Title

Gut microbiota parallelism and divergence associated with colonisation of novel habitats

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Running title: Gut microbiota parallelism and colonization

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Abstract

An organism's gut microbiota can change in response to novel environmental conditions, in particular when colonization of new habitats is accompanied by shifts in the host species' ecology. Here, we investigated the gut microbiota of three lizard species (*A. inornata*, *H. maculata*, *S. cowlesi*) from their ancestral-like habitat in the Chihuahuan desert and two colonized habitats with contrasting geological and ecological compositions: the White Sands and Carrizozo lava flow. The host species and the lizards' environment both shape gut microbiota composition, but host effects were overall stronger. Further, we found evidence that colonization of the same environment by independent host species led to parallel changes of the gut microbiota, whereas the colonization of two distinct environments by the same host species led to gut microbiota divergence. Some of the gut microbiota changes that accompanied the colonization of the White Sands were associated with shifts in diet (based on diet information from previous studies), which is congruent with the general observation that trophic ecology has a strong effect on gut microbiota composition. Our study provides insights into how shifts in host ecology accompanying colonization of novel environments can affect gut microbiota composition and diversity.

Keywords: gut microbiome, lizard, ecology, 16S rRNA sequencing, bacteria, reptiles

Introduction

When organisms colonize novel environments, the newly encountered abiotic and biotic conditions impose selective pressures which can lead to changes across a variety of phenotypic traits (Dormontt et al., 2011; Reznick et al., 2019) and ecological divergence of lineages (Grant et al., 1976; Parsons, 1983; Schluter, 2000) as the colonizing populations adapt to the new environment. Natural settings in which independent lineages repeatedly colonized ecologically similar environments and evolved similar phenotypic traits (i.e., parallel or convergent evolution) (Bolnick et al., 2018), can be particularly useful models to investigate the contributions of deterministic and stochastic components of evolutionary change (Colosimo et al., 2005; Reed et al., 2011; Rosenblum et al., 2017).

One important ecological factor that animals may need to cope with when colonizing new environments is a shift in availability and/or diversity of food resources. Trophic adaptation can be in part facilitated by the trillions of microbes inhabiting a host organism's gut (i.e., the gut microbiota) (Zepeda Mendoza et al., 2018) because microbes play a crucial function in nutrient metabolism (Sommer & Backhed, 2013; Turnbaugh et al., 2006). While gut microbiota composition is structured collectively by a range of environmental (e.g., temperature, pH, exposure to environmental microbes) and host-associated factors (e.g., genetics, physiology), diet is of particular importance (Amato et al., 2019; Benson et al., 2010; Moeller et al., 2020; Sepulveda & Moeller, 2020; Spor et al., 2011). Gut microbial communities are highly dynamic and their composition can shift rapidly in response to changes in their host's diet and/or environment (Baniel et al., 2021; Turnbaugh et al., 2009), which can help facilitate ecological niche shifts (Des Roches et al., 2015) and affect evolutionary trajectories of hosts (Kolodny & Schulenburg, 2020; Reznick & Ghalambor, 2001). Thus, uncovering the contributions of microbes to their hosts' evolution is essential for understanding how organisms adapt to novel environmental conditions.

We explored changes in gut microbiota composition and diversity by leveraging a natural system in New Mexico, where three lizard species (*Aspidoscleis inornata*, *Holbrookia maculata*, and *Sceloporus*

cowlesi) colonized the same environment, the White Sands, and one of these species (*S. cowlesi*) also colonized another environment, the Carrizozo lava flow (Figure 1) (Krohn et al., 2019; Rosenblum, 2006). The White Sands (less than 7,000 years old; Kocurek et al., 2007) and Carrizozo lava flow (roughly 5,000 years old; Dunbar, 1999) represent geologically young and unique habitats, which were presumably colonized from the surrounding Chihuahuan desert habitat (hereafter referred to as 'dark soil' to be consistent with previous research). While the highly contrasting substrate color is a striking characteristic across three habitats (Figure 1A & B), we know that many other factors differ between the White Sands and dark soil habitats (the natural history of the Carrizozo lava flow is less understood). For instance, the White Sands differ from the dark soil regarding temperature, water availability, and predation intensity (Dunbar, 1999; Gunderson et al., 2022; Rosenblum et al., 2017). Lizards inhabiting these contrasting environments show adaptive phenotypic differences in coloration (Rosenblum et al 2017), morphology (Robertson et al., 2011; Rosenblum, 2005), behavior (Robertson et al., 2011), and habitat use (Des Roches et al., 2011; Rosenblum & Harmon, 2011). Most relevant for this study, differences in the prey community and potentially trophic niche use between lizard populations in the White Sands and dark soil environments led to higher diet diversity occurring in White Sands lizard populations (Des Roches et al., 2015; Des Roches et al., 2016). Specifically, morphospecies richness is higher in the prey community of the White Sands habitat compared to the dark soil habitat; this was reflected in the diet diversity of the lizard populations as morphospecies richness in the stomach contents of lizards was higher in White Sands lizard populations than in dark soil lizard populations. However, lizard populations from both environments still consume a varied, arthropod-based, diet that includes many species (Des Roches et al., 2015; Des Roches et al., 2016). Further, although the trend is for greater diet diversity in the White Sands, the magnitude of dietary differentiation varies between species: the shift in diet is greater in *A. inornata* than in *S. cowlesi* (Des Roches et al., 2016). These shifts in diet make White Sands lizards a compelling system for investigating the gut microbiota dynamics accompanying ecological shifts associated with the repeated colonization of this unique habitat.

In the present study, we determined the relative contributions of host species and environment on structuring gut microbiota composition and diversity in lizard populations across desert habitats (Figure 1). Because environment and host ecology can be strong predictors of gut microbiota composition, we hypothesized that colonization of the same novel environment, the White Sands, by *A. inornata* and *S. cowlesi* would be accompanied by a pattern of gut microbiota parallelism whereas the isolated colonization of the Carrizozo lava flow by *S. cowlesi* would reflect a pattern of gut microbiota divergence (anti-parallelism). We further investigated three hypotheses motivated by previous studies of *A. inornata* and *S. cowlesi* trophic ecology: (i) a higher diet diversity in White Sands populations has led to higher within-population gut microbiota divergence compared to dark soil populations, (ii) stronger diet differentiation between dark soil and White Sands populations of *A. inornata* compared to *S. cowlesi* is reflected by stronger among-environment gut microbiota divergence in *A. inornata*, and (iii) higher dissimilarity in trophic position among White Sands populations is accompanied by higher among-species gut microbiota divergence.

Materials and Methods

Data collection

Adult male and non-gravid female lizards of three species (*A. inornata*, *H. maculata*, *S. cowlesi*) were collected from the White Sands National Park (*A. inornata*: n = 9, *H. maculata*: n = 15, *S. cowlesi*: n = 15), nearby dark soil sites (*A. inornata*: n = 8, *S. cowlesi*: n = 10), and the Carrizozo lava flow (*S. cowlesi*: n = 13) (Figure 1) in May and June of 2021 and 2022 using a pole and 'slipknot lasso' technique. Please note that one *A. inornata* individual from the dark soil environment was collected at a distinct site (close to the Carrizozo lava flow) compared to dark soil individuals of the same species (close to the White Sands) (Figure 1). We found that whether this host was included or excluded from our analyses did not affect our results qualitatively, hence, we included it in all of our analyses presented here. We were unable to find

dark soil *H. maculata* individuals during our study, hence, we only include data from this species when conducting analyses among White Sands populations.

After capture, sterile cotton swabs were used to sample microbial communities by gently inserting the tip into the cloaca and rotating several times. Lizards were then released at the site of capture. Cloacal swabs allow for standardized and reliable sampling across individuals (in contrast, fecal sampling from natural populations is more logistically challenging and can introduce noise due to variation in time since defecation etc.) and is a minimally invasive sampling technique commonly used in lizards (Colston et al., 2015; Jiang et al., 2017; Martin et al., 2010). Cotton swabs were immersed in RNAlater (Thermo Fisher Scientific, Waltham, MA) and stored at -20°C until DNA extraction. Sampling protocols were approved under permits WHSA-2022-SCI-0006, NMDGF Sci #3184, and IACUC AUP-2014-11-6857-2. Some studies have shown that microbial communities can differ across sampling techniques (i.e., cloacal swabs, feces, intestinal tissue), hence we warrant caution when comparing results among studies (Bunker et al., 2022; Videvall et al., 2018).

DNA was extracted under sterile conditions in a laminar flow hood using the QIAGEN DNeasy Blood & Tissue kit with some modifications (Qiagen, Hilden, Germany). Cotton swabs were air-dried and then fully immersed in 285 µl buffer ATL and 30 µl proteinase K and incubated for one hour at 65°C. After that, 315 µl buffer AL and 315 µl 100% ethanol were added, samples were thoroughly vortexed and applied to a DNeasy Mini spin column. The rest of our protocol followed the manufacturer's recommendations. Next, a 291 bp segment of the V4 region of the 16S rRNA gene was amplified using barcoded 515F and 806R primer (see https://github.com/SchlossLab/MiSeq_WetLab_SOP/blob/master/MiSeq_WetLab_SOP.md). All PCRs were done in triplicate in a 10 µl reaction volume using the Q5 High-Fidelity 2X Master Mix (New England Biolabs, Ipswich, MA) and subsequently pooled. We included negative controls of sterile swabs during DNA extraction and of sterile H_2O during PCR amplification; none of these yielded detectable DNA concentrations after PCR amplification. The PCR protocol consisted of an initial denaturation step for 60 s

at 98 °C, 35 amplification cycles with 10 s at 98 °C, 20 s at 56 °C and 60 s at 72 °C, and a final elongation at 72 °C for 10 min. We confirmed amplification specificity by gel electrophoresis (2% agarose). Using a Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA), we measured DNA concentrations of amplicons, and libraries were constructed by pooling barcoded samples in an equimolar manner. At the UC Davis Genome Center, libraries were purified by bead clean-up, quality checked using a Bioanalyzer and sequenced on the Illumina MiSeq 600 (PE300) platform.

Data filtering

We recovered low sequencing depths for several samples (Table S1) and merging of forward and reverse reads further decreased coverage due to filtering of reads during the merging step. Thus, we decided to use the forward reads for our analyses (250 bp), as these had consistently higher sequence quality compared to reverse reads yet encompassed 86% of the target locus (250 out of 291 bp). Our data set consisted of a total of 3,413,048 raw reads (mean: 48,758 reads/sample; Table S1), that were imported into QIIME2 (Bolyen et al., 2019). We used the QIIME2 plugin *dada2* to check the sequence data quality, correct sequencing reads, filter chimeric sequences and get amplicon sequencing variants (ASVs) (Callahan et al., 2016). Next, a bacterial phylogeny was produced with FastTree 2.1.3 (Price et al., 2010), and taxonomy was assigned based on the SILVA 138 ribosomal RNA (rRNA) database with a 99% similarity threshold (Quast et al., 2013). Before conducting downstream analyses, we removed low-abundance ASVs (< 10 reads) that were only present in one sample, could not be assigned below the phylum level, or were chloroplasts, mitochondria, cyanobacteria, or archaea (Table S1). After these filtering steps, a total of 2,471,580 reads remained (mean: 35,308 reads/sample; Table S1). ASV counts were normalized through scaling using ranked subsampling (SRS) with a C_{min} of 4642 reads (Beule & Karlovsky, 2020) and ASV richness ranged from 18-259 among hosts (mean: 94.3 ASVs/sample).

Gut microbiota alpha and beta diversity

To test for effects of host species identity, environment, host sex, and sampling year on alpha diversity (ASV richness, Faith's phylogenetic diversity, Shannon diversity), we used type III ANOVA followed by non-parametric Wilcoxon rank-sum tests for pairwise comparisons (Wilcoxon, 1945). P-values obtained from Wilcoxon rank-sum tests were corrected for multiple testing using Bonferroni correction. We used multiple alpha diversity metrics to capture different aspects of gut microbiota diversity. ASV richness is purely a richness measure that captures the number of bacterial ASVs, Faith's phylogenetic diversity further incorporates the phylogenetic diversity of bacterial lineages, and Shannon diversity takes into account evenness in abundance of bacterial lineages. For the visualization of microbiota taxonomic composition for host populations (Figure 2A) and individual hosts (Figure S1), only phyla that on average comprised more than one percent of the bacterial community in any of the populations were included. We further visualized gut microbiota composition based on bacterial families and, again, only families comprising more than one percent in any of the populations were included (Figure S2). Differential abundance of bacterial phyla was assessed by analysis of composition of microbiomes (ANCOM) (Table S2). For each colonization event, we further determined the ASVs present in ancestral and derived populations and 49 of these ASVs were shared across the three colonization events. We then assigned taxonomy on the family level and used Fisher's exact test to identify bacterial families that were overrepresented among these shared ASVs. Effects of the aforementioned factors on microbiota divergence (beta diversity; non-phylogenetic: Bray-Curtis dissimilarity, phylogenetic: unweighted and weighted UniFrac) were investigated by PERMANOVA using the *adonis2* function of the R package 'vegan' (Anderson, 2001; Lozupone & Knight, 2005; Lozupone et al., 2011). Similar to alpha diversity, we applied multiple beta diversity metrics which provides insights into the features of the gut microbiota (e.g., relative abundance and phylogenetic diversity) that might explain the observed patterns. To test for differences in the extent of gut microbiota divergence within and among populations, we calculated beta diversity dispersion by

calculating the distance of each host from the centroid of its respective group (*betadisper* function in vegan package). We used the *adonis2* function (vegan package) to calculate *P*-values for the comparison of beta diversity values between groups (Table S6).

Gut microbiota vector analysis

We determined microbiota (non)parallelism with multivariate vector analysis using the R package 'multivarvector' (Härer & Rennison, 2022; 2019). Multivariate vector analysis was originally developed for studying morphological traits (Adams & Collyer, 2009; Collyer & Adams, 2007), and has been used for the study of parallel evolution using both genetic and phenotypic data (Bolnick et al., 2018). This method has recently been proposed to be used more widely in microbiota research (Härer & Rennison, 2022), but thus far has only been applied for studying the gut microbiota of threespine stickleback fish (Rennison et al., 2019). Briefly, multivariate vectors were calculated by connecting the population means (centroids) of PCoA scores to test for (non)parallelism associated with (i) the repeated colonization of the White Sands by *S. cowlesi* and *A. inornata* (γ_1 in Figure 4A) and (ii) the colonization of the White Sands and lava flow by *S. cowlesi* (γ_2 in Figure 4A). Then, we measured angles between these pairs of vectors, whereas the vector directions were consistently estimated from ancestral (dark soil) to derived (White Sands or lava flow) environments. These angles represent quantitative measures of microbiota (non)parallelism associated with the colonization of novel environments; smaller angles (below 90°) indicate parallelism, angles around 90° indicate orthogonal change and larger angles (above 90°) indicate divergence (anti-parallelism). For more detailed discussions on interpreting the distribution of angles and the limitations of this method we refer to Bolnick et al. (2018) and Watanabe (2022). We compared our data to distributions of random angles in multidimensional space (which is centered at 90°) and used Monte Carlo simulations (with 10^5 iterations) to test for (anti-)parallelism. All statistical analyses were done in R v4.2.1 (R_Core_Team, 2021).

Results

To investigate gut microbiota changes associated with the colonization of novel environments, we tested whether alpha diversity (ASV richness, Shannon diversity, Faith's phylogenetic diversity) and beta diversity (Bray-Curtis dissimilarity, unweighted UniFrac, weighted UniFrac) differed between lizard populations from ancestral (dark soil) and derived (White Sands for *A. inornata*, White Sands and Carrizozo lava flow for *S. cowlesi*) environments. In addition, we included a White Sands population of *H. maculata* to test for differences in gut microbiota diversity among the three lizard species within a shared environment.

Host and environment affect alpha diversity, which might be reduced upon colonization of White Sands

The most abundant phyla were largely shared across host populations, but their relative abundances differed strongly across host species (Figure 2A; see Figure S2 for taxonomic bar plots of bacterial families) and some variation was also observed among individuals of the same population (Figure S1). Across all host populations, Actinobacteria, Epsilonbacteraeota, Firmicutes, Tenericutes, and Proteobacteria differed in their abundance among host species whereas only Chloroflexi were differentially abundant among environments (ANCOM; Table S2). When focusing specifically on the different colonization events, Chloroflexi were more abundant in the dark soil population compared to the White Sands and the Carrizozo lava flow in *S. cowlesi* (Table S2), but no phyla were differentially abundant between the dark soil and White Sands populations of *A. inornata*.

Across *A. inornata* and *S. cowlesi*, ASV richness differed between host species (ANOVA; $F = 7.863$, $P = 0.007$) and environments ($F = 3.332$, $P = 0.044$), but there were no effects of host sex or sampling year (Table S3). While ASV richness appeared to be lower for White Sands populations in both species (Figure 2B), pairwise tests revealed no differences in ASV richness for *A. inornata* and only suggestive evidence for differences between dark soil and White Sands populations for *S. cowlesi* (Wilcoxon rank-sum test; $W = 112.5$, $P = 0.080$). Faith's phylogenetic diversity was also affected by host species ($F = 5.856$, $P = 0.019$) and environment ($F = 4.571$, $P = 0.015$) (Table S3), and pairwise comparisons showed lower diversity in

White Sands populations compared to dark soil populations of *A. inornata* (W = 60, P = 0.021) and *S. cowlesi* (W = 119, P = 0.027) (Figure S3A). Results differed for Shannon diversity, where we detected effects of host species (F = 12.439, P < 0.001) and host sex (F = 4.115, P = 0.022) (Table S3), and no differences between ancestral and derived populations based on pairwise comparisons (Figure S3B). Overall, these results suggest that colonization of the White Sands might have been accompanied by a loss in alpha diversity in both host species (based on Faith's phylogenetic diversity), whereas we found no evidence for such an effect for the *S. cowlesi* colonization of the Carrizozo lava flow. Focusing on the lizard gut microbiota of the three species inhabiting the White Sands, we observed higher alpha diversity for two metrics in *S. cowlesi* compared to *A. inornata* (ASV richness: W = 25, P = 0.037, Faith's phylogenetic diversity: W = 19, P = 0.008) and potentially also compared to *H. maculata* (ASV richness: W = 57, P = 0.067, Faith's phylogenetic diversity: W = 46, P = 0.015), but no differences in Shannon diversity were detected.

Evidence for a core microbiota but the majority of bacteria were acquired in the colonized environment

Next, we quantified the proportion of bacterial lineages (ASVs) shared between populations from ancestral and derived environments of the same species to look at the changes in gut microbiota composition accompanying the colonization events. Overall, only a small proportion of ASVs were maintained in the derived populations (White Sands *A. inornata*: 20.49%, White Sands *S. cowlesi*: 18.47%, lava flow *S. cowlesi*: 23.25% (Figure S4). A total of 317 ASVs were shared between ancestral and derived populations, and 49 of these were found across all three colonization events (Figure S4, Table S4). Three bacterial families were enriched among these 49 core ASVs: the Burkholderiaceae (Fisher's exact test: P < 0.001), Corynebacteriaceae (P = 0.002), and Enterobacteriaceae (P < 0.001).

Host and environment shape differences in gut microbiota composition (beta diversity)

We then investigated whether colonization of novel environments was associated with divergence of gut microbiota composition (beta diversity). Patterns were largely similar across the three metrics used, and we present statistical results for Bray-Curtis dissimilarity in the main text unless results were inconsistent

(see Table S5 for test statistics for all three metrics). Bacterial communities differed between host species (PERMANOVA; Bray-Curtis dissimilarity: F = 12.453, r^2 = 0.167, P = 0.001) and environment (Bray-Curtis dissimilarity: F = 4.021, r^2 = 0.108, P = 0.001) for all three metrics (Figure 3 & Table S5), but not between sexes and only unweighted UniFrac showed differences between sampling years (F = 1.950, r^2 = 0.033, P = 0.004). When specifically testing between ancestral and derived populations of each colonization event separately, only dark soil and White Sands populations of *S. cowlesi* showed consistent differences across the three metrics (Bray-Curtis dissimilarity: F = 4.565, r^2 = 0.165, P = 0.005). Additionally, dark soil and White Sands *A. inornata* (F = 1.385, r^2 = 0.084, P = 0.032) as well as dark soil and lava flow *S. cowlesi* (F = 1.922, r^2 = 0.083, P = 0.004) differed based on unweighted UniFrac. Further, host sex had effects on gut microbiota composition in *S. cowlesi* between dark soil and White Sands (F = 3.150, r^2 = 0.113, P = 0.033) as well as lava flow populations (F = 2.660, r^2 = 0.111, P = 0.043) based on weighted UniFrac (Table S5). Considering only the three White Sands populations, gut microbiota composition differed across species for all three metrics (Bray-Curtis dissimilarity: F = 12.517, r^2 = 0.410, P = 0.001) (Table S5). Overall, there was evidence for effects of host species and the environment on bacterial community composition, but this appeared to be mainly driven by changes of the *S. cowlesi* gut microbiota after colonizing the White Sands.

Repeated colonization of White Sands was likely accompanied by gut microbiota parallelism

Next, we tested for gut microbiota (non)parallelism associated with the colonization of novel environments using multivariate vector analysis (Adams & Collyer, 2009; Bolnick et al., 2018; Collyer & Adams, 2007; Härer & Rennison, 2022; Rennison et al., 2019). In accordance with our prediction, we found some, but inconsistent, evidence that the repeated colonization of the White Sands from the dark soil in *S. cowlesi* and *A. inornata* (γ_1 in Figure 4A) was accompanied by parallel gut microbiota changes. For all three metrics, the angles were smaller than or at the lower end of a distribution of random angles but evidence for parallelism was only suggestive for Bray-Curtis dissimilarity (77.18°, P = 0.062) and stronger

for unweighted UniFrac (56.95°, $P < 0.001$) (Figure 4C). In contrast, and consistent with our prediction, the colonization of the White Sands and lava flow by *S. cowlesi* (γ_2 in Figure 4A) was accompanied by divergent gut microbiota changes based on Bray-Curtis dissimilarity (117.46°, $P < 0.001$) and weighted UniFrac (115.74°, $P < 0.001$) (Figure 4B & D). Yet, unweighted UniFrac provided conflicting results as there was strong evidence for gut microbiota parallelism (46.75°, $P < 0.001$) (Figure 4C). In sum, while results were somewhat inconsistent across metrics, we found some evidence for gut microbiota parallelism associated with the repeated colonization of the White Sands by *A. inornata* and *S. cowlesi* and gut microbiota divergence associated with the colonization of the contrasting habitats of the White Sands and the Carrizozo lava flow by *S. cowlesi*.

Some gut microbiota changes are consistent with shifts in diet after colonizing the White Sands

To test for potential associations between gut microbiota diversity and trophic ecology (obtained from previous work; Des Roches et al., 2015; Des Roches et al., 2016), we investigated the extent of gut microbiota divergence (beta diversity distances) within and among dark soil and White Sands populations. For Bray-Curtis dissimilarity, beta diversity was larger in dark soil populations compared to White Sands populations in *S. cowlesi* (PERMDISP; $F = 4.026$, $r^2 = 0.149$, $P = 0.044$) and there was suggestive evidence for a similar pattern in *A. inornata* ($F = 3.263$, $r^2 = 0.179$, $P = 0.081$) (Figure 5A & Table S6) which was contrary to our prediction based on increased diet diversity in the White Sands lizard populations. Yet, unweighted UniFrac showed the opposite trend with marginally larger distances within the White Sands population of *S. cowlesi* compared to the dark soil population ($F = 3.505$, $r^2 = 0.132$, $P = 0.062$) (Table S6) whereas Weighted UniFrac distances did not differ between environments for any of the two species. Among the White Sands populations of *A. inornata* vs. *S. cowlesi*, beta diversity was larger compared to among the dark soil populations only for unweighted UniFrac ($F = 4.080$, $r^2 = 0.093$, $P = 0.035$, Figure 5B), which was concordant with our prediction based on higher dissimilarity in trophic position among White Sands populations i, but no differences were found for the other two metrics. Lastly, , , beta diversity

distances among populations of the same species from dark soil and White Sands environments were larger in *A. inornata* compared to *S. cowlesi*, for Bray-Curtis dissimilarity (F = 4.919, r^2 = 0.110, P = 0.026, Figure 5C), which is concordant with our prediction based on stronger diet differentiation between dark soil and White Sands populations of *A. inornata* compared to *S. cowlesi*. However, no differences were detected for the other two metrics (Table S6).

Discussion

Host and environmental factors collectively shape microbial communities, but their relative contributions are still debated, and they can strongly vary across study systems (Goodrich et al., 2014; Rothschild et al., 2018; Youngblut et al., 2019). Our study provides the first insights into the gut microbiota dynamics associated with the independent colonization of the White Sands by two lizard species (*A. inornata* and *S. cowlesi*), allowing us to investigate if repeated adaptation to novel ecological niches is accompanied by predictable changes in gut microbial communities. Incorporating ecological data for these lizards (Des Roches et al., 2015; Des Roches et al., 2016), we found (i) that the gut microbiota is predominantly structured by host species and, to a lesser degree, by the environment and (ii) evidence that the independent colonization of the White Sands by *A. inornata* and *S. cowlesi* likely led to parallel shifts in the community composition of the gut microbiota. The low replication of lineages within species in our study prohibits any stronger conclusions regarding parallel shifts in the gut microbiota due to colonization and conclusions regarding the role of the gut microbiota during colonization writ large. However, our study is the first description of gut microbiota in this classic system for ecology and evolution (Rosenblum, 2006; Rosenblum & Harmon, 2011), shedding light on the various host-environment-microbiota interactions within it. Further, our experimental approach and results provide resources that future studies can use to investigate the drivers of gut microbiota changes and whether these changes might have any adaptive significance for lizards colonizing this novel environment and other novel environments.

Host and environment together shape gut microbiota composition in A. inornata and S. cowlesi

The host species (Amato et al., 2019; Brooks et al., 2016) and the environment (independent of host species) can affect the gut microbiota (Vasconcelos et al., 2022), and the relative contribution of both factors is often compared. Overall, we found the strongest evidence for gut microbiota divergence between host species, whereas the environmental effect was present but weaker (Table S5).

Across *A. inornata* and *S. cowlesi* from the ancestral and derived environments, the most abundant phyla in both species were Proteobacteria, Actinobacteria, Epsilonbacteraeota and Firmicutes, which is concordant with previous studies on lizards (Jiang et al., 2017; Kohl et al., 2017; Montoya-Ciriaco et al., 2020; Ren et al., 2016; Zhang et al., 2018). Yet despite the major phyla being shared across species, their relative abundances varied strongly *between* species (Figure 2A). In terms of the role of the environment in shaping the gut microbiota, we found contrasting evidence. Within species, relative abundances of the major microbiota phyla were similar among populations in different environments, suggesting that host species plays a strong role in shaping the gut microbiota across New Mexico lizards. However, at the level of ASVs, only 18.47% to 23.25% of ASVs were maintained by host populations after the colonization of a novel environment (Figure S4), suggesting that the environment can have a distinct influence on the gut microbiota. Among the ASVs that were shared between populations from the ancestral and derived environments across the three colonization events (Figure S4), Burkholderiaceae, Corynebacteriaceae, and Enterobacteriaceae were significantly enriched. Because the derived environments differ significantly in all three colonization events, it suggests that these bacterial families are tightly associated with their hosts, independent of ecological and environmental differences. Overall, these results suggest that in these study species, both the host and the environment shape gut microbiota composition. However, without understanding the significance of different microbial families on host function we cannot speculate as to whether these changes in the microbiota composition were reflective of an adaptive shift during or after the colonization process. This is because taxonomically distinct microbes can provide the same functional

role for their host (Louca et al., 2018).Hence, investigating the functional role of the microbial communities in this study (and in many microbiome systems) is an important avenue for future research.

Divergence in diet diversity partially explains gut microbiota variation within and among populations

One crucial factor that is consistently associated with gut microbiota composition is diet and even distantly related host lineages with similar diets can show convergent microbial communities (Brooks et al., 2016). There can also be gut microbiota divergence between host populations within the same species when those populations differ in diet, as is the case between populations of omnivorous and insectivorous *Podarcis* lizards (Lemieux-Labonte et al., 2022). To specifically investigate gut microbiota dynamics associated with changes in diet in our system, we focused on *A. inornata* and *S. cowlesi* populations from the White Sands and dark soil habitats because we could utilize data on their trophic ecology from prior studies (Des Roches et al., 2015; Des Roches et al., 2016). Because the diet data were collected years ago from different individuals than included in our study, we cannot make direct inferences about the association of diet and the microbiota at the individual host level, and rather consider population-level differences. Additionally, we cannot be sure that the diet of the individuals in our study matches the diet of the individuals sampled in the previous studies due to temporal variations in diet, which could weaken our ability to detect associations between diet and gut microbiota composition. However, we attempted to minimize these effects by sampling during the same season (late May to early July) and locations as the referenced diet studies. Further, we found no evidence for an effect of sampling year on our microbiome data (Table S3) which suggests annual effects may be minimal.

Based on increased diet variation in the White Sands populations compared to dark soils populations (Des Roches et al., 2015), we hypothesized that there is higher within-population microbiota divergence in White Sands populations. Support for this hypothesis was inconsistent across metrics. Counter to our hypothesis, there was evidence for higher microbiota divergence in the dark soil population compared to White Sands population in both species when using Bray-Curtis dissimilarity. However, the reverse pattern,

and thus support for our hypothesis, was found when using unweighted UniFrac in *S. cowlesi* (Figure 5A). Bray-Curtis dissimilarity incorporates information on the proportion of shared bacterial lineages and their abundance, the UniFrac metrics incorporate phylogenetic diversity of bacterial communities whereas weighted UniFrac also takes into account abundance. Thus, the discrepancies we found across metrics suggest that there is higher similarity in the overlap and relative abundance of bacterial communities in the dark soil environment for both species whereas the gut microbiota of the *S. cowlesi* White Sands population appears to be more phylogenetically diverse compared to the dark soil population. It is possible that environmental factors other than diet affect different aspects of the gut microbiota differently. The dark soil habitat is generally more variable than the White Sands across a myriad of ecological factors (temperature, water availability, flora/fauna community) (Rosenblum et al., 2017), which could have led to stronger differences in the identity and abundance of bacterial lineages (as measured by Bray-Curtis dissimilarity) but not so much in their phylogenetic diversity (as measured by UniFrac). Hypotheses with regards to which environmental and host-associated factors are impacting different aspects of the gut microbiota should be explicitly tested in the future.

Next, we hypothesized that there is higher between-species microbiota divergence in the White Sands lizard community compared to the dark soil lizard community due to increased trophic dissimilarity in the White Sands (Des Roches et al., 2016). Results matched our prediction only for unweighted UniFrac (Figure 5B), suggesting that the stronger divergence in trophic ecology among lizard species in the White Sands might have shaped the phylogenetic diversity of the gut microbiota, but did not necessarily have strong effects on the abundance of bacterial lineages. Our results based on unweighted UniFrac are in line with prior studies showing strong effects of host diet divergence on gut microbiota composition (Baldo et al., 2017; Youngblut et al., 2019). Lastly, *A. inornata* shows stronger diet differences between White Sands and dark soil habitats compared to *S. cowlesi* (Des Roches et al., 2016). Hence, we tested whether microbiota divergence is larger between White Sands and dark soil populations for *A. inornata* compared

to *S. cowlesi*. Results were variable across metrics, and we only detected lower divergence in *A. inornata* based on Bray-Curtis dissimilarity (Figure 5C) suggesting that identity and abundance of bacterial lineages but not the overall phylogenetic diversity of the gut microbiota differed between populations of these host species. In sum, due to inconsistencies across beta diversity metrics the observed gut microbiota changes were only partially in agreement with predictions based on diet, suggesting that other environmental or host factors such as geographic structuring, genetic divergence, and/or environmental heterogeneity might have stronger effects than diet on these lizards' gut microbiota.

White Sands colonization has led to parallel shifts in gut microbiota composition

The repeated colonization of the White Sands by *A. inornata* and *S. cowlesi* has led to a classic pattern of phenotypic parallelism (Rosenblum, 2006). This phenotypic parallelism appears to have been accompanied by parallel shifts in gut microbiota composition (Figure 4). In contrast, the colonization of White Sands and lava flow by *S. cowlesi* led to divergence of the gut microbiota between the two populations of this lizard species. The main assumption of this type of analysis is that shared selection pressures lead to more determinism in microbial community assembly and, therefore, stronger parallelism. Indeed, empirical and theoretical work has shown that genetic and morphological parallelism is expected to be stronger when parallelism of the selection landscape is high (Stuart et al., 2017; Thompson et al., 2019). In our study system, we predicted lizard populations colonizing the same novel White Sands environment would undergo parallel shifts of their gut microbial communities (Baldo et al., 2017; Ley et al., 2008; Muegge et al., 2011) as a result of a shift in their diet occurring because of a change in the prey community and/or trophic niche use in the White Sands (Des Roches et al., 2015; Des Roches et al., 2016). This prediction was supported as we found evidence for gut microbiota parallelism between 'ancestral' dark soil populations and White Sands populations in *S. cowlesi* and *A. inornata*. Further, we found divergent gut microbiota changes between independent colonization events of the White Sands and lava flow by dark soil *S. cowlesi*. This suggests that the parallelism we found at the White Sands is not simply due to the act of colonizing a

novel environment alone, but potentially a response to shared selection pressures in the colonized environment. It remains to be determined whether gut microbiota parallelism in the White Sands is mainly driven by parallelism of host factors such as trophic ecology or by a shift in shared abiotic conditions. However, because we detected evidence for parallel shifts of the gut microbiota by two species, and similar patterns have been found in threespine stickleback (Rennison et al., 2019), we hypothesize that some of the observed shifts might be adaptive.

Conclusion and future directions

Studying host populations that have adapted to similar conditions via convergent evolution offer a great opportunity to study the repeatability of gut microbiota changes (Laurentino et al., 2022). By leveraging the colonization of the White Sands by multiple lizard species from the dark soils habitat (*A. inornata* and *S. cowlesi* in this study), we investigated how host species, the environment, and diet/trophic ecology shape the gut microbiome. Generally, we found evidence that all these factors influenced the gut microbiota. Conflicting results between ways of measuring diversity and specific diversity metrics highlight that these factors are overly reductive and future studies are needed to understand which specific aspects of diet, the host species, and the environment affect the microbiota. Future work will also be necessary to determine whether the observed gut microbiota variation is advantageous for their hosts in the different environments (e.g., by improving the host's capability to metabolize a broader range of resources). Such studies could include reciprocal transplants or experimental manipulation of the gut microbiota (though these types of studies are challenging in non-model organisms and in natural populations). Such experiments will be critical in determining if the observed gut microbiota variation is due largely to the colonization process itself or rather subsequent local adaptation post-colonization. Ultimately, disentangling the relative contributions of host and environment in structuring the composition and diversity of gut microbial communities has the potential to improve our understanding of how host-microbiota interactions can affect the hosts' ecology and evolution, potentially facilitating or constraining

diversification. Natural colonization events, such as the ones of New Mexico lizards inhabiting geologically young environments, have the potential to greatly contribute to this field.

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References

Adams, D. C., & Collyer, M. L. (2009). A general framework for the analysis of phenotypic trajectories in evolutionary studies. *Evolution*, *63*(5), 1143-1154. https://doi.org/10.1111/j.1558-5646.2009.00649.x

Amato, K. R., Sanders, J. G., Song, S. J., Nute, M., Metcalf, J. L., Thompson, L. R., Morton, J. T., Amir, A., V, J. M., Humphrey, G., Gogul, G., Gaffney, J., A, L. B., G, A. O. B., F, P. C., Di Fiore, A., N, J. D., T, L. G., Gomez, A., . . . Leigh, R. S. (2019). Evolutionary trends in host physiology outweigh dietary niche in structuring primate gut microbiomes. *The ISME Journal*, *13*, 576-587. https://doi.org/10.1038/s41396-018-0175-0

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Australian Journal of Ecology*, *26*(1), 32-46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Baldo, L., Pretus, J. L., Riera, J. L., Musilova, Z., Bitja Nyom, A. R., & Salzburger, W. (2017). Convergence of gut microbiotas in the adaptive radiations of African cichlid fishes. *The ISME Journal*, *11*, 1975-1987. https://doi.org/10.1038/ismej.2017.62

Baniel, A., Amato, K. R., Beehner, J. C., Bergman, T. J., Mercer, A., Perlman, R. F., Petrullo, L., Reitsema, L., Sams, S., Lu, A., & Snyder-Mackler, N. (2021). Seasonal shifts in the gut microbiome indicate plastic responses to diet in wild geladas. *Microbiome*, *9*, 26. https://doi.org/10.1186/s40168-020-00977-9

Benson, A. K., Kelly, S. A., Legge, R., Ma, F. R., Low, S. J., Kim, J., Zhang, M., Oh, P. L., Nehrenberg, D., Hua, K. J., Kachman, S. D., Moriyama, E. N., Walter, J., Peterson, D. A., & Pomp, D. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and

host genetic factors. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(44), 18933-18938. https://doi.org/10.1073/pnas.1007028107

Beule, L., & Karlovsky, P. (2020). Improved normalization of species count data in ecology by scaling with ranked subsampling (SRS): application to microbial communities. *Peerj*, *8*, e9593. https://doi.org/10.7717/peerj.9593

Bolnick, D. I., Barrett, R. D. H., Oke, K. B., Rennison, D. J., & Stuart, Y. E. (2018). (Non)parallel evolution. *Annual Review of Ecology, Evolution, and Systematics*, *49*, 303-330. https://doi.org/10.1146/annurev-ecolsys-110617-062240

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodriguez, A. M., Chase, J., . . . Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852-857. https://doi.org/10.1038/s41587-019-0209-9

Brooks, A. W., Kohl, K. D., Brucker, R. M., van Opstal, E. J., & Bordenstein, S. R. (2016). Phylosymbiosis: relationships and functional effects of microbial communities across host evolutionary history. *PLOS Biology*, *14*(11), e2000225. https://doi.org/10.1371/journal.pbio.2000225

Bunker, M. E., Martin, M. O., & Weiss, S. L. (2022). Recovered microbiome of an oviparous lizard differs across gut and reproductive tissues, cloacal swabs, and faeces. *Molecular Ecology Resources*, *22*(5), 1693-1705. https://doi.org/10.1111/1755-0998.13573

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581-583. https://doi.org/10.1038/Nmeth.3869

Collyer, M. L., & Adams, D. C. (2007). Analysis of two-state multivariate phenotypic change in ecological studies. *Ecology*, *88*(3), 683-692. https://doi.org/10.1890/06-0727

Colosimo, P. F., Hosemann, K. E., Balabhadra, S., Villarreal, G., Jr., Dickson, M., Grimwood, J., Schmutz, J., Myers, R. M., Schluter, D., & Kingsley, D. M. (2005). Widespread parallel evolution in sticklebacks by repeated fixation of Ectodysplasin alleles. *Science*, *307*(5717), 1928-1933. https://doi.org/10.1126/science.1107239

Colston, T. J., Noonan, B. P., & Jackson, C. R. (2015). Phylogenetic Analysis of Bacterial Communities in Different Regions of the Gastrointestinal Tract of Agkistrodon piscivorus, the Cottonmouth Snake. *PLOS ONE*, *10*(6), e0128793. https://doi.org/10.1371/journal.pone.0128793

Des Roches, S., Brinkmeyer, M. S., Harmon, L. J., & Rosenblum, E. B. (2015). Ecological release and directional change in White Sands lizard trophic ecomorphology. *Evolutionary Ecology*, *29*(1), 1-16. https://doi.org/10.1007/s10682-014-9740-9

Des Roches, S., Robertson, J. M., Harmon, L. J., & Rosenblum, E. B. (2011). Ecological release in White Sands lizards. *Ecol Evol*, *1*(4), 571-578. https://doi.org/10.1002/ece3.50

Des Roches, S. D., Harmon, L. J., & Rosenblum, E. B. (2016). Colonization of a novel depauperate habitat leads to trophic niche shifts in three desert lizard species. *Oikos*, *125*(3), 343-353. https://doi.org/10.1111/oik.02493

Dormontt, E. E., Lowe, A. J., & Prentis, P. J. (2011). Is rapid adaptive evolution important in successful invasions. *Fifty years of invasion ecology: the legacy of Charles Elton*, 175-193.

Dunbar, N. W. (1999). Cosmogenic 36 Cl-determined age of the Carrizozo lava flows. *New Mexico Geology*, *21*(2), 25-29.

Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., Beaumont, M., Van Treuren, W., Knight, R., Bell, J. T., Spector, T. D., Clark, A. G., & Ley, R. E. (2014). Human genetics shape the gut microbiome. *Cell*, *159*(4), 789-799. https://doi.org/10.1016/j.cell.2014.09.053

Grant, P. R., Grant, B. R., Smith, J. N., Abbott, I. J., & Abbott, L. K. (1976). Darwin's finches: population variation and natural selection. *Proceedings of the National Academy of Sciences of the United States of America*, *73*(1), 257-261. https://doi.org/10.1073/pnas.73.1.257

Gunderson, A. R., Riddell, E. A., Sears, M. W., & Rosenblum, E. B. (2022). Thermal Costs and Benefits of Replicated Color Evolution in the White Sands Desert Lizard Community. *American Naturalist*, *199*(5), 666-678. https://doi.org/10.1086/719027

Härer, A., & Rennison, D. J. (2022). Quantifying (non)parallelism of gut microbial community change using multivariate vector analysis. *Ecol Evol*, *12*(12), e9674. https://doi.org/10.1002/ece3.9674

Jiang, H. Y., Ma, J. E., Li, J., Zhang, X. J., Li, L. M., He, N., Liu, H. Y., Luo, S. Y., Wu, Z. J., Han, R. C., & Chen, J. P. (2017). Diets Alter the Gut Microbiome of Crocodile Lizards. *Front Microbiol*, *8*, 2073. https://doi.org/10.3389/fmicb.2017.02073

Kocurek, G., Carr, M., Ewing, R., Havholm, K. G., Nagar, Y. C., & Singhvi, A. K. (2007). White Sands dune field, New Mexico: age, dune dynamics and recent accumulations. *Sedimentary Geology*, *197*(3-4), 313-331. https://doi.org/10.1016/j.sedgeo.2006.10.006

Kohl, K. D., Brun, A., Magallanes, M., Brinkerhoff, J., Laspiur, A., Acosta, J. C., Caviedes-Vidal, E., & Bordenstein, S. R. (2017). Gut microbial ecology of lizards: insights into diversity in the wild, effects of captivity, variation across gut regions and transmission. *Molecular Ecology*, *26*(4), 1175-1189. https://doi.org/10.1111/mec.13921

Kolodny, O., & Schulenburg, H. (2020). Microbiome-mediated plasticity directs host evolution along several distinct time scales. *Philosophical Transactions of the Royal Society B-Biological Sciences*, *375*, 20190589. https://doi.org/10.1098/rstb.2019.0589

Krohn, A. R., Diepeveen, E. T., Bi, K., & Rosenblum, E. B. (2019). Local adaptation does not lead to genome-wide differentiation in lava flow lizards. *Ecol Evol*, *9*(12), 6810-6820. https://doi.org/10.1002/ece3.5231

Laurentino, T. G., Dittmer, D. E., Grundler, M. R., Pina-Martins, F., Haddock, J., Hibbitts, T. J., & Rosenblum, E. B. (2022). Repeated evolution of blanched coloration in a lizard across independent white-sand habitats. *Ecol Evol*, *12*(12), e9555. https://doi.org/10.1002/ece3.9555

Lemieux-Labonte, V., Vigliotti, C., Tadic, Z., Wehrle, B., Lopez, P., Bapteste, E., Lapointe, F. J., German, D. P., & Herrel, A. (2022). Proximate drivers of population-level lizard gut microbial diversity: impacts of diet, insularity, and local environment. *Microorganisms*, *10*(8). https://doi.org/10.3390/microorganisms10081550

Ley, R., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., Schlegel, M. L., Tucker, T. A., Schrenzel, M. D., Knight, R., & Gordon, J. I. (2008). Evolution of mammals and their gut microbes. *Science*, *320*(5883), 1647-1651. https://doi.org/10.1126/science.1155725

Louca, S., Polz, M. F., Mazel, F., Albright, M. B. N., Huber, J. A., O'Connor, M. I., Ackermann, M., Hahn, A. S., Srivastava, D. S., Crowe, S. A., Doebeli, M., & Parfrey, L. W. (2018). Function and functional redundancy in microbial systems. *Nature Ecology & Evolution*, *2*(6), 936-943. https://doi.org/10.1038/s41559-018-0519-1

Lozupone, C., & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, *71*(12), 8228-8235. https://doi.org/10.1128/Aem.71.12.8228-8235.2005

Lozupone, C., Lladser, M. E., Knights, D., Stombaugh, J., & Knight, R. (2011). UniFrac: an effective distance metric for microbial community comparison. *The ISME Journal*, *5*(2), 169-172. https://doi.org/10.1038/ismej.2010.133

Martin, M. O., Gilman, F. R., & Weiss, S. L. (2010). Sex-specific symmetry within the cloacal microbiota of the striped plateau lizard, Sceloporus virgatus. *Symbiosis*, *51*, 97-105. https://doi.org/10.1007/s13199-010-0078-y

Moeller, A. H., Ivey, K., Cornwall, M. B., Herr, K., Rede, J., Taylor, E. N., & Gunderson, A. R. (2020). The lizard gut microbiome changes with temperature and is associated with heat tolerance. *Applied and Environmental Microbiology*, *86*(17), e01181-01120. https://doi.org/10.1128/AEM.01181-20

Montoya-Ciriaco, N., Gomez-Acata, S., Munoz-Arenas, L. C., Dendooven, L., Estrada-Torres, A., de la Vega-Perez, A. H. D., & Navarro-Noya, Y. E. (2020). Dietary effects on gut microbiota of the

mesquite lizard Sceloporus grammicus (Wiegmann, 1828) across different altitudes. *Microbiome*, *8*(1), 6. https://doi.org/10.1186/s40168-020-0783-6

Muegge, B. D., Kuczynski, J., Knights, D., Clemente, J. C., Gonzalez, A., Fontana, L., Henrissat, B., Knight, R., & Gordon, J. I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science*, *332*(6032), 970-974. https://doi.org/10.1126/science.1198719

Parsons, P. A. (1983). The evolutionary biology of colonizing species. *Cambridge University Press*, Cambridge.

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2-approximately maximum-likelihood trees for large alignments. *PLOS ONE*, *5*(3), e9490. https://doi.org/10.1371/journal.pone.0009490

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glockner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), 590-596. https://doi.org/10.1093/nar/gks1219

R_Core_Team. (2021). *R: A language and environment for statistical computing*, Vienna, Austria. Retrieved from https://www.R-project.org/.

Reed, R. D., Papa, R., Martin, A., Hines, H. M., Counterman, B. A., Pardo-Diaz, C., Jiggins, C. D., Chamberlain, N. L., Kronforst, M. R., Chen, R., Halder, G., Nijhout, H. F., & McMillan, W. O. (2011). optix drives the repeated convergent evolution of butterfly wing pattern mimicry [Research Support, Non-U.S. Gov't

Research Support, U.S. Gov't, Non-P.H.S.]. *Science*, *333*(6046), 1137-1141. https://doi.org/10.1126/science.1208227

Ren, T. T., Kahrl, A. F., Wu, M., & Cox, R. M. (2016). Does adaptive radiation of a host lineage promote ecological diversity of its bacterial communities? A test using gut microbiota of Anolis lizards. *Molecular Ecology*, *25*(19), 4793-4804. https://doi.org/10.1111/mec.13796

Rennison, D. J., Rudman, S. M., & Schluter, D. (2019). Parallel changes in gut microbiome composition and function during colonization, local adaptation and ecological speciation. *Proceedings of the Royal Society B-Biological Sciences*, *286*(1916), 20191911. https://doi.org/10.1098/rspb.2019.1911

Reznick, D. N., & Ghalambor, C. K. (2001). The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, *112*, 183-198. https://doi.org/10.1023/A:1013352109042

Reznick, D. N., Losos, J., & Travis, J. (2019). From low to high gear: there has been a paradigm shift in our understanding of evolution. *Ecol Lett*, *22*(2), 233-244. https://doi.org/10.1111/ele.13189

Robertson, J. M., Hoversten, K., Gründler, M., Poorten, T. J., Hews, D. K., & Rosenblum, E. B. (2011). Colonization of novel White Sands habitat is associated with changes in lizard anti-predator behaviour. *Biological Journal of the Linnean Society*, *103*(3), 657-667. https://doi.org/10.1111/j.1095-8312.2011.01644.x

Rosenblum, E. B. (2005). The role of phenotypic plasticity in color variation of Tularosa basin lizards. *Copeia*, *3*, 586-596. https://doi.org/10.1643/CP-04-154R1

Rosenblum, E. B. (2006). Convergent evolution and divergent selection: lizards at the White Sands ecotone. *American Naturalist*, *167*(1), 1-15. https://doi.org/10.1086/498397

Rosenblum, E. B., & Harmon, L. J. (2011). "Same same but different": replicated ecological speciation at White Sands. *Evolution*, *65*(4), 946-960. https://doi.org/10.1111/j.1558-5646.2010.01190.x

Rosenblum, E. B., Parent, C. E., Diepeveen, E. T., Noss, C., & Bi, K. (2017). Convergent phenotypic evolution despite contrasting demographic histories in the fauna of White Sands. *American Naturalist*, *190*(S1), S45-S56. https://doi.org/10.1086/692138

Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D., Costea, P. I., Godneva, A., Kalka, I. N., Bar, N., Shilo, S., Lador, D., Vila, A. V., Zmora, N., Pevsner-Fischer, M., Israeli, D.,

Kosower, N., Malka, G., Wolf, B. C., . . . Segal, E. (2018). Environment dominates over host genetics in shaping human gut microbiota. *Nature*, *555*(7695), 210-215. https://doi.org/10.1038/nature25973

Schluter, D. (2000). *The ecology of adaptive radiation*. Oxford University Press.

Sepulveda, J., & Moeller, A. H. (2020). The effects of temperature on animal gut microbiomes. *Frontiers in Microbiology*, *11*, 384. https://doi.org/10.3389/fmicb.2020.00384

Sommer, F., & Backhed, F. (2013). The gut microbiota - masters of host development and physiology. *Nature Reviews Microbiology*, *11*(4), 227-238. https://doi.org/10.1038/nrmicro2974

Spor, A., Koren, O., & Ley, R. (2011). Unravelling the effects of the environment and host genotype on the gut microbiome. *Nature Reviews Microbiology*, *9*(4), 279-290. https://doi.org/10.1038/nrmicro2540

Stuart, Y. E., Veen, T., Weber, J. N., Hanson, D., Ravinet, M., Lohman, B. K., Thompson, C. J., Tasneem, T., Doggett, A., Izen, R., Ahmed, N., Barrett, R. D. H., Hendry, A. P., Peichel, C. L., & Bolnick, D. I. (2017). Contrasting effects of environment and genetics generate a continuum of parallel evolution. *Nature Ecology & Evolution*, *1*, 0158. https://doi.org/10.1038/s41559-017-0158

Thompson, K. A., Osmond, M. M., & Schluter, D. (2019). Parallel genetic evolution and speciation from standing variation. *Evolution Letters*, *3*(2), 129-141. https://doi.org/10.1002/evl3.106

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, *444*(7122), 1027-1031. https://doi.org/10.1038/nature05414

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., & Gordon, J. I. (2009). The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Science Translational Medicine*, *1*(6), 6ra14. https://doi.org/10.1126/scitranslmed.3000322

Vasconcelos, D., Harris, D. J., Damas-Moreira, I., Pereira, A., & Xavier, R. (2022). What shapes the gut microbiome of lizards from different habitats? *ResearchSquare*, Preprint. https://doi.org/10.21203/rs.3.rs-1298977/v2

Videvall, E., Strandh, M., Engelbrecht, A., Cloete, S., & Cornwallis, C. K. (2018). Measuring the gut microbiome in birds: comparison of faecal and cloacal sampling. *Molecular Ecology Resources*, *18*(3), 424-434. https://doi.org/10.1111/1755-0998.12744

Watanabe, J. (2022). Detecting (non)parallel evolution in multidimensional spaces: angles, correlations and eigenanalysis. *Biol Lett*, *18*(2), 20210638. https://doi.org/10.1098/rsbl.2021.0638

Wilcoxon, F. (1945). Individual comparisons by ranking methods. *Biometrics Bulletin*, *1*(6), 80-83. https://doi.org/10.2307/3001968

Youngblut, N. D., Reischer, G. H., Walters, W., Schuster, N., Walzer, C., Stalder, G., Ley, R. E., & Farnleitner, A. H. (2019). Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. *Nature Communications*, *10*, 2200. https://doi.org/10.1038/s41467-019-10191-3

Zepeda Mendoza, M. L., Xiong, Z., Escalera-Zamudio, M., Runge, A. K., Theze, J., Streicker, D., Frank, H. K., Loza-Rubio, E., Liu, S., Ryder, O. A., Samaniego Castruita, J. A., Katzourakis, A., Pacheco, G., Taboada, B., Lober, U., Pybus, O. G., Li, Y., Rojas-Anaya, E., Bohmann, K., . . . Gilbert, M. P. T. (2018). Hologenomic adaptations underlying the evolution of sanguivory in the common vampire bat. *Nature Ecology & Evolution*, *2*(4), 659-668. https://doi.org/10.1038/s41559-018-0476-8

Zhang, W., Li, N., Tang, X., Liu, N., & Zhao, W. (2018). Changes in intestinal microbiota across an altitudinal gradient in the lizard Phrynocephalus vlangalii. *Ecol Evol*, *8*(9), 4695-4703. https://doi.org/10.1002/ece3.4029

Data Accessibility and Benefit-Sharing

We deposited all raw sequencing data (https://figshare.com/s/465d64120f6fc59aefc1), as well as data files and R scripts (https://figshare.com/s/28011077f41d832ecb5e) in the figshare database.

Author Contributions

A.H. and A.A.M. conceptualized the study. A.A.M. and T.G.L. collected fecal swabs in the field, A.H. conducted all molecular work and analyzed the data. A.H. and A.A.M. wrote the manuscript with input from all co-authors.

Figures

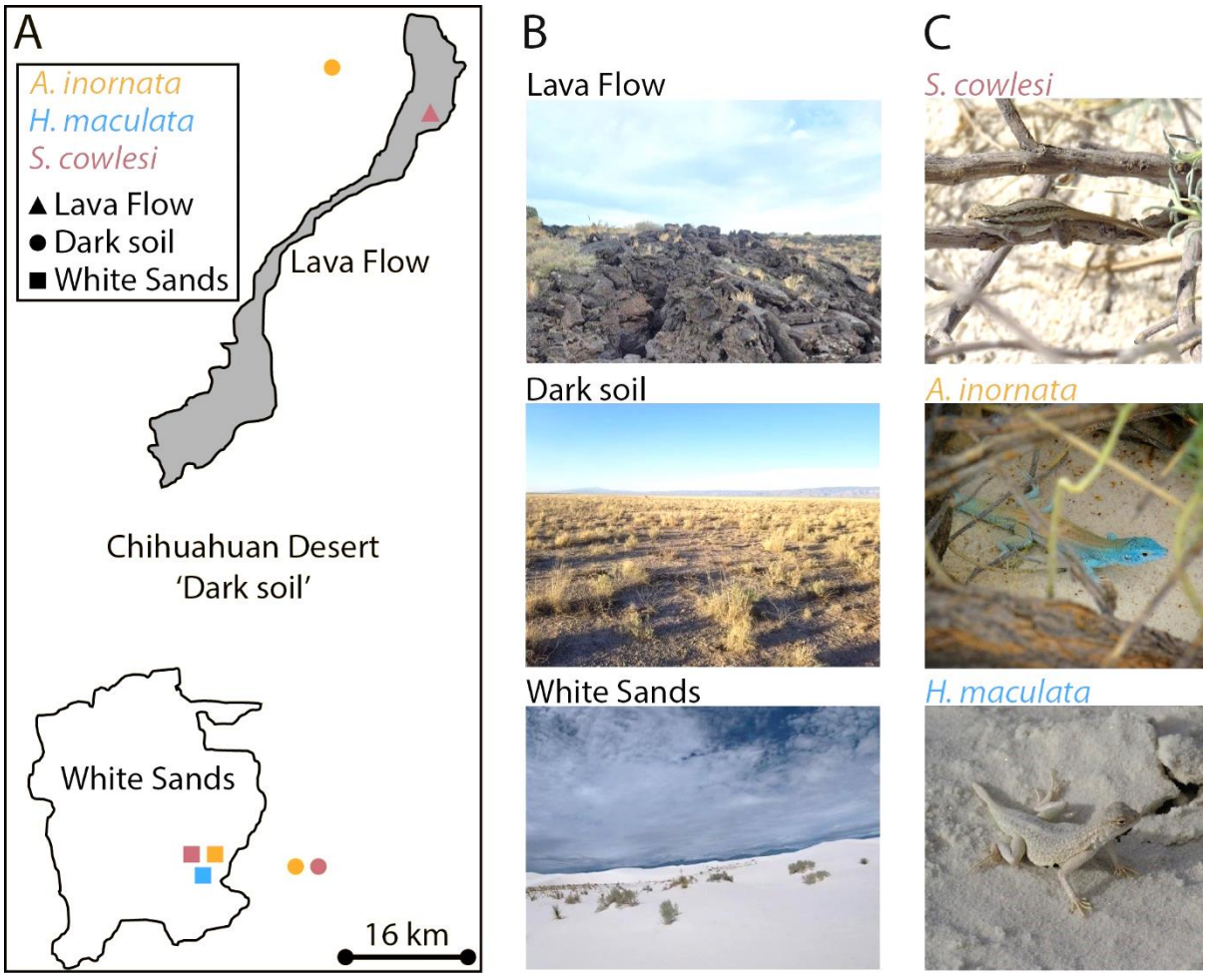

Figure 1: Map of the study area with sampling locations (A), examples of the respective habitats and photos of the three lizard species (B & C). The White Sands and Lava Flow habitats were colonized from

the surrounding desert within the last 7,000 years. The habitats where samples were collected are indicated by symbols and lizard species by colors (A).

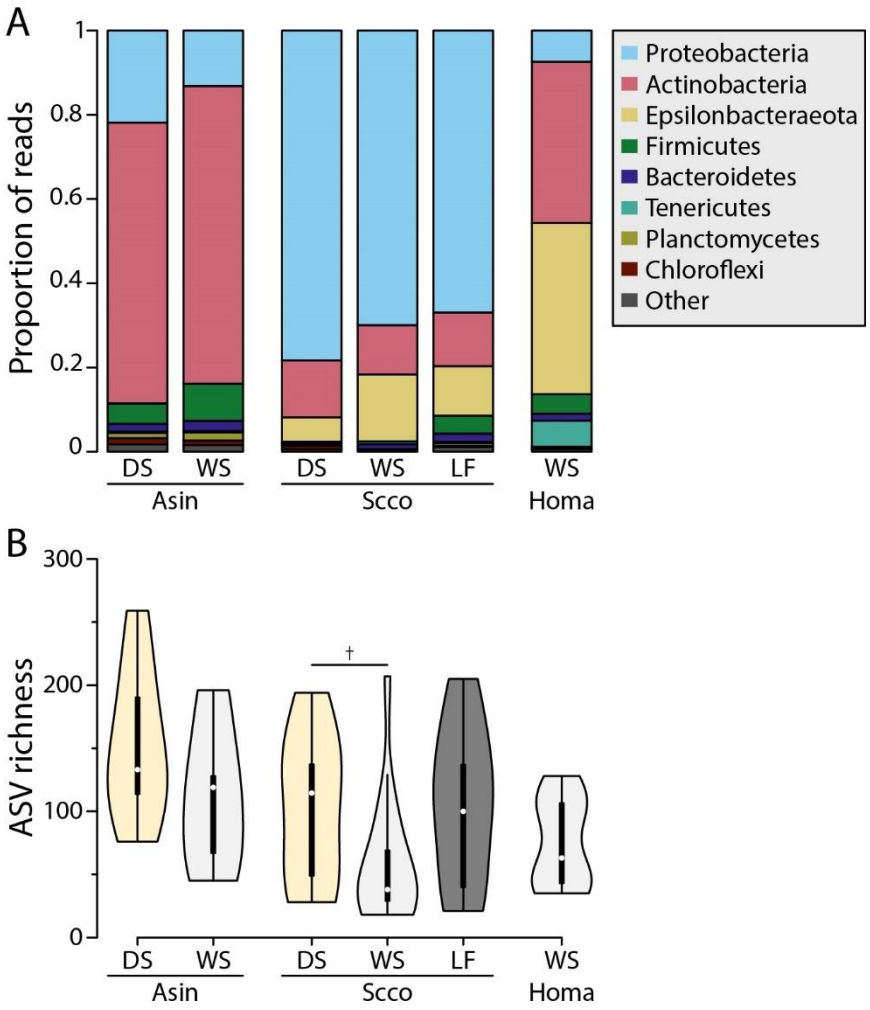

Figure 2: Taxonomic bar plots showing the relative abundances of the eight major phyla (mean proportion of >1% in any population). The lizards' gut microbiota were largely comprised of Proteobacteria, Actinobacteria, Epsilonbacteraeota and Firmicutes, but relative abundances differed across species. The position of phyla follows the order indicated in the box on the right (A). Further taxonomic bar plots are shown for individual hosts on the phylum level in Figure S1 and for host populations on the family level in Figure S2. Bacterial alpha diversity (ASV richness) was largely similar across populations from different habitats, but there was a tendency for lower values in the White Sands populations of *A. inornata* and *S. cowlesi* (B). DS: dark soil, WS: White Sands, LF: Carrizozo lava flow, Asin: *A. inornata*, Scco: *S. cowlesi*, Homa: *H. maculata*. $^{\dagger}P < 0.1$.

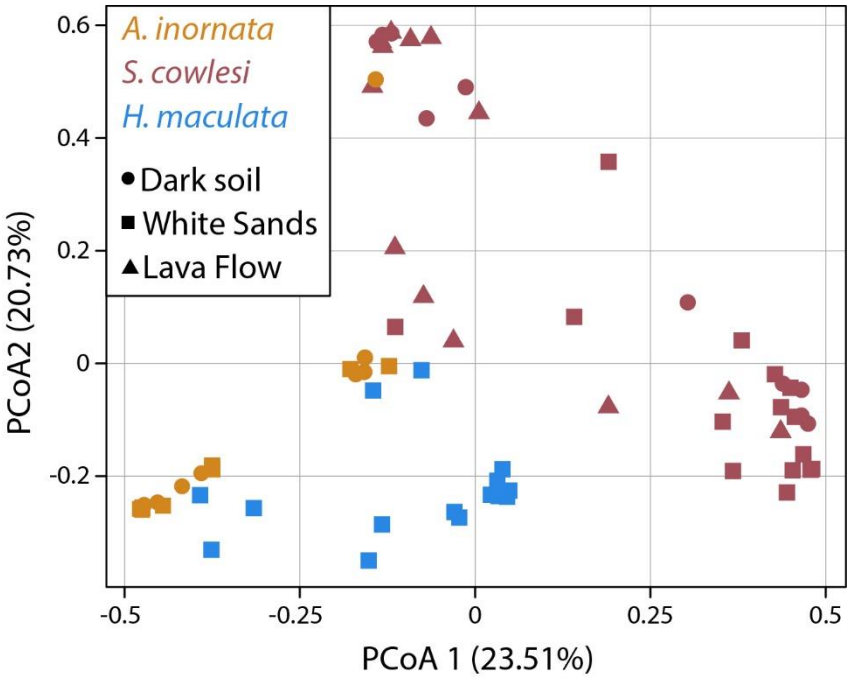

Figure 3: Principal coordinate analysis of the three lizard species (indicated by colors) from different habitats (indicated by symbols) based on Bray-Curtis dissimilarity. The three species are clearly separated along the first two axes, and there also is some clustering by habitat in *S. cowlesi*.

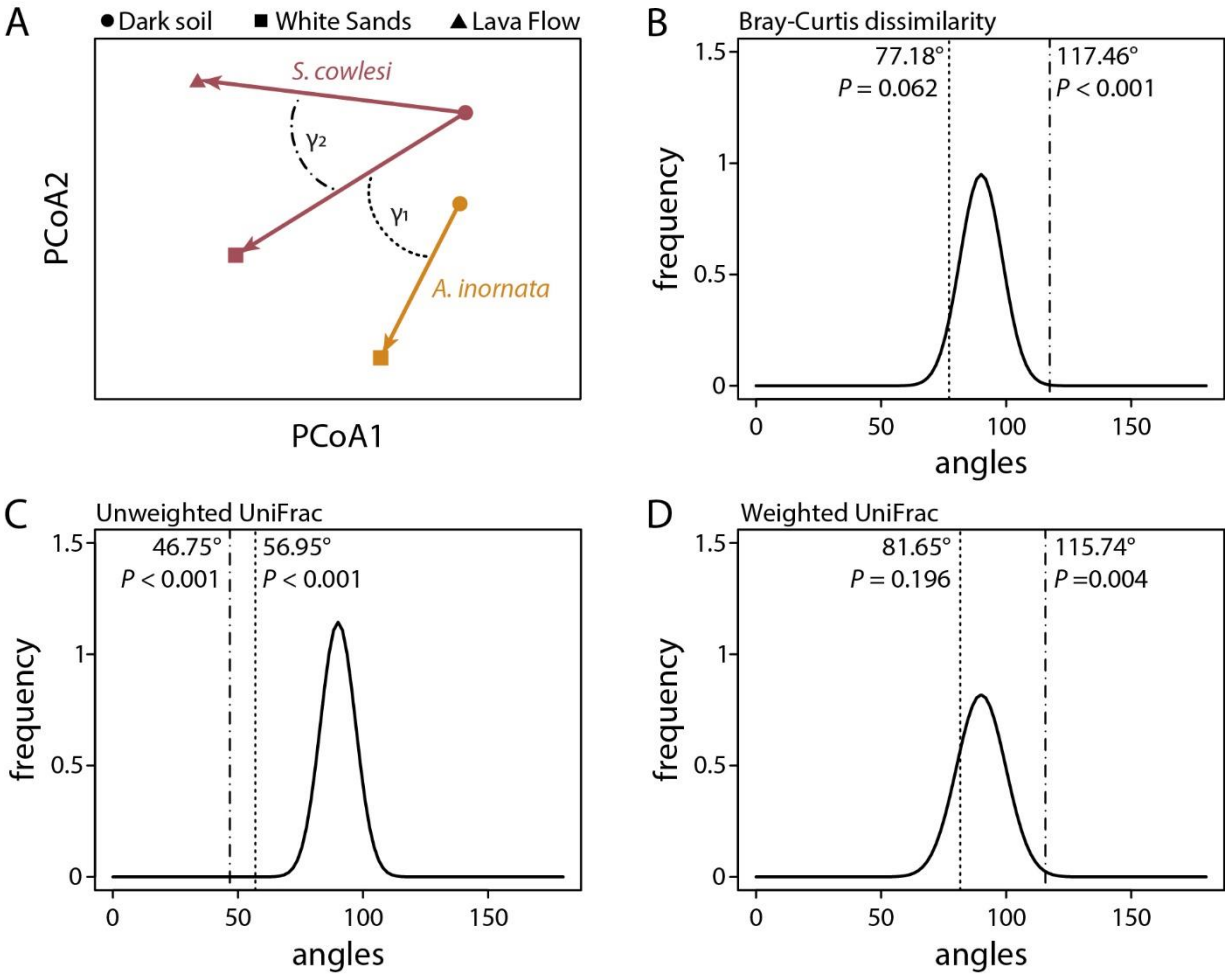

Figure 4: Multivariate vectors are calculated from PCoA scores based on gut microbiota beta diversity metrics. Vectors connect the population means (centroids) between 'ancestral' and colonizing populations to estimate a quantitative measure of microbiota (non)parallelism associated with the colonization of White Sands by *A. inornata* and *S. cowlesi* (γ_1) and White Sands and lava flow by *S. cowlesi* (γ_2) (A). The calculated angles (vertical dashed lines) were compared against the multidimensional distribution of random angles (solid curve), and the shape of the curves depends on the number of PCoA axes. There was suggestive evidence for parallelism during the colonization of White Sands by *A. inornata* and *S. cowlesi* based on Bray-Curtis dissimilarity (B), and stronger evidence based on unweighted UniFrac (C). For the colonization of White Sands and lava flow by *S. cowlesi*, divergence was detected for Bray-Curtis dissimilarity (B) and weighted UniFrac (D), but parallelism was found based on unweighted UniFrac (C).

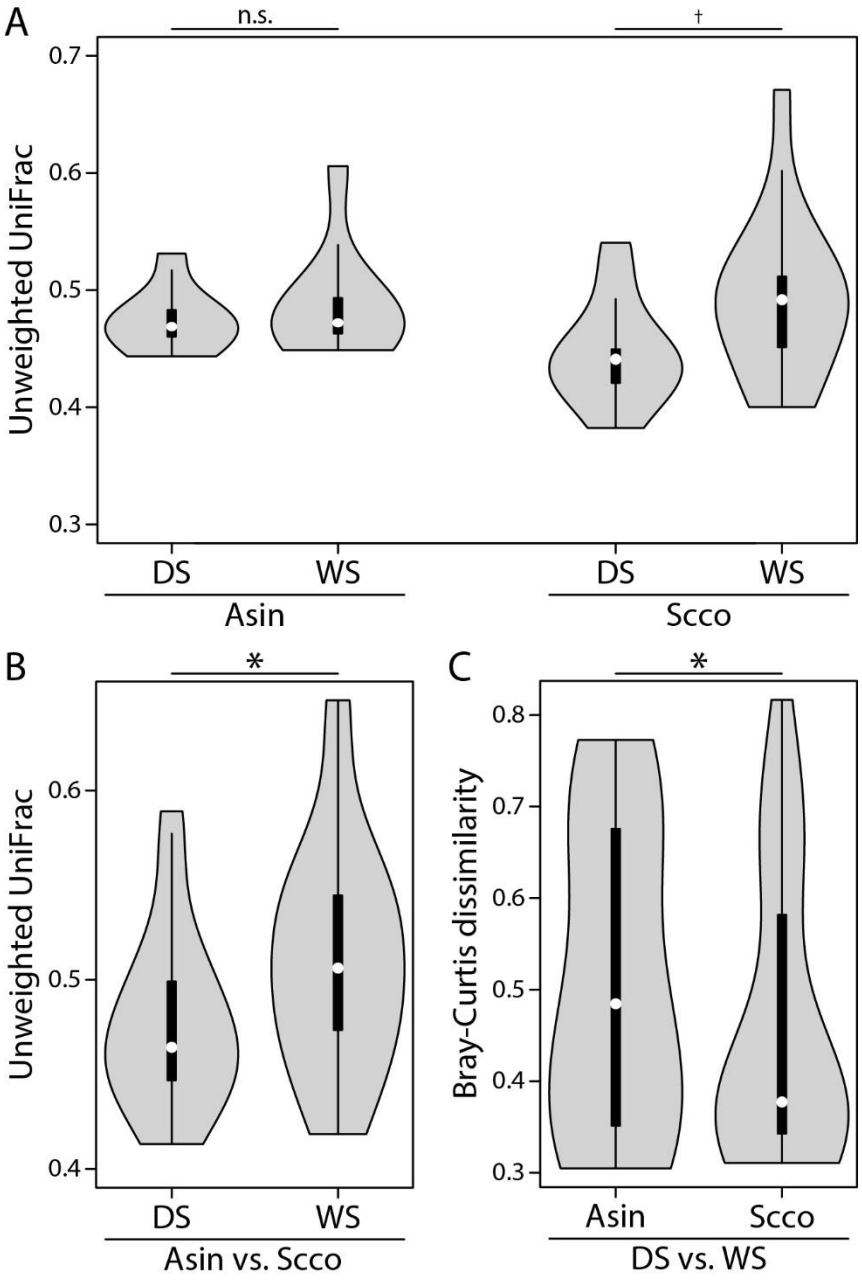

Figure 5: Solely analyzing beta diversity within and among *A. inornata* and *S. cowlesi* populations from the dark soil and White Sands habitats revealed some evidence that unweighted UniFrac distances might be larger within White Sands populations for *S. cowlesi* (A). Unweighted UniFrac distances were also larger among White Sands populations of the two species (B), and Bray-Curtis distances were larger among White Sands and desert populations of *A. inornata* compared to *S. cowlesi* (C). Yet, results differed across beta diversity metrics for these comparisons and here we illustrated results that were in accordance with our predictions (all test statistics can be found in Table S6). (Wilcoxon rank-sum test, † $P < 0.1$, *$P < 0.05$)