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Impact of endosymbionts on tick physiology and fitness

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Review Article

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Abstract

Ticks transmit pathogens and harbour non-pathogenic, vertically transmitted intracellular bacteria termed endosymbionts. Almost all ticks studied to date contain 1 or more of *Coxiella*, *Francisella*, *Rickettsia* or *Candidatus* Midichloria mitochondrii endosymbionts, indicative of their importance to tick physiology. Genomic and experimental data suggest that endosymbionts promote tick development and reproductive success. Here, we review the limited information currently available on the potential roles endosymbionts play in enhancing tick metabolism and fitness. Future studies that expand on these findings are needed to better understand endosymbionts' contributions to tick biology. This knowledge could potentially be applied to design novel strategies that target endosymbiont function to control the spread of ticks and pathogens they vector.

Introduction

Ticks are haematophagous ectoparasites of vertebrate animals worldwide. There are 2 main tick families Ixodidae (hard ticks), which possesses a sclerotized hard shield called scutum, and Argasidae (soft ticks), which lacks scutum (Anderson and Magnarelli, 2008). Both types of ticks are found worldwide, but the presence of a specific tick species in a given location is dependent on factors such as temperature, humidity, vegetation, altitude and the availability of reservoir hosts (Jongejan and Uilenberg, 2004; Estrada-Pena *et al.*, 2012). Ticks have significant impacts on human and animal health because they transmit pathogens that cause Lyme disease, anaplasmosis, babesiosis, ehrlichiosis, theileriosis, tick-borne encephalitis, Rocky Mountain spotted fever and many other diseases (Jongejan and Uilenberg, 2004; Piesman and Eisen, 2008; Petersen *et al.*, 2009; Dantas-Torres *et al.*, 2012; Sonenshine, 2018). Additionally, tick infestations cause considerable blood loss, allergic reactions and tick paralysis that could be fatal (Sonenshine and Roe, 2014). Tick control is generally based on the use of chemical acaricides; however, the frequent and incorrect use of acaricides has resulted in acaricide-resistant ticks and contamination of animal products and the environment (Jongejan and Uilenberg, 2004; Obaid *et al.*, 2022; Johnson, 2023). As an alternative, an integrated approach that involves the use of tick vaccines, administration of synthetic and plant-based acaricides to animals and the continuous surveillance and management of drug resistance in tick and host populations has been advocated (de La Fuente *et al.*, 2015).

Another potential approach to tick control is the use of 'anti-microbiota vaccines' that disrupt the functions of bacteria that enhance tick physiology and fitness (Mateos-Hernández *et al.*, 2020, 2021). Unlike extracellular bacteria found transiently on tick surfaces or midgut, a few lineages of intracellular bacteria have established long-term relationships with ticks. Loss of these so-called endosymbionts reduced tick reproductive success, indicating the potential to target them to control the spread of ticks. For this approach to be successful, a clear understanding of how bacteria promote critical processes in ticks is necessary. With this aim in mind, here we review currently available information that supports beneficial roles for tick endosymbionts.

Intracellular pathogens, reproductive parasites and endosymbionts in ticks

The advent of high-throughput sequencing has revealed a vast array of intracellular bacteria that associate with ticks (Andreotti *et al.*, 2011; Carpi *et al.*, 2011; Qiu *et al.*, 2014; Narasimhan and Fikrig, 2015). In addition to pathogens such as *Anaplasma* spp., *Ehrlichia* spp., *Rickettsia* spp., *Francisella tularensis* and *Coxiella burnetii* (Potgieter and Stoltsz, 1994; Parola and Raoult, 2001; Bown *et al.*, 2003; Jongejan and Uilenberg, 2004; Parola *et al.*, 2005; de la Fuente *et al.*, 2008; Dantas-Torres *et al.*, 2012; Kamani *et al.*, 2013; Latrofa *et al.*, 2014; Ereqat *et al.*, 2016; Regier *et al.*, 2017), ticks occasionally contain bacteria that are assumed to be reproductive parasites (Ahantarig *et al.*, 2013; Narasimhan and Fikrig, 2015; Bonnet *et al.*, 2017; Duron *et al.*, 2017). For instance, *Wolbachia* spp. that are closely related to those that manipulate insect reproduction have been detected in *Ixodes* and *Rhipicephalus* (Benson *et al.*, 2004; Zhang *et al.*, 2011; Hirunkanokpun *et al.*, 2018; Chao *et al.*, 2021). However, the impact *Wolbachia* have on tick reproduction is unknown and requires further investigation. In fact, detection of *Wolbachia* in *I. ricinus* has been linked

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to the presence of an endoparasitoid wasp, suggesting that ticks may not be natural hosts of *Wolbachia* (Plantard *et al.*, 2012; Lejal *et al.*, 2021).

Another suspected reproductive parasite present in ticks is *Rickettsiella* spp., which is prevalent in *Ixodes* and *Ornithodoros* ticks and is thought to cause sex ratio distortions in parthenogenetic *Ixodes woodi* (Kurtti *et al.*, 2002; Carpi *et al.*, 2011; Leclerque and Kleespies, 2012; Anstead and Chilton, 2014; Duron *et al.*, 2015, 2017; Bonnet *et al.*, 2017). Other tick-associated intracellular bacteria include *Arsenophonus* sp. that may decrease the questing success of *Dermacentor variabilis* and *Amblyomma americanum* ticks, and *Spiroplasma ixodetis*, *Cardinium* spp. and *Lariskella* spp. with unknown functions (Kurtti *et al.*, 1996; Grindle *et al.*, 2003; Benson *et al.*, 2004; Henning *et al.*, 2006; Clay *et al.*, 2008; Dergousoff and Chilton, 2010; Mediannikov *et al.*, 2012; Kagemann and Clay, 2013; Qiu *et al.*, 2014; Bell-Sakyi *et al.*, 2015; Duron *et al.*, 2017; Aivelo *et al.*, 2019).

Ticks also harbour intracellular bacteria that are thought to improve tick fitness. These 'endosymbionts' include *Coxiella* endosymbionts (CEs), *Francisella* endosymbionts (FEs), *Rickettsia* endosymbionts (REs) and *Candidatus* Midichloria mitochondrii (CMM) (Noda *et al.*, 1997; Sun *et al.*, 2000; Sassera *et al.*, 2006; Clay *et al.*, 2008). CEs are found in a variety of hard and soft ticks and are the most common endosymbionts identified in ticks worldwide (Andreotti *et al.*, 2011; Lalzar *et al.*, 2012; Qiu *et al.*, 2014; Duron *et al.*, 2017), FEs are present in soft ticks (e.g. *Ornithodoros moubata*, *O. porcinus porcinus*) and hard ticks (e.g. *Dermacentor* sp., *Amblyomma* sp.) (Noda *et al.*, 1997; Sun *et al.*, 2000; Gerhart *et al.*, 2016, 2018; Duron *et al.*, 2017) and REs are present in hard ticks of the genera *Ixodes*, *Dermacentor*, *Amblyomma*, *Haemaphysalis* and *Rhipicephalus* (Clay *et al.*, 2008; Ahantarig *et al.*, 2011; Carpi *et al.*, 2011; Lalzar *et al.*, 2012; Duron *et al.*, 2017; Gall *et al.*, 2017). CMM, which replicates within host mitochondria, was first detected in *I. ricinus*, but recent reports indicate its presence in other tick species as well (Lewis, 1979; Zhu *et al.*, 1992; Sacchi *et al.*, 2004; Epis *et al.*, 2008; Duron *et al.*, 2017). All ticks studied to date contain 1 or more of CE, FE, RE or CMM, suggestive of their importance to tick biology. Data currently available from genomic and experimental studies that support endosymbiont function are discussed below.

Genomic evidence for endosymbiont function

Genomes of bacteria that form long-term associations with eukaryotic hosts tend to lose genes that are not under selection while retaining genes that benefit the bacterium or host (McCutcheon and Moran, 2012; Bennett and Moran, 2015; McCutcheon *et al.*, 2019). Befitting this pattern, tick endosymbionts have degraded genomes with intact pathways for the production of B vitamins, suggesting a role for endosymbionts in provisioning these essential nutrients to ticks (Hunter *et al.*, 2015; Smith *et al.*, 2015; Gerhart *et al.*, 2016, 2018; Guizzo *et al.*, 2017; Tsementzi *et al.*, 2018; Olivieri *et al.*, 2019; Buysse *et al.*, 2021*b*) (Fig. 1). Reconstruction of the vitamin biosynthesis pathway of CEs in the ixodid tick *A. americanum* (CLEAA) and the argasid tick *O. amblus* (CLEOA) revealed that they have complete pathways to produce thiamine (vitamin B_1), riboflavin

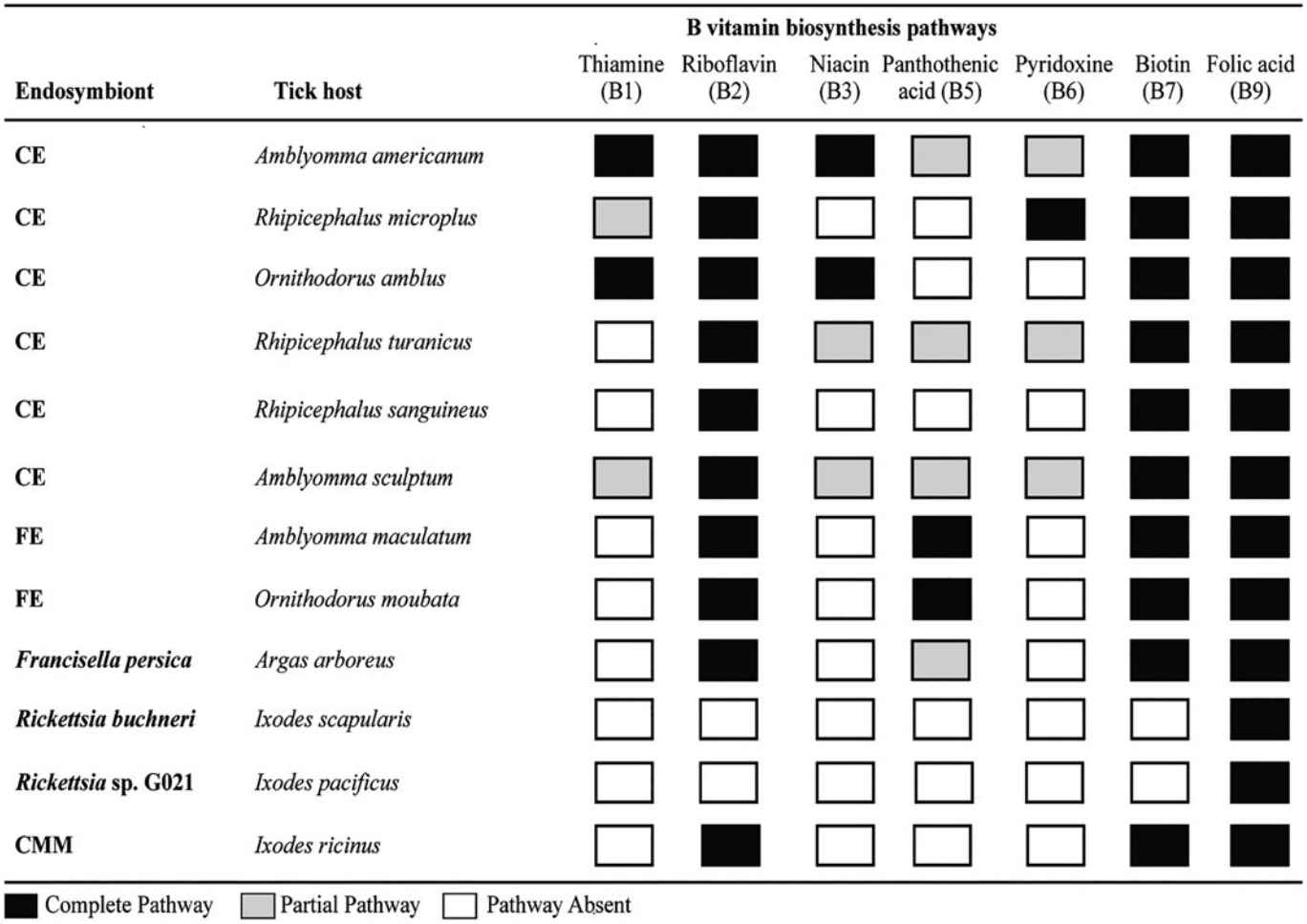

Figure 1. B vitamin biosynthesis pathways in tick endosymbionts. Pathway for the synthesis of cobalamin (vitamin B_{12}) was not detected in any tick endosymbiont genome. CE, *Coxiella* endosymbiont; FE, *Francisella* endosymbiont; CMM, *Candidatus* Midichloria mitochondrii.

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(vitamin B_2), niacin (vitamin B_3), biotin (vitamin B_7) and folate (vitamin B_9) (Smith *et al.*, 2015; Duron and Gottlieb, 2020). CLEAA also has partial pathways for the synthesis of pantothenic acid (vitamin B_5) and pyridoxine (vitamin B_6) (Smith *et al.*, 2015). The genomes of the CEs in *R. turanicus* (CRt) and *A. sculptum* (CeAS-UFV) have complete pathways to synthesize riboflavin, biotin and folate and partial pathways for niacin, pantothenic acid and pyridoxine (Gottlieb *et al.*, 2015; Duron and Gottlieb, 2020). The genome of CeAS-UFV also possesses a partial pathway for the synthesis of thiamine (Duron and Gottlieb, 2020), and the CE in *R. microplus* (CERM) has complete pathways for the synthesis of riboflavin, pyridoxine, biotin and folate and a partial pathway for the synthesis of thiamine (Gottlieb *et al.*, 2015; Smith *et al.*, 2015; Guizzo *et al.*, 2017).

Similar to CEs, analysis of the genomes of the FEs in *O. moubata* (FLE-Om), *A. maculatum* (FLE-Am) and *F. persica*, the endosymbiont of the soft tick *Argas arboreus*, showed that they possess complete pathways for the synthesis of riboflavin, biotin and folate (Gerhart *et al.*, 2018; Duron and Gottlieb, 2020) (Fig. 1). Furthermore, FLE-Am and FLE-Om may be able to utilize aspartate to synthesize pantothenic acid (vitamin B_5) but *F. persica* seems to only possess a partial pathway for this process (Gerhart *et al.*, 2018). In the case of *Rickettsia* symbionts, metabolic reconstruction suggests that *R. buchneri* in *I. scapularis* and *Rickettsia* sp. phylotype G021 in *I. pacificus* are likely able to synthesize folate but no other B vitamins (Hunter *et al.*, 2015; Bodnar *et al.*, 2018). For CMM, metabolic reconstructions showed that genomes encode complete pathways for the biosynthesis of riboflavin, biotin and folate suggesting that the intramitochondrial symbiont could also provide B vitamins to its tick partner (Sassera *et al.*, 2011; Olivieri *et al.*, 2019).

Many cofactors and coenzymes that are critical to the functioning of essential enzymes are derived from B vitamins (Douglas, 2017). Several CEs and FEs contain pathways for the production of cofactors and coenzymes from B vitamins (Guizzo *et al.*, 2017; Duron *et al.*, 2018; Gerhart *et al.*, 2018; Nardi *et al.*, 2020; Brenner *et al.*, 2021). For example, CERM, CLEAA and CRt could synthesize flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) from riboflavin and coenzyme A from pantothenate (Guizzo *et al.*, 2017). In addition, CLEAA encodes genes for the synthesis of nicotinamide adenine dinucleotide phosphate ($NADP^{+)}$ and CLEAA, CLEOA and CERM possess complete pathways to produce lipoic acid (Gottlieb *et al.*, 2015; Smith *et al.*, 2015; Guizzo *et al.*, 2017; Duron and Gottlieb, 2020). Analysis also found that the genome of FLE-Om possessed complete pathways for the biosynthesis of lipoic acid, FAD and coenzyme A (Duron *et al.*, 2018; Gerhart *et al.*, 2018). Additional analysis of FLE-Om and *F. persica* genomes using tools available in the microbial genome portal BioCyc (Karp *et al.*, 2017) revealed that both symbionts encode complete pathways for the biosynthesis of several other cofactors that could be useful to their tick hosts: iron-sulphur cluster [2Fe-2S], dipyrromethane, FMN, heme, molybdopterin, nicotinamide adenine dinucleotide (NAD^+), $NADP^+$, pyridoxal 5′-phosphate, S-adenosyl-L-methionine and thiamine diphosphate (A. Kolo, unpublished).

Apart from provisioning vitamins and cofactors, endosymbionts may also supply amino acids and other metabolites that boost tick fitness (Fig. 2). For instance, *in silico* flux balance analyses of CRt and CE of *R. sanguineus* (CRs) identified excessive production of the amino acid proline, which is thought to play a significant role in arthropods due to its involvement in energy production and nitrogen metabolism (O'Donnell and Donini, 2017; Tsementzi *et al.*, 2018). Similarly, CERM encodes genes for the production of essential amino acids, and FLE-Am appears to have the metabolic capacity to produce the amino acids cysteine, threonine, tyrosine, tryptophan, phenylalanine and serine from pyruvate (Gerhart *et al.*, 2016). Additionally, FEs encode enzymes that recycle nitrogen by incorporating ammonia, a metabolic waste product, into the synthesis of glutamine, as done by

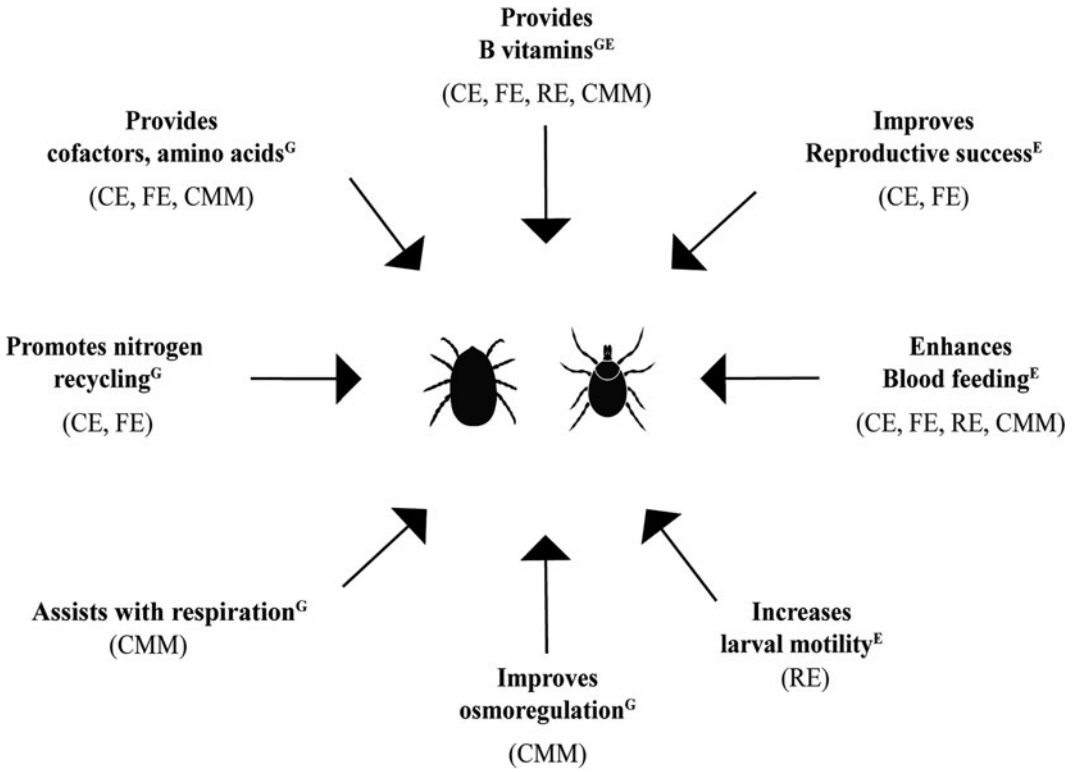

Figure 2. Putative functions of tick endosymbionts. [G] represents information based on genome sequences and [E] indicates data derived from experimental studies. CE, *Coxiella* endosymbiont; FE, *Francisella* endosymbiont; RE, *Rickettsia* endosymbiont; CMM, *Candidatus* Midichloria mitochondrii.

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several insect endosymbionts (Sabree *et al*., 2009; Hansen and Moran, 2011; Gerhart *et al*., 2018).

CMM genome encodes proteins that could assist *I. ricinus* in its response to oxidative stress and in energy metabolism (Olivieri *et al*., 2019) (Fig. 2). These proteins include the cytochrome cbb_3 oxidases that may support ATP production with reduced levels of oxygen concentration during oogenesis, heme exporter proteins, a protoheme ferro-lyase, superoxide dismutase, ferrochelatase, nucleotide tlc translocases and a pathway for the synthesis of lipoic acid, a cofactor that forms part of diverse enzyme complexes of electron transport chains in mitochondria (Packer *et al*., 1995; Sassera *et al*., 2011; Olivieri *et al*., 2019). Proteins such as constituents of the major facilitator superfamily and the drug/metabolite transporter superfamily potentially responsible for the transportation of fluids and ions such as sodium, protons and potassium and the uptake of organic compounds like amino acids were also annotated in the genome of CMM suggesting that the bacterium may play a role in the maintenance of osmoregulation and water balance in *I. ricinus* during blood feeding (Olivieri *et al*., 2019). Lastly, a recently acquired CMM in *Hyalomma marginatum* seems to compensate for the loss of biotin and heme biosynthesis genes in the co-infecting FE (Buysse *et al*., 2021*b*), probably because both endosymbionts together provide critical metabolites to ticks as observed in several insects (Husnik and McCutcheon, 2016; Santos-Garcia *et al*., 2018; Takeshita *et al*., 2019).

Experimental evidence for endosymbiont function

Administration of antibiotics that diminish endosymbiont populations leads to reduced reproductive success in ticks, suggesting a fitness-boosting role for endosymbionts (Fig. 2). For instance, exposure of *A. americanum* to tetracycline and rifampicin led to a decrease in CE load and concomitant reduction in reproductive fitness, as evidenced by a setback in the rate of oviposition and significantly lower number of ticks that emerged from eggs (Zhong *et al*., 2007). Similarly, injection of tetracycline into *R. microplus* and its cattle host led to reduced levels of CERM and a delay in the development of the tick at the metanymph phase (Guizzo *et al*., 2017), and administration of ofloxacin to *R. sanguineus* led to reduced burdens of CRs accompanied by an increase in time for adult females to feed to repletion, a reduced engorgement weight and a decreased capacity to lay eggs (Ben-Yosef *et al*., 2020; An *et al*., 2022). Likewise, tetracycline administration to female *H. longicornis* led to reduced densities of the *Coxiella* symbiont in the ovaries and malpighian tubules, which led to significant changes in tick engorgement weight, feeding time, number of eggs laid and the length of the oviposition period (Zhang *et al*., 2017).

The reduction in tick fitness associated with endosymbiont loss could be due to the diminished availability of B vitamins that are vital to tick development. Antibiotic treatment of *O. moubata* reduced the tick's FE burden and interrupted with normal nymph feeding and moulting, which forestalled the development of viable adult female ticks and significantly lowered the emergence of male ticks. Providing a B vitamin mixture (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folate and cobalamin) to *O. moubata* restored its reproductive fitness, indicating a role for FLE-Om in provisioning B vitamins required for normal tick development and reproduction (Duron *et al*., 2018).

Experiments also suggest that endosymbionts may contribute to the blood-feeding capacity of ticks (Fig. 2). Treatment of *H. longicornis* nymphs with tetracycline reduced the levels of the *Coxiella* symbiont (CHI), which in turn reduced the blood intake of the tick (Zhong *et al*., 2021). Intriguingly, it is the metabolite chorismate produced by CHI rather than B vitamins or cofactors that likely influences tick blood intake. The study showed that chorismate promotes the production of 5-hydroxytryptamine (serotonin), a bioamine whose stability in the midgut and synganglion of the tick regulates the amount of blood ingested by the tick (Zhong *et al*., 2021). Similar to *H. longicornis*, administration of antibiotics to *R. sanguineus* and *R. microplus* also reduced the density of CEs and tick blood intake, and transcriptomic analysis of CERM-free *R. microplus* metanymphs revealed that genes associated with tick blood-feeding capacity such as DAP-36, lipocalin, trypsin inhibitor-like family, Kunitz-type inhibitors, cystatin and evasins were significantly under-expressed (Guizzo *et al*., 2017; Zhang *et al*., 2017; Ben-Yosef *et al*., 2020; An *et al*., 2022). Collectively, these data indicate that CEs improve blood feeding across tick species, but further studies are required to confirm whether endosymbiont-produced chorismate is the key metabolite that drives this process in all ticks.

CMM is the most common endosymbiont associated with *I. ricinus* and feeding females with tetracycline-containing bovine blood produced CMM-free ticks within 2 generations. Larvae that hatched from eggs laid by CMM-free females consistently performed poorly during blood feeding, suggesting that CMM is required for the emergence of larvae with intact blood-feeding ability (Guizzo *et al*., 2023). Similar to the above findings, *I. ricinus* nymphs fed with gentamicin-treated blood had significantly lower engorgement weights, lower moulting proportions and lower weights of moulted unfed adult females in comparison to nymphs fed on antibiotic-free blood (Militzer *et al*., 2023). These studies show that CMM, in addition to CEs, enhances blood intake by ticks. Interestingly, increased blood feeding by ticks seems to benefit the endosymbionts as well. For example, *Francisella* symbionts in *H. doenitzi* significantly increased in number during blood feeding, and *Rickettsia* sp. phylotype G021 and CMM multiplied massively in *I. pacificus* and *I. ricinus*, respectively, following blood meals (Sassera *et al*., 2008; Cheng *et al*., 2013; Liu *et al*., 2016). Thus, improved intake of blood, which nourishes both tick and endosymbiont, seems to be one of the major benefits of long-term symbiosis between ticks and intracellular bacteria.

Finally, *D. variabilis* and *A. americanum* larvae infected with *Rickettsia* symbionts displayed increased motility than uninfected larvae [42]. The locomotive ability of newly hatched larvae was determined on flat and inclined surfaces and *Rickettsia*-containing larvae displayed increased locomotive speed relative to uninfected larvae. Tick motility plays a role in host-questing success; thus, infection with *Rickettsia* symbionts may impact the disease risk posed by tick-borne pathogens.

Conclusions and future directions

In summary, a major function of tick endosymbionts seems to be the provisioning of B vitamins, especially riboflavin, biotin and folate (Fig. 1). B vitamins are in short supply in vertebrate blood; hence, maintaining endosymbionts that reliably provide these vital nutrients could be an adaptation that allowed ticks to evolve a strictly blood-dependent lifestyle. Endosymbionts also seem to provide additional functions such as improved blood intake and increased motility that enhance tick physiology and reproductive success (Fig. 2). However, these roles have only been demonstrated in a few tick–endosymbiont systems, so more studies are needed to understand whether these features are lineage-specific or are more widespread. This information is especially relevant given that CE, FE and CMM phylogenies often do not agree with tick phylogenies (Epis *et al*., 2008; Cafiso *et al*., 2016; Duron *et al*., 2017; Binetruy *et al*., 2020). One of the underlying causes for this discordant evolutionary

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pattern could be that different tick species have acquired endosymbionts belonging to divergent *Coxiella*, *Francisella* and *Candidatus* Midichloria branches (Epis *et al.*, 2008; Cafiso *et al.*, 2016; Gerhart *et al.*, 2018; Brenner *et al.*, 2021). Thus, it is possible that depending on the lineages of their ancestors, endosymbionts in different tick species perform divergent functions.

Relatedly, another aspect of tick biology that we do not fully understand is how CEs and FEs evolved from pathogenic ancestors (Gerhart *et al.*, 2016; Brenner *et al.*, 2021). For example, pathogens such as *C. burnetii* and *F. tularensis* are not vertically transmitted (Genchi *et al.*, 2015; Buysse *et al.*, 2021*a*), but maternal transmission is a critical step in endosymbiosis. Thus, understanding how this process arose and is maintained in ticks would provide new insights into the biology of highly integrated tick–endosymbiont systems.

In addition to vertical transmission, an essential factor that sustains endosymbiosis is the presumed dependence of ticks on nutrients provided by endosymbionts. Going forward, functional studies to identify specific metabolites that sustain tick–endosymbiont relationships should be prioritized. Developing genetically tractable tick–endosymbiont model systems would accelerate this area of research by facilitating the disruption of endosymbiont genes to assess their impact on tick physiology and fitness. Although methodologies to culture and genetically manipulate CEs or FEs have not yet been developed, genetic tools and culture media are available for related pathogens *C. burnetii* and *F. tularensis* (Zogaj and Klose, 2010; Omsland *et al.*, 2011). These systems could be adapted to generate mutant CE or FE strains to assay the contributions of specific metabolites to tick physiology and reproductive success.

Future functional studies should also device alternatives to the current practice of using antibiotics to generate endosymbiont-free ticks. This is because antibiotics may eliminate other members of the tick microbiota, thus making it difficult to determine whether any observed effect is solely due to the loss of the endosymbiont. Another key aspect to consider while investigating endosymbiont function is the potential contributions made by the rest of the tick microbiota towards tick physiology. For instance, gut microbiota may modify metabolites present in blood meal to make them amenable for use by tick or endosymbiont. Similarly, antibacterial peptides produced by the tick innate immune system in response to gut bacteria could impact the location and functions of tick endosymbionts (Narasimhan *et al.*, 2021). Several recent studies have analysed the metabolic capabilities of tick microbiomes (Obregón *et al.*, 2019; Estrada-Peña *et al.*, 2020). These observations should be integrated with data from endosymbionts to gain a holistic view of how endosymbionts along with rest of the microbiota influence tick biology.

Lastly, targeting keystone taxa among tick microbiota is an innovative approach to inhibit the spread of ticks. Recent studies showed that microbiota were disrupted in ticks fed on blood from mice vaccinated against keystone taxa (Mateos-Hernández *et al.*, 2020, 2021). While the exact physiological consequences of such 'anti-microbiota' vaccines are yet to be elucidated, this approach holds promise as an alternative to the use of acaricides to control tick infestation and pathogen transmission. A complementary approach would be to identify/develop molecules that target biochemical processes that are unique to endosymbionts, which are stable members of tick microbiota. For this approach to succeed, it is necessary to gain a deeper understanding of how the enmeshed tick–endosymbiont metabolic pathways function to improve tick physiology and fitness. By developing new molecular tools to manipulate endosymbiont genes and by considering endosymbiont physiology in the context of the whole tick microbiota, future studies should make anti-endosymbiont strategies a reality.

Data availability. Not applicable.

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Competing interests. None.

Ethical standards. Not applicable.

References

Ahantarig A, Malaisri P, Hirunkanokpun S, Sumrandee C, Trinachartvanit W and Baimai V (2011) Detection of *Rickettsia* and a novel *Haemaphysalis shimoga* symbiont bacterium in ticks in Thailand. *Current Microbiology* **62**, 1496–1502.

Ahantarig A, Trinachartvanit W, Baimai V and Grubhoffer L (2013) Hard ticks and their bacterial endosymbionts (or would be pathogens). *Folia Microbiologica* **58**, 419–428.

Aivelo T, Norberg A and Tschirren B (2019) Bacterial microbiota composition of *Ixodes ricinus* ticks: the role of environmental variation, tick characteristics and microbial interactions. *PeerJ* **7**, e8217.

An L, Bhowmick B, Liang D, Suo P, Liao C, Zhao J and Han Q (2022) The microbiota changes of the brown dog tick, *Rhipicephalus sanguineus* under starvation stress. *Frontiers in Physiology* **13**, 932130. doi: 10.3389/fphys.2022.932130

Anderson JF and Magnarelli LA (2008) Biology of ticks. *Infectious Disease Clinics of North America* **22**, 195–215.

Andreotti R, Pérez de León AA, Dowd SE, Guerrero FD, Bendele KG and Scoles GA (2011) Assessment of bacterial diversity in the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* through tag-encoded pyrosequencing. *BMC Microbiology* **11**, 6.

Anstead CA and Chilton NB (2014) Discovery of novel *Rickettsiella* spp. in ixodid ticks from Western Canada. *Applied and Environmental Microbiology* **80**, 1403–1410.

Bell-Sakyi L, Palomar AM and Kazimirova M (2015) Isolation and propagation of a *Spiroplasma* sp. from Slovakian *Ixodes ricinus* ticks in *Ixodes* spp. cell lines. *Ticks and Tick-Borne Diseases* **6**, 601–606.

Ben-Yosef M, Rot A, Mahagna M, Kapri E, Behar A and Gottlieb Y (2020) *Coxiella*-like endosymbiont of *Rhipicephalus sanguineus* is required for physiological processes during ontogeny. *Frontiers in Microbiology* **11**, 493–493.

Bennett GM and Moran NA (2015) Heritable symbiosis: the advantages and perils of an evolutionary rabbit hole. *Proceedings of the National Academy of Sciences of the USA* **112**, 10169–10176.

Benson MJ, Gawronski JD, Eveleigh DE and Benson DR (2004) Intracellular symbionts and other bacteria associated with deer ticks (*Ixodes scapularis*) from Nantucket and Wellfleet, Cape Cod, Massachusetts. *Applied and Environmental Microbiology* **70**, 616–620.

Binetruy F, Buysse M, Lejarre Q, Barosi R, Villa M, Rahola N, Paupy C, Ayala D and Duron O (2020) Microbial community structure reveals instability of nutritional symbiosis during the evolutionary radiation of *Amblyomma* ticks. *Molecular Ecology* **29**, 1016–1029.

Bodnar JL, Fitch S, Rosati A and Zhong J (2018) The *folA* gene from the *Rickettsia* endosymbiont of *Ixodes pacificus* encodes a functional dihydrofolate reductase enzyme. *Ticks and Tick-Borne Diseases* **9**, 443–449.

Bonnet SI, Binetruy F, Hernández-Jarguín AM and Duron O (2017) The tick microbiome: why non-pathogenic microorganisms matter in tick biology and pathogen transmission. *Frontiers in Cellular and Infection Microbiology* **7**, 236.

Bown KJ, Begon M, Bennett M, Woldehiwet Z and Ogden NH (2003) Seasonal dynamics of *Anaplasma phagocytophila* in a rodent-tick (*Ixodes trianguliceps*) system, United Kingdom. *Emerging Infectious Diseases* **9**, 63–70.

Brenner AE, Muñoz-Leal S, Sachan M, Labruna MB and Raghavan R (2021) *Coxiella burnetii* and related tick endosymbionts evolved from pathogenic ancestors. *Genome Biology and Evolution* **13**(7), evab108.

Buysse M, Duhayon M, Cantet F, Bonazzi M and Duron O (2021a) Vector competence of the African argasid tick *Ornithodoros moubata* for the Q fever agent *Coxiella burnetii*. *PLoS Neglected Tropical Diseases* **15**, e0009008.

Buysse M, Floriano AM, Gottlieb Y, Nardi T, Comandatore F, Olivieri E, Giannetto A, Palomar AM, Makepeace BL, Bazzocchi C, Cafiso A,

https://doi.org/10.1017/S0031182023000793 Published online by Cambridge University Press

Sassera D and Duron O (2021b) A dual endosymbiosis supports nutritional adaptation to hematophagy in the invasive tick *Hyalomma marginatum*. *Elife* **10**, e72747.

Cafiso A, Bazzocchi C, De Marco L, Opara MN, Sassera D and Plantard O (2016) Molecular screening for Midichloria in hard and soft ticks reveals variable prevalence levels and bacterial loads in different tick species. *Ticks and Tick-Borne Diseases* **7**, 1186–1192.

Carpi G, Cagnacci F, Wittekindt NE, Zhao F, Qi J, Tomsho LP, Drautz DI, Rizzoli A and Schuster SC (2011) Metagenomic profile of the bacterial communities associated with *Ixodes ricinus* ticks. *PLoS ONE* **6**, e25604.

Chao L-L, Castillo CT and Shih C-M (2021) Molecular detection and genetic identification of *Wolbachia* endosymbiont in *Rhipicephalus sanguineus* (Acari: Ixodidae) ticks of Taiwan. *Experimental and Applied Acarology* **83**, 115–130.

Cheng D, Lane RS, Moore BD and Zhong J (2013) 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 and Tick-Borne Diseases* **4**, 421–426.

Clay K, Klyachko O, Grindle N, Civitello D, Oleske D and Fuqua C (2008) Microbial communities and interactions in the lone star tick, *Amblyomma americanum*. *Molecular Ecology* **17**, 4371–4381.

Dantas-Torres F, Chomel BB and Otranto D (2012) Ticks and tick-borne diseases: a One Health perspective. *Trends in Parasitology* **28**, 437–446.

de la Fuente J, Estrada-Pena A, Venzal JM, Kocan KM and Sonenshine DE (2008) Overview: ticks as vectors of pathogens that cause disease in humans and animals. *Frontiers in Bioscience* **13**, 6938–6946.

de La Fuente J, Kocan KM and Contreras M (2015) Prevention and control strategies for ticks and pathogen transmission. *Revue scientifique et technique (International Office of Epizootics)* **34**, 249–264.

Dergousoff SJ and Chilton NB (2010) Detection of a new *Arsenophonus*-type bacterium in Canadian populations of the Rocky Mountain wood tick, *Dermacentor andersoni*. *Experimental and Applied Acarology* **52**, 85–91.

Douglas AE (2017) The B vitamin nutrition of insects: the contributions of diet, microbiome and horizontally acquired genes. *Current Opinion in Insect Science* **23**, 65–69.

Duron O and Gottlieb Y (2020) Convergence of nutritional symbioses in obligate blood feeders. *Trends in Parasitology* **36**, 816–825.

Duron O, Noël V, Mccoy KD, Bonazzi M, Sidi-Boumedine K, Morel O, Vavre F, Zenner L, Jourdain E and Durand P (2015) The recent evolution of a maternally-inherited endosymbiont of ticks led to the emergence of the Q fever pathogen, *Coxiella burnetii*. *PLoS Pathogens* **11**, e1004892.

Duron O, Binetruy F, Noël V, Cremaschi J, McCoy KD, Arnathau C, Plantard O, Goolsby J, Pérez de León AA and Heylen DJ (2017) Evolutionary changes in symbiont community structure in ticks. *Molecular Ecology* **26**, 2905–2921.

Duron O, Morel O, Noël V, Buysse M, Binetruy F, Lancelot R, Loire E, Ménard C, Bouchez O, Vavre F and Vial L (2018) Tick-bacteria mutualism depends on B vitamin synthesis pathways. *Current Biology* **28**, 1896–1902.e1895.

Epis S, Sassera D, Beninati T, Lo N, Beati L, Piesman J, Rinaldi L, McCoy KD, Torina A, Sacchi L, Clementi E, Genchi M, Magnino S and Bandi C (2008) Midichloria mitochondrii is widespread in hard ticks (Ixodidae) and resides in the mitochondria of phylogenetically diverse species. *Parasitology* **135**, 485–494.

Ereqat S, Nasereddin A, Vayssier-Taussat M, Abdelkader A, Al-Jawabreh A, Zaid T, Azmi K and Abdeen Z (2016) Molecular evidence of *Bartonella* species in ixodid ticks and domestic animals in Palestine. *Frontiers in Microbiology* **7**, 1217.

Estrada-Pena A, Ayllon N and De La Fuente J (2012) Impact of climate trends on tick-borne pathogen transmission. *Frontiers in Physiology* **3**, 64.

Estrada-Peña A, Cabezas-Cruz A and Obregón D (2020) Behind taxonomic variability: the functional redundancy in the tick microbiome. *Microorganisms* **8**, 1829.

Gall CA, Scoles GA, Magori K, Mason KL and Brayton KA (2017) Laboratory colonization stabilizes the naturally dynamic microbiome composition of field collected *Dermacentor andersoni* ticks. *Microbiome* **5**, 133.

Genchi M, Prati P, Vicari N, Manfredini A, Sacchi L, Clementi E, Bandi C, Epis S and Fabbi M (2015) *Francisella tularensis*: no evidence for transovarial transmission in the tularemia tick vectors *Dermacentor reticulatus* and *Ixodes ricinus*. *PLoS ONE* **10**, e0133593.

Gerhart JG, Moses AS and Raghavan R (2016) A *Francisella*-like endosymbiont in the Gulf Coast tick evolved from a mammalian pathogen. *Scientific Reports* **6**, 33670.

Gerhart JG, Auguste Dutcher H, Brenner AE, Moses AS, Grubhoffer L and Raghavan R (2018) Multiple acquisitions of pathogen-derived *Francisella* endosymbionts in soft ticks. *Genome Biology and Evolution* **10**, 607–615.

Gottlieb Y, Lalzar I and Klasson L (2015) Distinctive genome reduction rates revealed by genomic analyses of two *Coxiella*-like endosymbionts in ticks. *Genome Biology and Evolution* **7**, 1779–1796.

Grindle N, Tyner JJ, Clay K and Fuqua C (2003) Identification of *Arsenophonus*-type bacteria from the dog tick *Dermacentor variabilis*. *Journal of Invertebrate Pathology* **83**, 264–266.

Guizzo MG, Parizi LF, Nunes RD, Schama R, Albano RM, Tirloni L, Oldiges DP, Vieira RP, Oliveira WHC, Leite MdS, Gonzales SA, Farber M, Martins O, Vaz IdS and Oliveira PL (2017) A *Coxiella* mutualist symbiont is essential to the development of *Rhipicephalus microplus*. *Scientific Reports* **7**, 17554.

Guizzo MG, Hatalová T, Frantová H, Zurek L, Kopáček P and Perner J (2023) *Ixodes ricinus* ticks have a functional association with Midichloria mitochondrii. *Frontiers in Cellular and Infection Microbiology* **12**, 1081666.

Hansen AK and Moran NA (2011) Aphid genome expression reveals host symbiont cooperation in the production of amino acids. *Proceedings of the National Academy of Sciences* **108**, 2849–2854.

Henning K, Greiner-Fischer S, Hotzel H, Ebsen M and Theegarten D (2006) Isolation of *Spiroplasma* sp. from an *Ixodes* tick. *International Journal of Medical Microbiology* **296**, 157–161.

Hirunkanokpun S, Ahantarig A, Baimai V and Trinachartvanit W (2018) A new record of *Wolbachia* in the elephant ticks from Thailand. *Science Asia* **44**, 44–47.

Hunter DJ, Torkelson JL, Bodnar J, Mortazavi B, Laurent T, Deason J, Thephavongsa K and Zhong J (2015) The *Rickettsia* endosymbiont of *Ixodes pacificus* contains all the genes of de novo folate biosynthesis. *PLoS ONE* **10**, e0144552.

Husnik F and McCutcheon JP (2016) Repeated replacement of an intrabacterial symbiont in the tripartite nested mealybug symbiosis. *Proceedings of the National Academy of Sciences* **113**, E5416–E5424.

Johnson N (2023) Controlling ticks and tick-borne disease transmission. In Johnson N (ed.), *Ticks: Biology, Ecology and Diseases*. Netherlands: Elsevier Science. pp. 193–215.

Jongejan F and Uilenberg G (2004) The global importance of ticks. *Parasitology* **129**, S3–S14.

Kagemann J and Clay K (2013) Effects of infection by *Arsenophonus* and *Rickettsia* bacteria on the locomotive ability of the ticks *Amblyomma americanum*, *Dermacentor variabilis*, and *Ixodes scapularis*. *Journal of Medical Entomology* **50**, 155–162.

Kamani J, Morick D, Mumcuoglu KY and Harrus S (2013) Prevalence and diversity of *Bartonella* species in commensal rodents and ectoparasites from Nigeria, West Africa. *PLoS Neglected Tropical Diseases* **7**, e2246.

Karp PD, Billington R, Caspi R, Fulcher CA, Latendresse M, Kothari A, Keseler IM, Krummenacker M, Midford PE, Ong Q, Ong WK, Paley SM and Subhraveti P (2017) The BioCyc collection of microbial genomes and metabolic pathways. *Briefings in Bioinformatics* **20**, 1085–1093.

Kurtti TJ, Munderloh UG, Andreadis TG, Magnarelli LA and Mather TN (1996) Tick cell culture isolation of an intracellular prokaryote from the tick *Ixodes scapularis*. *Journal of Invertebrate Pathology* **67**, 318–321.

Kurtti TJ, Palmer AT and Oliver JH (2002) *Rickettsiella*-like bacteria in *Ixodes woodi* (Acari: Ixodidae). *Journal of Medical Entomology* **39**, 534–540.

Lalzar I, Harrus S, Mumcuoglu KY and Gottlieb Y (2012) Composition and seasonal variation of *Rhipicephalus turanicus* and *Rhipicephalus sanguineus* bacterial communities. *Applied and Environmental Microbiology* **78**, 4110–4116.

Latrofa MS, Dantas-Torres F, Giannelli A and Otranto D (2014) Molecular detection of tick-borne pathogens in *Rhipicephalus sanguineus* group ticks. *Ticks and Tick-Borne Diseases* **5**, 943–946.

Leclerque A and Kleespies RG (2012) A *Rickettsiella* bacterium from the hard tick, *Ixodes woodi*: molecular taxonomy combining multilocus sequence typing (MLST) with significance testing. *PLoS ONE* **7**, e38062.

Lejal E, Chiquet J, Aubert J, Robin S, Estrada-Peña A, Rue O, Midoux C, Mariadassou M, Bailly X, Cougoul A, Gasqui P, Cosson JF, Chalvet-Monfray K, Vayssier-Taussat M and Pollet T (2021) Temporal patterns in *Ixodes ricinus* microbial communities: an insight into tick-borne microbe interactions. *Microbiome* **9**, 153.

Lewis D (1979) The detection of rickettsia-like microorganisms within the ovaries of female *Ixodes ricinus ticks*. *Zeitschrift fur Parasitenkunde* **59**, 295–298.

Liu JN, Yu ZJ, Liu LM, Li NX, Wang RR, Zhang CM and Liu JZ (2016) Identification, distribution and population dynamics of *Francisella*-like

https://doi.org/10.1017/S0031182023000793 Published online by Cambridge University Press

endosymbiont in *Haemaphysalis doenitzi* (Acari: Ixodidae). *Scientific Reports* **6**, 35178.

Mateos-Hernández L, Obregón D, Maye J, Borneres J, Versille N, de la Fuente J, Estrada-Peña A, Hodžić A, Šimo L and Cabezas-Cruz A (2020) Anti-tick microbiota vaccine impacts *Ixodes ricinus* performance during feeding. *Vaccines* **8**, 702.

Mateos-Hernández L, Obregón D, Wu-Chuang A, Maye J, Bornères J, Versillé N, de la Fuente J, Díaz-Sánchez S, Bermúdez-Humarán LG, Torres-Maravilla E, Estrada-Peña A, Hodžić A, Šimo L and Cabezas-Cruz A (2021) Anti-microbiota vaccines modulate the tick microbiome in a taxon-specific manner. *Frontiers in Immunology* **12**, 704621.

McCutcheon JP and Moran NA (2012) Extreme genome reduction in symbiotic bacteria. *Nature Reviews Microbiology* **10**, 13–26.

McCutcheon JP, Boyd BM and Dale C (2019) The life of an insect endosymbiont from the cradle to the grave. *Current Biology* **29**, R485–R495.

Mediannikov O, Subramanian G, Sekeyova Z, Bell-Sakyi L and Raoult D (2012) Isolation of *Arsenophonus nasoniae* from *Ixodes ricinus* ticks in Slovakia. *Ticks and Tick-Borne Diseases* **3**, 367–370.

Militzer N, Pinecki Socias S and Nijhof AM (2023) Changes in the *Ixodes ricinus* microbiome associated with artificial tick feeding. *Frontiers in Microbiology* **13**, 1050063.

Narasimhan S and Fikrig E (2015) Tick microbiome: the force within. *Trends in Parasitology* **31**, 315–323.

Narasimhan S, Swei A, Abouneameh S, Pal U, Pedra JHF and Fikrig E (2021) Grappling with the tick microbiome. *Trends in Parasitology* **37**, 722–733.

Nardi T, Olivieri E, Kariuki E, Sassera D and Castelli M (2020) Sequence of a *Coxiella* endosymbiont of the tick *Amblyomma nuttalli* suggests a pattern of convergent genome reduction in the *Coxiella* genus. *Genome Biology and Evolution* **13**(1), evaa253.

Noda H, Munderloh UG and Kurtti TJ (1997) Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals. *Applied and Environmental Microbiology* **63**, 3926–3932.

Obaid MK, Islam N, Alouffi A, Khan AZ, da Silva Vaz I Jr., Tanaka T and Ali A (2022) Acaricides resistance in ticks: selection, diagnosis, mechanisms, and mitigation. *Frontiers in Cellular and Infection Microbiology* **12**, 941831.

Obregón D, Bard E, Abrial D, Estrada-Peña A and Cabezas-Cruz A (2019) Sex-specific linkages between taxonomic and functional profiles of tick gut microbiomes. *Frontiers in Cellular and Infection Microbiology* **9**, 298.

O'Donnell MJ and Donini A (2017) Nitrogen excretion and metabolism in insects. In Weihrauch D and O'Donnell M (eds), *Acid-Base Balance and Nitrogen Excretion in Invertebrates: Mechanisms and Strategies in Various Invertebrate Groups with Considerations of Challenges Caused by Ocean Acidification*. Cham: Springer International Publishing, pp. 109–126.

Olivieri E, Epis S, Castelli M, Varotto Boccazzi I, Romeo C, Desirò A, Bazzocchi C, Bandi C and Sassera D (2019) Tissue tropism and metabolic pathways of Midichloria mitochondrii suggest tissue-specific functions in the symbiosis with *Ixodes ricinus*. *Ticks and Tick-Borne Diseases* **10**, 1070–1077.

Omsland A, Beare PA, Hill J, Cockrell DC, Howe D, Hansen B, Samuel JE and Heinzen RA (2011) Isolation from animal tissue and genetic transformation of *Coxiella burnetii* are facilitated by an improved axenic growth medium. *Applied and Environmental Microbiology* **77**, 3720–3725.

Packer L, Witt EH and Tritschler HJ (1995) Alpha-lipoic acid as a biological antioxidant. *Free Radical Biology and Medicine* **19**, 227–250.

Parola P and Raoult D (2001) Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. *Clinical Infectious Diseases* **32**, 897–928.

Parola P, Paddock CD and Raoult D (2005) Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clinical Microbiology Reviews* **18**, 719–756.

Petersen JM, Mead PS and Schriefer ME (2009) *Francisella tularensis*: an arthropod-borne pathogen. *Veterinary Research* **40**(2), 7.

Piesman J and Eisen L (2008) Prevention of tick-borne diseases. *Annual Review of Entomology* **53**, 323–343.

Plantard O, Bouju-Albert A, Malard MA, Hermouet A, Capron G and Verheyden H (2012) Detection of *Wolbachia* in the tick *Ixodes ricinus* is due to the presence of the hymenoptera endoparasitoid *Ixodiphagus hookeri*. *PLoS ONE* **7**, e30692.

Potgieter F and Stoltsz W (1994) Bovine anaplasmosis In Coetzer JAW and Tustin RC (eds), *Infectious Diseases of Livestock Diseases of Livestock, with Special Reference to Southern Africa*. Cape Town, South Africa: Oxford University Press. pp. 408-430.

Qiu Y, Nakao R, Ohnuma A, Kawamori F and Sugimoto C (2014) Microbial population analysis of the salivary glands of ticks; a possible strategy for the surveillance of bacterial pathogens. *PLoS ONE* **9**, e103961.

Regier Y, Ballhorn W and Kempf VAJ (2017) Molecular detection of *Bartonella henselae* in 11 *Ixodes ricinus* ticks extracted from a single cat. *Parasites and Vectors* **10**, 105.

Sabree ZL, Kambhampati S and Moran NA (2009) Nitrogen recycling and nutritional provisioning by *Blattabacterium*, the cockroach endosymbiont. *Proceedings of the National Academy of Sciences* **106**, 19521–19526.

Sacchi L, Bigliardi E, Corona S, Beninati T, Lo N and Franceschi A (2004) A symbiont of the tick *Ixodes ricinus* invades and consumes mitochondria in a mode similar to that of the parasitic bacterium *Bdellovibrio bacteriovorus*. *Tissue and Cell* **36**, 43–53.

Santos-Garcia D, Juravel K, Freilich S, Zchori-Fein E, Latorre A, Moya A, Morin S and Silva FJ (2018) To B or not to B: comparative genomics suggests *Arsenophonus* as a source of B vitamins in whiteflies. *Frontiers in Microbiology* **9**, 2254.

Sassera D, Beninati T, Bandi C, Bouman EAP, Sacchi L, Fabbi M and Lo N (2006) '*Candidatus* Midichloria mitochondrii', an endosymbiont of the tick *Ixodes ricinus* with a unique intramitochondrial lifestyle. *International Journal of Systematic and Evolutionary Microbiology*, **56**, 2535–2540.

Sassera D, Lo N, Bouman EAP, Epis S, Mortarino M and Bandi C (2008) *Candidatus* Midichloria endosymbionts bloom after the blood meal of the host, the hard tick *Ixodes ricinus*. *Applied and Environmental Microbiology* **74**, 6138–6140.

Sassera D, Lo N, Epis S, D'Auria G, Montagna M, Comandatore F, Horner D, Peretó J, Luciano AM, Franciosi F, Ferri E, Crotti E, Bazzocchi C, Daffonchio D, Sacchi L, Moya A, Latorre A and Bandi C (2011) Phylogenomic evidence for the presence of a flagellum and cbb(3) oxidase in the free-living mitochondrial ancestor. *Molecular Biology and Evolution* **28**, 3285–3296.

Smith TA, Driscoll T, Gillespie JJ and Raghavan R (2015) A *Coxiella*-like endosymbiont is a potential vitamin source for the Lone Star tick. *Genome Biology and Evolution* **7**, 831–838.

Sonenshine DE (2018) Range expansion of tick disease vectors in North America: implications for spread of tick-borne disease. *International Journal of Environmental Research and Public Health* **15**(3), 478.

Sonenshine DE and Roe RM (2014) *Biology of Ticks*, In Sonenshine DE and Roe RM (eds). Volume 2. New York, NY: Oxford University Press, pp. 3–16.

Sun LV, Scoles GA, Fish D and O'Neill SL (2000) *Francisella*-like endosymbionts of ticks. *Journal of Invertebrate Pathology* **76**, 301–303.

Takeshita K, Yamada T, Kawahara Y, Narihiro T, Ito M, Kamagata Y and Shinzato N (2019) Tripartite symbiosis of an anaerobic scuticociliate with two hydrogenosome-associated endosymbionts, a *Holospora* related alphaproteobacterium and a methanogenic archaeon. *Applied and Environmental Microbiology* **85**, e00854–e00819.

Tsementzi D, Castro Gordillo J, Mahagna M, Gottlieb Y and Konstantinidis KT (2018) Comparison of closely related, uncultivated *Coxiella* tick endosymbiont population genomes reveals clues about the mechanisms of symbiosis. *Environmental Microbiology* **20**, 1751–1764.

Zhang X, Norris DE and Rasgon JL (2011) Distribution and molecular characterization of *Wolbachia* endosymbionts and filarial nematodes in Maryland populations of the lone star tick (*Amblyomma americanum*). *FEMS Microbiology Ecology* **77**, 50–56.

Zhang C-M, Li N-X, Zhang T-T, Qiu Z-X, Li Y, Li L-W and Liu J-Z (2017) Endosymbiont CLS-HI plays a role in reproduction and development of *Haemaphysalis longicornis*. *Experimental and Applied Acarology* **73**, 429–438.

Zhong J, Jasinskas A and Barbour AG (2007) Antibiotic treatment of the tick vector *Amblyomma americanum* reduced reproductive fitness. *PLoS ONE* **2**, e405.

Zhong Z, Zhong T, Peng Y, Zhou X, Wang Z, Tang H and Wang J (2021) Symbiont-regulated serotonin biosynthesis modulates tick feeding activity. *Cell Host & Microbe* **29**, 1545–1557.e1544.

Zhu Z, Aeschlimann A and Gern L (1992) *Rickettsia*-like microorganisms in the ovarian primordial of molting *Ixodes ricinus* (Acari: Ixodidae) larvae and nymphs. *Annales de parasitologie humaine et comparée* **67**, 99–110.

Zogaj X and Klose KE (2010) Genetic manipulation of *Francisella tularensis*. *Frontiers in Microbiology* **1**, 142.

https://doi.org/10.1017/S0031182023000793 Published online by Cambridge University Press