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ORIGINAL RESEARCH

Parthenogenic hybrid geckos differ from their sexual counterparts in skin microbiomes but not in rates of water loss

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Abstract

The success of parthenogenic populations is facilitated by high fecundity, though studies show that parthenogenic hybrids can express traits of compromised fitness compared with their parent species, such as higher rates of evaporative water loss and greater susceptibility to ectoparasites. Recent works highlighting the importance of microbiomes on host health also show that both host lineage and the environment contribute to an animal's microbiome. In this study, we investigated how reproductive mode and the presence of mites affect evaporative water loss and if the skin bacterial microbiome differs between sympatric sexual and parthenogenic populations of the gecko *Heteronotia binoei* in central Australia. We collected sexual (n = 17) and parthenogenic (n = 66) *H. binoei* from six local sites (within 3 km of each other) to measure evaporative water loss, record mite infestations, and characterise skin bacterial communities from a subsample of individuals (n = 17 per reproductive mode). Only parthenogenic individuals had mites, and mite infestations were not severe at our site. We found that neither reproductive mode nor the presence of mites affected evaporative water loss in our study populations, contrary to prior studies elsewhere for the species. In microbiome analyses, we found that reproductive mode significantly predicted community structure and composition; however, local site explained more of the observed variation than other variables. While these findings challenge previously observed differences in physiology between parthenogenic and sexual *H. binoei*, we found support for ectoparasite susceptibility in parthenogenic individuals. Our microbiome results reinforce that even in sympatry, host lineages harbour unique microbiomes, although the environment largely influences the skin bacterial communities in our study system.

Introduction

Parthenogenic populations of reptiles resulting from hybridisation can exhibit distinct phenotypes from their sexual parent populations, which may improve or reduce fitness compared to the parent species (Arnold, 1997; Barton, 2001). In whiptail lizards (*Aspidocelis* spp.), parthenogens have reduced endurance compared to sexual individuals, though they perform equally in several other measures, such as burst speed and metabolic rate (Cullum, 1997). Studies of geckos (*Heteronotia binoei*) have found differences in some morphological and physiological parameters between parthenogenic and sexually reproducing populations, such as relatively larger heads, lower critical thermal maximum, and less sensitivity to incubation temperature in parthenogens (Kearney & Shine, 2004a, 2004b; Roberts et al., 2012). In contrast to whiptails, hybrid parthenogenic *H. binoei* have greater endurance, maximum aerobic speeds, and other activity measures at low temperatures (Kearney et al., 2005). Understanding how parthenogenic populations differ from their parent populations in physiology and ecology can give insight into the advantages and disadvantages of asexual reproductive strategies.

While clonal reproduction can increase individual fitness levels, parthenogenic populations may have a limited capacity to co-evolve and reduce susceptibility to parasites as they lack recombination and, thus, the capacity for rapid adaptation

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associated with sexual reproduction (Moritz et al., 1991; Van Valen, 1973). High parasite load can negatively impact a host's fitness, including reductions in endurance and sprint speed (Lee et al., 2023; Orton et al., 2020), while high ectoparasite load can also increase evaporative water loss (EWL), as found in both lizards and honeybees (Annoscia et al., 2012; Baldwin, 1999). Elevated water loss due to ectoparasites is attributed to excess moisture loss directly from the parasite or damage to the host's skin. Studies of host skin damage caused by mites reveal highly modified tissue associated with an inflammatory response, leading to ulcers and scabbing (Goldberg & Holshuh, 1992; Shatrov, 2009), which may cause damage by directly increasing skin permeability, compromising resistance to cutaneous water loss. Rates of EWL were positively correlated with mite load in parthenogenic geckos (Kearney & Shine, 2004b). However, elevated EWL in parthenogenic compared to sexual individuals carried over to captive-bred, parasite-free offspring, suggesting the observed physiological difference may have been independent of mite load (Kearney & Shine, 2004b; Moritz et al., 1991).

Alongside ectoparasites, hybrids may differ in other symbiotic relationships that contribute to or may be caused by physiological differences, such as their bacterial microbiomes. In many arthropods, the bacterium *Wolbachia* can impact hybrid success and sterility (Miller et al., 2021). Differences in gut microbiomes among vertebrates and their hybrids, including fish and mammals, may be associated with varying ecologies (e.g., diet) as well as physiological differences (Miller et al., 2021). Conceptually, the extent to which microbiomes in and on hybrid hosts overlap with those of the parent species depends on the broad ecological and physiological factors that impact microbiome assembly and maintenance (Camper et al., 2024). For some host–microbiome systems, such as gut microbiomes in diverse animal taxa or skin microbiomes on frogs (Rebollar et al., 2020; West et al., 2019), there is considerable data on the importance and roles of bacterial communities, and we may be able to predict causes and effects of shifts away from communities on parent species in hybrid taxa. However, little is known about bacterial communities on the skin of lizards, though research indicates that they can have greater diversity than sympatric frogs (Weitzman et al., 2018). Even geckos may harbour diverse skin communities (Weitzman et al., 2018), despite data suggesting self-cleaning, anti-bacterial properties of gecko skin (Li et al., 2016; Watson et al., 2015). Differences between skin bacterial communities on hybrid geckos living in sympatry with their parent species could suggest diverging ecological and physiological traits, such as shifts in microhabitat use or skin and scale anatomy.

We studied *H. binoei* at a site in central Australia, where hybrid parthenogens are sympatric with a sexually reproducing parent genotype, to test the hypothesis that these two reproductive modes exhibit differing physiology and ecology. We investigated four variables to test for these differences: body condition, rates of EWL, ectoparasitic mite infection, and skin bacterial communities. Based on previous studies (Kearney & Shine, 2004a, 2004b), we predicted that parthenogenic individuals would have greater body condition and higher rates of EWL than sympatric, sexually reproducing individuals. We further predicted that parthenogens have a greater prevalence of mites infecting their skin and that ectoparasitic mite infection would correspond with increased EWL rates, aligning with previous results in the system (Kearney & Shine, 2004b; Moritz et al., 1991). If the two reproductive modes sampled here differ in bacterial communities, it would strengthen the argument that the hybrid parthenogen lineage has ecologically diverged from its parental genotypes. In other systems, sympatric species support different skin microbiome communities (Christian et al., 2018), and such a difference could be impacted by both microhabitat and physiological properties supporting those communities.

Materials and methods

Study system and gecko capture

We compared sexual and parthenogenic races of *H. binoei*, a diverse but currently unresolved species complex of Australian geckos that consists of more than 20 sexually reproducing candidate species (Fujita et al., 2010; Moritz et al., 2016; Zozaya et al., 2022, 2025) and several clonally reproducing, triploid, parthenogenic races of hybrid origin (Moritz, 1984; Moritz et al., 1989; Moritz & Heideman, 1993). The parthenogenic races originated through hybridisation between two sexually reproducing, diploid lineages — CA6 and SM6 — that produced hybrid diploids that subsequently backcrossed with one of the two parental lineages, thus making the triploid races (Moritz, 1984; Moritz et al., 1989; Moritz & Heideman, 1993). Triploid races possess a double dose (i.e., two copies per chromosome) of the genome that the initial diploid hybrids backcrossed with, and they are phenotypically similar to whichever of the two sexual lineages contributed the double genome dose (Moritz, 1984). The CA6 lineage and all triploid races are restricted to the Australian arid zone, while the SM6 lineage occurs across the arid zone as well as tropical woodlands. Here, we compared a CA6 sexual population with sympatric parthenogenic hybrid clones (3N1 parthenogens, with CA6 double genome dosage) in the MacDonnell Ranges of central Australia (Fig. 1). Both CA6 and 3N1 geckos exhibit banded colour patterns and occupy Australia's central and western deserts. Comparing these populations, which are phenotypically similar and can be found side by side at a single site, reduces differences due to genome dosage and eliminates differences stemming from variation in local environments.

In March 2022 and May 2023, we hand-captured a total of 83 *H. binoei* geckos from the vicinity of Ross River Station, Hale, Northern Territory, from six local sites within 3 km of each other. Geckos were caught among multiple local sites to increase sample sizes, ensuring we caught enough of each reproductive type. Geckos captured in the evening (80/83) were processed the following morning. Processing was conducted in the following order: measuring EWL, swabbing the skin for microbiome samples, taking morphological measurements, and then collecting the tail-tip as a genetic sample, which was subsequently stored in RNAlater. The presence and relative load of mites were noted during measurement collection. For one individual (2022, sexual), we collected a

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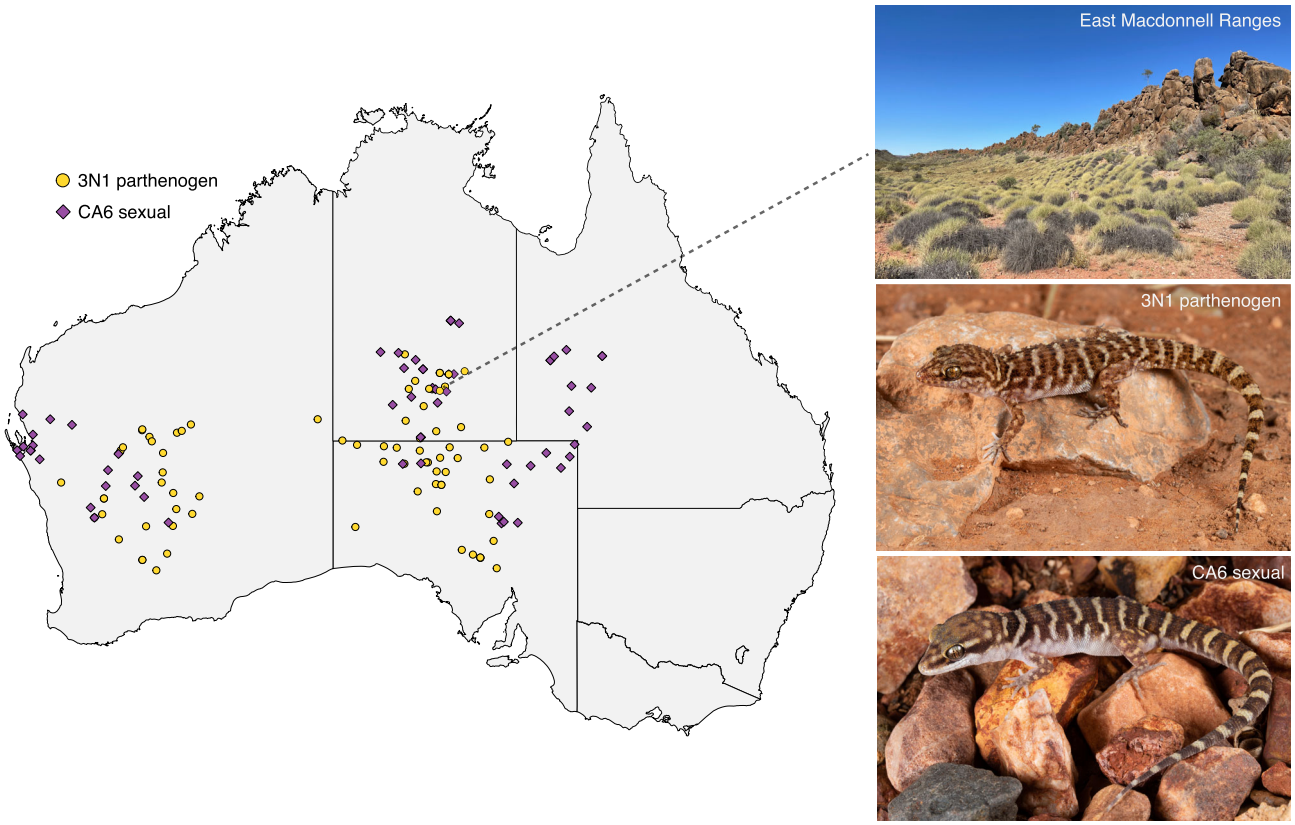

Figure 1 Map of mainland Australia (left) showing distributions of the two sampled *Heteronotia binoei* lineages: CA6 sexuals (purple diamonds) and 3N1 parthenogens (yellow circles). Dashed line indicates the location of the study site (Ross River Station), with a habitat photo of the area in the top right. An example 3N1 parthenogen is shown on the middle-right, while a CA6 sexual is shown on the bottom-right. At Ross River Station, the two reproductive modes look indistinguishable. Photo credits: Stephen Zozaya.

microbiome sample but no water loss data, as its skin was damaged during capture.

Evaporative water loss and measurements

Gecko EWL was measured on-site using a portable flow-through system (Blamires & Christian, 1999). Ambient room air was drawn through silica gel drying columns using low-flow samplers calibrated to 0.2 L min^{-1} (model LFS-113, Gilian®) to create desiccating conditions. We then pumped dry air through an experimental chamber (13.5 × 2.6 cm) that housed an individual gecko. A highly sensitive probe (model HMP 110, Vaisala) measured EWL by recording the relative humidity and temperature of the experimental air downstream from the gecko. We recorded continuous data using an Apple MacBook paired with an ADInstruments PowerLab and LabChart software (model PL3508, ADInstruments Pty Ltd, Bella Vista, Australia). We also recorded data from the running system without a gecko in the experimental chamber to record a baseline reading, and the small amount of water remaining in the air was subtracted from the experimental readings. The water loss system was housed across three incubators (model XHC-25, IVYX Scientific) set to 30°C to maintain a stable experimental temperature. Five experimental lines were operated simultaneously, and the geckos were left in the experiment until a 5-min reading of stable relative humidity was achieved, indicating a rest period. The gecko's skin temperature was immediately measured from the dorsum of the gecko using an infrared thermometer (Traceable© mini, Thomas Scientific) upon removing the gecko from the experimental chamber. Each gecko spent 30–60 min in the experiment. If a gecko defecated during the experiment, it was re-run in the following experiment group. Data files were processed by selecting 5 min from the gecko's lowest recorded relative humidity paired with the corresponding temperature data. A 5-min baseline measure was chosen to pair with each of the geckos' EWL data.

We collected a series of measurements with callipers to estimate surface area. Straight-line measurements (mm) included snout-vent length (SVL), tail length, body width, tail width, and forelimb and hindlimb length and width, along with mass (g). Width measurements were collected at the widest point of the respective body region. Surface area was estimated assuming the torso (snout to vent), tail, and legs were separate, single-ended cylinders (Belasen et al., 2017; Blamires & Christian, 1999; Chukwuka et al., 2020; Day et al., 2025). EWL

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rate, surface area, and skin temperature were used to calculate total resistance to water loss (Spotila & Berman, 1976).

Skin bacterial microbiome collection and sample processing

At the end of each round of EWL (up to 5 geckos per round), we collected skin swab samples to describe bacterial microbiomes. Geckos were rinsed with 75 mL high-pure water and swabbed with a nylon flocked swab (Copan Diagnostics Inc., cat. 520CS01, Murrieta, CA, USA) for approximately 10 s to sample the entire body, avoiding the head and cloaca. Swabs were broken into prepared microcentrifuge tubes with 300 μL Zymo DNA/RNA Shield (Zymo Research, Irvine, CA, USA) and chilled in the field until frozen at −20°C. Alongside gecko samples, we also collected environmental controls in the field, exposing swabs to the water and air at the swabbing location, storing and extracting these control samples alongside gecko samples. We extracted DNA from swab samples with the Norgen Microbiome DNA Isolation Kit (Norgen Biotek Corp., Thorold, ON, Canada). We verified the presence of bacterial DNA in the samples with quantitative PCR, amplifying a portion of the V4 region of bacterial 16S rRNA gene with the Earth Microbiome Project primers, 515F/806R (Apprill et al., 2015; Caporaso et al., 2011; Parada et al., 2016).

We used amplicon sequencing to describe the bacterial communities in our samples. Only samples from individuals without mites were included in microbiome assessments to remove ectoparasites as a confounding factor. DNA from an equal number of samples from the two reproductive modes (n = 17 each, Table 1) was sent to Ramaciotti Centre for Genomics for library prep using the Earth Microbiome Project primers (Apprill et al., 2015; Caporaso et al., 2011; Parada et al., 2016) and sequencing on an Illumina MiSeq (2 × 250 bp chemistry) alongside samples from other geckos. Four environmental control samples from this study were included in the sequencing run. We used DADA2 in QIIME2 v2023.7 (Bolyen et al., 2019; Callahan et al., 2016) to quality-filter, merge paired-end reads, and remove chimeric reads, trimming off primers and truncating reads to remove low-quality ends (218 bases forward, 166 bases reverse), with otherwise default parameters. Taxonomy of amplicon sequence variants (ASVs) was assigned with sklearn with the fitted classifier from the Silva v138 database (Pedregosa et al., 2011; Quast et al., 2012; Yilmaz et al., 2014). Only reads assigned as Bacteria were kept, further excluding those assigned as chloroplasts or mitochondria. With the decontam and phyloseq packages in R v4.4.2 (Davis et al., 2018; McMurdie & Holmes, 2013; R Core Team, 2024), we used the prevalence method to identify contaminant ASVs based on the four control samples. The probability threshold of 0.1 detected 17 contaminant ASVs representing 0.24% of the filtered gecko reads; those contaminant ASVs and the control samples were removed from further processing and analyses.

As spurious taxa likely remained after the initial quality processing, we removed ASVs that did not occur at a relative abundance above 0.1% in any one sample, and further removed ASVs present in only one sample (Reitmeier et al., 2021). For most analyses (see Statistics below), we normalised the data to the lowest sampling depth of the samples included in the study (14 050 reads per sample). With the normalised dataset, we calculated bacterial richness in the samples (ASV richness) and two metrics of beta diversity: Jaccard (based on the presence–absence of bacterial ASVs) and Bray–Curtis dissimilarity (based on the relative abundance of bacterial ASVs) for statistical analyses.

Table 1 Sample sizes of Bynoe's geckos (*Heteronotia binoei*) included in bacterial microbiome analyses

	2022		2023	
Local Site	Parthenogen	Sexual	Parthenogen	Sexual
Camp			4	4
Creek1	3	1		
Creek2			1	4
Dump	2	2	1	1
Homestead			5	2
River	1	3		

Local sites are <3 km from each other. River, Homestead, and Creek1 sites are all within 0.5 km of each other; Camp and Creek2 are 0.5 km apart. All samples for amplicon sequencing were collected from geckos free of mite infection.

Mitochondrial DNA sequencing and identification

The reproductive mode of individual geckos was determined using mitochondrial DNA sequencing and phylogenetic analysis. Parthenogenic races of *H. binoei* have mtDNA reflecting the lineage of the mother in the initial hybridisation event (3N1 for origin with CA6 mother; 3N2 for origin with SM6 mother; Moritz, 1984). While the mtDNA of parthenogens is phylogenetically nested within one of these two sexual populations, they each form a tightly clustered monophyletic group within them, making identification of triploids versus diploids simple with mtDNA data. We identified individuals to the population by sequencing 1041 bp of the *nd2* protein-coding locus, with PCR primers and protocols following Strasburg and Kearney et al. (2005), and with PCR products Sanger sequenced with an AB 3730xl DNA Analyzer at the ACRF Biomolecular Resource Facility, John Curtin School of Medical Research, Australian National University. Sequence chromatograms were trimmed in Geneious Prime 2021.2.2 with a 0.05 error probability threshold, followed by pairwise alignment using the Geneious Alignment to produce a consensus sequence for each individual. Newly generated sequences (n = 83; see Results) were aligned to 16 published sequences (Table S1; Kearney et al., 2006; Moritz et al., 2016; Strasburg & Kearney, 2005) representing samples of the CA6 (n = 4) and SM6 (specifically SM6C; n = 4) sexual lineages, and the 3N1 (n = 4) and 3 N2 (n = 4) parthenogenic triploid races. Sequence alignment was performed using Clustal Omega 1.2.3 implemented in Geneious 2021.2.2 with default settings, followed by visual inspection to check for unexpected gaps or frame shifts. Finally, we calculated pairwise distances among

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all sequences with the 'dist.dna' function in ape v5.7–1 (Paradis & Schliep, 2019) and used the resulting matrix to create a neighbour-joining tree using the 'nj' function. We use a neighbour-joining tree here because we simply needed to determine the population identity of each sample, rather than accurately infer deeper relationships and branch lengths among mtDNA haplotypes.

Statistical analyses

Data were analysed using R v4.4.2 in RStudio v2024.09.0 (Posit Team, 2024; R Core Team, 2024) unless otherwise stated. Where relevant, we calculated partial *P*-values using the 'Anova' function (type II tests) in the *car* package to determine significance (Fox & Weisberg, 2019).

Linear models were used for all analyses of rates of EWL (log-transformed), with surface area as a covariate. First, we determined whether the two sympatric reproductive modes of *H. binoei* differed in rates of EWL. Sampling year was initially included in the analysis of reproductive mode but was not significant and was removed from the final analysis. Because mites were only found on parthenogenic individuals, we analysed the impact of the presence of mites on EWL with a data subset that included only the parthenogens.

Next, we used body condition to determine whether the two reproductive modes differ in shape and robustness, defining body condition as mass divided by SVL (Sion et al., 2021). We removed juveniles (SVL < 30 mm; retaining $n = 66$ adults) for this analysis to remove ontogenetic factors and compared body condition values between the two reproductive modes with an ANOVA. Adults of the two reproductive modes did not differ in SVL.

Using skin bacterial microbiome data, we conducted multiple analyses to detect differences in communities between the two reproductive modes, as well as influences of other factors that could explain variation in skin bacteria. First, we used an ANOVA to assess the effects of reproductive mode and sampling year on bacterial richness. The remaining analyses of beta diversity and differential abundance were performed in QIIME2 v2023.7 (Bolyen et al., 2019). For each beta diversity metric, we used 'adonis2' to conduct permutational MANOVAs (PERMANOVA) to test the effects of sampling year, local site, reproductive mode, and the interaction between year and reproductive mode. This method accounts for the impacts of sampling year and local site before measuring the difference between the two reproductive modes. For significant predictor variables, we further tested whether this difference was impacted by a difference in dispersion.

As another way of assessing the degree to which bacterial communities differ between the two reproductive modes, we ran analyses of differential abundance (ANCOM-bc; Lin & Peddada, 2020) to determine if relative abundances of specific bacterial taxa were greater on one reproductive mode of geckos versus the other. We ran these analyses with the complete data set (reads not normalised) at the levels of bacterial genus and phylum. We focus the results of these analyses on taxa that represent at least 0.1% of the dataset.

Lastly, we aimed to investigate whether there is an interplay between the geckos' water loss physiology and their skin bacterial communities. We addressed this question using total resistance to water loss as the physiological predictor variable, as the value accounts for differences in size among individuals. We removed the individual that was not included in the water loss experiment and ran PERMANOVAs on both beta diversity metrics to detect the significance of total resistance on community structure. Analyses included local site as a covariate.

Results

We sampled 66 parthenogenic (2022 $n = 37$, 2023 $n = 29$) and 17 sexually reproducing (2022 $n = 6$, 2023 $n = 11$) *H. binoei* from Ross River Station, NT, verified by neighbour-joining phylogenetic analysis of mitochondrial *nd2* sequences (Fig. S1). Two parthenogens from 2023 were shedding during the water loss experiment and had the two highest rates of water loss of all those measured (>0.1 mg min^{-1}); therefore, we removed these individuals from water loss analyses.

The two reproductive modes did not differ in rates of EWL (Fig. 2a; $F_{(1,77)} = 0.75$, $P = 0.4$). Though previous work found that infection with mites can increase rates of EWL (Kearney & Shine, 2004b), we found that the parthenogenic geckos with mites did not have differing water loss rates from those without mites ($F_{(1,61)} = 0.52$, $P = 0.5$); however, we only found mites on 10 individuals at relatively low infection intensity, with mites only observed around the eyes (Fig. S2). In adult geckos, body condition differed between the reproductive modes (Fig. 2b; $F_{(1,62)} = 9.35$, $P = 0.003$), with parthenogenic individuals on average having 27% greater body condition.

Bacterial communities on the geckos were largely comprised of the phyla Bacteroidota, Proteobacteria, and Firmicutes (Fig. S3), in particular, *Burkholderia* (Proteobacteria, family Burkholderiaceae) and uncultured Flavobacteriaceae (Bacteroidota) on sexually reproducing individuals and *Bacteroides* (Bacteroidota, family Bacteroidaceae) and *Parabacteroides* (Bacteroidota, family Tannerellaceae) on parthenogens (Fig. S4).

In our analyses, only beta diversity, but not alpha diversity, was correlated with our predictor variables. Bacterial richness did not differ between the two reproductive modes or sampling years (Fig. S5; reproductive mode: $F_{(1,31)} = 1.11$, $P = 0.3$; sampling year: $F_{(1,31)} = 2.80$, $P = 0.1$). For Jaccard dissimilarity, reproductive mode was a significant predictor of community structure (Fig. 3a; $F_{(1,25)} = 1.48$, $R^2 = 0.040$, $P = 0.02$) as was sampling year ($F_{(1,25)} = 2.29$, $R^2 = 0.062$, $P = 0.001$), with no significant interaction between the two ($F_{(1,25)} = 1.19$, $R^2 = 0.032$, $P = 0.1$). Local site explained more variation than the other predictor variables (Fig. 3b; $F_{(5,25)} = 1.41$, $R^2 = 0.190$, $P = 0.001$), with many significant differences in pairwise analyses (Table S2). Dispersion did not differ between groups for any of the variables (year: $F = 0.70$, $P = 0.6$; site: $F = 0.99$, $P = 0.7$; mode: $F = 0.49$, $P = 0.5$). For Bray–Curtis dissimilarity, year, reproductive mode, and their interaction were significant predictors of beta diversity (Fig. 3c; year: $F_{(1,25)} = 3.86$, $R^2 = 0.096$, $P = 0.001$; reproductive mode: $F_{(1,25)} = 1.54$, $R^2 = 0.038$, $P = 0.048$; interaction:

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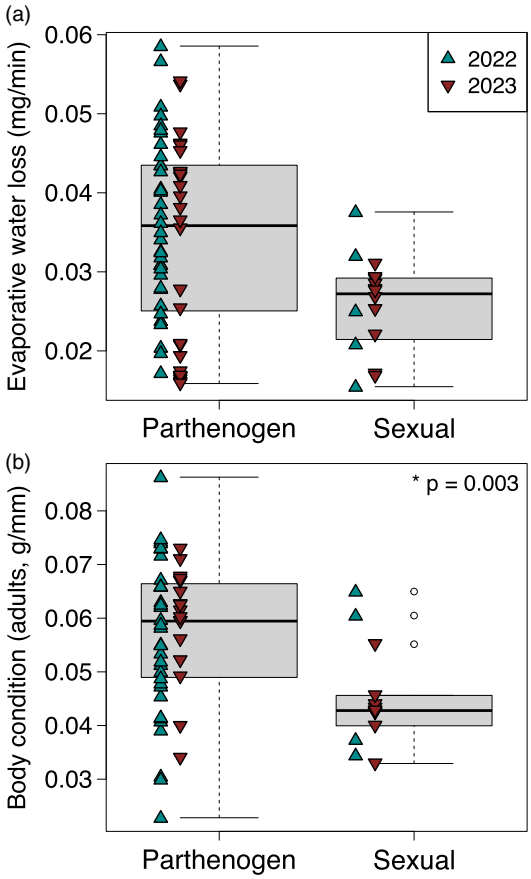

Figure 2 *Heteronotia binoei* geckos representing two reproductive modes did not differ in rate of evaporative water loss (a), but did differ in body condition (b). Boxplots of evaporative water loss are predicted values from the linear model to account for surface area. The shape and colour of points denote the sampling year. The year was not significant in either metric. On average, adult parthenogenic geckos had 27% greater body condition (mass/snout-vent length) than sexually reproducing adults (from estimated marginal means of linear model results).

$F_{(1,25)} = 1.64$, $R^2 = 0.040$, $P = 0.04$). Post-hoc pairwise analyses indicated that the two reproductive modes differed in the first year of the experiment but not the second (2022 comparison: pseudo-$F = 2.52$, adjusted-$P = 0.004$; 2023 comparison: pseudo-$F = 1.12$, adjusted-$P = 0.3$). Again, the local site explained more variation than the other predictor variables (Fig. 3d; $F_{(5,25)} = 1.67$, $R^2 = 0.207$, $P = 0.001$), with many significant differences in pairwise analyses (Table S2). Sampling years and local sites did not differ in beta dispersion (year: $F = 0.34$, $P = 0.6$; local site: $F = 1.36$, $P = 0.2$), though dispersion was lower in communities on sexually reproducing geckos than parthenogens ($F = 4.33$, $P = 0.02$).

We found that 13 bacterial genera differed in abundance between the two reproductive modes in a differential abundance analysis, with five genera enriched in parthenogenic individuals and eight enriched in sexually reproducing individuals (Fig. S6). Most of these genera had over 4× the relative abundance in the enriched group compared with the other reproductive mode. Four phyla were enriched on parthenogenic geckos, with two of these, Bacteroidota and Firmicutes, accounting for a large proportion of the communities, on average 45% and 17%, respectively (Figs S3 and S6). Members of these phyla likely account for a significant portion of the differences observed between the reproductive modes.

Resistance to water loss was a significant predictor in the analysis of Jaccard dissimilarity ($F_{(1,26)} = 1.33$, $P = 0.048$) and only marginally significant for Bray–Curtis dissimilarity ($F_{(1,26)} = 1.52$, $P = 0.07$), though it only accounts for ~4% of the variation in either metric (Fig. S7).

Discussion

We found mixed support for our hypothesis that *H. binoei* hybrid parthenogens and their sympatric, sexually reproducing parent genotype differ in physiology and ecology (specifically, symbioses). At a single site in central Australia, parthenogens exhibited hybrid vigour through increased body condition, corresponding with previous data of larger mass-adjusted head sizes and hatchling SVL in *H. binoei* hybrids (Kearney & Shine, 2004a, 2004b). Mites were only found on parthenogenic geckos, consistent with earlier studies (Kearney & Shine, 2004b; Moritz et al., 1991). However, our predictions that EWL differs between the two reproductive modes and is influenced by mites were not supported. Our results on the reproductive modes harbouring divergent skin bacterial communities suggest further differences in their ecology.

Although we did not find a difference in EWL between parthenogenic geckos with and without mites, the mite load was generally limited to a few around the eyes. Mite loads in the region are lower than found elsewhere and vary temporally, with very low loads observed in the past, making our results not out of the ordinary (Kearney & Shine, 2004b). Greater infestations are likely to result in more open wounds and higher rates of EWL (Kearney & Shine, 2004b). Although Kearney and Shine (2004b) found higher rates of EWL in parthenogenic *H. binoei* without mites compared to the sexual form, there was no difference between the two reproductive modes at Ross River. This may be attributable to the two different approaches to measuring EWL used in our study and the previous study. In this study, the continuous measurements of water loss allowed us to select periods of inactivity for comparative measures of EWL, because activity resulted in high and variable rates of EWL, whereas the measurements from resting geckos were reproducibly low and constant. The method of measuring mass loss over a 16 or 24-h period (Kearney & Shine, 2004b) incorporates periods of activity with periods of rest. Thus, higher rates of EWL by parthenogens could be due to higher rates of activity rather than differences in cutaneous permeability. Additionally, the study sampled geckos from multiple sites, grouping individuals within regions; perhaps there was an influence of geography, climate, or lineage on the significant difference in EWL previously found.

Parthenogenic and sexually reproducing geckos differed in their bacterial microbiomes on the skin, with enrichment and

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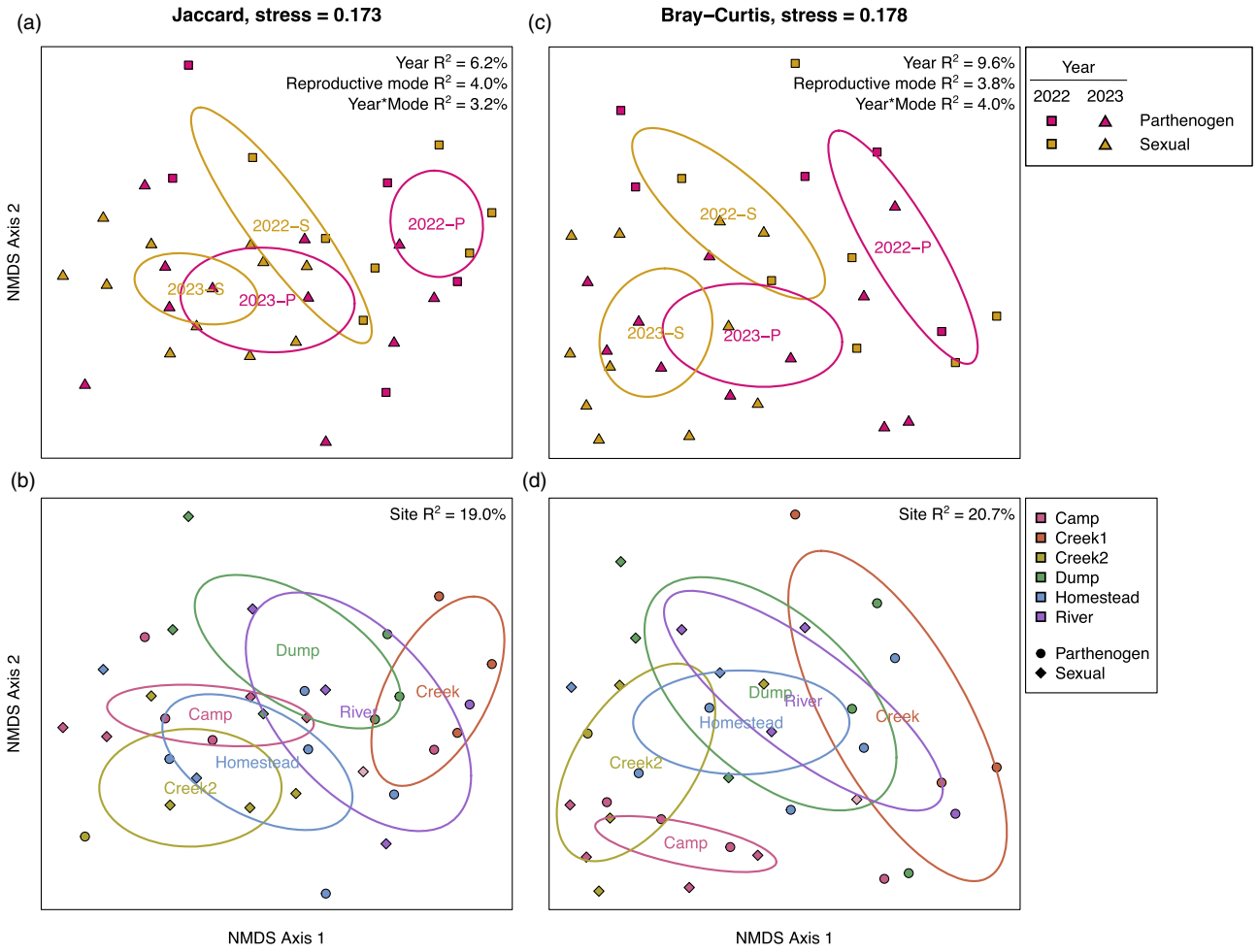

Figure 3 Ordinations of bacterial communities on *Heteronotia binoei* geckos from a site in Central Australia show impacts of reproductive mode, sampling year, and local sampling site. Reproductive mode and year account for relatively small amounts of variation in (a) Jaccard and (c) Bray–Curtis dissimilarity compared with the variation explained by local site [(b) Jaccard, (d) Bray–Curtis]. R^2 values are from PERMANOVAs with adonis2.

depletion of specific taxa, despite being found in sympatry within the sampled area in central Australia. As in other hosts with limited parental care, we presume that gecko skin bacterial communities are largely comprised of taxa filtered from the environment (McGrath-Blaser et al., 2021; Walke et al., 2014). Our results may suggest divergent microhabitat use in the two gecko groups studied here, as well as differing taxa retained from the environment. Though unstudied, we presume that scale microstructure plays an important part in skin bacterial colonisation and maintenance. Consequently, if the two reproductive modes of *H. binoei* differ in skin morphology and physiology, that could create divergent environments for bacterial assemblage. Alternatively, the two reproductive modes may differ in immunological parameters that impact skin communities. In amphibians, hosts interact closely with their skin communities via immunological feedback through their mucosal skin surfaces (Woodhams et al., 2023). We are aware of no immunological studies in this system that can explain the divergent skin environments and communities, or whether such close interactions occur in reptiles. Although there is emerging literature on the reptile skin microbiome focusing on microbial shifts associated with pathogen invasion (Ross et al., 2019), the mechanisms for direct feedback between host and skin taxa in reptilian systems remain unclear. Thus far, research suggests that skin microbes can aid in resistance or pathogen clearance, as microbes on snakes can inhibit the fungal pathogen *Ophidiomyces ophidiicola* (Hill et al., 2018), and probiotics can help control bacterial skin ulcer disease in crocodile lizards (*Shinisaurus crocodilurus*; Xiong et al., 2022). Interactions between symbiotic microbes and invading pathogens can lead to shifts in bacterial communities, for instance, when invading *O. ophidiicola* alters snake skin bacterial communities, resulting in dysbiosis (Allender et al., 2018; Romer et al., 2022, 2024). In contrast, we lack data on whether skin communities impact reptilian host susceptibility to invasion, and whether hosts select for beneficial taxa or if they are present in the

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community by chance (as has been explored in amphibians; Loudon et al., 2016). Notably, however, the link we found between resistance to water loss and bacterial community structure reveals a possible interplay between the gecko host and its skin bacteria.

Despite differences in reproductive modes, sampling year and, especially, local sampling site, explained more variation in the gecko skin microbiomes. The effect of year may be impacted by differing seasons sampled (March 2022 vs. May 2023), considering our growing understanding of the role of weather on gecko skin bacteria (C. Weitzman and K. Christian, *unpublished data*). Local site, however, was the best predictor of bacterial communities, explaining approximately 20% of the variation. This phenomenon may be more influenced by different microhabitats at the sites than by an effect of geographical distance, since microbiomes from the nearby sites of homestead, river, and creek, have little overlap in ordination space. Other studies tend to group samples collected within a few kilometres of each other into host populations, comparing microbiomes among more distant sites. A large-scale investigation of snakes revealed the impacts of time and location (over a greater distance) on skin communities, although their predictive power depends on the species and geographic scale (Walker et al., 2019). Frogs, however, harbour divergent communities from others just a few kilometres away (Albecker et al., 2019; Christian et al., 2018).

Our sampling included individuals of a hybrid-parthenogenic clone and one parent lineage (CA6) but not the second parent lineage (SM6). The study of the patterns, causes, and effects of bacterial communities in and on hybrid lineages is still nascent (Camper et al., 2024; Miller et al., 2021) in comparison to physiological studies in hybrid systems. Future work sampling both parental lineages and their hybrids would reveal the relative influences and evolutionary shifts away from the parent types, with bacterial studies further uncovering physiological and ecological patterns in these systems, as well as shedding light on the processes and factors determining host-associated microbiome assembly (Camper et al., 2024).

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Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

KC conceived the study. CLW, KD, SMZ, KS, and KC collected the data. CLW and SMZ analysed the data. CLW, KD, SMZ, and KC led the writing of the manuscript. GPB, KG, and KC provided funding. KG and KC provided resources. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

Bacterial amplicon sequence data are available on NCBI's Sequence Read Archive (BioProject accession: PRJNA1150660). Sequences for newly sequenced samples of the *nd2* locus have been deposited to GenBank (accession nos. PV600652–PV600734). All remaining data are available in the supplementary materials (Table S3).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Neighbour-joining phylogeny based on 1041 bp of the mitochondrial ND2 locus used to assign sampled geckos to their respective populations.

Figure S2. Parthenogenic *H. binoei* moderately infected with mites near the eye.

Figure S3. Bacterial phyla in skin communities on *H. binoei*.

Figure S4. Bacterial genera (or lowest taxon identified) in skin communities on *H. binoei*.

Figure S5. Richness of bacterial ASVs on parthenogenic and sexually reproducing *H. binoei* geckos did not differ between the two reproductive modes or the two sampling years.

Figure S6. Differential abundance analyses (ANCOM-bc) detected multiple bacterial taxa enriched or depleted in parthenogenic *H. binoei* compared with sympatric, sexually-reproducing conspecifics.

Figure S7. Ordinations depict the direction of association between skin bacterial communities on *H. binoei* and the skin's total resistance to water loss.

Table S1. Published ND2 sequences were used as references to identify samples in our phylogenetic analysis.

Table S2. Pairwise beta diversity analysis (PERMANOVA) results between local sites.

Table S3. Evaporative water loss data and metadata used in analyses to compare parthenogenic and sexually-reproducting *H. binoei* geckos.

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