Title: Quantifying how evolutionary history and diet shape the mammalian gut microbiome

Authors: Sara B. Weinstein*[1,2], Tess E. Stapleton[1], Rodolfo Martínez-Mota[1,4], Dylan Klure[1], Robert Greenhalgh[1], Teri J. Orr[1,5], Kevin D. Kohl[3], M. Denise Dearing[1]

Introduction

Intact microbial communities are critical for animal development and survival (McFall-Ngai et al. 2013). Although dysbiotic and low diversity microbiomes are often associated with disease (Kriss et al. 2018), healthy hosts also exhibit substantial temporal and interindividual variation (Lozupone et al. 2012, Flores et al. 2014). These differences are influenced by numerous bacterial, host, and environmental factors, with bacterial dispersal, host diet and genetics often the most influential features. These factors are fundamental to bacterial community composition, yet their relative contributions to natural microbiome structure and stability remain uncertain.

Like all ecological communities, animal associated microbiomes assemble through both neutral and selective processes (Sloan et al. 2006, Burns et al. 2016). Hosts acquire bacteria from the environment and, when bacteria are dispersal limited, more distantly located hosts have more disparate microbiomes (Moeller et al. 2017). While geography can be the primary factor structuring microbial communities (e.g., Lankau et al. 2012, Linnenbrink et al. 2013), host genetic effects are often stronger (e.g. Knowles et al. 2019). Related hosts frequently harbor similar microbial communities, producing a pattern termed “phylosymbiosis” (Lim & Bordenstein 2020). Congruence between host phylogeny and microbiome composition can result from host-microbe co-diversification (Moeller et al. 2016, Groussin et al. 2017) or by related hosts selecting similar microbes from the environment (Mazel et al. 2018).

Phylosymbiosis is widely documented in mammals (e.g., Brooks et al. 2016, Nishida & Ochman 2018, Amato et al. 2019). However, related hosts often have similar gut morphology and diet, and these attributes can also influence microbiome structure. Diet substantially alters the microbiome (David et al. 2014) and the microbiome contributes to digestive functions such as fiber fermentation, vitamin synthesis (Stevens & Hume 1998), and toxin metabolism (Kohl et al. 2014c). Similar diets can select for similar microbiota, with examples of convergent communities seen among herbivores (Muegge et al. 2011) and ant feeding mammals (Delsuc et al. 2014). This suggests that diet effects can overshadow host evolutionary history, at least for broad diet classifications (e.g. herbivore v carnivore) and captive hosts. Studies of wild animals from diverse locations, with fully characterized diets, are needed to understand how geography, diet, and host evolutionary history contribute microbiome assembly.

Once assembled, bacterial communities are thought to be relatively stable (Lozupone et al. 2012). However, perturbations (e.g., altered diet, antibiotic exposure) can rapidly change microbiome structure (Turnbaugh et al. 2009, Dethlefsen & Relman 2011), potentially shifting communities to less functional, dysbiotic states (Sommer et al. 2017). Resistant communities retain their structure and function when perturbed, with multiple factors potentially contributing to stability (Shade et al. 2012). For example, more diverse communities are thought to be more resistant and less invadable due to increased functional redundancy and fewer empty niches, respectively (Shade 2012). Alongside community attributes, past exposure might also influence stability. For instance, consistent environments can produce more sensitive microbiomes (Hawkes & Keitt 2015), perhaps explaining why captivity results in greater changes to the microbiota of dietary specialists (Kohl et al. 2014b). To date, microbial

community stability and resilience has primarily been studied in free-living, soil communities or captive model organisms (Shade et al. 2012). It remains unclear whether predictions from macroecological systems, soil microbiota, and model organisms extends to naturally assembled animal microbiomes, and how the stability of these communities is influenced by host diet and evolutionary history.

The widespread rodent genus *Neotoma* (“ woodrats”) provides an ideal system to examine how geography, diet, and host evolutionary history influence the structure and stability of the mammalian gut microbiome. *Neotoma* species are found throughout North America, and multiple species often occur in sympatry (e.g. Dial 1988). Across the genus, the behavior and morphology of these herbivorous, solitary, midden-dwelling rodents is highly conserved. Body sizes are similar (120-600 g) and all species have the same gut morphology, with a fermenting foregut and large cecum (Reid 2006, Kohl et al. 2014a). Although similar in morphology, *Neotoma* spp. exhibit extensive variation in diet composition and specialization, with repeated diet convergence across species. Diets typically include plants with high levels of secondary compounds. For examples, diets heavy in cactus, creosote, juniper expose animals to high concentrations of oxalate, nordihydroguaiaretic acid and terpenes, respectively (Cameron & Rainey 1972, Atsatt & Ingram 1983, Dial 1988). These compounds are toxic to most mammals and many microbes, and woodrat digestion is aided by specialized, toxin degrading, gut microbiomes (Kohl et al. 2014c, Miller et al. 2016). As different diets expose microbiota to distinct substrates and antimicrobial compounds, and novel diets rapidly alter the captive woodrat gut microbiota (Martínez-Mota et al. 2020), natural diet differences are predicted to effect wild woodrat microbiome structure.

To examine how geography, diet, and host evolutionary history influence natural microbiome structure and stability, we quantified diet components and bacterial communities from *Neotoma* spp. throughout the US Southwest. After collecting wild samples, we brought individuals into captivity, exposing all animals to an identical diet and environment. Captivity created a perturbation, allowing us to both examine factors influencing microbiome stability and test whether phylosymbiosis strength increased in the absence of site and diet differences. Results from this extensively replicated study of wild and captive bacterial communities provide novel insight into the neutral and selective factors shaping the diversity, composition, and stability of the mammalian gut microbiome.

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