

# Review

# Grappling with the tick microbiome

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*Ixodes scapularis* and *Ixodes pacificus* are the predominant vectors of multiple human pathogens, including *Borrelia burgdorferi*, one of the causative agents of Lyme disease in North America. Differences in the habitats and host preferences of these closely related tick species present an opportunity to examine key aspects of the tick microbiome. While advances in sequencing technologies have accelerated a descriptive understanding of the tick microbiome, molecular and mechanistic insights into the tick microbiome are only beginning to emerge. Progress is stymied by technical difficulties in manipulating the microbiome and by biological variables related to the life cycle of Ixodid ticks. This review highlights these challenges and examines avenues to understand the significance of the tick microbiome in tick biology.

## The hematophagous arthropod microbiome

There is increasing interest in determining the role of the microbiomes of hematophagous arthropod vectors of disease in pathogen acquisition and transmission as well as in the life cycle of the vector itself. Of particular interest has been the arthropod gut microbiome, since acquiring and utilizing a bloodmeal is central to the life cycle of the vector and hinges on the physical and functional integrity of the gut. Further, pathogens acquired by the vector most often have to transit through the gut and this brings the vector, the microbiome, and the pathogen into close proximity, a crucible of interactions that may impact vectorial capacity. **Hematophagous** (see [Glossary](#)) arthropods have a restricted diet, feeding predominantly on vertebrate blood. Comparison of the microbiomes of different hematophagous arthropods demonstrates that dietary bloodmeal is not the only determinant of their composition [1] and that the composition of the microbiome is broadly related to specific arthropod genera. In hematophagous arthropods the microbiome composition is also determined by whether they are obligate or facultative blood-feeders [2]. Obligate blood-feeders, such as ticks, bed bugs, and tsetse flies, have evolved to rely on microbial endosymbionts to supplement several B vitamins – such as biotin, folate, and riboflavin – that are deficient in blood [2,3]. Thus, in obligate feeders, we observe a convergent evolution or co-cladogenesis [2,3] that favors associations with microbial endosymbionts – such as *Wigglesworthia* in tsetse flies, *Wolbachia* in bed bugs, and *Rickettsia*, *Francisella*, *Candidatus Midichloria*, or *Coxiella*-like symbionts in ticks – that encode analogous functions critical to circumvent nutritional deficiencies in the blood meal [2,3]. These endosymbionts reside in **bacteriocytes** associated with the gut or with reproductive organs [2,3]. Facultative blood feeders, such as mosquitoes, obtain their nutrients from additional food sources and do not appear to demonstrate nutritional dependence on specific bacteria [4].

Eubacterial organisms in the microbiota of hematophagous arthropods have been shown to have significant effects on arthropod evolution and ecology [1]. It is important to note that the microbiome also includes viral and eukaryotic microbes [2,3]. Although examination of the virome and eukaryotic microbiome has been hampered by cumbersome analysis and bioinformatic pipelines, technological advancements are providing the momentum to describe the viromes and eukaryotic microbiomes of arthropod vectors to enhance our understanding of the arthropod microbiome in further detail [5–9]. Functional understanding of arthropod microbiota has largely come from

## Highlights

*Ixodes scapularis* and *Ixodes pacificus* harbor a simple primary microbiome composed predominantly of the endosymbiont *Rickettsia buchneri*.

The tick microbiome also includes microbiota acquired from the environment and represents a transient microbiome.

The tick microbiome composition is in a state of flux and is likely influenced by multiple biotic and abiotic factors.

The microbiome composition is regulated by immune responses of the tick at the vector–host and environment–vector interfaces.

Understanding the interactions between the tick microbiota, tick-borne pathogens and tick immune responses will reveal new insights in tick biology.

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studies on hematophagous dipteran and hemipteran insect vectors of disease. Important functions associated with the arthropod microbiome include the production of **B vitamins** [2,3], manipulation of reproductive behaviors – including cytoplasmic incompatibility [10], parthenogenesis, and male killing – and protection of the host against infection by pathogens and parasites [11–13], possibly via modulation of innate immune responses [14–17].

Ticks are obligate blood-feeding members of the phylum Arthropoda, class Arachnida, subclass Acari, and are evolutionarily distant from the class Insecta; they transmit human and livestock pathogens worldwide [18]. The two primary families of tick species are Ixodidae (hard ticks) and Argasidae (soft ticks) and a monospecific family, Nuttalliellidae [19]. The feeding habits of hard and soft ticks vary significantly, with hard ticks feeding once in each developmental stage on a limited number of hosts, and soft ticks feeding on several different hosts at more frequent intervals [20]. While hard and soft ticks harbor microbiomes of varying complexities [21], a detailed understanding of their composition, role in tick biology, and vectorial capacity is only beginning to emerge. This review focuses on *I. pacificus* and *I. scapularis*, two *Ixodes* species that are endemic to North America [22]. These ticks transmit multiple human pathogens [18], and indeed these pathogens represent frequent microbial residents of the tick microbiome. This review dwells only on the nonpathogenic components of the microbiome and summarizes our current understanding of the bacterial microbiome of these tick species; it highlights the knowledge gaps that remain to be bridged in order to achieve a functional understanding of the tick microbiome.

#### *Ixodes scapularis* and *Ixodes pacificus*

*I. scapularis* and *I. pacificus* are the principal vectors of *B. burgdorferi*, one of the causative agents of Lyme disease in the USA [22]. *I. scapularis* is endemic to the Northeast, upper Midwest of the USA, and southeastern parts of Canada. *I. pacificus* is endemic predominantly to the west coast of the USA. In addition to *B. burgdorferi*, both species have also been known to carry *Anaplasma phagocytophilum* (which causes human anaplasmosis), *Babesia microti*, the agent of babesiosis [18], and *Borrelia miyamotoi* which causes a relapsing-fever-like disease [23]. *I. scapularis* also serves as a vector of Powassan virus that causes encephalitis [24], *Borrelia mayonii*, that causes Lyme disease, and *Ehrlichia euclairensis*, a minor causative agent of ehrlichiosis [25]. *I. pacificus* has been shown to carry spotted fever group *Rickettsia* [26], although there is no reported transmission of *Rickettsia* from *I. pacificus* to vertebrate hosts. Both *I. scapularis* and *I. pacificus* larval and nymphal stages tend to feed on small- to medium-sized mammals, birds, mice, squirrels, deer, and humans; notably, *I. pacificus* also feeds on lizards [27]. Differences in host preferences and geographic distributions between these two species significantly influence their microbiome compositions (Figure 1) and offer novel opportunities to gain mechanistic insights into tick-microbiome interactions.

#### Opportunities for acquisition of microbiota

Despite their comparatively long life spans, *I. scapularis* and *I. pacificus* have limited and discrete opportunities to acquire or lose microbes over their life span. The first opportunity for establishing the microbiome is seeded from the adult female tick to her offspring through **transovarial transmission**. After that, ticks can also acquire microbes from their environment and from blood feeding on vertebrate hosts. Before and after feeding to repletion on a vertebrate host, *I. scapularis* and *I. pacificus* ticks come into direct contact with soil microbes. Bacterial entry into the tick is predominantly through transovarial, oral, or cuticular routes. The majority of the soil microbiome is comprised of Acidobacteria, with bacteria from the phyla Verrucomicrobia, Bacteroidetes, Alphaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Betaproteobacteria, Planctomycetes, and Actinobacteria seen at lower relative abundances [28] and some of these have been found to be associated with the

#### Glossary

**B vitamins:** water-soluble vitamins synthesized by microorganisms. Folate and biotin are examples of this class of eight vitamins.

**Bacteriocytes:** specialized giant cells observed in certain arthropods that harbor endosymbionts. Endosymbionts provide nutrients such as vitamins and amino acids to the host.

**Endosymbionts:** microorganisms that live within the body cells of another organism. They may or may not live in specialized cells of the host organism, and there may or may not always be a mutualistic relationship. Endosymbionts may be transferred vertically (from parent to offspring) or horizontally (free living symbionts are acquired by the host organism from the environment).

**Hematophagous:** feeding on vertebrate hosts to obtain blood as the predominant source of nutrition.

**IMD pathway:** a conserved immune signaling pathway in arthropods that is involved in the activation of antibacterial responses predominantly towards Gram-negative bacteria.

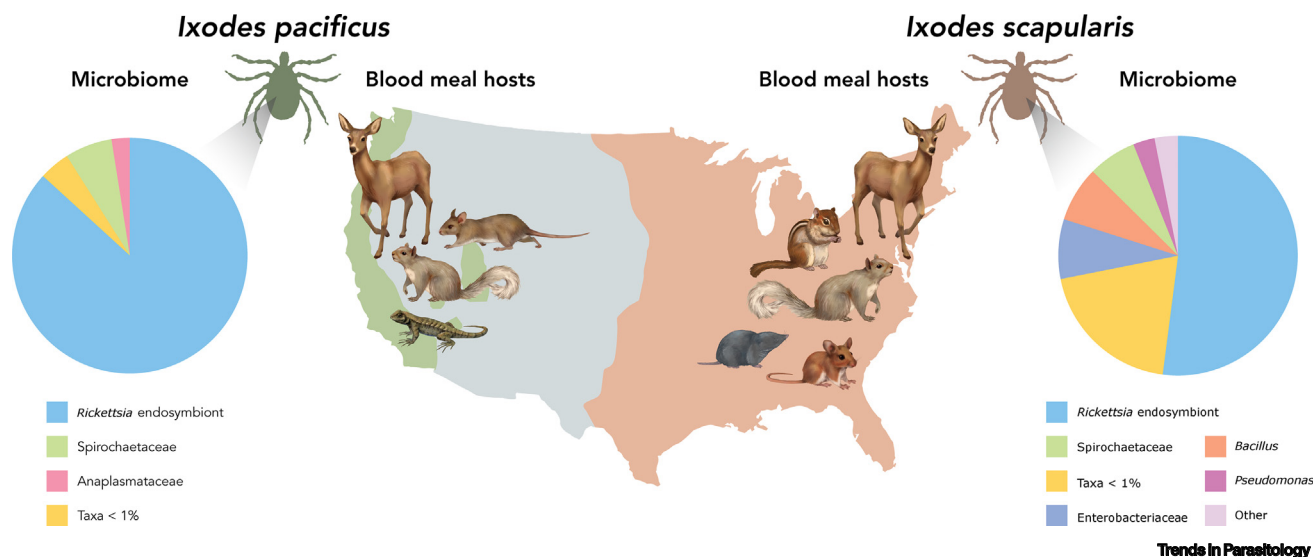
**JAK/STAT pathway:** a conserved signaling pathway found in arthropods and mammals; it is involved in key events, including immunity, cell division, repair, and remodeling. The main components of this pathway include a transmembrane receptor that engages with signals in the external milieu, an intracellular Janus kinase (JAK) that is associated with the transmembrane receptor, and signal transducer and activator of transcription (STAT) proteins.

**Microbiome:** a community of microorganisms that inhabit a specific niche, such as within an animal host; it includes commensal as well as mutualistic and parasitic organisms.

**Transovarial transmission:** transmission of microorganisms through oocytes from mother to offspring.

**Upd:** a secreted protein, encoded by the *upd* gene in *Drosophila*, that activates the JAK/STAT signaling pathway by binding to the transmembrane receptor of the JAK/STAT pathway.

**16S rRNA:** ribosomal RNA component of the 30S small subunit of the prokaryotic ribosome.



**Figure 1. Microbiome composition and distribution map of *Ixodes pacificus* and *Ixodes scapularis*.** *I. pacificus* and *I. scapularis*, the two vectors of Lyme disease in the USA, with the most commonly reported blood-meal hosts for juvenile and adult stages of the tick. Summaries of major eubacterial components of the adult tick microbiomes are displayed in the pie charts for each tick species. Illustration created by Ms Mona Luo.

tick microbiome [29]. There has been ongoing debate as to whether these shared soil bacteria are environmental contaminants or whether they constitute an important part of the intrinsic tick microbiome [30]. The microbiome of the mammalian skin plays a vital role in shaping mammalian immunobiology [31]. Therefore, we must be careful not to dismiss the role of microbiota on the surface of ticks.

In a field experiment, Couper *et al.* [32] directly tested the influence of environmental exposure time on the *I. pacificus* microbiome by placing larval *I. pacificus* in field enclosures buried in the soil for different amounts of time, ranging from 0 to 6 weeks, and found that field-exposed larvae acquire microbial richness. This was also supported by another study on *I. scapularis* that coupled microbiome analysis with midgut immunostaining; it found that some taxa isolated from external wash samples were also detected internally – such as *Bacillus*, Enterobacteriaceae, and *Pseudomonas* [33]. Further, laboratory-reared *I. scapularis* and *I. pacificus* display distinct microbiomes compared to field-collected ticks and are generally characterized by lower microbial diversity – presumably due to a more sterile environment compared to field-collected ticks [15,21,34,35].

### Host blood meal and tick microbiome

The intimate relationship between *Ixodes* spp. and their blood-meal hosts prompted investigations on whether the host influences the tick's microbiome. Several studies found that there is little correlation between host skin or blood and the microbiomes of hard ticks such as *I. scapularis* and *Dermacentor variabilis* [35–37]. Instead, those studies found that the most important factor in structuring tick microbiomes was tick species [36]. When the microbiomes of *D. variabilis* and *I. scapularis* that fed on two rodent blood-meal hosts – prairie voles (*Microtus ochrogaster*) and white-footed mice (*Peromyscus leucopus*) – were analyzed, the overwhelmingly dominant factor influencing microbiome composition was the tick species [37]. The study of Landesman *et al.* [38] indicates that host species can also affect overall microbiome composition as well as the relative proportion of the endosymbiont, *Rickettsia buchneri*. The life history of *I. pacificus* may be well suited to address this question because its dominant blood-meal host is not a rodent

but a reptile [39–41], the western fence lizard (*Sceloporus occidentalis*). This species is *Borrelia* refractory [42], meaning that infected *I. pacificus* feed on *S. occidentalis* are cleared of their *B. burgdorferi* infections [42]. It is perhaps because of this unusual property of *S. occidentalis* that Swei and Kwan [43] observed that nymphal *I. pacificus* that fed on lizards as larvae had significantly lower microbiome species richness and higher relative abundance of the rickettsial endosymbiont compared to ticks that fed on *Peromyscus* mice as larvae. A recent study of several species of hard ticks found that generalist ticks that feed on diverse hosts have more diverse microbiomes than nest-dwelling or one-host tick species [44]. These findings suggest that the host bloodmeal does have an impact on the composition of the tick microbiome, due perhaps to as-yet undefined factors in the bloodmeal that may modulate it directly or indirectly by regulating the tick's innate immune responses.

### Interstadial changes in the microbiome

How the tick's microbiome changes throughout its life is not well characterized. The transmission of obligate endosymbionts, a core component of the tick microbiome, from one generation to the next and between tick life stages is a well-documented phenomenon in hard ticks [45,46]. In *I. scapularis*, the most commonly reported endosymbiont to date is *R. buchneri* [47–50]. Meanwhile, *I. pacificus* is frequently, and potentially ubiquitously, associated with *Rickettsia* genomospecies G021 which clusters closely with *R. buchneri* based on several folate-synthesis loci [51]. *Rickettsia* genomospecies G021 is distinct from the spotted fever group *Rickettsia* genomospecies G022 observed by Phan *et al.* in *I. pacificus* [26]. The importance of rickettsial endosymbionts is believed to lie in the nutritional benefit they provide to the tick, particularly of B vitamins, such as folate, that ticks lack from having a strictly hematophagous diet [52]. Gillespie *et al.* [53] have shown that the genome of *R. buchneri* encodes two functional biotin operons not observed in other rickettsial species. *R. buchneri*, the predominant endosymbiont of *I. scapularis* [54], may therefore be unique in having the potential to provide biotin in addition to folate to the tick host. Many microbiome studies have documented a pattern of increasing relative abundance of endosymbionts through development of *Ixodes* spp. from larva, to nymph, to adult stages [35,47,55–57]. *Rickettsia* accumulation by adult female ticks is thought to be an adaptation to facilitate transovarial transmission of the endosymbiont to eggs, underlining the importance of endosymbionts to tick survival and development [46,51,58]. It is worth noting that *I. pacificus* and *I. scapularis* are associated with rickettsial endosymbionts but not with *Coxiella* or *Francisella*-like endosymbionts observed in other tick species [59] (Table 1). The biological

Table 1. Bacterial endosymbionts of selected hard-tick vectors of human diseases in the USA

| Tick vector                      | Predominant endosymbionts                                                                                  | Localization                                               | Refs      |
|----------------------------------|------------------------------------------------------------------------------------------------------------|------------------------------------------------------------|-----------|
| <i>Ixodes scapularis</i>         | <i>Rickettsia buchneri</i>                                                                                 | Ovaries, salivary glands, guts                             | [98,99]   |
| <i>Ixodes pacificus</i>          | <i>Rickettsia buchneri</i> -like (G021)                                                                    | Ovary, midgut                                              | [34,100]  |
| <i>Ixodes ricinus</i>            | <i>Candidatus</i> Midichloria, <i>Francisella</i> , <i>Spiroplasma</i> , <i>Rickettsia</i>                 | Ovaries, trachea, salivary glands, Malpighian tubules      | [73,101]  |
| <i>Dermacentor andersoni</i>     | <i>Rickettsia peacockii</i> , <i>Rickettsia belli</i> , <i>Francisella</i> spp., <i>Arsenophonus</i> spp., | Salivary glands, guts                                      | [102]     |
| <i>Amblyomma americanum</i>      | <i>Coxiella</i> -like, <i>Rickettsia</i> spp. <sup>a</sup>                                                 | Ovaries, Malpighian tubules, salivary glands, muscles, gut | [103,104] |
| <i>Amblyomma maculatum</i>       | <i>Francisella</i> -like endosymbiont and <i>Candidatus</i> Midichloria mitochondrii                       | Salivary glands, gut, ovaries                              | [105,106] |
| <i>Haemaphysalis longicornis</i> | <i>Coxiella</i> -like, <i>Francisella</i> -like                                                            | Malpighian tubules and ovaries                             | [107,108] |

<sup>a</sup>*Amblyomma americanum* also harbors *Rickettsia amblyommatis*, a potential pathogen [109].

significance of this preferential association of rickettsial endosymbionts with *I. scapularis* and *I. pacificus* is not understood.

In multiple studies of *I. scapularis* that examined the relative abundance within sequenced 16S rRNA amplicons, adult female ticks were observed to exhibit a higher proportion of *Rickettsia* endosymbionts than males and to have lower microbiome diversity as measured by Shannon diversity or overall richness [35,47,55,56]. However, Tokarz *et al.* performed detailed qPCR analysis of male and female ticks separately and did not observe significant differences in bacterial abundance between male and female *I. scapularis* [47]. In contrast to *I. scapularis*, *I. pacificus* males and females have more similar microbiome profiles based on species diversity and the proportions of *Rickettsia* endosymbiont [34]. Looking to other *Ixodes* species, *Ixodes ricinus* resembles *I. scapularis* in exhibiting a higher relative abundance of *Rickettsia* in female ticks relative to males [60], but other species, such as *Ixodes ovatus* and *Ixodes persulcatus*, exhibited higher alpha diversity and lower relative abundance of endosymbionts in female ticks [61]. Thus, there is evidence that the patterns of overall microbial diversity and the relative abundance of a key endosymbiont at the adult stage can vary depending on the species of tick and the methods used for quantification. When drawing conclusions on general patterns across life stages it is also important to distinguish between field- and laboratory-collected ticks and to identify the stage of blood feeding (i.e., questing or engorged ticks) [62]. When ticks are sampled from their host at various stages of engorgement, the *Rickettsia* endosymbiont dominates the tick microbiome because it replicates prolifically during blood feeding [36,45].

In another study that examined several hard-tick species, Chicana *et al.* [44] reported similar patterns of reduced microbiome species richness and diversity through life stages of both *I. pacificus* and two *Dermacentor* species (*D. occidentalis* and *D. variabilis*). At the same time, the relative abundance of the dominant endosymbiont abundance increased along life stage development in several of the species examined [44]. On average, adult stages had 50% of larval richness, while nymphs had intermediate levels of richness. This pattern may be due to competitive interactions between the components of the tick microbiome or could reflect the gradual loss of transient, environmentally acquired microbes typically associated with the larval stage [32]. There was no evidence of competition between microbial species based on checkboard score (C-score) analysis which compares the co-occurrence of operational taxonomic units (OTUs) with random simulations of microbiome community assembly [32]. The loss of species through time within a single life stage suggests that environmental microbes can assimilate into the tick microbiome but that host filtration through immune or physiological processes may remove the vast majority of microbes [32,33]. Gene function analysis of microbiomes through the duration of this experiment did not find functionally different microbiomes here or in another study [62], suggesting that the tick microbiome is functionally stable and potentially redundant [32,63].

### Geographic changes and regional patterns in microbiome composition

Thus far, there has been limited evidence of microbiome structuring in *I. scapularis* or *I. pacificus* based on habitat or region [37,44] but investigation of microbiomes across a broader spatial scale has not been systematically attempted (Box 1). A comparative genomic study of six Ixodid tick species has suggested the importance of ecogeographical fauna on the distribution of pathogenic bacteria in ticks [64]. Consideration of the spatial scale in designing microbiome experiments will be important in future studies to draw more general conclusions [65]. *Rickettsia* species, the most commonly reported endosymbionts in *I. scapularis* and *I. pacificus* microbiomes, are not observed in equal abundance in ticks from all geographic regions [36,37,44,56,66]. In the southeastern USA, *Rickettsia* spp. were not common in *I. scapularis* and, instead, an uncharacterized member of the family Enterobacteriaceae was the most



**Box 1. Addressing disparities in the composition of tick microbiomes**

The disparate compositions of the *Ixodes* microbiome observed by different studies could be explained in part by technical and sample processing differences. Even with a unified protocol, differences may be observed due to the differential impact of biotic and abiotic factors on the tick. Careful assessment of the microbiome's composition under different conditions may help to resolve the inconsistencies.

- (i) A systematic assessment of seasonal variations in the composition of the microbiome may serve as a prologue to how climate change may impact the tick's microbiome.
- (ii) A systematic analysis of the composition of the tick's microbiome in the context of geographic variations, ecology, and host preference may reveal source(s) of variation.
- (iii) A detailed examination of genetic changes in *Ixodes* ticks may reveal a correlation with the composition of the microbiome.

common element of the microbiome, and while some specimens had low abundance, some samples had no measurable reads corresponding to *Rickettsia* [56]. Meanwhile, in Canada, *Rickettsia* was more common in the Atlantic region (New Brunswick and Nova Scotia) but further west in Ontario, members of the families Pseudomonadaceae and Enterobacteriaceae appear to be the most abundant elements in the *I. scapularis* microbiome [66]. Some of this geographic variation may be traced to genetic history or life-cycle differences in the tick population. Although *I. scapularis* is considered to be one species from the southeast and north through the upper Midwest and eastern Canada, there are considerable life-cycle differences between populations of *I. scapularis* in the northeast versus the southern Atlantic coast, including different questing modality [67], and host associations [68]. *I. scapularis* from the northeastern USA tends to quest above the leaf litter, feed on small mammals and shrews, and is more likely to attach to people. Genetic analyses suggest that there is some genetic structuring that limits gene flow from the northern and southern populations of *I. scapularis* in the eastern USA [69,70] which may influence reported microbiome differences. A detailed comparative genomic study [64] also highlighted the multifactorial impact of ecogeographic fauna on the bacterial distribution in ticks. Therefore, studies that examine the alignment of tick population genetics with microbiome profiles could help to shed new light on microbial diversity in ticks from different regions (Box 1).

**Disparities in assessing the composition of microbiomes**

An important decision in the preparation of amplicon-based microbiome libraries is the selection of the gene target. Most microbiome analyses use amplicon-based methods targeting the **16S rRNA** region because it is highly conserved in prokaryotes but also has several regions of hypervariability, termed V1–V9. Of the nine hypervariable regions, V1, V3, and V4 were the most informative [56,66] while V9 gave the least reliable estimates of diversity [71,72]. Most tick microbiome studies focus on the V3 and V4 regions, making cross-study comparisons more feasible. Metagenomic sequence analysis [64], and metatranscriptomic RNA-seq approaches in combination with metaproteomic approaches are also being utilized to obtain a comprehensive description of the microbiota [73].

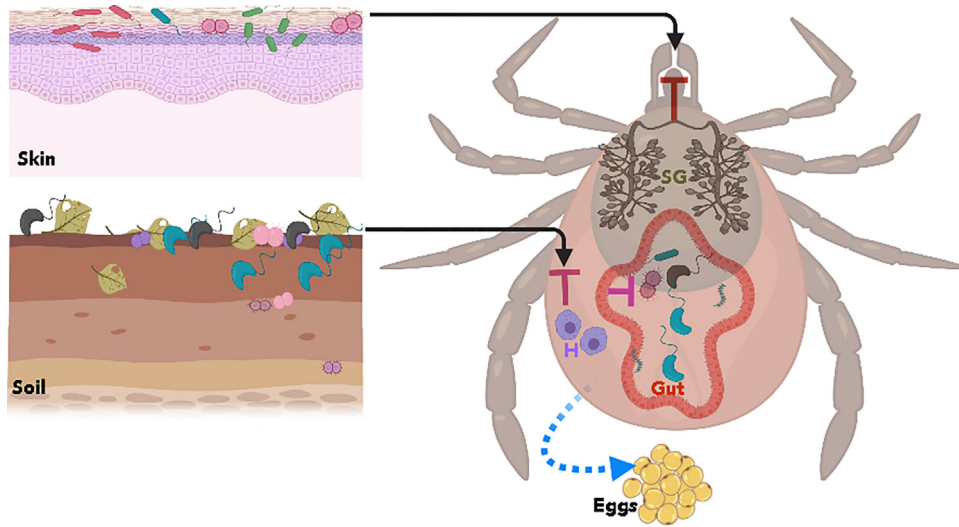
There is considerable debate on the complexity of the composition of the microbiome in *Ixodes* species, with some reporting tens of different genera [60,74,75] and some reporting significantly less diversity [33,76]. It is critical to understand that the tick microbiome is influenced at the macro (population) and micro (organ/tissue) level in the context of biotic and abiotic factors [56,65,77]. The external surface of ticks can be contaminated by environmental microbes and cloud our assessment of bona fide members of the microbiome [33], and stringent surface sterilization protocols – including bleach, hydrogen peroxide, and 70% ethanol – may be required [17,78]. Despite stringent surface sterilization, Couper *et al.* [32] identified likely contaminants such as *Propionibacterium*, a common resident on human skin. Whether these bacteria should be

discarded as contaminants or regarded as transient, yet relevant, passengers, remains a conundrum. Instead of using whole ticks, dissection of specific tissues – such as guts, or salivary glands – in conjunction with robust visualization strategies, pooling multiple low-input samples [33], adding internal positive controls [79], and incorporating negative controls [80–82], may also help to clarify the true composition of the tick microbiome. Analysis of core taxa (defined as occurring at more than 5% of sequence reads in a majority of the samples) in the tick microbiome can be employed to assess important taxa that constitute a consistent and important presence [83].

### Microbiome–tick immunome interactions

There is general consensus that the *Ixodes* microbiome is composed predominantly of rickettsial endosymbionts [33,54,78,84], and members of other bacterial genera – such as *Enterococcus*, *Pseudomonas*, *Staphylococcus*, *Lysinibacillus*, and *Bacillus* – occur at much lower abundance [32,33,76]. Bacterial members that associate with the tick, even if transiently and in much lower abundance, may impact the tick and the pathogens it harbors directly, or indirectly. A study in 2014 demonstrated that the microbiome plays an important role in facilitating *B. burgdorferi* colonization of the tick gut [15]. *I. scapularis* larvae raised in sterile containers associated with a significantly decreased relative abundance of *Acinetobacter* spp., *Brevibacterium* spp., *Lysinibacillus* spp., and *Staphylococcus* spp. compared to that in normal laboratory-reared ticks. Dysbiosed larvae were also less effectively colonized by *B. burgdorferi* compared to larvae raised in normal containers. The microbiome was suggested to impact the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway of the tick and to modulate the peritrophic matrix (PM), a key component of gut barrier integrity [15]. The PM was shown to provide an effective barricade against luminal contents as spirochetes colonized the gut epithelium [15]. Ross *et al.* [33] showed that *B. burgdorferi* lacks interbacterial effector immunity genes that would be critical for it to survive in a polymicrobial milieu, indicating the need for *B. burgdorferi* to escape the gut lumen and take cover under the PM. Consistent with this rationale, the abundance of *Pseudomonas*, *Bacillus*, or Enterobacteriaceae was negatively correlated with *B. burgdorferi* abundance [85].

The **JAK/STAT pathway** is an evolutionarily conserved and key signaling pathway invoked in repair and remodeling of the gut epithelial cells and in activating immune responses in arthropods [86,87]. The tick genome encodes all the key components of this pathway [88] except **Upd** (unpaired), a cytokine-like molecule, released upon damage to the epithelial cells, that is essential for activation of the JAK/STAT pathway [89]. The observation that the tick gut microbiota modulates the JAK/STAT pathway [15] raises the possibility that bacterial components may also activate this pathway either by serving as Upd surrogates or by other mechanisms that remain to be understood. The study of Chou *et al.* has shown that *I. scapularis*–microbiota associations are ancient [90] and that, during the course of evolution, *I. scapularis* likely co-opted and domesticated a type VI secretion amidase effector gene (Dae2) from one of its gut-associated bacteria to protect itself from invading bacteria, including *B. burgdorferi*. A recent study by Hayes *et al.* [91] has shown that tick salivary Dae2 has broad spectrum antibacterial activity and is delivered into the bite site during feeding. Dae2 acts on skin commensal bacteria, such as *Staphylococcus* spp., to pre-empt their entry into the tick gut. RNAi-mediated silencing of Dae2 resulted in an increased abundance of skin-resident *Staphylococcus* in the gut and impaired tick feeding. Effector molecules such as these antimicrobial peptides may control the abundance of environmental bacteria that enter the tick and tick gut from the soil, bloodmeal, or even the host skin (Figure 2). This may explain the relatively simple microbiome of the tick relative to the complex microbiomes of the environment, including soil and host skin that the tick intimately associates with during its off-host and on-host phases, respectively.



Trends in Parasitology

**Figure 2. Environmental encounters that facilitate the acquisition of microbiota.** *Ixodes scapularis* and *Ixodes pacificus* come into contact with environmental bacteria, including soil microbiota, during their off-host phase, and with vertebrate skin microbiota during feeding. Defense responses of hemocytes (H) in the hemolymph, such as phagocytosis and the production of antimicrobial peptides, may thwart some of these microbiotas from infecting the tick. Salivary defense responses, including antimicrobial peptides secreted by the salivary glands (SG), may prevent skin microbiota from infecting the tick. Immune responses of the gut may also control microbiota that enter the gut. The stand-off between microbiota and the tick helps to modulate the immune milieu, barrier integrity, nutrient status, tick biology, and consequently the vectorial capacity. Broken arrow indicates transovarial transmission of specific microbiota that inoculates eggs laid by mated females. Illustration created by [Biorender.com](https://www.biorender.com).

*I. scapularis* has also been shown to express proteins such as IAFGP (*Ixodes* antifreeze glycoprotein). IAFGP was originally identified as an antifreeze protein that was increased in *A. phagocytophilum*-infected ticks [92] and was suggested to provide a survival advantage during overwintering of nymphal ticks. Careful analysis revealed that IAFGP was also an antibacterial protein that effectively impaired bacterial biofilm formation [93]. Abraham *et al.* [94] showed that increased IAFGP expression during *A. phagocytophilum* acquisition from the mammalian host altered the composition of the tick-gut microbiota. This, in turn, resulted in compromised barrier integrity, facilitating the exit of *A. phagocytophilum* from the gut to the salivary glands [94]. While the integrity of the peritrophic matrix was critical for successful *B. burgdorferi* colonization [15], the converse was invoked in *A. phagocytophilum* infection [94]. This suggests that the composition of the microbiota may also determine the frequency and success of coinfections with these bacterial pathogens in endemic areas. Demonstrating a thematic pattern in controlling the composition of the microbiota in the tick gut, it was observed that tick feeding induced the expression of a protein of *I. scapularis* with a Reeler domain, known as PIXR, that inhibits the formation of Gram-positive bacterial biofilms [76]. Abrogation of PIXR function by antibodies, or by RNAi-mediated silencing, resulted in increased biofilm formation, altered microbiome composition, and impaired *B. burgdorferi* colonization of the tick. PIXR abrogation-mediated changes in the composition of the gut microbiome had no impact on the JAK/STAT pathway [76] nor on the integrity of the PM, invoking other interactions between microbiota and tick that influence *B. burgdorferi* colonization and remain to be deciphered.

The immunodeficiency pathway, or **IMD pathway**, is an evolutionarily conserved innate immune signaling pathway that activates the transcription factor NF- $\kappa$ B, leading to the expression of antimicrobial peptides that predominantly control Gram-negative bacteria in arthropods [95]. Key components of



the canonical IMD pathway are not represented in the *I. scapularis* genome [88]. This may seem an evolutionary adaptation to maintain critical Gram-negative bacteria, such as *Rickettsia* [54], in the tick and to avoid inadvertent activation of the IMD pathway. However, Shaw *et al.* [96] showed that, despite lacking several components of the IMD pathway, including PGRPLC, that plays a critical role in sensing the diaminopimelic acid (DAP)-type peptidoglycans that decorate Gram-negative bacterial cell walls, the IMD pathway in *Ixodes* is functional, and is activated by infection-derived lipid components of bacteria, including *B. burgdorferi* and *A. phagocytophilum*. As a better understanding of the unique IMD pathway of the *Ixodes* tick unfolds, we may gain new insights into microbiota that may or may not have adapted to this unusual IMD pathway.

Tokarz *et al.* [8] and Sakamoto *et al.* [7] have used unbiased deep sequencing to characterize the virome of several tick species, including *I. scapularis*, and they show that viruses of the families Bunyaviridae, Rhabdoviridae, and Chuviridae are predominant members of the tick microbiome. Detailed and targeted studies will be required to understand interactions between these viruses, the bacteria in the microbiome, and tick immune pathway/s (Box 2). Importantly, to make progress in our understanding of the tick microbiome, and its unique interactions with its microbial partners, we must develop robust tools to generate germ-free ticks and gnotobiotic ticks – this remains a major challenge in this field (Box 2). Kurlavs *et al.* [97] utilized various antibiotics, including ciprofloxacin, to reduce rickettsial endosymbiont levels in *I. pacificus* and observed no impact on fecundity or egg hatching. In recent studies, Oliver *et al.* [98] similarly used microinjection and artificial feeding strategies to introduce the antibiotic ciprofloxacin into female *I. scapularis* to effectively eliminate *R. buchneri* from the tick microbiome. Interestingly, their initial studies also suggest that *R. buchneri* elimination has no impact on fecundity and tick development, raising questions regarding the role of this endosymbiont in tick reproduction and development and paving the way for determining the vectorial competence of *R. buchneri*-deficient ticks.

## Concluding remarks

The long life cycle of the tick, variations in developmental stage, age, and gender of ticks, and the spatial distribution of ticks, present disparate microbiome compositions and confound conclusive determination of the bona fide members of the tick microbiome. The core tick microbiome is simple, and the reported diversity of bacterial genera in the tick microbiome likely represents transient associations with bacteria in the environment, including from soil, leaf litter, host skin, or a host blood meal. These transient microbial associations are under surveillance by tick innate immune responses and are cleared by effector molecules such as antibacterial peptides, or the microbes are potentially excreted due to the absence of cognate adhesins to engage with the tick gut. Nevertheless, it is in the context of this constant friction with environment-associated microbes that ticks also encounter tick-transmitted pathogens of human disease. Increased immune surveillance may clear the environmental microbes, but this is likely to be energetically costly for the tick and must warrant careful fine-tuning of the immune responses. Presumably, tick-borne

## Outstanding questions

Pathogens such as *B. burgdorferi* are stably maintained, unlike environmental/transient bacteria that appear to be cleared. What are the mechanisms that drive this differential infection of ticks?

Does the microbiome composition change when specific tick innate immune pathways are impaired or abrogated?

Does the microbiome composition change in the context of specific tick-borne pathogens under similar environmental exposures?

### Box 2. Tools for manipulating the tick's microbiome

In order to obtain a mechanistic understanding of the impact of specific bacteria on tick biology it will be critical to improve strategies to robustly manipulate the tick's microbiota.

- (i) As artificial/membrane feeding systems for feeding ticks become more amenable for routine use in many laboratories, strategies for generating ticks with little or no environmentally acquired microbiota may be feasible simply by adding combinations of antibiotics to the medium. Given the long life cycle of ticks, long-term maintenance will require germ-free isolators in conjunction with artificial membrane feeding systems.
- (ii) Optimizing robust strategies for generating gnotobiotic ticks, and developing the ability to add and remove specific microbiota and correlate the cause and effect of specific microbiota on tick biology, will be further important milestones. This will facilitate the discovery of biocontrol strategies for preventing tick-borne diseases.

pathogens have evolved ways to suppress, circumvent, or even co-opt these immune responses. Indeed, this highlights a fundamental difference between these transient microbial passengers and the stable pathogenic microbial inhabitants that survive through the tick's developmental stages, and it raises important questions about tick–microbe interactions (see [Outstanding questions](#)). The time is ripe to direct scientific efforts to unravel a functional and mechanistic understanding of the tick microbiome, albeit transient, and its interactions with the tick, and implicit in this is also the understanding of how tick-borne pathogens are sustained and transmitted.

### Acknowledgments

Parts of the research described in this review were supported by grants from the National Institutes of Health/National Institute of Allergy and Infectious Diseases (AI138949, AI126033).

### Declaration of interests

The authors declare no competing interests.

### References

- Degli Esposti, M. and Martínez Romero, E. (2017) The functional microbiome of arthropods. *PLoS One* 12, e0176573
- Duron, O. and Gottlieb, Y. (2020) Convergence of nutritional symbioses in obligate blood feeders. *Trends Parasitol.* 36, 816–825
- Rio, R.V.M. *et al.* (2016) Grandeur alliances: symbiont metabolic integration and obligate arthropod hematophagy. *Trends Parasitol.* 32, 739–749
- Strand, M.R. (2018) Composition and functional roles of the gut microbiota in mosquitoes. *Curr. Opin. Insect Sci.* 28, 59–65
- Stanojevic, M. *et al.* (2020) Depicting the RNA virome of hematophagous arthropods from Belgrade, Serbia. *Viruses* 12, 975
- Chandler, J.A. *et al.* (2015) RNA shotgun metagenomic sequencing of northern California (USA) mosquitoes uncovers viruses, bacteria, and fungi. *Front. Microbiol.* 6, 185
- Sakamoto, J.M. *et al.* (2016) Bunyaviruses are common in male and female *Ixodes scapularis* ticks in central Pennsylvania. *PeerJ* 4, e2324
- Tokarz, R. *et al.* (2018) Identification of novel viruses in *Amblyomma americanum*, *Dermacentor variabilis*, and *Ixodes scapularis* ticks. *mSphere* 3, e00614-17
- Tokarz, R. *et al.* (2014) Virome analysis of *Amblyomma americanum*, *Dermacentor variabilis*, and *Ixodes scapularis* ticks reveals novel highly divergent vertebrate and invertebrate viruses. *J. Virol.* 88, 11480–11492
- Sicard, M. *et al.* (2019) *Wolbachia* prevalence, diversity, and ability to induce cytoplasmic incompatibility in mosquitoes. *Curr. Opin. Insect Sci.* 34, 12–20
- Zindel, R. *et al.* (2011) Arthropod symbioses: a neglected parameter in pest- and disease-control programmes. *J. Appl. Ecol.* 48, 864–872
- Werren, J.H. *et al.* (2008) *Wolbachia*: master manipulators of invertebrate biology. *Nat. Rev. Microbiol.* 6, 741–751
- Taylor, M.J. *et al.* (2018) Microbe profile: *Wolbachia*: a sex selector, a viral protector and a target to treat filarial nematodes. *Microbiology (Reading)* 164, 1345–1347
- Shokal, U. *et al.* (2016) Effects of co-occurring *Wolbachia* and *Spiroplasma* endosymbionts on the *Drosophila* immune response against insect pathogenic and non-pathogenic bacteria. *BMC Microbiol.* 16, 16
- Narasimhan, S. *et al.* (2014) Gut microbiota of the tick vector *Ixodes scapularis* modulate colonization of the Lyme disease spirochete. *Cell Host Microbe* 15, 58–71
- Cirimotich, C.M. *et al.* (2011) Native microbiota shape insect vector competence for human pathogens. *Cell Host Microbe* 10, 307–310
- Binetruy, F. *et al.* (2019) Surface sterilization methods impact measures of internal microbial diversity in ticks. *Parasit. Vectors* 12, 268
- Goodman, J.L. *et al.* (2005) *Tick-Borne Diseases of Humans*, ASM Press
- Estrada-Pena, A. (2015) Ticks as vectors: taxonomy, biology and ecology. *Rev. Sci. Tech.* 34, 53–65
- Anderson, J.F. and Magnarelli, L.A. (2008) Biology of ticks. *Infect. Dis. Clinics North Am.* 22, 195–215 v
- Greay, T.L. *et al.* (2018) Recent insights into the tick microbiome gained through next-generation sequencing. *Parasit. Vectors* 11, 12
- Barbour, A.G. and Fish, D. (1993) The biological and social phenomenon of Lyme disease. *Science* 260, 1610–1616
- Krause, P.J. *et al.* (2015) *Borrelia miyamotoi* infection in nature and in humans. *Clin. Microbiol. Infect.* 21, 631–639
- Hernance, M.E. and Thangamani, S. (2017) Powassan virus: an emerging arbovirus of public health concern in North America. *Vector Borne Zoonotic Dis.* 17, 453–462
- Eisen, L. (2018) Pathogen transmission in relation to duration of attachment by *Ixodes scapularis* ticks. *Ticks Tick Borne Dis.* 9, 535–542
- Phan, J.N. *et al.* (2011) Molecular detection and identification of *Rickettsia* species in *Ixodes pacificus* in California. *Vector Borne Zoonotic Dis.* 11, 957–961
- Couper, L.I. *et al.* (2020) Comparative vector competence of North American Lyme disease vectors. *Parasit. Vectors* 13, 29
- Fierer, N. (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* 15, 579–590
- Narasimhan, S. and Fikrig, E. (2015) Tick microbiome: the force within. *Trends Parasitol.* 31, 315–323
- Varela-Stokes, A.S. *et al.* (2017) Microbial communities in North American ixodid ticks of veterinary and medical importance. *Front. Vet. Sci.* 4, 179
- Byrd, A.L. *et al.* (2018) The human skin microbiome. *Nat. Rev. Microbiol.* 16, 143–155
- Couper, L.I. *et al.* (2019) Drivers and patterns of microbial community assembly in a Lyme disease vector. *Ecol. Evol.* 9, ece.3.5361
- Ross, B.D. *et al.* (2018) *Ixodes scapularis* does not harbor a stable midgut microbiome. *ISME J.* 12, 2596–2607
- Kwan, J.Y. *et al.* (2017) Vertical versus horizontal transmission of the microbiome in a key disease vector, *Ixodes pacificus*. *Mol. Ecol.* 26, 6578–6589
- Zolnik, C.P. *et al.* (2016) Microbiome changes through ontogeny of a tick pathogen vector. *Mol. Ecol.* 25, 4963–4977
- Rynkiewicz, E.C. *et al.* (2015) Concordance of bacterial communities of two tick species and blood of their shared rodent host. *Mol. Ecol.* 24, 2566–2579
- Hawlena, H. *et al.* (2013) The arthropod, but not the vertebrate host or its environment dictates bacterial community composition of fleas and ticks. *ISME J.* 7, 221–223
- Landesman, W.J. *et al.* (2019) Potential effects of blood meal host on bacterial community composition in *Ixodes scapularis* nymphs. *Ticks Tick Borne Dis.* 10, 523–527

39. Eisen, R.J. *et al.* (2001) Prevalence and abundance of *Ixodes pacificus* immatures (Acari: Ixodidae) infesting western fence lizards (*Sceloporus occidentalis*) in northern California: Temporal trends and environmental correlates. *J. Parasitol.* 87, 1301–1307
40. Lane, R.S. and Loye, J.E. (1989) Lyme disease in California USA interrelationship of *Ixodes pacificus* (Acari: Ixodidae) the western fence lizard *Sceloporus occidentalis* and *Borrelia burgdorferi*. *J. Med. Entomol.* 26, 272–278
41. Swei, A. *et al.* (2012) Impacts of an introduced forest pathogen on the risk of Lyme disease in California. *Vector Borne Zoonotic Dis.* 12, 623–632
42. Lane, R.S. and Quistad, G.B. (1998) Borreliacidal factor in the blood of the western fence lizard (*Sceloporus occidentalis*). *J. Parasitol.* 84, 29–34
43. Swei, A. and Kwan, J.Y. (2017) Tick microbiome and pathogen acquisition altered by host blood meal. *ISME J.* 11, 813–816
44. Chicana, B. *et al.* (2019) Comparative microbiome profiles of sympatric tick species from the far-western United States. *Insects* 10, 353
45. Brinkerhoff, R.J. *et al.* (2020) Factors affecting the microbiome of *Ixodes scapularis* and *Amblyomma americanum*. *PLoS One* 15, e0232398
46. Noda, H. *et al.* (1997) Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals. *Appl. Environ. Microbiol.* 63, 3926–3932
47. Tokarz, R. *et al.* (2019) Microbiome analysis of *Ixodes scapularis* ticks from New York and Connecticut. *Ticks Tick Borne Dis.* 10, 894–900
48. Benson, M.J. *et al.* (2004) Intracellular symbionts and other bacteria associated with deer ticks (*Ixodes scapularis*) from Nantucket and Wellfleet, Cape Cod, Massachusetts. *Appl. Environ. Microbiol.* 70, 616–620
49. Moreno, C.X. *et al.* (2006) Molecular analysis of microbial communities identified in different developmental stages of *Ixodes scapularis* ticks from Westchester and Dutchess Counties, New York. *Environ. Microbiol.* 8, 761–772
50. Felsheim, R.F. *et al.* (2009) Genome sequence of the endosymbiont *Rickettsia peacockii* and comparison with virulent *Rickettsia rickettsii*: identification of virulence factors. *PLoS One* 4, e8361
51. Hunter, D.J. *et al.* (2015) The *Rickettsia* endosymbiont of *Ixodes pacificus* contains all the genes of *de novo* folate biosynthesis. *PLoS One* 10, e0144552
52. Duron, O. *et al.* (2018) Tick–bacteria mutualism depends on B vitamin synthesis pathways. *Curr. Biol.* 28, 1896–1902 e1895
53. Gillespie, J.J. *et al.* (2012) A *Rickettsia* genome overrun by mobile genetic elements provides insight into the acquisition of genes characteristic of an obligate intracellular lifestyle. *J. Bacteriol.* 194, 376–394
54. Kurti, T.J. *et al.* (2015) *Rickettsia buchneri* sp. nov., a rickettsial endosymbiont of the blacklegged tick *Ixodes scapularis*. *Int. J. Syst. Evol. Microbiol.* 65, 965–970
55. Chauhan, G. *et al.* (2019) Combining citizen science and genomics to investigate tick, pathogen, and commensal microbiome at single-tick resolution. *Front. Genet.* 10, 1322
56. Van Treuren, W. *et al.* (2015) Variation in the microbiota of *Ixodes* ticks with regard to geography, species, and sex. *Appl. Environ. Microbiol.* 81, 6200–6209
57. Thapa, S. *et al.* (2019) Bacterial microbiomes of *Ixodes scapularis* ticks collected from Massachusetts and Texas, USA. *BMC Microbiol.* 19, 138
58. Buchner, P. (1953) *Endosymbiose der Tiere mit pflanzlichen Mikroorganismen*, Verlag Birkhauser
59. Ahantari, A. *et al.* (2013) Hard ticks and their bacterial endosymbionts (or would be pathogens). *Folia Microbiol. (Praha)* 58, 419–428
60. Carpi, G. *et al.* (2011) Metagenomic profile of the bacterial communities associated with *Ixodes ricinus* ticks. *PLoS One* 6, e25604
61. Obregon, D. *et al.* (2019) Sex-specific linkages between taxonomic and functional profiles of tick gut microbiomes. *Front. Cell. Infect. Microbiol.* 9, 298
62. Zolnik, C.P. *et al.* (2018) Transient influence of blood meal and natural environment on blacklegged tick bacterial communities. *Ticks Tick Borne Dis.* 9, 563–572
63. Estrada-Pena, A. *et al.* (2020) Resistance of tick gut microbiome to anti-tick vaccines, pathogen infection and antimicrobial peptides. *Pathogens* 9, 309
64. Jia, N. *et al.* (2020) Large-scale comparative analyses of tick genomes elucidate their genetic diversity and vector capacities. *Cell* 182, 1328–1340 e1313
65. Pollet, T. *et al.* (2020) The scale affects our view on the identification and distribution of microbial communities in ticks. *Parasit. Vectors* 13, 36
66. Sperling, J.L. *et al.* (2017) Comparison of bacterial 16S rRNA variable regions for microbiome surveys of ticks. *Ticks Tick Borne Dis.* 8, 453–461
67. Arsnoe, I.M. *et al.* (2015) Different populations of blacklegged tick nymphs exhibit differences in questing behavior that have implications for human Lyme disease risk. *PLoS One* 10, e0127450
68. Oliver Jr., J.H. *et al.* (1993) Isolation and transmission of the Lyme disease spirochete from the southeastern United States. *Proc. Natl. Acad. Sci. U. S. A.* 90, 7371–7375
69. Van Zee, J. *et al.* (2015) Nuclear markers reveal predominantly north to south gene flow in *Ixodes scapularis*, the tick vector of the Lyme disease spirochete. *PLoS One* 10, e0139630
70. Sakamoto, J.M. *et al.* (2014) Population and demographic structure of *Ixodes scapularis* Say in the eastern United States. *PLoS One* 9, e101389
71. Barb, J. *et al.* (2016) Development of an analysis pipeline characterizing multiple hypervariable regions of 16S rRNA using mock samples. *PLoS One* 11, e0148047
72. Yang, B. *et al.* (2016) Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinform.* 17, 135
73. Hernandez-Jarguin, A. *et al.* (2018) Integrated metatranscriptomics and metaproteomics for the characterization of bacterial microbiota in unfed *Ixodes ricinus*. *Ticks Tick Borne Dis.* 9, 1241–1251
74. Clay, K. *et al.* (2008) Microbial community ecology of ticks: prokaryotic diversity, distribution and interactions in the lone star tick, *Amblyomma americanum*. *Mol. Ecol.* 17, 4371–4381
75. Thapa, S. *et al.* (2019) Effects of temperature on bacterial microbiome composition in *Ixodes scapularis* ticks. *Microbiologyopen* 8, e00719
76. Narasimhan, S. *et al.* (2017) Modulation of the tick gut milieu by a secreted tick protein favors *Borrelia burgdorferi* colonization. *Nat. Commun.* 8, 184
77. Adegoke, A. *et al.* (2020) Tick-borne pathogens shape the native microbiome within tick vectors. *Microorganisms* 8, 1299
78. Couper, L.I. and Swei, A. (2018) Tick microbiome characterization by next-generation 16S rRNA amplicon sequencing. *J. Vis. Exp.* 138, e58239
79. Galan, M. *et al.* (2016) 16S rRNA Amplicon sequencing for epidemiological surveys of bacteria in wildlife. *mSystems* 1, e00032-16
80. Salter, S.J. *et al.* (2014) Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biol.* 12, 87
81. Hornung, B.V.H. *et al.* (2019) Issues and current standards of controls in microbiome research. *FEMS Microbiol. Ecol.* 95, f02045
82. Davis, N.M. *et al.* (2018) Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome* 6, 226
83. Shade, A. and Handelsman, J. (2012) Beyond the Venn diagram: the hunt for a core microbiome. *Environ. Microbiol.* 14, 4–12
84. Stewart, P.E. and Bloom, M.E. (2020) Sharing the Ride: *Ixodes scapularis* symbionts and their interactions. *Front. Cell. Infect. Microbiol.* 10, 142
85. Landesman, W.J. *et al.* (2019) Cross-kingdom analysis of nymphal-stage *Ixodes scapularis* microbial communities in relation to *Borrelia burgdorferi* infection and load. *FEMS Microbiol. Ecol.* 95, f02167
86. Herrera, S.C. and Bach, E.A. (2019) JAK/STAT signaling in stem cells and regeneration: from *Drosophila* to vertebrates. *Development* 146, 167643
87. Buchon, N. *et al.* (2009) Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*. *Genes Dev.* 23, 2333–2344

88. Palmer, W.J. and Jiggins, F.M. (2015) Comparative genomics reveals the origins and diversity of arthropod immune systems. *Mol. Biol. Evol.* 32, 2111–2129
89. Agaisse, H. and Perrimon, N. (2004) The roles of JAK/STAT signaling in *Drosophila* immune responses. *Immunol. Rev.* 198, 72–82
90. Chou, S. *et al.* (2015) Transferred interbacterial antagonism genes augment eukaryotic innate immune function. *Nature* 518, 98–101
91. Hayes, B.M. *et al.* (2020) Ticks resist skin commensals with immune factor of bacterial origin. *Cell* 183, 1562–1571 e1512
92. Neelakanta, G. *et al.* (2010) *Anaplasma phagocytophilum* induces *Ixodes scapularis* ticks to express an antifreeze glycoprotein gene that enhances their survival in the cold. *J. Clin. Invest.* 120, 3179–3190
93. Heisig, M. *et al.* (2014) Antivirulence properties of an antifreeze protein. *Cell Rep.* 9, 417–424
94. Abraham, N.M. *et al.* (2017) Pathogen-mediated manipulation of arthropod microbiota to promote infection. *Proc. Natl. Acad. Sci. U. S. A.* 114, E781–E790
95. Myllymaki, H. *et al.* (2014) The *Drosophila* imd signaling pathway. *J. Immunol.* 192, 3455–3462
96. Shaw, D.K. *et al.* (2017) Infection-derived lipids elicit an immune deficiency circuit in arthropods. *Nat. Commun.* 8, 14401
97. Kurlovs, A.H. *et al.* (2014) *Ixodes pacificus* ticks maintain embryogenesis and egg hatching after antibiotic treatment of *Rickettsia* endosymbiont. *PLoS One* 9, e104815
98. Oliver, J.D. *et al.* (2021) Growth dynamics and antibiotic elimination of symbiotic *Rickettsia buchneri* in the tick *Ixodes scapularis* (Acari: Ixodidae). *Appl. Environ. Microbiol.* 87, e01672-20
99. Al-Khafaji, A.M. *et al.* (2020) *Rickettsia buchneri*, symbiont of the deer tick *Ixodes scapularis*, can colonise the salivary glands of its host. *Ticks Tick Borne Dis.* 11, 101299
100. Bagheri, G. *et al.* (2017) Enhanced detection of *Rickettsia* species in *Ixodes pacificus* using highly sensitive fluorescence *in situ* hybridization coupled with tyramide signal amplification. *Ticks Tick Borne Dis.* 8, 915–921
101. Olivieri, E. *et al.* (2019) Tissue tropism and metabolic pathways of *Mitochondria* suggest tissue-specific functions in the symbiosis with *Ixodes ricinus*. *Ticks Tick Borne Dis.* 10, 1070–1077
102. Gall, C.A. *et al.* (2016) The bacterial microbiome of *Dermacentor andersoni* ticks influences pathogen susceptibility. *ISME J.* 10, 1846–1855
103. Maldonado-Ruiz, L.P. *et al.* (2021) The bacterial community of the lone star tick (*Amblyomma americanum*). *Parasit. Vectors* 14, 49
104. Klyachko, O. *et al.* (2007) Localization and visualization of a *Coxiella*-type symbiont within the lone star tick, *Amblyomma americanum*. *Appl. Environ. Microbiol.* 73, 6584–6594
105. Budachetri, K. *et al.* (2014) An insight into the microbiome of the *Amblyomma maculatum* (Acari: Ixodidae). *J. Med. Entomol.* 51, 119–129
106. Budachetri, K. *et al.* (2018) The tick endosymbiont *Candidatus Mitochondria* and selenoproteins are essential for the growth of *Rickettsia parkeri* in the Gulf Coast tick vector. *Microbiome* 6, 141
107. Wang, M. *et al.* (2018) Tissue Localization and variation of major symbionts in *Haemaphysalis longicornis*, *Rhipicephalus haemaphysaloides*, and *Dermacentor silvarum* in China. *Appl. Environ. Microbiol.* 84, e00029-18
108. Wang, Y. *et al.* (2018) A novel *Francisella*-like endosymbiont in *Haemaphysalis longicornis* and *Hyalomma asiaticum*, China. *Vector Borne Zoonotic Dis.* 18, 669–676
109. Karpathy, S.E. *et al.* (2016) *Rickettsia amblyommatis* sp. nov., a spotted fever group *Rickettsia* associated with multiple species of *Amblyomma* ticks in North, Central and South America. *Int. J. Syst. Evol. Microbiol.* 66, 5236–5243