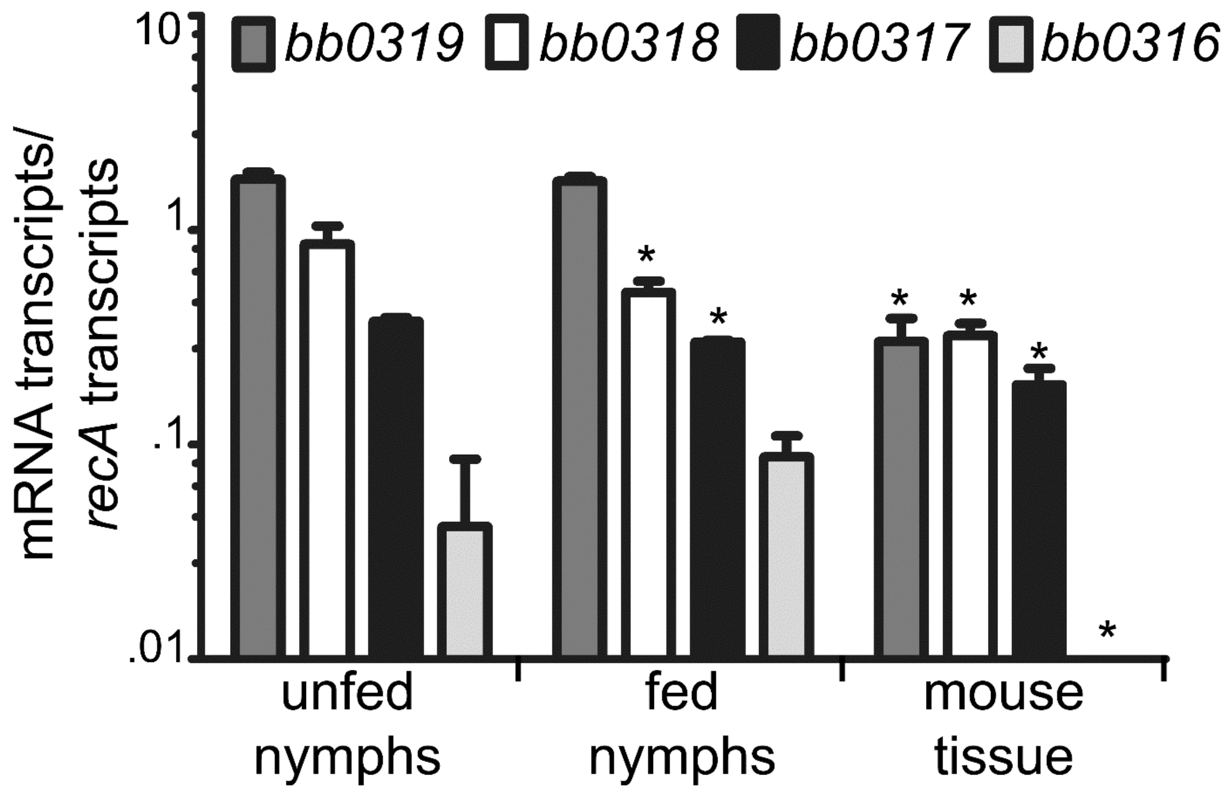

Figure S1. The *bb0318* gene is expressed throughout the tick-mouse infectious cycle. RNA was isolated from *B. burgdorferi* B31 A3 infected unfed nymphs, fed nymphs and mouse tissue. Expression of genes *recA*, *bb0319*, *bb0318*, *bb0317*, and *bb0316* was quantified by reverse transcriptase qPCR. The data are expressed as the number of mRNA transcripts per number of *recA* transcripts, and are the averages of three biological replicates. The expression of levels of each gene in the different environments were compared using one-way ANOVA and Tukey's post-test (GraphPad Prism 6.0). Statistical comparisons were not found to be significant unless otherwise noted. * denotes $p < 0.05$ compared to gene-specific expression in unfed nymphs.

Supplemental Materials and Methods

Mouse and Tick Infections

The University of Central Florida (UCF) is accredited by the International Association for Assessment and Accreditation of Laboratory Animal Care, and all experiments were approved by the Institutional Animal Care and Use Committee of UCF. All spirochetes used for mouse infection studies were grown to the same stationary phase density (~$1x10^8$/ ml) in BSKII medium and then diluted to the desired inoculum density just prior to use. All inoculum densities were determined using a Petroff-Hausser counting chamber and verified by colony forming unit (cfu) counts in solid BSK medium. All inoculum cultures were analyzed for the endogenous plasmid content by PCR (1). All inoculum cultures carried the expected endogenous plasmid content. Six 6-8 week old female C3H/HeN mice (Envigo) were needle inoculated intraperitoneally (80%) and subcutaneously (20%) with 1×10^5 B31 A3. Three weeks post inoculation infection was by serology as previously described (2). Approximately 200 uninfected *Ixodes scapularis* larvae (BEI resources) were allowed to feed to repletion on the *B. burgdorferi* infected mice, as previously described (1, 3). Individuals from a subset of fed larvae were each homogenized in BSKII medium and plated for cfus in solid BSK medium, as previously described (Jewett 2009). These analyses indicated that approximately 90% of the larvae had acquired *B. burgdorferi* during feeding. The remaining fed larvae were maintained at room temperature under 98% humidity and allowed to molt to nymphs. Three groups of 25 unfed, *B. burgdorferi* infected nymphs were processed for RNA isolation. Three groups of 25 nymphs each were fed to repletion on individual naïve 6-8 week old female C3H/HeN mice, as previously described (1, 3). Three groups of 15 fed

nymphs were processed for RNA isolation. For isolation of spirochete RNA from mouse tissues, six 6-8 week old female C3H/HeN mice (Envigo) were needle inoculated intraperitoneally (80%) and subcutaneously (20%) with 1 x 10^5 B31 A3. Inoculum cultures were quantitated and verified as described in the Materials and Methods in the text of the paper. Ten days post inoculation mice were assessed for infection by serology and reisolation of spirochetes from joint tissue (4). Bladder tissue was harvested for RNA isolation.

RNA Isolation

Triplicate groups of 25 unfed B31 A3 infected nymphs, 15 fed B31 A3 infected nymphs or bladder tissue isolated from two B31 A3 infected mice at the time of dissection were snap frozen in liquid nitrogen and homogenized in approximately 1 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 8) containing 0.5 mg/ml lysozyme and 1% SDS in gentleMACS™ M Tubes using a gentleMACS™ Dissociator (Miltenyi Biotech). RNA was isolated as described in the Materials and Methods in the text of the paper.

Gene Expression Analysis

cDNA was synthesized from 1 µg of RNA isolated from unfed nymph-, fed nymph- or mouse tissue-derived spirochetes using the iScript Select cDNA synthesis kit and random hexamers (Bio-Rad), according to the manufacturer's instructions. Parallel cDNA reactions were carried out in the absence of reverse transcriptase. Real-time quantitative PCR reactions were performed and analyzed as described in the Materials and Methods in the text of the paper. The expression of levels of each gene in the different environments were compared using one-way ANOVA and Tukey's post-test (GraphPad Prism 6.0). Statistical comparisons were not found to be significant unless

otherwise noted. * denotes $p < 0.05$ compared to gene-specific expression in unfed nymphs.

Supplemental References

1. **Jewett, M. W., K. A. Lawrence, A. Bestor, R. Byram, F. Gherardini, and P. A. Rosa.** 2009. GuaA and GuaB are essential for *Borrelia burgdorferi* survival in the tick-mouse infection cycle. J Bacteriol **191:**6231-6241.
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