The ISME Journal (2017) 11, 813–816

www.nature.com/ismej

SHORT COMMUNICATION

Tick microbiome and pathogen acquisition altered by host blood meal

Andrea Swei and Jessica Y Kwan
Department of Biology, San Francisco State University, San Francisco, CA, USA

Lyme disease, a zoonotic disease, is the most prevalent vector-borne disease in the Northern Hemisphere. Diversity of the vector (tick) microbiome can impact pathogen transmission, yet the biotic and abiotic factors that drive microbiome diversity are largely unresolved, especially under natural, field conditions. We describe the microbiome of *Ixodes pacificus* ticks, the vector for Lyme disease in the western United States, and show a strong impact of host blood meal identity on tick microbiome species richness and composition. Western fence lizards, a host that is refractory to the Lyme disease pathogen, significantly reduces microbiome diversity in ticks relative to ticks that feed on a mammalian reservoir host. Host blood meal-driven reduction of tick microbiome diversity may have lifelong repercussions on *I. pacificus* vector competency and ultimately disease dynamics.
The ISME Journal (2017) **11,** 813–816; doi:10.1038/ismej.2016.152; published online 18 November 2016

Animal hosted microbiome communities are now recognized as essential assemblages that can affect host health, phenotype and disease susceptibility (Gosalbes *et al*., 2012; Jumpstart Consortium Human Microbiome Project Data Generation Working Group, 2012; Christian *et al*., 2015). Further, the microbiomes of arthropod vectors of human pathogens can affect the transmission of zoonotic pathogens (Weiss and Aksoy, 2011; Hughes *et al*., 2014; Narasimhan *et al*., 2014). Laboratory experiments have found that higher tick microbiome diversity is negatively correlated with colonization success of the Lyme disease pathogen, *Borrelia burgdorferi* (Narasimhan *et al*., 2014). But it remains unclear what factors drive natural variation of the tick microbiome and whether such variation would affect pathogen transmission in the wild and therefore regulate disease risk. Prior field studies have identified microbiome differences by tick species (Hawlena *et al*., 2013), sex (Williams-Newkirk *et al*., 2014; Zhang *et al*., 2014) and region (Carpi *et al*., 2011; van Treuren *et al*., 2015) but have not found correlations with host blood or the immediate environment (Hawlena *et al*., 2013; Rynkiewicz *et al*., 2015).

Tick larvae must feed on an infected blood meal source in order to acquire *B. burgdorferi* and then molt into an infected nymph. This simple life history and feeding strategy provides an ideal system to investigate the natural drivers of microbiome diversity and its effect on pathogen transmission in a disease vector. A previous study did not find a correlation between host blood meal and *Ixodes scapularis* microbiomes (Rynkiewicz *et al*., 2015), yet *Ixodes pacificus*, the focal tick of this study, has a distinct natural history (Lane and Loye, 1989; Eisen *et al*., 2001). As a generalist, *I. pacificus* feeds on numerous vertebrate species that may be pathogen reservoirs, but the primary blood meal host for juvenile *I. pacificus* is the western fence lizard, *Sceloporus occidentalis*, a *Borrelia*-refractory host (Lane and Quistad, 1998; Kuo *et al*., 2000). Infected ticks that feed on *S. occidentalis* are cleared of their *B. burgdorferi* infections and are no longer infective as a result of complement proteins in the lizard's innate immune system (Kuo *et al*., 2000).

We collected all stages of *I. pacificus*, the vector of Lyme disease in the western United States, from the field and analyzed the interactions between tick microbiome diversity, host blood meal and pathogen infection using amplicon-based deep sequencing analyses. *I. pacificus* of all life stages were collected from the field and nymphs were tested for *B. burgdorferi* infection (Lane *et al.,* 2005). Engorged larvae were collected from a reservoir host (mice) or a non-reservoir host (lizards) and molted into nymphs for microbiome analysis (Swei *et al*., 2012). Then 16S rRNA microbiome libraries were prepared for individually barcoded samples and sequenced on an Illumina MiSeq (San Diego, CA, USA). Sequences were quality-filtered, clustered at 97% sequence similarity and analyzed in QIIME (Caporaso *et al*., 2010).

Our analysis found a significant loss of microbiome species richness and evenness as field-collected *I. pacificus* mature (Figures 1a and b).

Correspondence: A Swei, Department of Biology, San Francisco State University, 1600 Holloway Ave, San Francisco, CA 94132, USA.
E-mail: aswei@sfsu.edu
Received 15 July 2016; revised 16 September 2016; accepted 28 September 2016; published online 18 November 2016

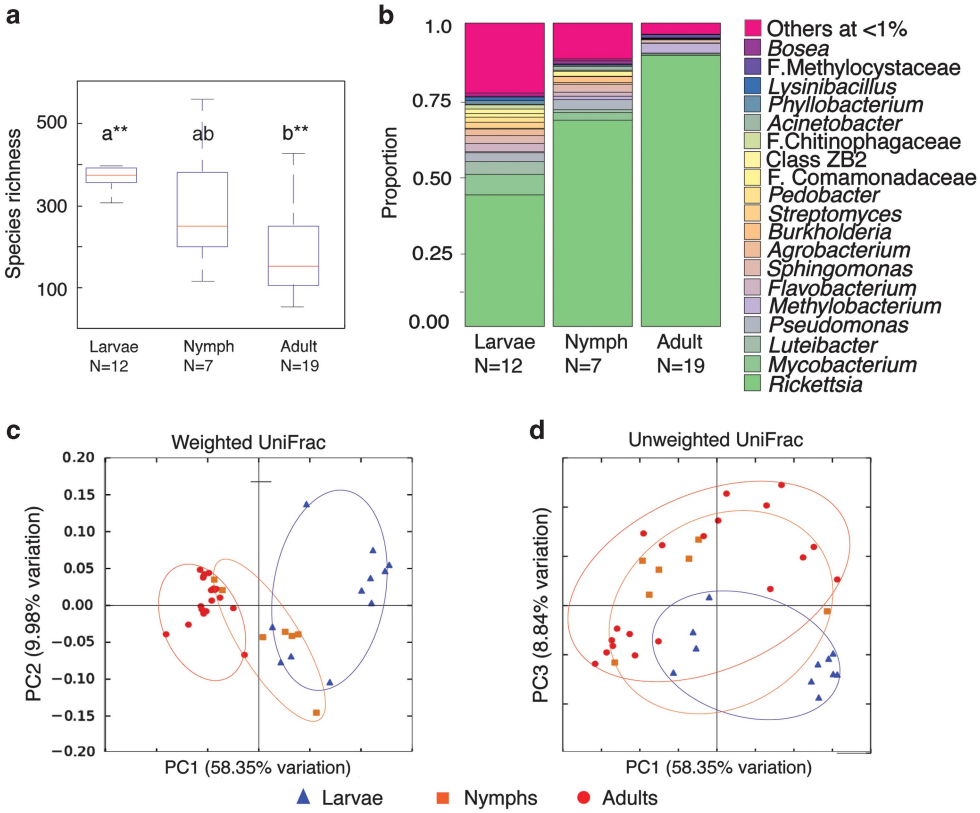

Figure 1 Microbiome results of field-collected, host-seeking *Ixodes pacificus* life stages depicting (**a**) alpha diversity as measured by observed OTUs. Group significance is indicated above the bars with ** indicating *P*-value < 0.01. (**b**) Life stage differences in genus-level taxonomic assignment based on 97% OTU phylogroups. Genera present at <1% were binned together into the 'Others at <1%' group. (**c**) Weighted and (**d**) unweighted PCOA analysis of life stage beta diversity.

The loss of species richness in microbiome communities across vector life stage has not been observed in other arthropod species and may reflect unique factors that drive microbiome diversity in *I. pacificus*. Beta diversity was also significantly different between the life stages measured by relative abundance (weighted Unifrac distance, *t*-test = −18.40, *P* = 0.001; Figure 1c) and occurrence (unweighted UniFrac distance, *t*-test = −7.35, *P* = 0.001, Figure 1d). At all stages, tick microbiomes were dominated by one bacterial OTU belonging to the genus *Rickettsia* and identified as the endosymbiont *Rickettsia* G021 based on 16S sequence (Supplementary Figure 1; Hunter *et al*., 2015). The pathogenicity of *Rickettsia* G021 is unknown but it is believed to be nonpathogenic. In addition, *I. pacificus* is not known to harbor pathogenic *Rickettsia* species (Cheng *et al*., 2013). Our analysis found that *Rickettsia* was ubiquitous in *I. pacificus* and becomes increasingly dominant at older life stages simultaneous with loss of microbiome species richness and evenness (Supplementary Table 2). Thus, environmental exposure (for example, age) does not have a strong role in the introduction of microbes into the tick microbiome.

We examined *I. pacificus* ticks that were *B. burgdorferi* positive or negative and found no significant differences in microbiome species richness or composition (Supplementary Figure 2A). While *B. burgdorferi* positive ticks likely fed on a mammal reservoir, negative ticks may be negative because they either fed on a *Borrelia*-refractory lizard or an uninfected mammal. To specifically examine how host blood meal affects the nymphal tick microbiome, we evaluated the microbiome of nymphs known to have fed on mice or lizards as larvae (Figures 2a and b, Supplementary Figure 4). *I. pacificus* nymphs that fed on lizards as larvae were characterized by significantly lower species richness relative to mouse-fed ticks (*t*-test = 5.41, *P* = 0.003 Figure 2a). Microbiome community composition was distinct between lizard-fed and mouse-fed nymphs by unweighted UniFrac (*t*-test = −5.14, *P* = 0.001, Figure 2b) and weighted UniFrac (*t*-test = −5.47, *P* = 0.001, Figure 2c). *Rickettsia* comprises a significantly greater portion of the microbiome in lizard-fed ticks relative to mouse-fed ticks primarily as a function of reductions in richness of other microbiome species (*P* = 0.002, Supplementary Figure S1). These results show that *S. occidentalis* reduces the diversity of other bacteria comprising the tick microbiome but does not reduce the relative abundance of *Rickettsia* perhaps because it is an intracellular bacteria not found in the midgut where interaction with host blood would occur (Supplementary Table 1).

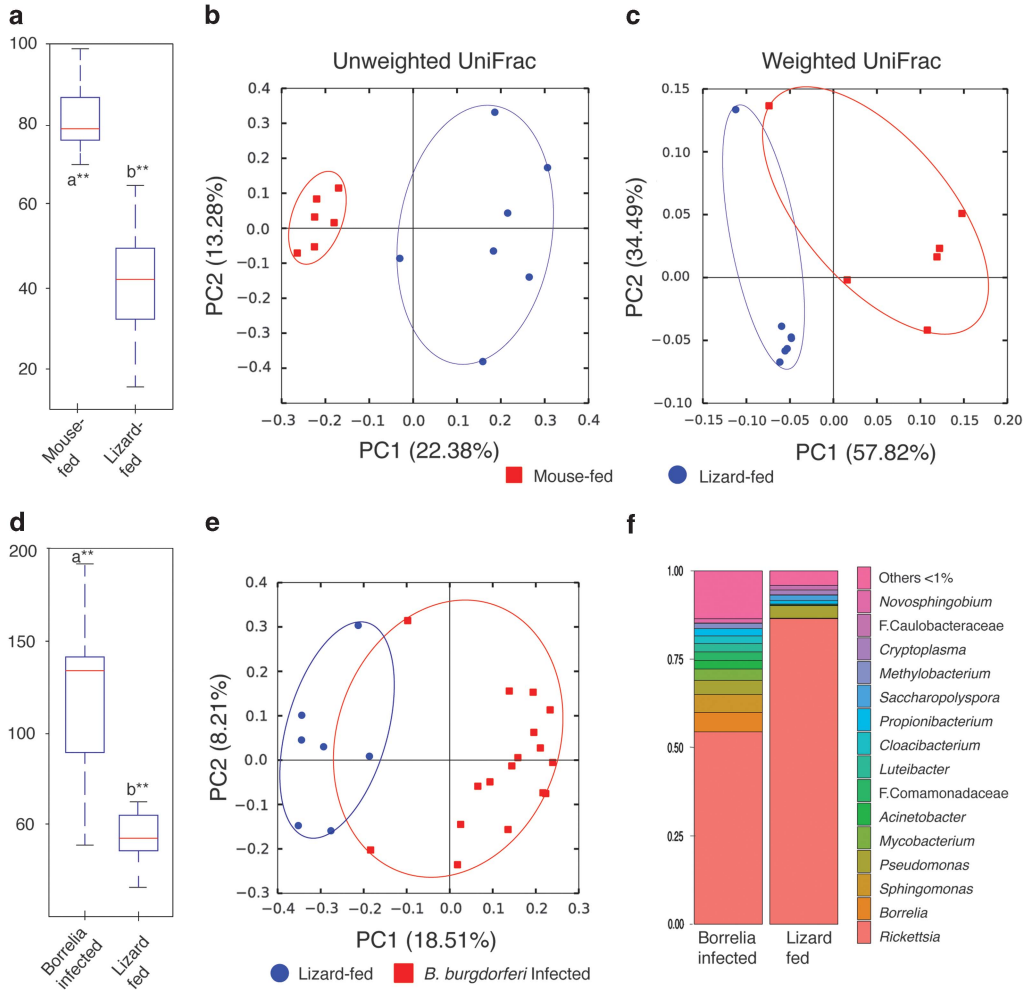

Figure 2 Host blood meal impacts on tick microbiome diversity shown by (**a**) alpha diversity as measured by observed OTUs, and group significance indicated by the box plots with ** indicating *P*-value < 0.01. (**b**) Beta diversity analysis by unweighted UniFrac analysis and (**c**) Weighted UniFrac analysis of nymphal *Ixodes pacificus* that fed on wild lizards or mice as larvae. Microbiome summaries of nymphal ticks that fed on *Sceloporus occidentalis* ('Lizard-fed') as larvae and questing nymphs that were infected with *Borrelia burgdorferi* ('Borrelia infected') are shown by (**d**) species richness as measured by observed OTUs. Group significance is indicated by the box plots with ** indicating *P*-value < 0.01. (**e**) Unweighted UniFrac analysis of lizard-fed versus *Borrelia*-infected ticks shown along PC1 and PC2. (**f**) Genus level taxonomic composition of lizard-fed versus *Borrelia*-infected ticks showing relative proportion of dominant taxa at the genus level.

B. burgdorferi-infected *I. pacificus* nymphs were distinct from lizard-fed ticks both in terms of species richness and composition (Figure 2d–f) and had a lower proportion of *Rickettsia* reads (Supplementary Table 1, Supplementary Figure 3). Although the source of infection is unknown, infected nymphs must have fed on a pathogen reservoir such as a small mammal. Western fence lizards host an overwhelming proportion of juvenile ticks, especially nymphs (Eisen *et al.*, 2001), therefore, the loss of species richness from subadult to adult ticks is unique to *I. pacificus* (Zolnik *et al.*, 2016) and appears to be driven by lizard blood meals. We find that when ticks feed on mice or other pathogen reservoirs, microbiome species richness is significantly higher. These results are intriguing because when microbiome diversity of *I. scapularis* was experimentally lowered, colonization success of *Borrelia burgdorferi* was impaired (Narasimhan *et al.*, 2014). Thus, ticks with reduced microbiome richness due to feeding on lizards may exhibit decreased ability to acquire *B. burgdorferi* in subsequent blood meals.

We show that *S. occidentalis* significantly alters the composition of the tick microbiome. Laboratory studies (Narasimhan *et al.*, 2014) suggest that reduction of tick microbiome diversity may affect future acquisition of *B. burgdorferi* by *I. pacificus.* These results demonstrate that ecological factors (that is, blood meal source) can significantly influence vector microbiomes and may fundamentally impact pathogen transmission in natural systems.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

We thank Caitlin Miller and Betsabel Chicana for field and laboratory assistance. We thank Frank Cipriano for technical support for sequencing assistance. This research was supported by an NSF grant #1427772 and CSUPERB New Investigator Grant to AS. The microbiome sequence data are archived at Sequence Read Archive under the BioProject ID PRJNA352452.

References

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK *et al.* (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**: 335–336.

Carpi G, Cagnacci F, Wittekindt NE, Zhao FQ, Qi J, Tomsho LP *et al.* (2011). Metagenomic profile of the bacterial communities associated with *Ixodes ricinus* ticks. *PLoS One* **6**: e25604.

Cheng D, Vigil K, Schanes P, Brown RN, Zhong J. (2013). Prevalence and burden of two rickettsial phylotypes (G021 and G022) in *Ixodes pacificus* from California by real-time quantitative PCR. *Ticks Tick Borne Dis* **4**: 280–287.

Christian N, Whitaker BK, Clay K. (2015). Microbiomes: unifying animal and plant systems through the lens of community ecology theory. *Front Microbiol* **6**: 869.

Eisen RJ, Eisen L, Lane RS. (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.

Gosalbes MJ, Abellan JJ, Durban A, Perez-Cobas AE, Latorre A, Moya A. (2012). Metagenomics of human microbiome: beyond 16s rDNA. *Clin Microbiol Infect* **18**: 47–49.

Hawlena H, Rynkiewicz E, Toh E, Alfred A, Durden LA, Hastriter MW *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.

Hughes GL, Dodson BL, Johnson RM, Murdock CC, Tsujimoto H, Suzuki Y *et al.* (2014). Native microbiome impedes vertical transmission of *Wolbachia in Anopheles mosquitoes*. *Proc Natl Acad Sci USA* **111**: 12498–12503.

Hunter DJ, Torkelson JL, Bodnar J, Mortazavi B, Laurent T, Deason J *et al.* (2015). The *Rickettsia* endosymbiont of *Ixodes pacificus* contains all the genes of *de novo* folate biosynthesis. *PLoS One* **10**: e0144552.

Jumpstart Consortium Human Microbiome Project Data Generation Working Group. (2012). Evaluation of 16S rDNA-based community profiling for human microbiome research. *PLoS One* **7**: e39315.

Kuo M, Lane RS, Giclas PC. (2000). A comparative study of mammalian and reptilian alternative pathway of complement-mediated killing of the Lyme disease spirochete (*Borrelia burgdorferi*). *J Parasitol* **86**: 1223–1228.

Lane RS, Loye. JE. (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.

Lane RS, Mun J, Eisen RJ, Eisen L. (2005). Western gray squirrel (Rodentia: Sciuridae): a primary reservoir host of *Borrelia burgdorferi* in Californian oak woodlands? *J Med Entomol* **42**: 388–396.

Lane RS, Quistad GB. (1998). Borreliacidal factor in the blood of the western fence lizard (*Sceloporous occidentalis*). *J Parasitol* **84**: 29–34.

Narasimhan S, Rajeevan N, Liu L, Zhao YO, Heisign J, Pan J *et al.* (2014). Gut microbiota of the tick vector *Ixodes scapularis* modulate colonization of the Lyme disease spirochete. *Cell Host Microbe* **15**: 58–71.

Rynkiewicz EC, Hemmerich C, Rusch DB, Fuqua C, Clay K. (2015). Concordance of bacterial communities of two tick species and blood of their shared rodent host. *Mol Ecol* **24**: 2566–2579.

Swei A, Briggs CJ, Lane RS, Ostfeld RS. (2012). Impacts of an introduced forest pathogen on the risk of Lyme disease in California. *Vector Borne Zoonot Dis* **12**: 624–632.

van Treuren W, Ponnusamy L, Brinkerhoff RJ, Gonzalez A, Parobek CM, Juliano JJ *et al.* (2015). Variation in the microbiota of *Ixodes* ticks with regard to geography, species, and sex. *Appl Environ Microbiol* **81**: 6200–6209.

Weiss B, Aksoy S. (2011). Microbiome influences on insect host vector competence. *Trends Parasitol* **27**: 514–522.

Williams-Newkirk AJ, Rowe LA, Mixson-Hayden TR, Dasch GA. (2014). Characterization of the bacterial communities of life stages of free living lone star ticks (*Amblyomma americanum*). *PLoS One* **9**: e102130.

Zhang X, Yang Z, Lu B, Ma X, Zhang C, Xu H. (2014). The composition and transmission of microbiome in hard tick, *Ixodes persulcatus*, during blood meal. *Ticks Tick Borne Dis* **5**: 864–870.

Zolnik CP, Prill RJ, Falco RC, Daniels TJ, Kolokontronis SO. (2016). Microbiome changes through ontogeny of a tick pathogen vector. *Mol Ecol* **25**: 4963–4977.

Supplementary Information accompanies this paper on The ISME Journal website (http://www.nature.com/ismej)