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Validating the use of trap-collected feces for studying the gut microbiota of a small mammal (*Neotoma lepida*)

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Gut microbes can largely influence the ecology and evolution of mammalian hosts. As research in this area increases, it will be necessary to collect fecal samples from nature to inventory microbial populations. Here, we tested the appropriateness of using feces collected from live-traps for microbiome studies. We found that feces collected from the traps containing the desert woodrat (*Neotoma lepida*) did not differ from aseptically collected feces in terms of microbial community structure, abundances of bacterial phyla, or measurements of α-diversity. Roughly 83% of the microbes in trap-collected feces represented the endogenous microbiota. Thus, we suggest that feces collected from small mammal traps are acceptable for studying the microbiota of wild, small mammals.

Key words: host–microbe interactions, microbiome, *Neotoma*, Sherman trap

The role that gut microbial communities play in the ecology and evolution of mammals is an emerging field of research. Studies on captive mammals have revealed that microbial communities are driven by diet, evolutionary history, and gut anatomy (Ley et al. 2008; Muegge et al. 2011). Targeted studies of samples collected from wild-caught animals hold promise for revealing the dynamics of these relationships in nature. However, feces collected from nature may be contaminated with environmental microbes. Thus, the validity of these samples must be verified.

Several studies suggest the potential for contamination by environmental microbes. A recent large-scale comparative study of the microbiomes of myrmecophagous mammals discarded more than one-third (37 of 92) of samples due to likely contamination by soil or rain (Delsuc et al. 2014). Additionally, we have investigated changes in microbial diversity of woodrats (*Neotoma* spp.) before and during captivity (Kohl and Dearing 2014; Kohl et al. 2014a). Fecal samples from woodrats collected from the live-traps used at capture exhibited higher microbial diversity than samples collected from the same animals after a 2-week period of captivity. We attributed this difference to loss of microbial diversity in captivity (Kohl and Dearing 2014; Kohl et al. 2014a). However, another explanation is environmental contamination of field-collected samples.

Therefore, we designed an experiment to examine the use of trap-collected feces for studying the microbiota of wild-caught mammals. We focused our study on woodrats, wild herbivores that tend to specialize on toxic plants. Previous research by our group has shown that gut microbes are crucial in allowing woodrats to consume toxic plants (Kohl et al. 2014b). Thus, to facilitate comparison of microbial communities across woodrat species and populations, we conducted a controlled study in the lab to validate the use of trap-collected feces. We compared the microbial communities of feces collected aseptically in the lab to those collected after the same animals were placed in live-traps with bait and cotton and held overnight. We also inventoried the microbial communities of potential environmental sources (bait, cotton, trap walls) and investigated their contribution to the microbiota of trap-collected feces.

Methods

Animals and sample collection.—Seven individuals of *Neotoma lepida* were collected from the Great Basin desert at White Rocks, Tooele County, Utah (40°19′N, 112°54′W) in November 2012. Animals were transported to the University of Utah Department of Biology Animal Facility and housed in individual cages (48 × 27 × 20 cm) under a 12:12-h light:dark cycle, with 28ºC ambient temperature and 20% humidity. Woodrats were fed high-fiber rabbit chow daily (Formula 2031, Harlan Teklad, Madison, Wisconsin).

In February 2014, we collected a suite of samples to investigate whether trap-collected feces represent the existing microbiome. Our "control" samples were collected by placing animals in ethanol-sterilized cages for 2 h at the beginning of their light cycle. Feces were collected with sterile forceps, immediately frozen, and stored at −80°C. These feces were fresh and collected in sterile conditions. Woodrats were then placed back in shoebox cages and fed rabbit chow for 2 nights.

The "trap-collected" feces were collected the following evening by placing woodrats in live-traps (aluminum Sherman traps: 3×4×12 inches) that had last been used and sprayed with bleach 4 months prior to this experiment. We added cotton batting, an apple slice, and a bait mixture of peanut butter and oatmeal to the traps. These additional items were to mimic the typical trapping protocol. Animals were removed from the traps the following morning, and contents of the traps were emptied on to a standard, unsterilized paper towel. We used sterile forceps to collect feces that appeared fresh by visual inspection and avoided feces that had obviously been urinated on. The "trap-collected" samples had the potential to come in contact with unsterile sources of contamination and be several hours old. Feces were immediately frozen and stored at −80°C. All procedures followed guidelines approved by the American Society of Mammalogists (Sikes et al. 2011).

We also collected several environmental sources of microbes to investigate potential microbial contamination of trap-collected feces. We collected samples from the cotton batting, apple slices, and the bait mixture of peanut butter and oatmeal that were not placed in the traps. Additionally, the inside walls of the traps were sampled with a sterile cotton swab. All samples were collected before animals were placed in the traps. We analyzed 3 samples each of cotton, apple, and peanut butter and oatmeal bait, and 4 swabs from the inside walls of traps.

Microbial inventories and analysis.—We isolated DNA from feces and all environmental samples using the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, California). A previously established technique was used to amplify the V4 region of the 16S rRNA gene with primers *515F* and *806R* (Caporaso et al. 2012). The reverse primer also contained a 12-base barcode sequence, which allowed for pooling of samples. PCRs were conducted in triplicate and the resulting products were pooled within a single sample. DNA was quantified using PicoGreen (Life Technologies, Carlsbad, California) and a plate reader and cleaned using the UltraClean PCR Clean-Up Kit (MoBIO, Carlsbad, California). Amplicons were sequenced on the MiSeq platform (Illumina, San Diego, California) using previously described techniques (Caporaso et al. 2012). Sequences were analyzed using the QIIME version 1.6.0 (Caporaso et al. 2010). Sequences underwent standard quality control and were split into libraries using default parameters in QIIME. Sequences were grouped into operational taxonomic units (OTUs) using UCLUST (Edgar 2010) with a minimum sequence identity of 97%. The most abundant sequences within each OTU were designated as a "representative sequence" and aligned against the Greengenes core set (DeSantis et al. 2006) using PyNAST (Caporaso et al. 2009) with default parameters set by QIIME. A PH Lane mask supplied by QIIME was used to remove hypervariable regions from aligned sequences. FastTree (Price et al. 2009) was used to create a phylogenetic tree of representative sequences. OTUs were classified using the Ribosomal Database Project classifier with a standard minimum support threshold of 80% (Wang et al. 2007). Sequences identified as chloroplasts or mitochondria were removed from the analysis.

We calculated the relative abundances of bacterial phyla and genera in fecal samples and compared them between control and trap-collected samples using paired *t*-tests and the false discovery rate correction. Several measurements of α-diversity were calculated. We calculated Chao1, which estimates microbial species richness by calculating the asymptote on a species accumulation curve. We also calculated evenness of bacterial species, the Shannon index, which integrates both evenness and richness, and Faith's phylogenetic diversity (Faith 1992), which measures the cumulative branch lengths from randomly sampling OTUs from each sample. For each sample, we calculated the mean of 20 iterations for a subsampling of 40,300 sequences. We also conducted principal coordinates analysis (PCoA) on UniFrac distances to investigate similarities between control and trap-collected feces. The use of unweighted UniFrac distances documents the presence or absence of microbial OTUs but does not use relative abundances of microbial taxa, and thus we refer to this as "community membership." The use of weighted UniFrac distances takes relative abundance into account, and thus we refer to this as "community structure." Separation of samples based on individual animal, or between control and trap-collected feces was tested using the analysis of similarity (ANOSIM) function within QIIME with 999 permutations. Last, we used SourceTracker version 0.9.6 (Knights et al. 2011) to estimate the percentage of microbial OTUs in trap-collected feces that originated from the endogenous microbiota, cotton batting, apple slices, the bait mixture, or the trap wall.

All sequences were deposited in the Sequence Read Archive of NCBI under accession number PRJNA252885.

Results

Our sequencing effort resulted in an average of 63,396 ± 4,930 sequences per fecal sample. Sequencing effort did not differ between control and trap-collected feces (paired *t*-test: $P = 0.25$). These sequences were grouped into 23,274 OTUs. Sequencing effort for environmental samples was as follows: cotton (20,030 ± 1,460); peanut butter and oatmeal bait (1,060 ± 54); trap wall (22,991 ± 1,369); apple (8,677 ± 899).

We did not find any significant changes in the relative abundances of microbial phyla or genera between control and trap-collected feces. Statistics and relative abundances of bacterial phyla and genera can be found in Supporting Information S1. Similarly, we did not observe any significant differences in measurements of α-diversity (paired *t*-tests: $P > 0.78$ for Chao1, evenness, Shannon index, and Faith's phylogenetic diversity). In the PCoA plot estimating community membership, samples grouped strongly by individual animal (Fig. 1; ANOSIM: $P < 0.001$) but did not segregate between control and trap-collected samples ($P = 0.94$). We found similar results for community structure, where samples also grouped by individual animal (ANOSIM: $P < 0.001$), but not between control and trap-collected samples ($P = 0.81$).

SourceTracker revealed that trap-collected feces do not contain a large proportion of environmental contaminants. The endogenous microbiota constituted about four-fifths of the community of trap-collected feces (Fig. 2). Microbes found

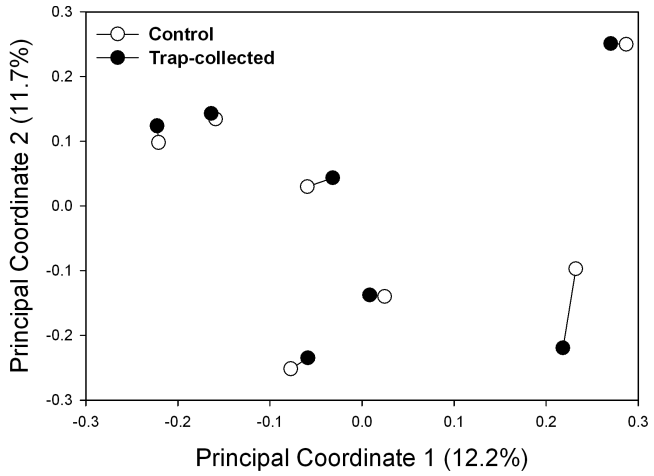

Fig. 1.—Principal coordinate analysis of control and trap-collected feces using unweighted UniFrac distances. Samples connected by lines were collected from the same individual.

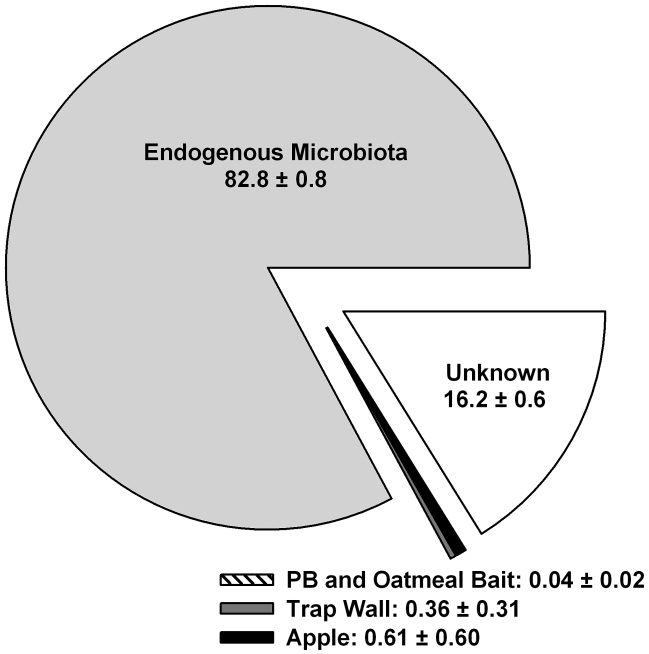

Fig. 2.—Sources of microbes in trap-collected feces.

on cotton were not detected in any of the trap-collected feces. Together, the remaining potential environmental sources (peanut butter and oatmeal bait, trap wall, apple) only comprised 1% of the microbes in trap-collected feces. Roughly one-sixth of the community of trap-collected feces was from unknown origin (Fig. 2).

Discussion

As microbiome research becomes more popular, fecal collection will likely be a regular practice when trapping small mammals. For such fecal samples to be used accurately, it must first be determined if they represent the endogenous microbiota of mammals and not environmental contaminants. Indeed, the microbial communities of control and trap-collected feces in our study exhibited remarkable similarities. These data suggest that trap-collected feces are acceptable for studying the microbiome of small mammals.

The microbial communities of control and trap-collected feces were strikingly similar. We were unable to detect any changes in microbial community membership, community structure, relative abundances of microbial taxa, or measurements of α-diversity. Within our studies of the woodrat microbiome, we have detected changes in microbial diversity as a result of captivity and dietary changes, often using smaller sample sizes than the current study (Kohl and Dearing 2012, 2014; Kohl et al. 2014a). Thus, we are fairly confident that the similarities presented in this study are real and not due to inabilities to detect changes.

We found that a large proportion of the microbes in trap-collected feces originate from the endogenous microbiota. This result is in contrast to opportunistic collection of feces from the wild, where sometimes less than 0.1% of microbes can be attributed to the mammalian gut microbiota, or 100% of microbes originate from soil (Delsuc et al. 2014). In our study, roughly 16% of microbes originated from an "unknown" source. These microbes may actually be in the "endogenous microbiota," but due to randomness in sequencing and detectability, they may not have overlapped between our 2 samples. However, these microbes may also be contamination from other sources, such as the air, skin of the animals, or urine. Regardless, these microbes did not largely impact the overall community structure of feces collected from traps.

Our results also help to clarify the loss of microbial diversity when animals are brought into captivity. In previous work from our group, feces collected from the contents of traps exhibited higher diversity than samples collected from the same animals in the laboratory (Kohl and Dearing 2014; Kohl et al. 2014a). The results from this study suggest that this higher diversity was not an artifact of environmental contamination within the traps. However, our study was conducted in the lab and thus it is unknown whether soil or air may contain higher loads of microbial contamination in nature. Animals likely lose microbial diversity as they enter the controlled environment of the lab. This loss of microbial diversity may have implications for captive breeding of endangered mammals (Redford et al. 2012).

Overall, our results suggest that feces collected from live-traps are representative of the microbiota of small mammals. However, there are a number of trapping practices that may confound the use of trap-collected feces in other studies. First, our traps are disinfected with bleach between trapping events in accordance with the American Society of Mammalogists' guidelines for handling rodents (Kelt and Hafner 2010) due to the fact that woodrats have the potential to transmit hantavirus (Dearing et al. 1998). The traps used in this study had last been used and sprayed with bleach 4 months prior to this experiment. It is unclear whether a trapping protocol that does not involve sterilizing traps after trapping events would be more likely to experience microbial contamination. Additionally, our study was conducted in the lab. It is unknown whether soil or air may contain higher loads of microbial contamination

in nature. Last, we predict that our results are likely only applicable to close-sided traps such as Sherman traps, and not necessarily mesh traps such as Tomahawk or pitfall traps, especially given the high incidence of soil contamination in opportunistic collection of samples (Delsuc et al. 2014). Future studies could investigate the acceptability of feces from these trapping protocols.

Supporting Information

The Supporting Information documents are linked to this manuscript and are available at Journal of Mammalogy online (jmammal.oxfordjournals.org). The materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supporting data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Supporting Information S1.— Relative abundances of bacterial phyla and genera in cage- and trap-collected feces.

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