from: Jani, Andrea J., Roland A. Knapp, and Cheryl J. Briggs. 2017. *Epidemic and endemic pathogen dynamics correspond to distinct host population microbiomes at a landscape scale*. Proceedings of The Royal Society B-Biological Sciences.

BIOINFORMATIC AND STATISTICAL DETAILS

16S sequencing and bioinformatic processing: Detailed methods for 16S sequencing are described in [1]. Briefly, the V1-V2 regions of the 16S gene were amplified using primers 8f and 338r with sample-specific barcodes and Roche FLX amplicon adapters. DNA concentrations of PCR products were measured and amplicons from each sample were pooled in equimolar quantities for sequencing on a Roche/454 GS FLX using Titanium Chemistry. Sequences were bioinformatically processed using the program Mothur (v1.30) as described in Jani and Briggs (2014). Briefly, sequences were quality-filtered (de-noised and screened for short, potentially low-quality, or chimeric sequences), aligned to a non-redundant representative subset of the SILVA v111 SSU Ref 16S curated alignment database [2], and clustered into operational taxonomic units (95% identity OTUs) and phylotypes. Sequences were classified using the Bayesian classifier of Wang *et al.* [3] and each OTU was assigned a consensus taxonomy from SILVA v111. Pairwise phylogenetic community distances among all samples were calculated using relative abundance-weighted Unifrac [4]. Richness and diversity of each sample was estimated using four metrics after randomly subsampling 500 sequences per sample to equalize detection effort among samples. Metrics used were: observed OTU richness (S_{OBS}), Chao's richness estimate [5], Shannon diversity, and Shannon evenness.

Statistical details: All statistical analyses were performed using JMP v. 10 (SAS Institute Inc., Cary, NC, USA, 1989-1212), with the following exceptions: Multivariate bacterial community data were analyzed using nonparametric, permutation-based methods (NMDS, ANOSIM, PERMANOVA, DistLM, and Mantel tests) in the software package Primer-E v6 [6]. Data were transformed to approximate the Gaussian distribution of errors, as follows: Bd load data were $\log_{10}$ transformed [$\log_{10}$(Bd load +1)]; For analyses of individual bacterial OTUs, relative abundances were arcsine(square root) transformed.

In analyzing beta diversity (multivariate community composition), for complex (2-way and/or nested) analyses, we used PERMANOVA. For simple single-factor tests, we use ANOSIM.

Identifying OTUs that differ between Enzootic and Epizootic populations: Patterns in the relative abundances of individual OTUs were tested for only common OTUs, which we define as any OTU comprising on average at least 0.1% of the total sequence reads within surveys, for a total of 47 common OTUs. To prevent inflation of Type I statistical error due to testing multiple OTUs (i.e., multiple comparisons), we calculated the false discovery rate (Q) using the program Qvalue [7]. To test for differences in relative abundance between enzootic and epizootic populations, we used a linear mixed model (fixed effect: population type, random effect: population survey, response variable: relative abundance of OTU). Tests with $P<0.05$ are reported as significant if $Q<0.05$, and marginally significant if $Q<0.1$. We also tested for correlations between Bd load and OTU relative abundance. For each common OTU, we tested for a correlation between Bd load and OTU relative abundance. Bd load is a predictor variable that varies among frogs within a population, and here we were interested only in among-population patterns. Therefore, to avoid spuriously significant results, we used the survey-means of Bd load as input data. We also used survey-means of OTU relative abundances to maintain a one-to-one relationship among data in correlation tests.

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EFFECT OF RINSING ON *R. SIERRAE* MICROBIOME SWAB DATA

We compared bacterial communities from 9 wild-caught frogs before and after rinsing. We also compared frog swabs, frog rinsates, and lake water samples. Microbiome samples were collected from frog skin (swabs) and lake water (filters) as described in Methods in this paper and in Jani and Briggs (2014), with the modification that frogs were swabbed both before and after rinsing, with one half of the body swabbed for each sample. Specifically, before rinsing, a randomly chosen side (left or right) of the body was swabbed. Frogs were then rinsed twice with 60 ml sterile water, and then swabbed again (on the side of the body that was not swabbed prior to rinsing). New nitrile gloves were worn for handling each frog. The water used to rinse frogs ("rinsate") was collected in sterile 50 ml conical polypropelene tubes and 50 ml of rinsate per frog was passed through a 0.22 micron filter to collect rinsate bacteria. All frogs were sampled from a single lake and sampling date to avoid spatial and temporal confounding. Bacterial communities were characterized by terminal restriction fragment length polymorphism (TRFLP) analysis [8]. Bacterial profiles from swabs, rinsates, and lake water all differed from each other (PERMANOVA, model P=0.0001, swabs versus rinsates P=0.0001, swabs versus lake water P=0.0003, lake water versus rinsates P=0.0032) but there was no difference between rinsed and unrinsed frogs (PERMANOVA, P=0.2934; Figure S1 below). This result indicates that rinsing does not affect the bacterial community detected on frog skin, at least in this study system. Notably, we did find that bacterial communities from rinsates and frog swabs differed, conistent with a previous study [9], but despite this we found that rinsing did not alter the skin microbiome samples. Rinsates probably contain a combination of frog-associated bacteria and "transient" bacteria derived from lake water. Rinsing with sterile water had no effect on the bacterial profile of swab samples, probably because the density of lake water bacteria on frogs is very low compared to the density of resident bacteria on the skin. The importance of rinsing may depend on the study system; for example, it is possible that *R. sierrae* microbiome sampling is insensitive to rinsing because these frogs inhabit oligotrophic, relatively homogenous lake water.

Figure S1. Rinsing with sterile water does not affect bacterial community profiles from skin swabs. NMDS ordination of bacterial communities collected from frogs that were rinsed (open triangles) or not rinsed (filled triangles), rinsates (crosses), and lake water samples (filled dots).

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Figure S2. Bacterial communities on frog skin are distinct from surrounding aquatic bacterial communities. NMDS ordination shows separation between bacterial communities of frog skin and bacterial communities sampled from lake water. PERMANOVA: P=0.0001. ANOSIM: P=0.0001; Global R=0.98. Each data point represents one population survey. Frog skin microbiome data are pooled within frog population surveys, such that each frog data point is a pooled sample for all frogs swabbed in a given lake on a given date.

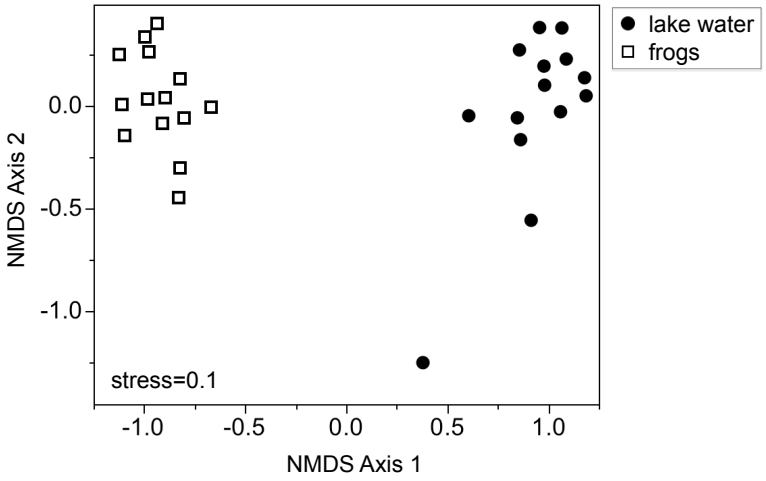

Figure S3. Phylogenetic composition of bacterial communities from (a) frog skin and (b) lake water. Graphics depict mean relative abundances of bacterial phylotypes across frogs or lake water samples. Phylotypes that make-up at least 1% of mean per-sample relative abundance are named. Phylotypes with less than 1% relative abundance and unclassified taxa are pooled and shown as "unassigned". Plots constructed using the program Krona [1].

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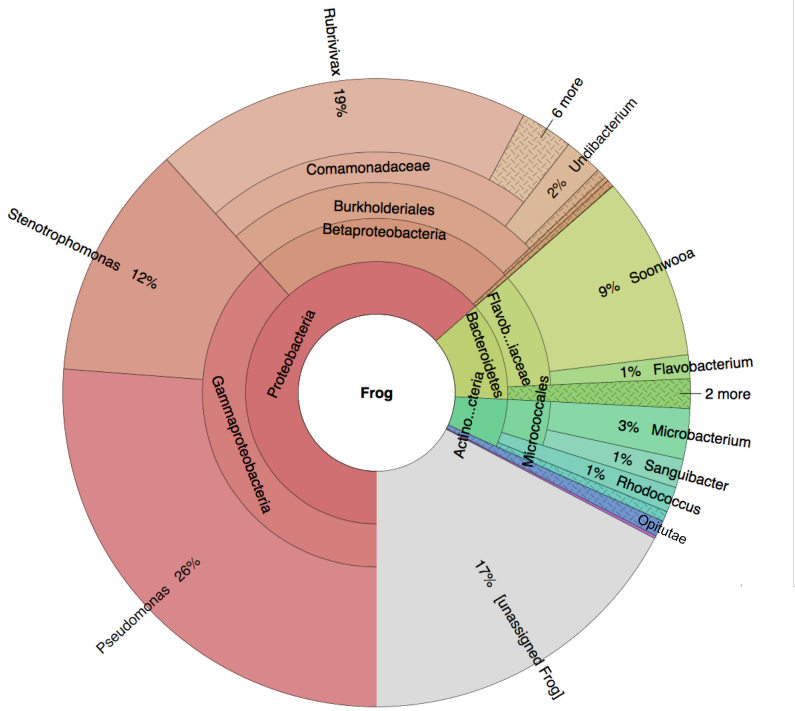
a



b

Table S1. *R. sierrae* population surveys.

population name	region	survey date	sample size [a] (Bd load)	sample size [a] (microbiome)	disease status in 2010 [b]	Eventual response to infection [c]	year epizootic observed	mean Bd load (log_{10}) (st err)
Enz-1	North	7/9/10	27		enzootic	persist (enzootic)	-	1.06 (0.17)
		8/18/10	30	18	enzootic			2.55 (0.22)
		9/10/10	30		enzootic			2.25 (0.15)
Enz-4	North	8/6/10	26		enzootic	persist (enzootic)	-	0.73 (0.16)
		8/27/10	30	8	enzootic			1.94 (0.25)
Enz-3	North	7/7/10	6		enzootic	persist (enzootic)	-	0.63 (0.24)
		8/11/10	20		enzootic			1.81 (0.24)
		9/1/10	33	8	enzootic			2.09 (0.25)
		9/16/10	40	18	enzootic			2.17 (0.21)
Enz-2	North	7/28/10	19		enzootic	persist (enzootic)	-	0.45 (0.15)
		8/29/10	31	7	enzootic			1.9 (0.16)
		9/13/10	37	18	enzootic			2.03 (0.18)
Pre-2	South	7/24/10	11		pre-epizootic	die-off (epizootic)	2011	0.04 (0.04)
		8/22/10	22	8	pre-epizootic			0 (0)
		9/9/10	20	10	pre-epizootic			0.73 (0.14)
Epi-2	South	8/23/10	18	8	epizootic	die-off (epizootic)	2010	4.73 (0.09)
Pre-3	South	8/14/10	43		pre-epizootic	die-off (epizootic)*	2015	0.01 (0.01)
		9/4/10	29	8	pre-epizootic			0.04 (0.03)
Epi-1	South	7/16/10	30		pre-epizootic	die-off (epizootic)	2010	0 (0)
		8/8/10	30		pre-epizootic			0.06 (0.04)
		8/30/10	30	20	epizootic			3.31 (0.18)
		9/14/10	14	10	epizootic			4.6 (0.1)
		9/15/10	29		epizootic			4.38 (0.07)
Pre-4	South	7/15/10	15		pre-epizootic	predicted epizootic	-	0 (0)
		9/6/10	30	8	pre-epizootic			0.01 (0.01)
Pre-1	South	8/9/10	1		pre-epizootic	die-off (epizootic)	2011	0
		8/31/10	24	8	pre-epizootic			0.05 (0.03)
		9/15/10	14		pre-epizootic			0.36 (0.09)

(a) # of frog swabs processed for Bd load or microbiome. (b) Disease status denotes status at the time of the specified survey. (c) Response to Bd infection denotes eventual response to Bd infection, including observations made after 2010.

* Bd invasion and massive population die-off characteristic of an epizootic event began in 2015. Ongoing intervention is attempting to prevent extirpation which would otherwise be the most likely outcome for this population based on die-offs observed in 2015.

Table S2. Factors predicting the *R. sierrae* skin microbiome.

PREDICTOR VARIABLES ----------RESPONSE VARIABLES----------

variable	description	Observed OTUs	Chao's richness	Shannon diversity	Shannon evenness	beta diversity
Bd load	mean $\log_{10}$(Bd load) for each population survey	NS	NS	NS	NS	P=0.0004
geographic region	north or south	P=0.0033 (North>South)	P=0.0049 (North>South)	P=.0144 (North>South)	NS	NS alone. (Significant only if added to model after Bd, P=0.0134 .)
elevation	elevation (m)	P=0.0302	NS	P=0.0468	NS	NS
lake area	$\log_{10}$(lake area), in m^2	NS	NS	NS	NS	NS
aquatic bacterial richness	# OTUs observed in lake water	NS	NS	NS	NS	NS

* Elevation covaries with geographic region (P=0.0004)
NS, Not statistically significant.

Table S3. OTUs that differed in relative abundance between enzootic and epizootic populations, or that were correlated with Bd load.

OTU	Classification	Type of population where OTU was more abundant	enzootic mean relative abundance	epizootic mean relative abundance	Correlation with Bd load	Correlation Coefficient	Bd correlation within populations for OTUs in this genus (a)
F-70	Alphaproteobacteria-Sphingomonadaceae-Sandarakinorhabdus		0.00195945	0.00044969	negative *	-0.7645	
F-6	Betaproteobacteria-Burkholderiaceae-Polynucleobacter		0.00434229	0.00172776	negative *	-0.7758	negative
F-23	Betaproteobacteria-Comamonadaceae-Ideonella (b)	enzootic *	0.01293786	0.00102758	negative *	-0.7671	
F-44	Betaproteobacteria-Comamonadaceae-Rhizobacter		0.00382501	0.00118247	negative *	-0.7411	
F-20	Betaproteobacteria-Methylophilaceae-Methylotenera		0.0021662	0.00137443	negative *	-0.7746	negative
F-96	Betaproteobacteria-Oxalobacteraceae-Undibacterium	epizootic *	0.00017336	0.00323619	positive **	0.9033	positive
F-1	Gammaproteobacteria-Pseudomonadaceae-Pseudomonas		0.24397411	0.07000079	negative *	-0.7333	negative
F-17	Bacteroidetes-Cytophagaceae-Arcicella		0.00211116	0.00107299	negative *	-0.7581	
F-14	Bacteroidetes-Cytophagaceae-Arcicella		0.0044397	0.00025633	negative *	-0.7454	
F-4	Bacteroidetes-Flavobacteriaceae-Soonwooa		0.06431263	0.29072695	positive *	0.8516	positive
*	marginally significant (P<0.05, Q<.1) ** significant (P<0.05, Q<0.05)						
(a)	reference: [1]						
(b)	OTU F-23 could only be classified to family (Comamonadaceae) by Wang classifier. We used a BLAST search to refine taxonomy of this OTU to Ideonella.						

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Table S4. Comparisons of *R. sierrae* microbiome composition for all possible pairwise combinations of the 14 surveys in this study. Data are P-values from ANOSIM test for each comparison. P-values are adjusted for multiple tests, see footnote. Bold-type p-values show significant differences between two surveys. Each survey is identified by site name and collection date. ŧ indicates comparison of two surveys from the same population on different dates.

Epi-1	Aug. 30	**0.013**												
Enz-2	Aug. 29	0.270	**0.006**											
Enz-4	Aug. 27	0.072	**<0.001**	0.441										
Epi-2	Aug. 23	**0.001**	**<0.001**	**<0.001**	**<0.001**									
Pre-2	Aug. 22	**0.048**	**0.001**	**0.036**	0.057 ŧ	**<0.001**								
Enz-1	Aug. 18	**<0.001**	**<0.001**	**0.007**	**0.013**	**<0.001**	0.162							
Enz-3	Sept. 16	0.065	**<0.001**	0.081	0.074	**<0.001**	**0.029**	**<0.001**						
Epi-1	Sept. 14	**0.027**	**<0.001 ŧ**	**0.006**	**0.001**	**0.009**	**0.001**	**<0.001**	**0.020**					
Enz-2	Sept. 13	**0.016**	**<0.001**	0.055 ŧ	0.055	**<0.001**	0.229	**0.024**	**0.006**	**<0.001**				
Pre-2	Sept. 9	**0.009**	**<0.001**	**0.006**	**0.007**	**<0.001**	**<0.001 ŧ**	**0.002**	0.081	**<0.001**	0.084			
Pre-4	Sept. 6	0.143	**0.004**	0.060	**0.035**	**<0.001**	**0.001**	**<0.001**	**0.034**	**0.002**	**0.049**	**0.028**		
Pre-3	Sept. 4	0.268	0.176	**0.029**	**0.001**	**<0.001**	**0.002**	**<0.001**	**0.007**	**0.004**	**<0.001**	**<0.001**	0.089	
Enz-3	Sept. 1	0.227	**0.029**	**0.013**	**<0.001**	**0.002**	**<0.001**	**<0.001**	**0.044 ŧ**	**0.022**	**<0.001**	**<0.001**	**0.001**	**0.049**
		Pre-1 Aug. 31	Epi-1 Aug. 30	Enz-2 Aug. 29	Enz-4 Aug. 27	Epi-2 Aug. 23	Pre-2 Aug. 22	Enz-1 Aug. 18	Enz-3 Sept. 16	Epi-1 Sept. 14	Enz-2 Sept. 13	Pre-2 Sept. 9	Pre-4 Sept. 6	Pre-3 Sept. 4

P-values were adjusted to account for multiple tests using the Benjamini-Hochberg method [1], rather than directly estimating the false discovery rate, Q [2] as was done for other analyses in this study (see methods). The reason for using the Banjamini-Hochberg method for this specific analysis is that the P-value distribution in this case was skewed toward small values, and the Q-value calculation failed to identify any tests as false positives.

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Figure S4. Bacterial richness and Bd loads in enzootic, epizootic, and pre-epizootic populations. Populations in the northern region (Enzootic) have higher richness than populations in the south (Epizootic and Pre-epizootic). Epizootic and pre-epizootic populations have similar bacterial richness (indicated by both a t-test comparing epizootic and pre-epizootic, and ANOVA: richness ~ population class; Tukey HSD). Note that richness groups by region (north, south), rather then Bd load class. Each data point is mean Bd load or bacterial richness for one population survey.