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Promiscuity in Mice is Associated with Increased Vaginal Bacterial Diversity

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Abstract

Differences in the number of sexual partners (i.e., mating system) have the potential to exert a strong influence on the bacterial communities present in reproductive structures like the vagina. Because this structure serves as a conduit for gametes, bacteria present there may have a pronounced, direct effect on host reproductive success. As a first step towards the identification of the relationship between sexual behavior and potentially pathogenic bacterial communities inhabiting vital reproductive structures—as well as their potential effects on fitness, I sought to quantify differences in bacterial diversity in a promiscuous and monogamous mammal species. To accomplish this, I used 2 sympatric species of *Peromyscus* rodents—*P. californicus* and *P. maniculatus* that differ with regard to numbers of sexual partners per individual to test the hypothesis that bacterial diversity should be greater in the promiscuous *P. maniculatus* relative to the monogamous *P. californicus*. As predicted, phylogenetically controlled and operational taxonomic unit-based indices of bacterial diversity indicated that diversity is greater in the promiscuous species. These results provide important new insights into the effects of mating system on bacterial diversity in free-living vertebrates, and may suggest a potential cost of promiscuity.

Introduction

Infection by parasites and pathogens represents a strong and ubiquitous selective pressure, as animals suffering from such infections often have reduced survival and fitness (Fuller & Blaustein 1996; Vandegrift et al. 2008; Schwanz 2008). Sexual contact is a particularly efficient mechanism for the transmission of some pathogens (Padian et al. 1997; Potterat et al. 1999). Because sex involves inter-individual physical contact of mucosal tissues that are highly optimized for the transfer of biological materials (gametes), this event represents a time in which other materials (e.g., microbes) may also be transferred. Furthermore, because sex represents a type of contact that is assured to happen in a large proportion of individuals in sexually reproducing populations, microbes capable of taking advantage of this opportunity for transfer are likely to experience higher fitness than other less-easily transmitted organisms.

Because sexual contact is frequent and by definition involves reproductive structures commonly serving as reservoirs of transmissible bacteria, sexual transmission of pathogens is common amongst sexually reproducing animals (Gray et al. 2001; Wawer et al. 2005; Padian et al. 1997). Given that patterns of transmission are related to the frequency (Gray et al. 2001; Wawer et al. 2005) and duration of contact (Kraut-Becher & Aral 2003), variation in sexual behavior between individuals and species (i.e., differences in mating systems) may

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influence the sexual transmission of bacteria (Mitsunaga et al. 2005; Poiani & Gwozdz 2002), and thus bacterial communities of relevant reproductive structures, leading to significant inter-specific and inter-individual differences in microbiota of specific anatomical structures. A considerable amount of research, at least in humans, has been devoted to understanding how patterns of sexual contact can modify the risk of pathogen acquisition. For instance, Gregson and others found that increasing the number of sexual partners (=increased promiscuity) increases the risk of HIV infection in African teenagers (Gregson et al. 2002). In another study of more than 600 men and women, Manhart and colleagues found that an increase in the number of concurrent sexual partners (=increased promiscuity) was associated with an increased risk for contraction of a sexually transmitted pathogen (Manhart et al. 2002). In contrast to the amount of research done in this single species, there has been surprisingly little research done regarding how differences in sexual behavior (i.e., number of partners and patterns of concurrency) affect bacterial (pathogenic and non-pathogenic) community composition in non-model taxa (but see (Poiani & Gwozdz 2002) and (Webberley et al. 2002)). This is important, as existing data, from a limited number of species, already suggest that large differences in microbiota, may exist (Rivera, Frank, et al. 2010b; Spear et al. 2010).

Novel methods for the sampling and identification of bacteria (i.e., metagenomics (Hugenholtz & Tyson 2008)) have revolutionized our ability to study bacterial communities of both randomly selected environments and host organisms (Kvennefors et al. 2010; Green & Barnes 2010). Because these methods rely on molecular methods such as PCR (Weisburg et al. 1991; Yarza et al. 2008; Coolen et al. 2005), and therefore eliminate certain biases related to bacterial growth (e.g., some bacteria are unculturable, or only grow in very restrictive conditions (Hattori & Taylor 2009; Handelsman 2004)), we now have a much better understanding of how bacterial diversity, spatiotemporal dynamics, and community structure are related to complex host phenotypes. However, although molecular methods have undoubtedly advanced the field of community microbiology, other biases may be introduced, including those associated with PCR and cloning (Pontes et al. 2007; Hill et al. 2010). Newer studies using PCR-based methods have revealed extremely complex microbial communities, some with clear associations with health-related conditions like obesity (Turnbaugh et al. 2009), cigarette use (Erb-Downward et al. 2011), and oral hygiene (Paster et al. 2001). Other studies have linked sexual behavior in humans (De et al. 2004; Manhart et al. 2002) and other vertebrate taxa (Poiani & Gwozdz 2002) to differences in bacterial communities of the lower reproductive tract. Specifically, microbial communities, including obligate pathogens (e.g., *Chlamydia spp.*) and opportunistic pathogens (e.g., many *Pseudomonas spp.*) have been described in the lower reproductive tract of females of multiple species including humans (Kim et al. 2009; Witkin et al. 2007; Brown et al. 2007), red-winged blackbirds (Hupton et al. 2003), and the koala (Weigler et al. 1988). Although explicit tests of the host fitness consequences of these infections are generally lacking, given that bacterial infections have been shown to be able to influence host phenotype (Brook 2005; Servin 2004), we might expect to see fitness effects associated with the bacterial colonization of reproductive structures.

Mice of the genus *Peromyscus* provide an ideal opportunity to explore the effects of mating system on vaginal bacterial diversity. Because these animals have long been targets of study, many aspects of their sexual behavior are known. The genus includes examples of promiscuity (Birdsall & Nash 1973; Ribble & Millar 1996), polygyny (Shurtliff et al. 2005), and monogamy (Gibernick & Teferi 2000; Ribble 1992). *Peromyscus californicus*, which exhibits lifetime social (=lifelong monogamous pair bond) and genetic (=offspring belong to social partner) monogamy (Gibernick & Teferi 2000; Ribble 1992), is sympatric across its range with the socially and genetically promiscuous *Peromyscus maniculatus* (Birdsall & Nash 1973; Ribble & Millar 1996). This pronounced difference in reproductive behavior

among otherwise similar congeners offers a rare opportunity to explore the effects of differences in mating system – specifically, the contrast between monogamy and promiscuity – on vaginal bacterial diversity.

To characterize the relationship between mating system and vaginal bacterial diversity, I compared bacterial communities in the monogamous *P. californicus* and the promiscuous *P. maniculatus*. If promiscuity increases exposure to sexually transmitted bacteria, then *P. maniculatus* should show increased bacterial diversity relative to *P. californicus*. I tested this prediction using data obtained from sympatric populations of these species from central coastal California. Aside from sexual behavior, multiple other factors are likely involved in the regulation of host bacterial communities; ecology (Zhou et al. 2007) and evolutionary history (Rivera, Frank, et al. 2010b) may be amongst the strongest, and are effectively controlled using this study design.

This study is the first to describe the relationship between mating system and vaginal bacterial diversity using a comparative approach and mice captured in a single location—effectively controlling for differences in environmental exposure to bacteria. The results provide new insights into the potential effects of mating system on bacterial diversity in free-living vertebrates, which, as a result, may have important effects on host fitness.

Materials and Methods

Study populations and tissue sampling

Field research was conducted on Landels-Hill Big Creek Reserve (36.011156°, –121.518644°), located 40 kilometers south of Big Sur, Monterey County, California, USA. Big Creek Reserve is a part of the University of California Natural Reserve System, and consists of 15.57 km² of coastal scrub, redwood forest, and oak woodlands. This locality was selected because both *P. maniculatus* and *P. californicus* are abundant and can be captured in the same habitat using a single trapping grid.

Fieldwork was conducted in 2009 during the months of May and June. Live trapping was conducted using a single rectangular 100m × 100m trapping grid comprised of 120 standard aluminum small Sherman traps (H.B. Sherman Trap Company). Traps were baited with an apple-oat mixture, opened at dusk, and checked every 4 hours until dawn, at which time they were closed until the subsequent evening.

To facilitate handling, all animals captured were lightly anesthetized with isoflurane. Sterile scissors were used to remove a 3mm² section of the distal tip of the right ear pinna for genetic species identification. Tissue was stored in 70% ethanol and frozen at –20° C within 2 hours of collection. For adult females, reproductive condition was assessed via visual inspection of the external genitalia; when a female was found to be sexually active (=perforate vagina), a sample of vaginal bacteria was collected from that individual. To do this, using sterile procedure, I inserted a sterile, small cotton-tipped swab (Medical Wire & Equipment Co. LTD) into the vagina to approximately 3mm depth, then removed the tip and placed it into a sterile 1.5ml microcentrifuge tube. A negative control swab was also obtained during a single rodent trapping event. This negative control was treated identically to the sample swabs, except that it did not come into contact with the host species. Swabs were stored dry, and frozen at –20° C, within 2 hours of collection. Lastly, each animal was individually marked using a small (Monel 1005, National Band & Tag Company) numbered ear tag placed in the right ear. After handling, animals were allowed to recover from anesthesia (typically < 5 min) and were then released at the site of capture. All procedures were approved by the University of California, Berkeley Animal Care and Use Committee

(Protocol Number R224-0309) and were in accord with the guidelines of the American Society of Mammalogists (Sikes & Gannon 2011).

Bacterial DNA extraction, 16S rRNA PCR, cloning, sequencing

I extracted bacterial DNA from swabs from 10 randomly selected females per species and negative control swab under sterile conditions following a protocol modified from protocols contained within (Green & Barnes 2010; Coolen et al. 2005). In brief, swabs were immersed in 200 μ L sterile extraction buffer, after which several sterile small glass beads (2mm diameter) were added to enhance mechanical disruption of the bacterial cell wall. The collection tube was then heated to 95° C for 10 minutes, with vortexing every 2 minutes. The resultant extract was then subjected to enzymatic digestion of bacterial cell walls with a mixture of lysozyme (20mg/ml) and lysostaphin (10mg/ml) for 30 minutes at 37°C. To concentrate and purify the bacterial DNA, a Qiagen DNeasy Blood and Tissue DNA Extraction kit (Qiagen # 69504) was used as a final step to DNA extraction. Final DNA concentration was measured on the Nanodrop system (Thermo Scientific), and ranged from 10-40ng/uL.

Polymerase chain reaction amplification of the bacterial 16S rRNA gene from each sample (n=10 per species, negative control) was then carried out using undiluted DNA extract. The primer pair E321 and U1177 were chosen because together, they were shown *in silico* to amplify a part of the 16S rRNA gene of a large proportion of all known bacterial species (Wang & Qian 2009). The resultant PCR product was subjected to gel electrophoresis using a 1% agarose gel and visualized using ethidium bromide. Amplification was considered successful if a well-resolved band of the expected size (~700bp) was observed.

Because the resultant PCR product contained PCR amplicons from all bacteria collected from the vagina of a specific animal, successful PCR were cloned to separate different bacterial phylotypes. Cloning was completed using a TOPO TA Cloning Kit for Sequencing (Invitrogen, K4575-01) following the manufacturer's instructions. For each sample (n=10 per species and negative control), 48 positive clones were randomly selected for PCR amplification, using the vector-specific primers M13R and M13F and conditions specified by the manufacturer. The resultant PCR product was then subjected to gel electrophoresis to confirm the correct insert size.

Successfully cloned PCR products were cleaned using ExoSap and cycle sequenced using ABI BigDye Terminator v3.1 Cycle Sequencing Kits (ABI #4337456). Sequencing in both forward and reverse directions was carried out on an ABI3730 automated DNA sequencer. Sequences were edited and assembled using the program Geneious version 5.41 (Drummond et al. 2010). This process included the removal of primer sequences, base pairs corresponding to the cloning vector, and bases below a specific quality threshold (PHRED < 20). Sequences identified as bacterial in origin using the BLAST algorithm (Altschul et al. 1990; Camacho et al. 2009) as implemented in NCBI Genbank (Benson et al. 2007) (<http://www.ncbi.nlm.nih.gov/genbank/>) were exported in a standard FASTA format. Any sequence recovered in both the negative control and vaginal swabs were removed from the dataset as likely contaminants. All unique non-contaminate sequences have been deposited to Genbank (Accession Numbers JN653750-JN654219, release upon publication).

Bacterial 16S gene tree construction

Phylogenetic analysis of all recovered 16S rRNA gene sequences was completed using RAxML version 7.2.6 (Stamatakis 2006) using the GTRGAMMA model of molecular evolution. Tree topology was assessed using 1000 bootstrap replicates. Bootstrapping analyses were conducted on one of the TeraGrid supercomputing system resources (Ranger)

located at the Texas Advanced Computing Center (TACC) at the University of Texas at Austin (<http://www.tacc.utexas.edu/>). The tree was rooted using mid-point rooting. Branches were labeled according to their host-species of origin. When a specific phylotype was identified in both host-species, it was assigned to the host in which that phylotype was more common based on simple counting of sequences.

Analysis of Bacterial Diversity

The 16S rRNA gene sequences recovered from each of the 20 females (n=10 per species) were grouped for analyses at the species level. Sequences were then aligned against the SILVA database (Pruesse et al. 2007) using the NAST algorithm (DeSantis et al. 2006) within the program mothur (Schloss et al. 2009). Alignments were checked for chimeric sequences using Bellerophon (Huber et al. 2004), and when detected, such sequences were removed from the dataset. I generated a matrix of uncorrected pairwise distances between all remaining non-chimeric aligned sequences. Sequences were then clustered into operational taxonomic units (OTU's) based on a 90%, 95%, 97% and 99% sequence similarity cutoff.

To evaluate sampling depth, I generated rarefaction curves using a re-sampling without replacement approach and a 95% and 97% sequence similarity cutoff for each host species (n=2, promiscuous, *P. maniculatus*, monogamous, *P. californicus*) as well as on the combined dataset. Lastly, I identified bacterial sequences from each host species at the phylum and order levels using a Bayesian method and 60% bootstrap value cutoff. To evaluate bacterial diversity, I calculated both Simpson's (Simpson 1949) and Shannon's (Shannon 1948) indices of diversity within the program mothur. However, because differences in diversity may be the product of differences in either richness or evenness ($\approx$ abundance), these were calculated as well. Species richness was estimated using Chao1 (Chao 1984), jackknife (Burnham & Overton 1979), ACE (Chao 1992; 1993) and bootstrap (E Smith & VanBelle 1984) methods. Evenness was estimated using the formula $E=e^{D/N}$, where D=diversity index, and N=number of sequences in the sample (Bik et al. 2010). To assess differences in community diversity and structure, I calculated weighted and unweighted uniFrac scores (Lozupone & Knight 2005). I compared community composition using three complementary, yet independent tests. Analysis of molecular variance (AMOVA) (Martin 2002) compares whether two communities have different centroids (*sensu* (Schloss 2008)), while the homogeneity of molecular variance (HOMOVA) method (Martin 2002; Schloss 2008) tests the hypothesis that genetic diversity is the same in both communities. To better understand how additional sequence data would add to the number of phylotypes uncovered, I calculated Good's coverage estimator (Good 1953). Lastly the phylodiversity test (Faith 1992), which determines if libraries have differences in branch lengths, was calculated.

Host species identification

The study species were easily distinguishable in the field based external morphology but, because genetic analyses were critically dependent on accurate taxonomic identification, species identity was confirmed for all animals sampled by sequencing an 850bp segment of cytochrome B. To accomplish this, I extracted genomic DNA from vertebrate tissue samples using a salt extraction method (Miller et al. 1988); the resultant extract was tested for purity and concentration using the Nanodrop system (Thermo Scientific). From this DNA stock solution, 50ng/ μ L dilutions were prepared.

The cytochrome B gene was PCR amplified using a set of primers known to work in a variety of rodents (MF Smith & Patton 1993). Successful PCR reactions were sequenced in both forward and reverse directions as described above using internal primers. Sequences

were identified to species using the BLAST algorithm (Camacho et al. 2009; Altschul et al. 1990) implemented on NCBI Genbank.

Results

The bacterial flora from the vaginas of 10 sexually active adult females per species was characterized. This work yielded 423 partial 16S rRNA gene sequences from *P. maniculatus*, and 479 sequences from *P. californicus*. After removal of environmental contaminants, low quality sequences, those not matching known bacterial sequences and putative chimeric sequences, the resultant data set contained 322 and 345 sequences from *P. maniculatus* and *P. californicus* respectively. A rarefaction analysis indicated that increasing the depth of coverage would potentially result in the addition of novel operational taxonomic units (95% and 97% similarity) (Figure 1), however, because the rate of increase (=slope of the curve) was similar between monogamous and promiscuous groups, increasing the depth of sampling should not alter the relationships presented here. Additionally, using the boneh estimator of unseen species (Boneh et al. 1998) at the 98% sequence similarity level, with an additional 500 sequences I would have uncovered an estimated 13.7 new phylotypes in the monogamous species, and 13.8 in the promiscuous species. Good's coverage estimator indicated that at the species level (98% sequence similarity, I sampled approximately 84.5% of the vaginal bacterial community in the monogamous *P. californicus* and 80.1% in the promiscuous *P. maniculatus*.

Host species identification

The identities of the 10 individuals per species included in this study were identified to species based on field methods and confirmed via sequencing of the *cytB* gene. In no instance did the identification based on sequencing differ from those made in the field.

Analysis of Bacterial Diversity

The resultant gene tree containing all unique (n=469) sequences from both species is presented in Figure 2. The vaginal bacteria community of the two *Peromyscus* species contained three phyla of bacteria—the Firmicutes, the Proteobacteria, and the Actinobacteria (Figure 3). Within these phyla, 8 orders of bacteria were present between the two species. When looking at orders that comprised >1% of the total communities of each species, 7 were recovered from both groups with the Xanthomonadales present only in the promiscuous *P. maniculatus* and the Legionellales unique to the monogamous *P. californicus* (Figure 4). Additionally, there was an increase in number of sequences assigned to Lactobacillales, and Clostridiales in the promiscuous species (Figure 4) relative to the monogamous, while the Actinomycetales and Bacillales were more common in the monogamous species.

The two measures of bacterial diversity (Figure 5A-B) both indicated enhanced diversity in the promiscuous species. Because differences in diversity may be the product of differences in either richness or evenness, these were calculated as well. While richness (Figure 5C-F) was greater across all biologically meaningful levels of sequence similarity in the promiscuous *P. maniculatus*, evenness (Figure 5G) showed a more complicated pattern, with higher evenness in the promiscuous *P. maniculatus*, but only when analyzing more similar, potentially biologically relevant, groups. Finally, an index of phylodiversity was calculated, and found to be greater in promiscuous species (monogamous=3.5739, promiscuous=6.0443).

Differences in bacterial community structure between the two host-species was assessed using both weighted and unweighted uniFrac analyses. Both tests yielded highly significant

results (weighted, $D=0.369631$, $p<.001$, unweighted $D=0.790383$, $p<.001$) indicating that community phylogenetic structure was different. Additionally, AMOVA and HOMOVA tests yielded significant differences (AMOVA, $F_s=97.7931$, $p<.001$, HOMOVA $B=48.8487$, $p<.001$) in sequence diversity.

Taken together, these analyses suggest that at all levels of sequence similarity; there is increased bacterial community diversity in the vagina of the promiscuous *P. maniculatus* relative to its closely related congener, the monogamous *P. californicus*.

Discussion

My results provide convincing evidence that bacterial communities differ between the monogamous *P. californicus* and the promiscuous *P. maniculatus*. Specifically, multiple analyses indicate bacterial diversity, evenness, and richness is greater in the promiscuous taxon. This finding was supported by an increase in phylogenetic diversity in promiscuous mice, as well as significant differences in centroid location and phylogenetic structure.

Both Good's coverage estimator and the boneh estimator of unseen species indicate that with the acquisition of additional sequence data, additional phylotypes would be revealed. Specifically, Good's estimator suggests that approximately 15% more species would be uncovered in the monogamous taxa, and 20% more in the promiscuous. Similarly, the boneh estimator indicates that 13.7 and 13.8 (monogamous and promiscuous respectively) additional phylotypes would be recovered with additional sequencing. While both estimates suggest sampling is incomplete, both also suggest that the patterns reported here would be further exaggerated with additional sequencing, and thus would not change the conclusions presented here.

Whether increased bacterial diversity indicates a greater potential for the development of a transmissible disease state is debated. Indeed, the opposite of diversity—i.e. monoculture, has typically been associated with several types of disease states including *Clostridium difficile* infection of the lower intestinal tract (Kelly et al. 1994), and *Helicobacter pylori* infection of the stomach (Suerbaum & Michetti 2002). Furthermore, a more diverse bacterial community may confer significant benefits to its host by reducing the opportunity for invasion by more pathogenic bacteria (Flanagan et al. 2007; Dethlefsen et al. 2008). More recently however, it has been suggested that the potential for infection of host tissues by pathogenic bacteria may be enhanced in highly diverse bacterial communities. Because bacteria are either pathogenic, potentially pathogenic (i.e., opportunistic), or non-pathogenic, increasing diversity likely increases the numbers of pathogens and potential pathogens in a given bacterial community (Dionne et al. 2007). Bacterial vaginosis for example, is a disease state where bacterial community diversity is increased over non-diseased states (Fredricks et al. 2005). Importantly, no known bacterial infection is associated with a reduction (but not elimination), of bacterial diversity in a given anatomical structure. In my study, no individual in either species exhibited a monoculture. Both species exhibited a highly diverse community, with the promiscuous species being more diverse. I argue that this may be a direct result of differences in mating behavior, with the promiscuous species likely mating with many partners, while the monogamous *P. californicus* mates with only one. Whether increased diversity results in fitness effects either related to disease or differential mating opportunity or success is unknown.

Aside from sexual behavior, several other factors have been shown to influence vaginal bacterial community structure. For instance in humans, community structure has been shown to vary with cultural or dietary practices (Zhou et al. 2007) and hygiene (Myer et al. 2005). Additionally, differences in vaginal bacterial communities may be related to evolutionary

and or historical demographic processes (Rivera, Stumpf, et al. 2010a). Although I have attempted to control for many of these factors by using closely related, ecologically similar, sympatric species, the variation in vaginal bacterial community structure may be related to another uncontrolled variable.

In addition to the biology and evolutionary history of *Peromyscus*, the distribution of mammalian monogamy limits our ability to perform a phylogenetically controlled multi-species examination of the relationship between sexual behavior and vaginal bacterial communities. Within Mammalia, monogamy is thought to occur in less than 5% of all species (Kleiman 1977), and in the genus *Peromyscus* only two examples are known (*P. polionotus* and *P. californicus*) (Foltz 1981; Ribble 1991). The range of *P. polionotus* does not overlap that of *P. californicus*, occurring exclusively in the Southeastern United States, and therefore, controlling for environmental exposure would not have been possible. Although lab studies using mice of the genus *Peromyscus* are possible, it is well known that important behavioral parameters (e.g. mating behavior) are likely to vary between wild and captive animals (Calisi & Bentley 2009), which in turn may alter the very relationship under study (Wienemann et al. 2011).

An examination of vaginal bacterial community structure has been conducted in several species including humans (Ravel et al. 2011; Jin et al. 2007; Kim et al. 2009), Rhesus macaques (Spear et al. 2010), and Criollo Limonero cows (Zambrano-Nava et al. 2010). These studies illustrate the differences in bacterial species composition across a functionally and anatomically similar structure. For instance, the human vagina is typically dominated by *Lactobacillus* species—while in the vagina of both captive and wild-caught Rhesus macaques and in cows it is essentially absent. In both *Peromyscus* species included in this study, the common commensal *Lactobacillus* species are present, but differ in frequency, being more prevalent in the promiscuous *P. maniculatus*. Armed with an understanding of how ecology and behavior affect bacterial communities of reproductive structures, future studies should attempt to link differences in microbiota to differences in host fitness. Indeed, although linking phenotype to fitness has been one a major focus of study within evolutionary biology, how bacterial community structure impacts host fitness is completely unknown. This study attempts to serve as the 1st step in linking host bacterial community structure to fitness, by demonstrating differences over an ecologically relevant phenotype (mating behavior). Future studies of these relationships, perhaps using a set of tightly controlled laboratory experiments that complement field-based studies of wild animals, will likely add yet another layer of complexity to our understanding of phenotype-fitness interactions.

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Figure 1.

Rarefaction analysis of the clone libraries from both host species at the 95% and 97% sequence similarity cutoff. PECA indicates the monogamous *P. californicus* while PEMA indicates the promiscuous *P. maniculatus*.

Figure 2.

Phylogeny of the 16S rRNA sequences used in this study. Red branches correspond to the bacterial sequences recovered from the monogamous *P. californicus*, while the black branches related to the promiscuous *P. maniculatus*. Phylotypes recovered in both host-species were relegated to the host in which they were more common.

Figure 3.

Bacterial phylotypes recovered from each species were assigned to phyla using a Bayesian method and a 60% posterior probability. Together, the Firmicutes, Proteobacteria, and Actinobacteria are recovered from the vaginas of the mice included in this study.

Figure 4.

Bacterial phylotypes recovered from each species were assigned to order using a Bayesian method and a 60% posterior probability. Eight orders are recovered, 7 from each host species.

Figure 5.

Plots of various indices of diversity, richness, and evenness. Y-axis refers to the value of the specific index. The X-axis, “seq-sim” indicates the sequence similarity cutoff used. Figures 5A-B plot bacterial diversity using 2 different indices, Shannon’s (H') and Simpson’s (D) diversity indices. Figure 5C-F plot bacterial richness. Figure 5G plots community evenness using Shannon’s evenness index E.