Dispersal limitation promotes the diversification of the mammalian gut microbiota

Andrew H. Moeller[a,b,c,1], Taichi A. Suzuki[b,c], Dana Lin[b,c], Eileen A. Lacey[b,c], Samuel K. Wasser[d], and Michael W. Nachman[b,c]

[a]Miller Institute for Basic Research in Science, University of California, Berkeley, CA 94720; [b]Museum of Vertebrate Zoology, University of California, Berkeley, CA 94720; [c]Department of Integrative Biology, University of California, Berkeley, CA 94720; and [d]Center for Conservation Biology, University of Washington, Seattle, WA 98195

Edited by James J. Bull, The University of Texas at Austin, Austin, TX, and approved October 16, 2017 (received for review January 3, 2017)

The gut bacterial communities of mammals have profound effects on host fitness, but the processes that generate and maintain gut bacterial diversity remain poorly understood. We mapped compositional variation (i.e., β-diversity) in the gut microbiotas of 136 pairs of wild mammalian species living throughout the Americas to assess how the distribution of mammals across geographic space influences the diversification of their gut bacteria. Comparing the gut microbiotas of sympatric and allopatric mammalian populations provided insights into the flow of gut bacteria within and between mammalian communities, revealing that spatial limits on bacterial dispersal promote β-diversity between the gut microbiotas of mammalian species. Each geographic locale displayed a unique gut-microbiota composition that could not be fully explained by the diets and phylogenetic histories of the resident mammalian hosts, indicating that some gut bacteria are geographically restricted. Across the western hemisphere, the compositional overlap between the gut microbiotas of allopatric mammalian populations decayed exponentially with the geographic distance separating the hosts. The relationship between geographic distances among hosts and compositional differences among their gut microbiotas was independent of dietary and phylogenetic divergence among hosts. Within mammalian communities, we observed widespread sharing of gut bacteria between predator–prey host-species pairs, indicating horizontal transfer of gut bacteria through mammalian food chains. Collectively, these results indicate that compositional differences between the gut microbiotas of mammalian taxa are generated and maintained by limits to bacterial dispersal imposed by physical distance between hosts.

microbiome | vertebrate | biogeography | food web | metacommunity

The gut bacterial communities of mammals profoundly influence host fitness (1), but the processes that generate and maintain variation in gut-microbiota composition (i.e., β-diversity) among host species remain poorly understood. All ecological communities, including mammalian gut microbiotas, are shaped by the dispersal of organisms into the habitat followed by natural selection (i.e., habitat filtering), drift, and in situ diversification (2). Comparisons across the mammalian phylogeny have revealed that differences in selective pressures between the gut environments of mammalian species have promoted the diversification of gut-microbiota compositions: Each mammalian species maintains a compositionally distinct gut microbiota that reflects host diet, physiology, and genetics (3–15). However, the degree to which the diversification of gut-microbiota compositions across mammalian species has been influenced by spatial limits on bacterial dispersal has been less widely explored.

If mammalian gut bacteria are dispersal limited, then increasing the physical distance between host species should attenuate bacterial transmission and increase β-diversity between the hosts' gut microbiotas. This hypothesis yields several predictions. First, co-occurring (i.e., sympatric) populations of different host taxa should harbor gut microbiotas that are more compositionally similar to one another than are the microbiotas of geographically separated (i.e., allopatric) populations. Second, β-diversity between the gut microbiotas of populations of host taxa living in allopatry should increase with the geographic distance separating the hosts. Third, within host assemblages, host-species pairs that have a long history of direct interactions (e.g., predator–prey relationships) should harbor gut microbiotas that are more compositionally similar than would be expected based on host phylogenetic divergence and dietary differences. Theory suggests that these predictions can manifest even if selective pressures, such as those imposed by host diet and immune response, vary among host species, given that bacterial dispersal between host species is sufficiently frequent (e.g., mass effects) (16, 17).

Several studies have shown that limitations on bacterial dispersal can generate differences between the gut microbiotas of individuals within mammalian species (18, 19), but few have explored whether dispersal limitation promotes differences in gut-microbiota composition among mammalian species. Most interspecific comparisons of mammalian gut microbiotas have focused on host species living in isolation from one another, either in captivity (2, 3, 19) or in the wild (4–14), and little attention has been paid to how spatial relationships among host species influence patterns of gut-microbiota β-diversity. A study of humans and dogs found little evidence for an effect of living together versus separately on the compositional similarity between the gut microbiotas of

Significance

Mammals harbor communities of gut bacteria that regulate host health, requiring an understanding of the processes that govern the evolution of gut microbiotas. We investigated the diversification of mammalian gut microbiotas by surveying the gut microbiotas of 136 pairs of wild mammalian species living throughout the Americas. These comparisons indicated that physical distance produces barriers to bacterial dispersal that appear to accelerate compositional divergence between the gut microbiotas of mammalian species over evolutionary time. In contrast, contact between host species, such as that between predators and prey, leads to widespread bacterial transmission and the homogenization of microbiotas within mammalian communities. Our findings suggest that spatial limits on bacterial dispersal generate and maintain mammalian gut bacterial diversity across the western hemisphere.

Author contributions: A.H.M. designed research; A.H.M., T.A.S., D.L., E.A.L., S.K.W., and M.W.N. performed research; A.H.M., T.A.S., D.L., E.A.L., S.K.W., and M.W.N. contributed new reagents/analytic tools; A.H.M. analyzed data; and A.H.M. and M.W.N. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: Sequence data were submitted to the European Nucleotide Archive, https://www.ebi.ac.uk/ena (accession no. PRJEB23639).

[1]To whom correspondence should be addressed. Email: andrew.moeller@berkeley.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1700122114/-/DCSupplemental.

the two host species (20). In contrast, a study of sympatric and allopatric populations of chimpanzees and gorillas reported that sympatric host populations shared a greater proportion of gut bacterial phylotypes than did allopatric populations (15), suggesting that spatial separation and dispersal limitation have promoted the divergence of the gut microbiotas of allopatric populations. However, because sympatric chimpanzees and gorillas inhabit overlapping ecological niches and consume many of the same food resources, it is not clear whether the compositional similarity of the gut microbiotas of sympatric hosts was due to increased bacterial dispersal between these species or to shared selective pressures in the gut environments of the host species. Isolating the effects of dispersal limitation on the compositional diversification of mammalian gut microbiotas would be better accomplished by comparing the microbiotas of sympatric and allopatric populations of host species that experience little or no dietary overlap in sympatry.

To quantify the degree to which bacterial dispersal limitation has accelerated the diversification of the gut microbiotas of mammalian species, we surveyed the gut microbiotas of sympatric and allopatric populations of carnivore, artiodactyl, and rodent species residing in the wild throughout the Americas, focusing on mammalian assemblages in western North America. These data provide insights into the tempo of compositional change in the gut microbiotas of free-living mammals over evolutionary time under various ecological scenarios. In particular, our results support central roles for spatial separation among hosts and bacterial dispersal limitation in generating and maintaining compositional differences between the gut microbiotas of mammalian species.

Results

A total of 15,251,957 reads were produced for 204 fecal samples collected from 17 mammalian species. After quality filtering, a total of 37,347 operational taxonomic units (OTUs) were detected across all samples. A phylogeny of the host species sampled obtained from TimeTree (21) and a map of all sampling locations are presented in Fig. 1. Host species' binomial names, common names, and sample counts are presented in Table S1. Metadata for all samples analyzed are presented in Dataset S1. The mean Bray–Curtis dissimilarities and weighted UniFrac distances between the microbiotas of all host-species pairs are presented in Dataset S2. For each host species, gut microbiotas were on average compositionally more similar among conspecifics than among hosts of different species (Dataset S2). A principal coordinate plot of 99% OTU Bray–Curtis dissimilarities among samples is shown in Fig. S1. All subsequent analyses of gut-microbiota composition were based on the 99% OTU Bray–Curtis dissimilarities among samples.

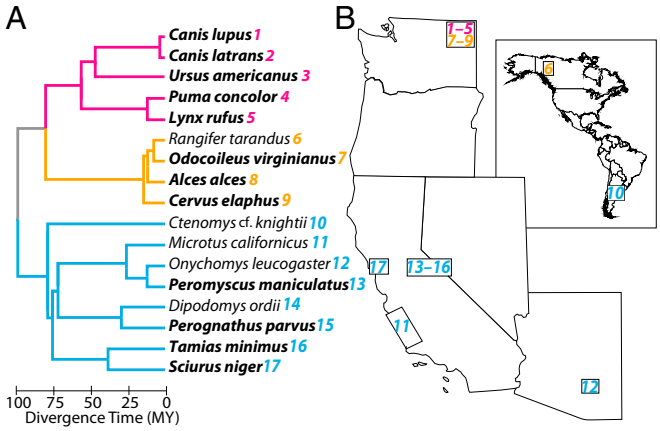

Fig. 1. Phylogenetic and geographic sampling of mammalian gut microbiotas. (*A*) Time-calibrated phylogeny of mammalian species sampled in the present study. Branches are colored by host taxonomic order: Carnivora (red), Artiodactyla (yellow), or Rodentia (blue). Bold species names indicate species whose geographic ranges overlap in northeast Washington. (*B*) Map of sampling locations. Numbers correspond to mammalian species in *A*. Boxes containing multiple numbers represent geographic regions inhabited by multiple sympatric host populations.

Allopatry Promotes Divergence Among the Gut Microbiotas of Host Populations. Comparisons of 136 host-species pairs revealed that the compositional overlap between the gut microbiotas of host species has decayed exponentially throughout the diversification of mammals (Fig. 2). A Mantel test indicated that compositional overlap (1 − Bray–Curtis dissimilarity) between the gut microbiotas of host populations was negatively associated with the evolutionary time separating the hosts ($P = 0.0001$), and Vuong's test indicated that an exponential decay function fit the data better than did a linear function ($P < 2.2 \times 10^{-16}$). Consistent with the hypothesis that bacterial dispersal limitation promotes β-diversity between the gut microbiotas of host species, the rate of microbiota diversification with evolutionary time was lowest between sympatric host populations linked by predator–prey relationships (Fig. 2*A*, dashed dark gray curve), higher between sympatric host populations that do not engage in predator–prey relationships (Fig. 2*A*, solid dark gray curve), and highest between host populations living in allopatry (Fig. 2*A*, light gray curve). Sympatric predator and prey hosts represented in the dashed dark gray curve in Fig. 2*A* include *Canis lupus*, *Puma concolor*, *Alces alces*, *Odocoileus virginianus*, and *Cervus elaphus*.

We tested for significant differences between the three exponential decay curves displayed in Fig. 2*A* using Vuong's closeness test, which indicated that each of the three models fits its respective subset of the data better than did either of the two alternatives. Specifically, these tests rejected the null hypotheses that the exponential decay curve derived from comparisons of sympatric non-predator–prey populations fit the predator–prey comparisons as well as did the exponential decay curve derived from the predator–prey comparisons ($P = 0.0358$) and that the exponential decay curve derived from comparisons of sympatric populations fit the allopatric comparisons as well as did the exponential decay curve derived from the allopatric comparisons ($P = 0.0002$). Nonlinear least squares analysis indicated that compositional overlap between microbiotas has decayed with a half-life of 81.68 My between sympatric predator–prey host populations, 49.16 My between sympatric host populations that do not engage in predator–prey relationships, and 13.39 My between allopatric host populations. Taxonomic assignments of bacterial phylotypes that display geographic distributions independent of their hosts' phylogenetic histories (i.e., phylotypes shared by sympatric host populations but absent from allopatric host populations phylogenetically nested within the clade of sympatric hosts) are presented in Dataset S3.

The pairwise comparisons presented in Fig. 2*A* are not phylogenetically independent because each host population is included in multiple pairwise comparisons and shares phylogenetic history with every other host population. To evaluate the impact of allopatry on the compositional divergence (i.e., Bray–Curtis dissimilarity) between gut microbiotas while controlling for host phylogenetic divergence, we identified in our dataset each set of pairwise comparisons between host populations that included comparisons between both sympatric and allopatric host populations and in which each pairwise comparison contained host populations separated by the same amount of evolutionary time. This filtering of the data yielded 13 sets of comparisons representing 31 tests evaluating whether allopatry influences compositional divergence between the gut microbiotas of host populations independently of host phylogenetic divergence (Fig. S2). In 29 of 31 comparisons, allopatric host populations harbored gut microbiotas that were more compositionally divergent than were the gut microbiotas of sympatric host populations (nonparametric $P < 0.01$). The other two

EVOLUTION

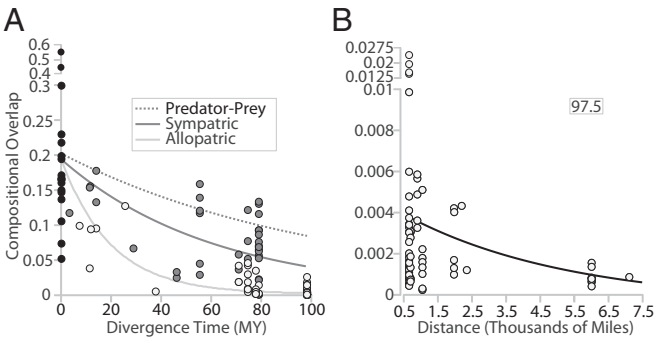

Fig. 2. Geographic distance accelerates gut microbiota divergence over evolutionary time. (*A*) Curves display the exponential decay of compositional overlap (1 − Bray–Curtis dissimilarity) between the gut microbiotas of diverging host species over evolutionary time under three ecological scenarios. Each point represents the mean compositional overlap calculated from all pairwise comparisons within a host population (black), between two sympatric host populations (dark gray), and between two allopatric host populations (light gray). The light-gray curve represents the best-fit exponential-decay function for all comparisons within host populations and between allopatric host populations. The solid dark-gray curve represents the best-fit exponential-decay function for all comparisons within host populations and between sympatric host populations. The dashed dark-gray curve represents the best-fit exponential-decay function for all comparisons within host populations and between sympatric populations that engage in a predator–prey relationship. (*B*) The plot displays the relationship between geographic distance between allopatric Scrotifera and Rodentia host-species pairs and compositional divergence between their gut microbiotas. Each point represents the mean compositional overlap between the gut microbiotas of a Scrotifera population and an allopatric Rodentia population. The curve displays the best-fit exponential-decay function. The number within the box indicates the divergence time (in millions of years) between Scrotifera and Rodentia.

comparisons indicated no significant effect of allopatry (nonparametric $P > 0.05$) (Fig. S2).

In some cases, whether host populations occurred sympatrically or allopatrically was a better predictor of the compositional divergence between their gut microbiotas than was host phylogenetic divergence. For example, *Alces* and *Cervus* living in sympatry harbored more similar gut microbiotas than did *Alces* and *Rangifer* living in allopatry, even though *Alces* and *Rangifer* are more closely related than are *Alces* and *Cervus* (nonparametric $P = 0.001$; Fig. S2 and Dataset S2). Similarly, *Odocoileus* harbored microbiotas that were more similar to those of sympatric *Alces* and *Cervus* than to those of allopatric *Rangifer* (nonparametric $P = 0.001$) (Dataset S2), despite *Odocoileus* being more closely related to *Rangifer* than to *Alces* or *Cervus*. In contrast, host phylogenetic divergence was a better predictor of microbiota dissimilarity between rodent populations than was whether the hosts lived sympatrically or allopatrically. *Peromyscus* microbiotas were more similar to those of allopatric *Onychomys* and *Microtus* than to those of sympatric *Dipodomys*, *Perognathus*, and *Tamias* (nonparametric $P = 0.001$) (Fig. S2 and Dataset S2). That phylogenetic distance was a better predictor of microbiota dissimilarity than was geographic distribution in rodents but not in artiodactyls might reflect that the sympatric rodent species sampled were more phylogenetically divergent from one another than were the sympatric artiodactyl species sampled (Fig. 1).

Geographic Distance Promotes Divergence Between the Gut Microbiotas of Allopatric Host Populations. To test whether the degree of geographic distance separating allopatric host populations promotes compositional divergence between the gut microbiotas of the hosts, we compared the gut microbiotas of Scrotifera (Artiodactyla and Carnivora) populations living in northeast Washington and Canada with those of Rodentia populations living in North and South America. This analysis allowed us to isolate the effect of geographic distance between host populations on microbiota divergence while controlling for host divergence times and dietary differences, because in each pairwise comparison host populations were separated by ∼97.5 My of evolution and consumed largely nonoverlapping diets. The compositional overlap between Scrotifera and Rodentia gut microbiotas decreased with the geographic distance separating host populations (Mantel test, $P = 0.055$), and Vuong's test indicated that an exponential decay function fit the data better than did a linear function ($P = 0.0071$) (Fig. 2*B*). Nonlinear least squares analysis indicated that the compositional overlap between the gut microbiotas of Scrotifera–Rodentia species-pairs was halved for every ∼3,550 miles separating the hosts. Moreover, across our entire dataset, likelihood ratio tests indicated that compositional overlap between the gut microbiotas of allopatric host populations was better modeled as a linear combination of the geographic distances and phylogenetic divergences among host populations than as linear functions of only geographic distances ($P < 2.2 \times 10^{-16}$) or only phylogenetic divergences ($P = 0.0488$). Similarly, variance partitioning analysis indicated that host divergence times and geographic distances each independently explained a portion of the variation in Bray–Curtis dissimilarity among host populations (*Supporting Information*). These results suggest that geographic distance between allopatric host populations positively influences the compositional divergence between their microbiotas.

Transmission of Gut Bacteria Through Mammalian Food Chains. The observation that the gut microbiotas of sympatric predator–prey host populations were more compositionally similar than were the gut microbiotas of non-predator–prey sympatric host populations (Fig. 2*A*) suggests that predator–prey interactions provide routes for the transmission of gut bacteria between host species. To explore this possibility further, we evaluated the consistency with which predators and their preferred prey harbored more similar gut microbiotas than expected based on their phylogenetic relationships and geographic distributions. A diagram of the preferred prey of the predators sampled in this study is presented in Fig. S3. For these analyses, we included the gut microbiotas of prey populations whose species ranges overlap with the sampling region in northeast Washington State even if the specific prey populations sampled were not located in this region. In *Supporting Information*, we discuss how this sampling scheme allowed us to isolate the effect of predator–prey interactions on the compositional overlap between the gut microbiotas of host species while controlling for the potentially confounding effects of host body size.

Every pairwise Carnivora–Artiodactyla and Carnivora–Rodentia comparison supported the hypothesis that predators acquire gut bacteria from their prey. *Puma* and *Canis lupus* harbored gut microbiotas that were on average more compositionally similar to those of their preferred artiodactyl prey than were the gut microbiotas of *Lynx* and *Canis latrans* (Fig. 3 and Fig. S4). Conversely, *Lynx* and *Canis latrans* harbored gut microbiotas that were on average compositionally more similar to those of their preferred rodent prey than were the gut microbiotas of *Puma* and *Canis lupus* (Fig. 3 and Fig. S4). Six of the 14 individual comparisons displayed in Fig. 3 reached significance (Bonferroni-corrected $P < 0.05$). Sign tests across comparisons indicated that the microbiotas of large-bodied predators were significantly more similar to the microbiotas of large-bodied prey than were the microbiotas of small-bodied predators (six of six comparisons; $P = 0.0156$) and that the microbiotas of small-bodied predators were significantly more similar to the microbiotas of small-bodied prey than were the microbiotas of large-bodied predators (eight of eight comparisons; $P = 0.0039$). Similarly, the gut microbiotas of *Puma*, *Lynx*, *Canis lupus*, and *Canis latrans* were each more compositionally similar to the gut microbiotas of their preferred prey species than were the gut microbiotas of *Ursus*

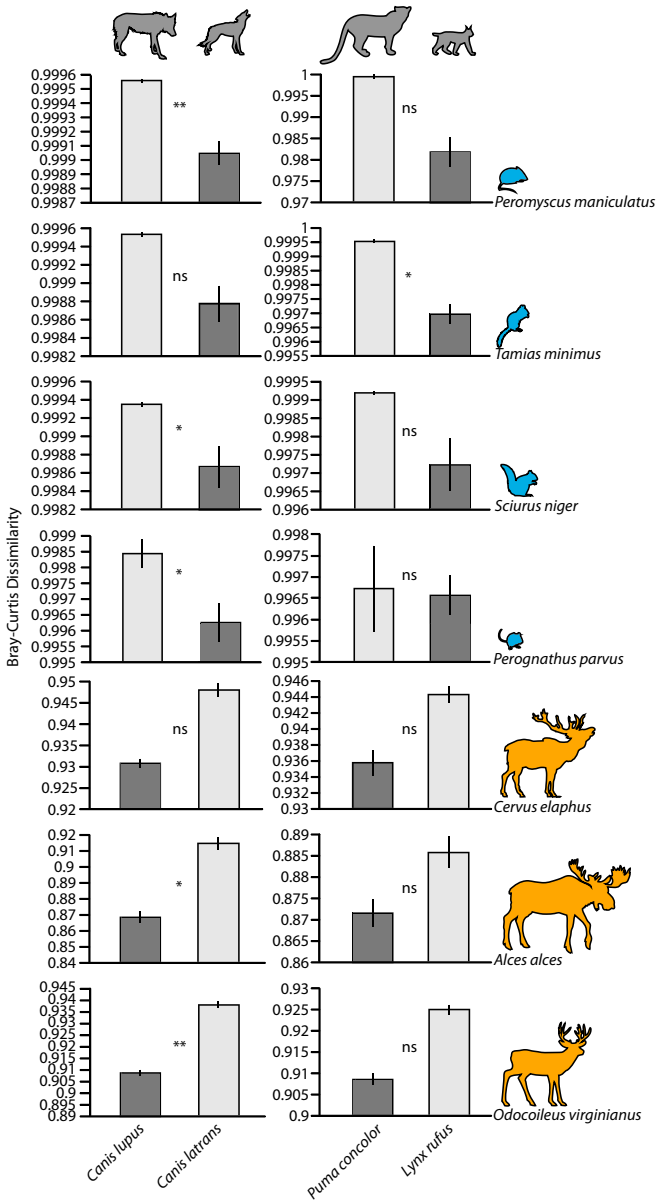

Fig. 3. Predators and their preferred prey harbor compositionally similar gut microbiotas. Bar plots show the mean Bray–Curtis dissimilarities between the microbiotas of predator and prey host populations. Each bar corresponds to a pairwise comparison between predator (columns) and prey (rows). Colors of animals correspond to host taxonomic orders as in Fig. 1. Error bars indicate SEs of means. Within each plot, bars representing higher and lower mean Bray–Curtis dissimilarities are colored in light and dark gray, respectively. Note that in each comparison the difference between means is in the direction expected if predator–prey relationships promote the exchange of gut microbiota (six of six comparisons including rodents, $P = 0.0156$, and eight of eight comparisons including artiodactyls, $P = 0.0039$). The significance of differences between means in individual comparisons, based on Bonferroni-corrected P values, is denoted as not significant (ns), $*P < 0.05$, and $**P < 0.01$. Data underlying each plot are presented in Dataset S2.

(Fig. S2 and Dataset S2). For example, Bray–Curtis dissimilarities between the gut microbiotas of *Puma* and *Alces* were on average lower than the Bray–Curtis dissimilarities between the gut microbiotas of *Ursus* and *Alces* (Bonferroni-corrected $P = 0.002$) (Fig. S2 and Dataset S2).

In some cases, individual hosts belonging to different orders of mammals but living sympatrically and connected by a predator–prey relationship harbored microbiotas that were compositionally more similar to one another than to the microbiotas of other individuals belonging to their respective taxonomic orders (Fig. S5). The combined effects of sympatry and predator–prey interactions have also led to the convergence of the gut microbiotas of host populations. For example, *Alces* harbored gut microbiotas that were on average compositionally more similar to those of sympatric *Canis lupus* (nonparametric $P = 0.001$) and *Puma* (nonparametric $P = 0.001$) than to those of allopatric *Rangifer* (Fig. S2 and Dataset S2). Similarly, *Canis lupus* and *Puma* harbored gut microbiotas that were on average compositionally more similar to those of sympatric *Alces*, *Cervus*, and *Odocoileus* than to those of sympatric *Ursus* (nonparametric $P = 0.001$ for each pairwise comparison; Fig. S2 and Dataset S2).

To identify the bacterial phylotypes underlying the convergence of predator and prey microbiotas, we identified all phylotypes shared by *Lynx*, *Canis latrans*, *Tamias*, *Perognathus*, *Peromyscus*, and *Sciurus* but not by *Puma* and *Canis lupus* as well as all phylotypes shared by *Puma*, *Canis lupus*, *Alces*, *Odocoileus*, and *Cervus* but not by *Lynx* and *Canis latrans*. These analyses revealed 419 putatively prey-derived phylotypes within carnivores, constituting 0–41% of the microbiota of individual carnivore fecal samples. These phylotypes represent a diversity of bacterial taxa, including some of the most common taxa of mammalian gut bacteria (Dataset S4). Moreover, most individual carnivore fecal samples contained bacterial phylotypes derived from multiple prey species (*Supporting Information* and Dataset S5), suggesting that prey-derived bacterial phylotypes colonize and proliferate within individual carnivores. SILVA (22) taxonomic assignments of bacterial phylotypes included in Datasets S4 and S5 are presented in Dataset S6.

The putatively prey-derived phylotypes within carnivores belonged primarily to the Clostridia (Dataset S4). In predators, Clostridia constituted 70.17% of the reads that mapped to the 420 putatively prey-derived phylotypes. In contrast, in prey, Clostridia constituted only 52.11% of the reads that mapped to the 420 phylotypes. This observation suggests that prey-derived Clostridia phylotypes display higher fitness within recipient predator hosts than do prey-derived phylotypes belonging to different taxonomic orders ($P < 0.0001$; χ^2 test with Yates' correction). Several of the putatively prey-derived Clostridia phylotypes belonged to species that have been associated with disease in humans, including *Clostridium neonatale* (23), *Clostridium perfringens* (24), *Veillonella parvula* (25), and *Ruminococcus gnavus* (26). More generally, prey-derived phylotypes within carnivores displayed phylum-level compositional profiles that more closely resembled a typical carnivore microbiota phylum-level compositional profile than they did a typical prey microbiota phylum-level compositional profile (Fig. S6), suggesting host-level selection by carnivores on the relative abundances of prey-derived phylotypes.

Discussion

Comparing gut microbiotas of mammals residing throughout the Americas revealed that geographic proximity and predator–prey interactions enable the flow of gut bacteria between distantly related host species (Figs. 2 and 3 and Fig. S4). In some cases, the combined effects of shared geography and predator–prey interactions have led to the convergence of the gut microbiotas of carnivorous and herbivorous mammals belonging to different taxonomic orders (Figs. S2 and S5). These results indicate that the compositional diversification of mammalian gut microbiotas has been promoted by barriers to bacterial dispersal generated by physical separation between diverging mammalian species.

Our results demonstrate that ecological networks connecting mammalian species provide routes for transmission of gut bacteria within mammalian communities. Predator–prey interactions were associated with increased overlap between the gut microbiotas of host species: The microbiotas of prey species were always more similar to those of their primary predator species

than to those of other Carnivora species (Fig. 3 and Fig. S4). The compositional convergence of predator and prey microbiotas cannot be explained by body size effects (i.e., smaller and larger predators tend to prefer smaller and larger prey, respectively) (*Supporting Information*), which have been shown to influence gut-microbiota composition in vertebrates (27). These results suggest that predators acquire gut bacteria from their prey, mirroring previous observations that New World vultures appear to acquire gut microbiota from the carcasses on which they feed (28). Together, these findings demonstrate that the gut microbiotas of tetrapod species can form metacommunities, i.e., sets of local communities linked by dispersal of multiple potentially interacting species (16), challenging the hypothesis that the fitnesses of host and gut bacterial lineages tend to be tightly linked (29).

One possible explanation for the compositional convergence of predator and prey microbiotas is the transient presence of prey-derived phylotypes within predators following feeding events. The detection within individual predator fecal samples of bacterial phylotypes derived from multiple prey species (Dataset S5) suggests that prey-derived phylotypes colonize and proliferate within predators. Moreover, the observation that the relative abundances of prey-derived phylotypes within predators shift to more closely resemble a carnivore-like phylum-level compositional profile (Fig. S6) suggests that these phylotypes experience different selection pressures within predator and prey species. Together, these results indicate that some of the most common taxa of gut bacteria are capable of proliferating across distantly related orders of mammalian hosts.

Microbial transmission from prey into predators has been implicated in the emergence of pathogens. For example, the recombination of the endogenous viruses from two prey species of monkey gave rise to *Simian immunodeficiency virus* in chimpanzees (30), and, ultimately, HIV-1 in humans. Similarly, the prevalence of plague (i.e., *Yersinia pestis*) in carnivore species in North America has been associated with the degree of reliance on rodents as prey items (31). Whereas strict maternal transmission of bacteria can enable the evolution of obligate mutualisms (32, 33), the opportunity for frequent transfer between host species may promote the evolution of selfish phenotypes in bacterial lineages. Intriguingly, several of the bacterial phylotypes that appear to have been transferred from prey into predators have been associated with disease in humans, such as *Clostridium neonatale* (23) and *Clostridium perfringens* (24).

Sympatric populations of carnivore, artiodactyl, and rodent species harbor more similar gut microbiotas than would be expected based on host evolutionary divergence, even when the hosts do not enter into predator–prey relationships (Fig. 2*A* and Fig. S2). Previous work has shown that sympatric chimpanzees and gorillas share on average 53% more bacterial phylotypes than do allopatric hosts (14), and a survey of two sympatric species of *Peromyscus* revealed no consistent compositional differences between their gut microbiotas (34). In contrast, the gut microbiotas of cohabiting humans and dogs do not appear to share significantly more phylotypes than do the gut microbiotas of humans and dogs living separately, although the skin microbiotas of dog owners do appear to contain phylotypes derived from the gut microbiotas of their dogs (20). Together, these results indicate that the probability of bacterial proliferation between the gut microbiotas of co-occurring host species may depend on the phylogenetic divergence between the hosts.

We observed that the compositional overlap between the gut microbiotas of allopatric mammalian populations decayed exponentially with the geographic distance separating the hosts across the western hemisphere independently of host phylogenetic divergence (Fig. 2 and Fig. S3). This distance-decay of microbiota similarity mirrors observations of free-living microbial communities in salt-marsh sediment (35, 36), indoor (37), and hydrothermal vent (38) environments. Distance-decay of community similarity (39) can arise from the decreasing probability of dispersal with distance (40) as well as from ecological gradients in which more proximate habitat patches select for more similar sets of species (41). That the overlap between the gut microbiotas of mammalian species decayed with the geographic distance between hosts independently of host phylogenetic divergence, which is associated with the divergence of selective pressures within the gut (e.g., gut physiology, host immune system, and host diet), suggests that dispersal limitation contributes to the distance-decay of mammalian gut microbiotas. Dispersal limitation has been previously shown to promote β-diversity between the gut microbiotas of individuals of the same host species (18, 19, 42, 43); our results indicate that dispersal limitation also contributes to β-diversity between gut microbiotas of populations of different host species.

We also observed that the compositional overlap between the gut microbiotas of mammalian species decayed exponentially with the evolutionary time separating the host species independently of geographic distance. This result corroborates previous studies that have reported associations between host phylogenetic history and gut-microbiota composition across a diversity of mammalian taxa (3–15). However, our observations indicate that phylosymbiosis (i.e., congruence between a dendrogram of gut microbiota dissimilarity and the host phylogeny) (44), which has been observed in hominids (5), may be the exception rather than the rule across mammalian species, owing to effects of host geography, interspecific interactions (e.g., predator–prey relationships), and potentially other factors. For example, patterns of gut-microbiota dissimilarity among artiodactyl species did not mirror the host phylogeny but instead were better explained by geography (Figs. S2 and S5 and Dataset S2).

We have shown that compositional differences among the gut microbiotas of mammalian species arise in part due to limits on bacterial dispersal imposed by physical distance. That free-living microbial taxa exhibit biogeographic patterns has been well-established (45). Our study indicates that many gut bacteria exhibit geographic distributions that are independent of that of any individual host species and that the composition of the mammalian gut microbiota is determined in part by the locale inhabited by the host. These results set the stage for future investigations into the biogeography of vertebrate gut microbiotas.

Materials and Methods

Sample Collection and Processing. Fecal samples ($n = 205$) were collected from field sites throughout the Americas (Fig. 1 and *Supporting Information*). Host-derived DNA was extracted from samples as described previously, and species of origin was confirmed by mtDNA D-loop sequencing (46, 47). Variation in measurements of gut-microbiota composition due to differences in the sample collection procedures used in this study is expected to be low relative to the biological variation among the microbiotas of host species (48, 49). Total DNA from fecal material was extracted via a bead-beating procedure, and PCR amplifications of the V4 region of the 16S rRNA gene were conducted using the universal 515F/806R primer pair as described previously (50). Amplicons were sequenced on an Illumina MiSeq at the Microbial Analysis, Resources and Services (MARS) facility at the University of Connecticut. All sequence data produced were deposited in the European Nucleotide Archive, https://www.ebi.ac.uk/ena (accession no. PRJEB23639).

Sequence Processing. All 16S reads were processed in QIIME v1.9 (51). Split_libraries.py was employed to filter raw FASTQ files for quality using default settings, and 99% OTUs were generated via uclust (52). OTUs represented by a single read were removed from downstream analyses. Representative sequences from each remaining OTU were assigned to taxonomic groups through comparisons with the Greengenes database May 2013 release and the SILVA 128 database using the uclust algorithm as implemented in *assign_taxonomy.py*. One fecal sample that contained an uncharacteristically high frequency (53%) of Chloroflexi (sample NA0004279074) was removed from downstream analysis due to potential contamination. To enable comparisons across samples, each sample was rarefied to an even depth of 10,000 reads. Although rarefaction can produce false positives when testing for differential abundance of specific OTUs across sample groups (53), we chose to rarefy our data because our subsequent analyses focused on overall patterns of β-diversity, which may be sensitive to differences in library size between sample groups. Bray–Curtis dissimilarities and weighted UniFrac distances were calculated through *beta_diversity.py*.

Statistical Analyses. To quantify and to visualize gut-microbiota β-diversity, pairwise Bray–Curtis dissimilarities were calculated among all samples in QIIME, and principal coordinates plots were produced (Fig. S1). Full details about the statistical analyses performed in this paper are presented in *Supporting Information*. Statistical significance of differences between exponential decay functions fit to different subsets of the data were assessed using Vuong's closeness tests (54) as implemented in the nonnest2 package in R. For these analyses, collecting localities were grouped into seven distinct nonoverlapping geographic regions (Fig. 1*B*), and comparisons between species sampled from different regions were classified as allopatric while comparisons between species sampled from the same region were classified as sympatric. The maximum distance between collecting localities within a region was 100 miles, and the minimum distance between regions was 180 miles. Some comparisons classified as allopatric include host populations that were sampled at distant locations but whose species ranges overlap (e.g., *Canis latrans* and *Peromyscus maniculatus*).

To determine whether predator–prey interactions between host species provide routes for bacterial transmission, we compared the gut microbiotas of *Puma*, *Lynx*, and *Canis* hosts residing in northeast Washington State with those of their primary prey species (i.e., either artiodactyl or rodent species). Determinations of the primary prey species for each carnivore species were based on field observations of predator behavior in Washington State (55). In these analyses, we included the gut microbiotas of all prey populations in our dataset whose species ranges overlap with the sampling region in northeast Washington State, even if the specific populations of prey sampled were located outside this region.

ACKNOWLEDGMENTS. We thank the editor and three anonymous reviewers for their constructive comments; Veronica Valentenuzzi and Juan Amaya from Centro Regional de Investigaciones Científicas y Transferencia Tecnológica for aiding in the collection of *Ctenomys* samples; and the Leonard X. Bosack and Bette M. Kruger Charitable Foundation for aiding in the collection of the Artiodactyl and Carnivora samples. This work was funded by a Miller Research Fellowship from the Miller Institute for Basic Research in Science at the University of California, Berkeley (to A.H.M.) and NIH Grant R01 GM074245 (to M.W.N.).

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