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Wild-caught rodents retain a majority of their natural gut microbiota upon entrance into captivity

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Summary

Experiments conducted on captive animals allow scientists to control many variables; however, these settings are highly unnatural. Previous research has documented a large difference in microbial communities between wild animals and captive-bred individuals. However, wild-caught animals brought into captivity might retain their natural microbiota and thus provide a better study system in which to investigate the ecology of the gut microbiome. We collected individuals of the desert woodrat (*Neotoma lepida*) from nature and investigated changes in the microbial community over 6 months in captivity. Additionally, we inventoried potential environmental sources of microbes (food, bedding) from the wild and captivity. We found that environmental sources do not make large contributions to the woodrat gut microbial community. We documented a slight decrease in several biodiversity metrics over 6 months in captivity, yet the magnitude of change was small compared with other studies. Wild and captive animals shared 64% of their microbial species, almost twice that observed in other studies of wild and captive-bred individuals (≤ 37% shared). We conclude that wild-caught animals brought into captivity retain a substantial proportion of their natural microbiota and represent an acceptable system in which to study the gut microbiome.

Introduction

The gut microbiome and its role in the ecology and evolution of animals is a burgeoning area of interest (McFall-Ngai *et al*., 2013). For ease of study, most comparative and experimental studies regarding the microbiota house animals in captive settings (Ley *et al*., 2008; Kohl and Dearing, 2012). However, it is possible that captivity may alter the microbiota. Indeed, many studies have compared wild and captive individuals and found significant differences in microbial community composition (Uenishi *et al*., 2007; Ley *et al*., 2008; Scupham *et al*., 2008; Villers *et al*., 2008; Xenoulis *et al*., 2010; Wienemann *et al*., 2011; Nelson *et al*., 2013). However, all these studies compared animals born in captivity with animals born in the wild, with some individuals living on different continents. Thus, these studies cannot exclude the possibility that differences were the result of unique microbial sources. Only a single study, conducted on Atlantic cod, documented a decrease in microbial diversity as animals entered captivity (Dhanasiri *et al*., 2011). Such as study has not been conducted on a wild tetrapod species.

The desert woodrat, *Neotoma lepida*, is a model system to study interactions between dietary plant toxins and the gut microbiota. We previously demonstrated that plant toxins significantly alter microbial community structure and diversity of woodrats in captivity (Kohl and Dearing, 2012). However, the effect of captivity on these microbial communities was not studied. To address this deficiency in our understanding of the system, we conducted a study to examine the effects of captivity. Several *N. lepida* were collected from the Mojave desert in an area dominated by creosote bush (*Larrea tridentata*), black brush (*Coleogyne ramosissima*) and rabbit brush (*Chrysothamnus paniculatus*). Because woodrats are herbivorous and known to feed primarily on creosote bush (Karasov, 1989), these plants may serve as environmental sources of microbes. In captivity, woodrats were fed commercial rabbit chow (Harlan Teklad 2031) and kept in plastic cages with wood shavings as bedding material. While our study does not isolate the effects of captivity and dietary changes, the use of commercial diets is common, and so this design is of more relevance to comparative biologists.

We investigated environmental sources of microbes in wild and captive settings by inventorying the microbial communities from three dominant plant species in the wild, commercial rabbit chow and wood shavings, and then comparing these with woodrat fecal microbial communities. Additionally, we monitored changes in microbial diversity by inventorying the fecal microbial communities of four *N. lepida* in the wild and over three time points in captivity (2 weeks, 3 months and 6 months).

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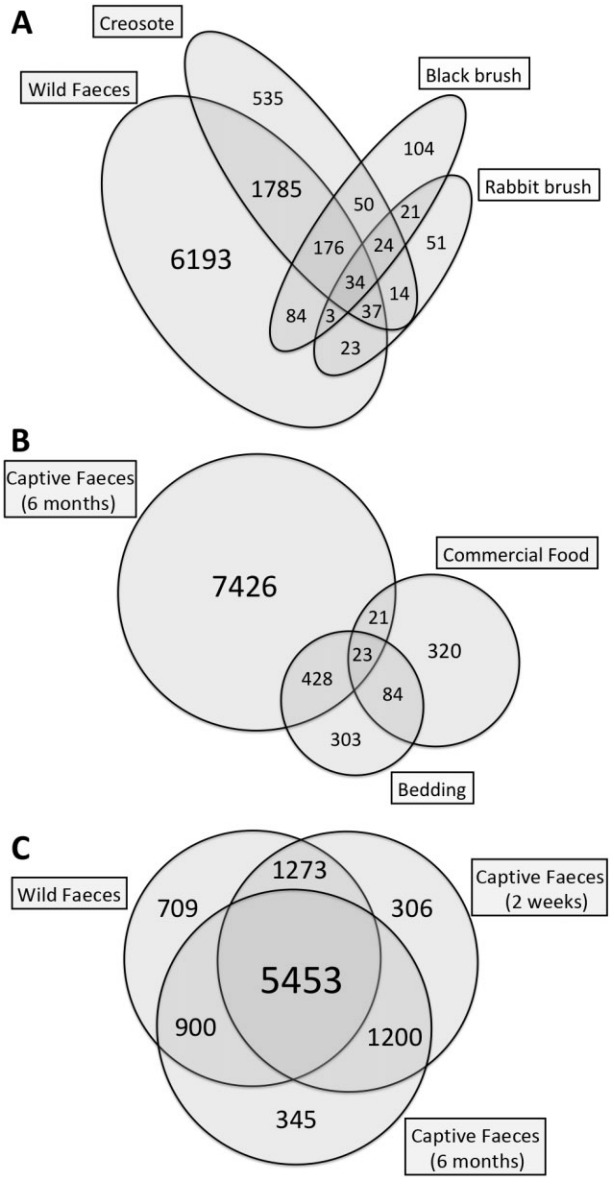

Fig. 1. Shared microbial OTUs between (A) faeces collected from the wild and the surfaces of three dominant plants from where animals were collected; (B) faeces collected after 6 months in captivity and commercial food and bedding; and (C) faeces collected from the wild and after 2 weeks and 6 months in captivity.

Results and discussion

Microbial inventories were conducted by isolating microbial DNA from plant surfaces, food, bedding and faeces, and amplifying and sequencing the 16S rRNA gene on an Illumina MiSeq platform (Caporaso *et al.*, 2012). Over 2 million microbial sequences were produced, which were classified into operational taxonomic units (OTUs) based on 97% sequence identity using QIIME (Quantitative Insights Into Microbial Ecology; Caporaso *et al.*, 2010). Sequences were deposited in the National Center for Biotechnology Information's Sequence Read Archive under accession SRP029350. Details regarding animal collection, sequencing and data analysis can be found in the Supplementary Material.

The gut microbial communities of woodrats in nature were not solely determined by environmental sources. Only ~25% of OTUs detected in the faeces of wild woodrats were also detected on leaf surfaces (Fig. 1A). The largest proportion of these microbes were also present on creosote bush, which is the predominant species consumed by *N. lepida*. After 6 months in captivity, there was minimal inoculation by new environmental microbes. Only 6% of OTUs detected in the faeces of captive woodrats were also detected on commercial food and bedding (Fig. 1B). These results are consistent with a number of studies suggesting that diet and other environmental sources are not the main determinants of the gut microbiome. As examples, less than 1% of the gut microbes found in the Burmese python gut are derived from a rodent meal (Costello *et al.*, 2010), and roughly 3% of seal gut microbes are acquired from sea water (Nelson *et al.*, 2013).

We also compared microbial OTUs between faeces collected in the wild and along three time points up to 6 months in captivity. Woodrats lost 19% of their natural microbes after 2 weeks in captivity (1609 of 8335) and 24% after 6 months (1982 of 8335; Fig. 1C). This effect may have been due to the removal of the natural diet of creosote bush. However, of this proportion of microbes lost in captivity, only a quarter of them were also detected on environmental sources (wild plants). Thus, these lost microbes might represent transient microbes that originate from other environmental sources in the wild that we did not inventory (other plants, soil, etc.) or microbes that were lost because of changes in host physiology in captivity. After 6 months in captivity, woodrats harboured 1545 new OTUs that were not detected in the wild, although only 38 of these were detected on commercial food or bedding. These newly acquired microbes may have come from other sources that we did not inventory (researchers, water, etc.) or they may have been resident in the wild microbiota, and only increased to detectable levels after 6 months in captivity.

Overall, 68% of microbial OTUs were present both in fecal samples from the wild and after 2 weeks in captivity (6726 of 9841), and decreased to 64% after 6 months in captivity (6353 of 9880; Fig. 1C). This overlap is much greater than that observed in previous studies comparing animals born in captivity to animals born in the wild. Both captive leopard seals and parrots only share 4% of OTUs with their wild counterparts (Xenoulis *et al.*, 2010; Nelson *et al.*, 2013). Likewise, wild and captive turkeys only share 37% of their microbes (Scupham *et al.*, 2008). Our study demonstrates that wild-caught animals maintain the

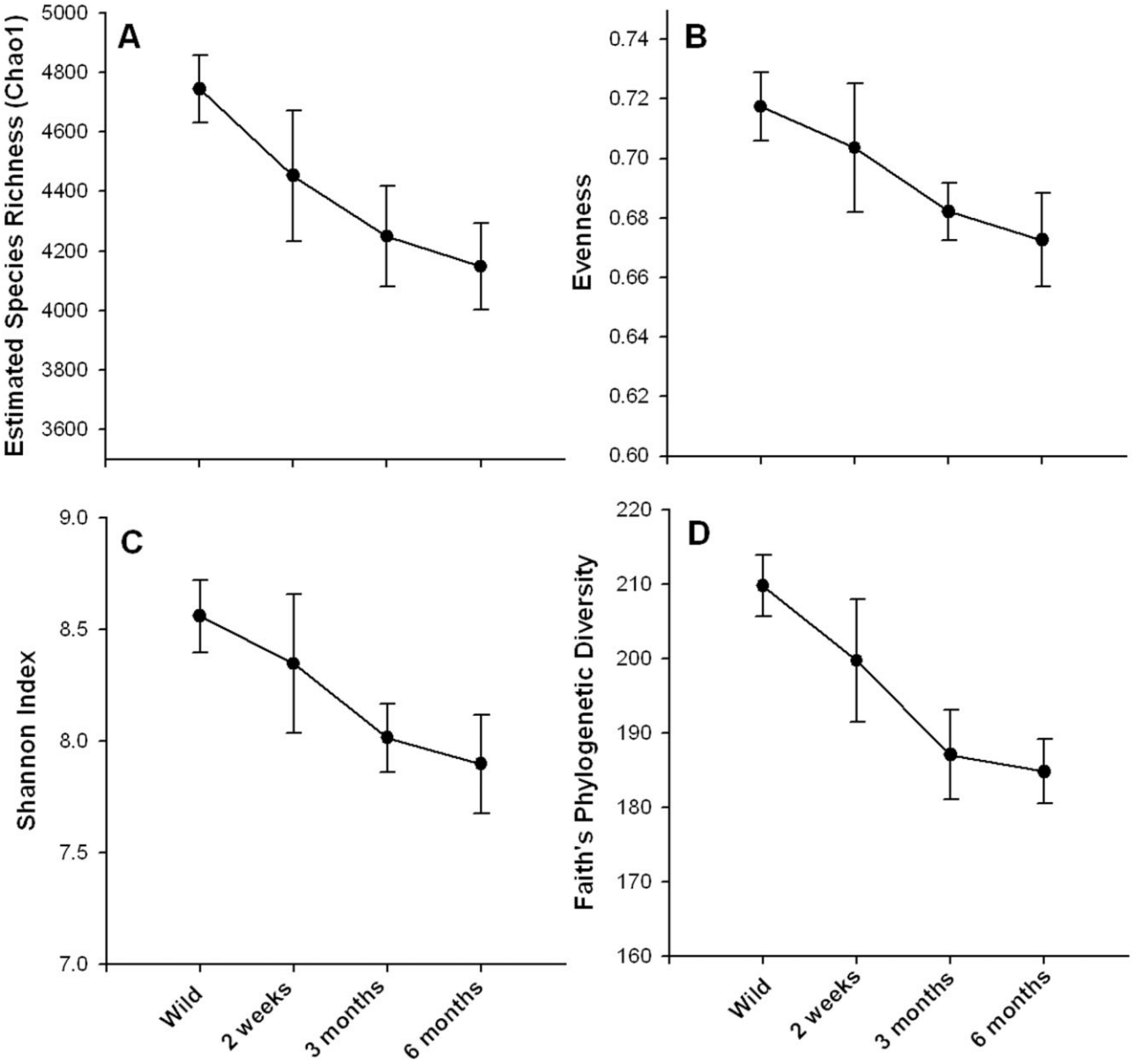

Fig. 2. Measurements of alpha diversity over time in captivity. (A) Estimated species richness (Chao1); (B) Evenness; (C) Shannon Index; (D) Faith's phylogenetic diversity.

majority of their native microbiota after being in captivity for a substantial period of time.

The gut microbial community exhibited a minimal change in biodiversity as animals entered captivity. We monitored changes in several metrics of biodiversity, such as estimated species richness, evenness, Shannon index and Faith's phylogenetic diversity index (Fig. 2). Across all biodiversity measures, only evenness decreased significantly over time in captivity (repeated measures analysis of variance: $P = 0.03$; Fig. 2B). This loss in biodiversity was small relative to previously documented changes. For example, the addition of the plant toxins of creosote bush to the diet can alter microbial diversity by 20–30% in *N. lepida* (Kohl and Dearing, 2012). For comparison, 6 months in captivity woodrats resulted in only a 6–12% decrease in biodiversity depending on the metric.

We also investigated how captivity altered overall community membership (the presence and absence of certain microbes) and community structure (their relative abundances). We conducted principal coordinates analysis of unweighted or weighted UniFrac data to investigate changes in community membership and structure, respectively, and compared clustering based on either individual animal or time in captivity using the adonis

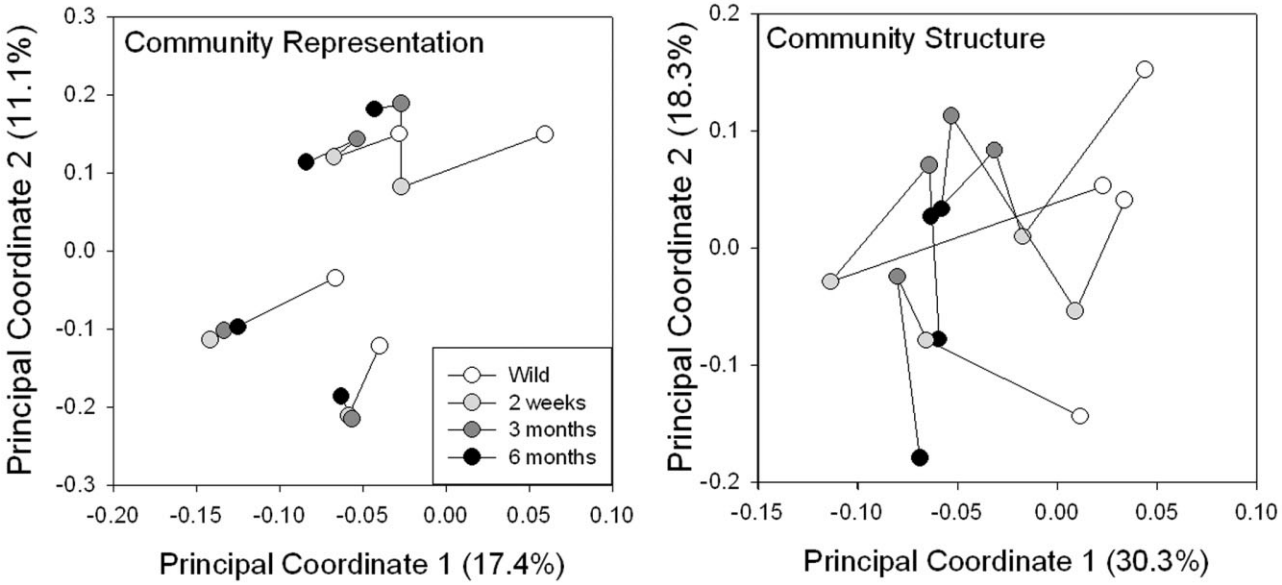

Fig. 3. The effect of captivity on (A) micriobial community representation, and (B) microbial community structure. Community representation uses unweighted UniFrac distances, while structure uses weighted distances. Lines connect an individual animal over time.

function within QIIME. Microbial community memberships clustered by individual animal ($P = 0.006$; Fig. 3A) but not by length of time in captivity ($P = 0.3$). In contrast, community structures clustered significantly according to time in captivity ($P = 0.008$; Fig. 3B) but not by individual animal ($P = 0.2$). These data suggest that as animals enter captivity, they retained the unique microbial community harboured in the wild, while the relative abundances of the communities shifted over time. These changes in community structures are most likely driven by the change in diet that woodrats experience upon entering captivity (Turnbaugh *et al*., 2009). Also, captivity is known to affect many physiological traits that might influence microbial community structure, such as immune function (Martin *et al*., 2011), stress physiology (Dickens *et al*., 2009) and gut anatomy (Millan *et al*., 2001).

The shifts in community structure were driven by changes in the relative abundances of certain microbial taxa. We conducted paired *t*-tests on the abundances of microbial taxonomic groups between faeces collected in the wild and after 6 months in captivity, and corrected *P*-values using the false discovery rate control. While there were no significant changes in the abundances of any identified microbial taxa, there were several near-significant trends. There was a decrease, although not significant, in the abundance of the phylum *Tenericutes* after 6 months in captivity ($P = 0.09$), from 1.6% of the total community in the wild to roughly 0.3% in captivity. At the genus level, the *Ruminococcus* (wild: $7.4 \pm 2.9\%$; captive $15.9 \pm 2.6\%$; $P = 0.08$) and *Coprococcus* (wild: $0.7 \pm 0.4\%$; captive: $1.4 \pm 0.3\%$; $P = 0.06$) slightly increased, whereas *Adlercreutzia* (wild: $0.15 \pm 0.03\%$; captive: $0.02 \pm 0.01\%$; $P = 0.06$) slightly decreased. There were significant changes in abundance of many unidentified microbes that drove the changes seen in overall community structure.

Studying animals in captivity offers the obvious benefit of being able to isolate a few variables of interest and their effects on the study organism. The main downfall is that laboratory conditions are often unnatural. These advantages and disadvantages also pertain to the study of gut microbial communities, where these communities may experience loss or gain of microbes. Overall, we found that bringing *N. lepida* into captivity from the wild significantly altered the microbiota. However, these changes were smaller than predicted based on previous studies that compared animals born in captivity with animals born in the wild. We documented a markedly higher overlap in microbial OTUs between wild and captive samples than previous studies. Additionally, changes in biodiversity over 6 months in captivity were relatively small compared with changes seen as the result of small dietary changes. We conclude that the use of wild-caught individuals in gut microbial studies is acceptable for short periods of time in captivity, while subsequent studies should monitor microbial diversity over longer periods of captivity. Additionally, future studies should investigate changes in microbial gene expression caused by captivity, which may exhibit larger changes and greatly impact host physiology.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Supplementary methods.