

Supplementary figures for the article:

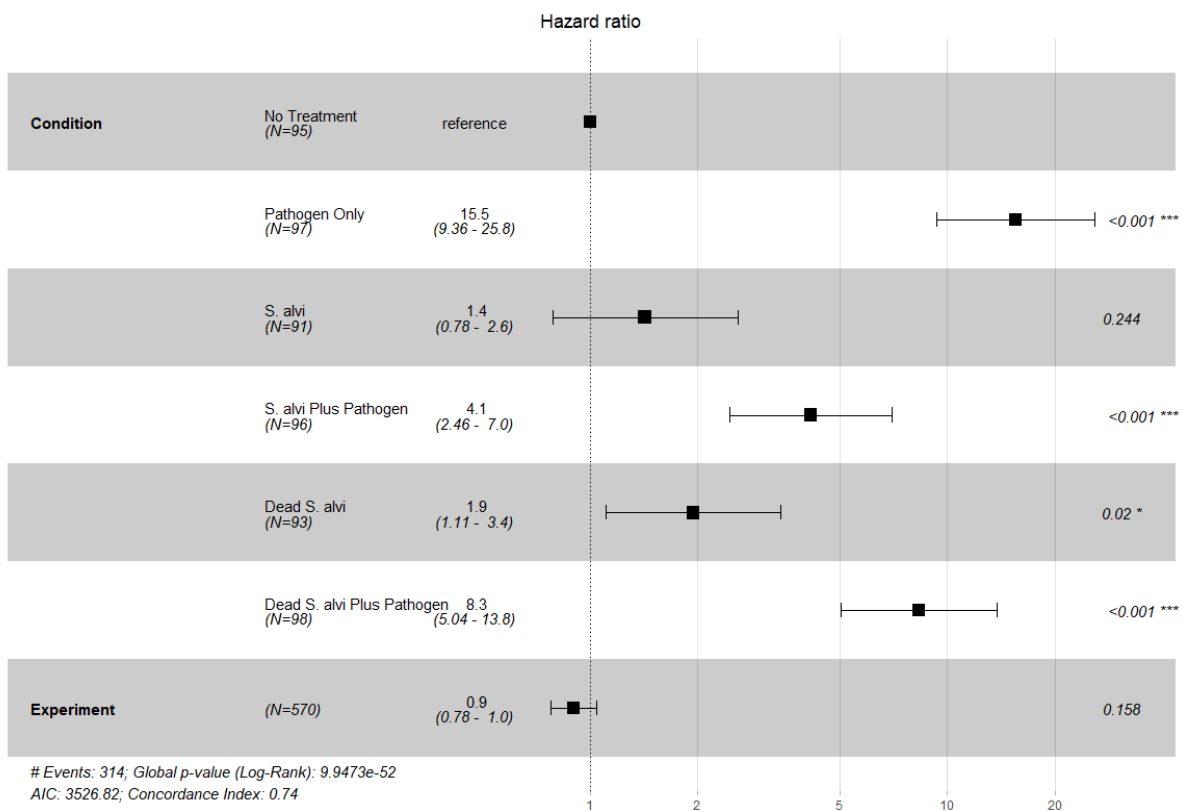
Title: Symbionts shape host innate immunity in honey bees

Authors: Richard D. Horak^{1*}, Sean P. Leonard¹, Nancy A. Moran^{1*}

Affiliations: ¹Department of Integrative Biology, The University of Texas at Austin, Austin, Texas 78712, United States

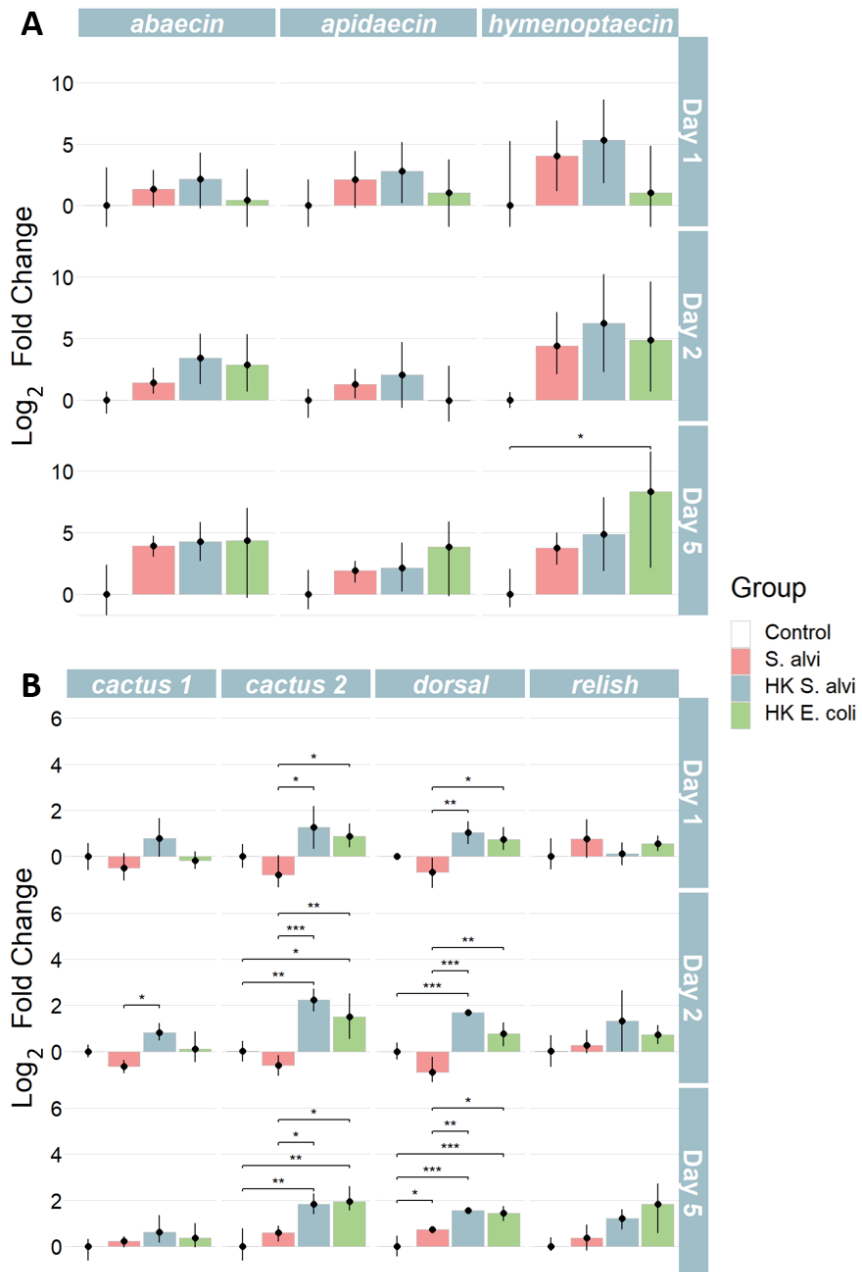
*Correspondence to: rdhorak1@gmail.com, nancy.moran@austin.utexas.edu

DOI: 10.1098/rspb.2020.1184



Supplementary Figure 1 Bees challenged by the pathogen *S. marcescens* have higher survival when pre-inoculated with *S. alvi*

Hazard plot detailing effect of honey bee treatment and replicate experiments on honey bee survival after inoculation with PBS or 5 ul of the pathogen *S. marcescens* at OD₆₀₀ = 1. Honey bees given no bacterial inoculum and no pathogen exposure used as a reference group. Bees treated with live *S. alvi* or heat-killed *S. alvi* without pathogen exposure were affected much less than honey bees exposed to the pathogen, regardless of prior treatment. Total N = 570 bees.



Supplementary Figure 2 Live and heat-killed *S. alvi* trigger differential host immune gene expression

Bee gene expression relative to the housekeeping gene *RPS18* measured using qPCR from cDNA derived from bee whole abdomens 1,2, and 5 day(s) post treatment. HK = heat-killed. (A) Bees inoculated with live *S. alvi*, heat-killed *S. alvi*, and heat-killed *E. coli* trigger non-significant upregulation of AMPs compared to the uninoculated control group. (B) Bees inoculated with heat-killed *S. alvi* or *E. coli* had significantly higher expression of *cactus2* and *dorsal* than the control or live *S. alvi* groups. Total N = 54 bees from one hive. *** = P < 0.001, ** = P < 0.01, * = P < 0.05. P-values obtained using Tukey Honest Significant Difference method.

Supplementary Table 1 qPCR primer sequences for gene expression analysis

Gene	Source	Forward	Reverse
Abaecin	[23]	TCGGATTGAATGGTCCTGAC	ATCTTCGCACTACTCGCCAC
Apidaecin	[23]	GTAGGTCGAGTAGGCCGATCT	TTTTGCCTTAGCAATTCTTGTTG
Cactus-1	[53]	CTATCGTGGAGAACTGCGTAT	TCAGGAAGTGGTTCTGGTATTG
Cactus-2	[53]	ATCAGACGGCTCTGCTCTAT	TCGTCTTCGTCAGTGGTATCT
Dorsal	[54]	AGAGATGGAACGCAGGAAAC	TGACAGGATATAGGACGAGGTAA
Dredd	[54]	GCGTCATAAAGAAAAGGATCA	TTTCGGGTAATTGAGCAACG
Hymenoptaecin	[55]	GTCGTCCATCCTTGGACATT	TTTCCCAAACCTCGAATCCTG
PGRP-LC	[54]	TCCGTCAGCCGTAGTTTTTC	CGTTTGTGCAAATCGAACAT
Pirk	This Study	GCCCGAAATCTAGCAAGGATAA	TTCCTCTCCTCGTCCATCTT
Relish	[55]	GGAGCTGATCCAAATCGAAC	AGTGGCATCCATCCATCATT
RPS18	[53]	AGGTGTTGGTCGTCGTTAT	CATTCTCCAGCACGCTTAT
Toll	[54]	TAGAGTGGCGCATTGTCAAG	ATCGCAATTTGTCCCAAAC