. 3), facultative symbionts and pathogens need to hide their cells from the host immune system and/or to be resistant to its antimicrobial peptides. Outer membrane proteins are generally hypothesized to be responsible for hiding bacterial cells from the host immunity and therefore allowing widespread persistence of facultative symbionts in insects (Weiss et al. 2008). Even when recognized, cells of facultative symbionts were shown to be much more resistant to antimicrobial peptides of their hosts than bacteria from different hosts such as *Escherichia coli*. For example, *Sodalis glossinidius* forms biofilms in the host tissue that reduce the effect of antimicrobial peptides (Maltz et al. 2012). Since *Sodalis* gene expression can be modulated in accordance with the bacterial cell density by quorum sensing (Pontes et al. 2008; Enomoto et al. 2017), it can rapidly adapt when targeted by the host immune system to either become less or more virulent depending on its host. Understanding these density-dependent interactions with the host or other microorganisms will be essential to fully take advantage of facultative

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**Fig. 2.** B-vitamin and co-factor biosynthetic pathways encoded in the genomes of endosymbionts in blood-feeding parasites. Only species harbouring intracellular symbionts are shown for simplicity. Genome sequences available for the human louse, tsetse fly and bed bug do not suggest that host-derived enzymes of blood-feeding parasites complement partial biosynthetic pathways of their intracellular symbionts.

**Fig. 3.** Host-microbiome gene exchange and immune cross-talk hot spots in blood-feeding parasites (melting pots and intracellular arenas of evolution) highlighted for one model blood-feeding species, *Glossina* sp.
symbionts such as *Sodalis* (De Vooght et al. 2014) or *Wolbachia* (Hoffmann et al. 2011) for the elimination of causative agents of sleeping sickness, malaria and dengue or other viruses.

Blood-feeding arthropods form a peritrophic matrix in their gut to separate the blood meal from their gut tissue. This noncellular membrane is composed of chitin and many diverse proteins and proteoglycans (Shao et al. 2001). The matrix likely has several functions from digestion improvement to mechanical, chemical and pathogen protection (Lehane, 1997; Shao et al. 2001). Interestingly, reducing the permeability of this matrix was shown to reduce immune response to bacteria in some blood-feeding animals. For example, *Anopheles gambiae* mosquitoes form a dityrosine network in a mucus layer under the peritrophic matrix and this mucus prevents activation of immunity by bacteria ingested with a blood meal (Kumar et al. 2010). Whether this or similar mechanisms blocking access from the gut lumen to epithelial tissue are common in blood-feeding animals is currently unknown. What is certain is that the matrix is a constant battle field where many microbes such as *Plasmodium* sp. or *S. glossinidius* use chitinases to penetrate the membrane during their development (Langer and Vinetz, 2001; Rose et al. 2014).

**Horizontal gene transfer in microbiomes of blood-feeding parasites**

A concept of ‘melting pots of evolution’ was originally raised to highlight environments with much increased opportunity for horizontal gene transfer (HGT) among organisms living in such environments (e.g. bacteria and viruses co-infecting vacuoles of amoebae) (Moliner et al. 2010). Very similar concept was described for oocytes of multicellular eukaryotes as ‘intracellular arenas’ (Bordenstein and Wernegreen, 2004). Incidentally, oocytes (or any segregated germline cells) represent so-called ‘weak links’ allowing vertical inheritance of foreign genes in multicellular organisms (Huang, 2013), and it is probably not a coincidence that such environments in which primarily prokaryotes exchange genes, simply by chance, also seem to support higher frequency of prokaryote-to-eukaryote HGT (Husnik and McCutcheon, 2018). In terms of melting pots of HGT in blood-feeding parasites, there are at least three tissues (Fig. 3) that serve as microbiome meeting points: salivary glands, digestive tracts and reproductive tissues (such as oocytes or ‘milk glands’ in tsetse flies).

Oocytes are germline cells that are analogous to amoebal cells in a way that they are quite often shared by several different microorganisms that take advantage of oocytes for vertical transmission (Husnik and McCutcheon, 2018). For example, genomes of obligate *Wolbachia* and *Legionella* symbionts in bed bugs and *Polyplax* lice contain a biotin operon acquired horizontally from either *Cardinium*, *Wolbachia* or *Rickettsia* (Gerth and Bleidorn, 2016). This operon likely assisted these symbionts and a protist pathogen. A phospholipase of bacterial origin was likely transferred from the *S. glossinidius* genome to the *T. brucei* genome in the gut environment of their tsetse fly vector (Richmond and Smith, 2007). Genomes of bacterial pathogens such as *Bartonella*, *Rickettsia*, *Borrelia*, *Coxiella*, *Francissella* or *Yersinia* that are transmitted by blood-feeding vectors are notoriously known to be replete with pathogenicity-related genes between facultative or pathogenic microorganisms transmitted by blood-feeding parasites likely take place in these tissues (Fig. 3). For example, genomes of mosquito-associated *Spiroplasma* spp. contain multiple gene acquisitions from the Mycoides–Entomoplasmataceae clade of ruminant pathogens (Lo and Kuo, 2017). HGT can also occur between a facultative bacterial symbiont and a protist pathogen. A phospholipase of bacterial origin was likely transferred from the *S. glossinidius* genome to the *T. brucei* genome in the gut environment of their tsetse fly vector (Richmond and Smith, 2007). 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which some model species with relatively species-poor, but stable microorganisms (e.g., tsetse flies or lice) have well-studied microorganisms, but other model species with more species-rich and less stable microorganisms (e.g., many dipterans) have less-studied microorganisms. This review highlights the importance of microorganisms for some blood-feeding parasites and advocates for taxonomic breadth in parasite microbiome research, particularly to understand microorganisms of vector species with richer communities of loosely associated (and sometimes larva-specific) microorganisms.

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Conflicts of Interest. None.

Ethical Standards. Not applicable.

References


Michalkova V, et al. (2014) Obligate symbiont-generated vitamin B6 is critical to maintain proline homeostasis and fecundity in tsetse flies. Applied and Environmental Microbiology 80, 5844–5853.


Pachet JA, et al. (2013) Draft genome sequence of Rhodococcus rhodnii strain LMG5362, a symbiont of Rhodnius prolixus (Hemiptera, Reduviidae, Triatominae), the vector principle of Trypanosoma cruzi. Genome Announcements 1, 1–3.


