Received: 10 February 2022 | Revised: 1 September 2022 | Accepted: 6 September 2022

DOI: 10.1111/mec.16696

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Adapting to climate with limited genetic diversity: Nucleotide, DNA methylation and microbiome variation among populations of the social spider *Stegodyphus dumicola*

Anne Aagaard[1] | Shenglin Liu[1] | Tom Tregenza[2] | Marie Braad Lund[3] | Andreas Schramm[3] | Koen J. F. Verhoeven[4] | Jesper Bechsgaard[1] | Trine Bilde[1]

[1]Section for Genetics, Ecology & Evolution, Department of Biology, Aarhus University, Aarhus C, Denmark

[2]Centre for Ecology & Conservation, School of Biosciences, University of Exeter, Penryn Campus, UK

[3]Section for Microbiology, Department of Biology, Aarhus University, Aarhus C, Denmark

[4]Terrestrial Ecology Department, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands

Correspondence
Anne Aagaard, Section for Genetics, Ecology & Evolution, Department of Biology, Aarhus University, Aarhus C, Denmark.
Email: anneaagaard@bio.au.dk

Funding information
Natur og Univers, Det Frie Forskningsråd, Grant/Award Number: 6108-00565; Novo Nordisk Foundation Interdisciplinary Synergy Grant, Grant/Award Number: NNF16OC0021110

Handling Editor: Victoria L. Sork

Abstract

Understanding the role of genetic and nongenetic variants in modulating phenotypes is central to our knowledge of adaptive responses to local conditions and environmental change, particularly in species with such low population genetic diversity that it is likely to limit their evolutionary potential. A first step towards uncovering the molecular mechanisms underlying population-specific responses to the environment is to carry out environmental association studies. We associated climatic variation with genetic, epigenetic and microbiome variation in populations of a social spider with extremely low standing genetic diversity. We identified genetic variants that are associated strongly with environmental variation, particularly with average temperature, a pattern consistent with local adaptation. Variation in DNA methylation in many genes was strongly correlated with a wide set of climate parameters, thereby revealing a different pattern of associations than that of genetic variants, which show strong correlations to a more restricted range of climate parameters. DNA methylation levels were largely independent of *cis*-genetic variation and of overall genetic population structure, suggesting that DNA methylation can work as an independent mechanism. Microbiome composition also correlated with environmental variation, but most strong associations were with precipitation-related climatic factors. Our results suggest a role for both genetic and nongenetic mechanisms in shaping phenotypic responses to local environments.

KEYWORDS
adaptation, DNA methylation, low evolutionary potential, microbiome, phenotypic plasticity, social spiders

1 | INTRODUCTION

The environment fluctuates at a range of timescales and in space across species ranges. If environmental changes occur over periods that are many multiples of species generation times, or if there are restrictions on gene flow between locations, organisms can evolve naturally selected adaptations to this variation (Charlesworth et al., 2017). Additionally, and even in the absence of local

adaptation, organisms may be able to cope with environmental variation through the capacity of a single genotype to express a range of phenotypes. This phenotypic plasticity gives organisms the potential to respond to environmental variation at short spatial or temporal scales (Fox et al., 2019; Ghalambor et al., 2007, 2015; Gonzalo-Turpin & Hazard, 2009). Phenotypes that are modulated plastically can be expressed very briefly, for example in behavioural reactions, or the rapid production of heat shock proteins (Dahlgaard et al., 1998; Gong et al., 2012), or they can last over multiple generations (Hanson et al., 2017; Nätt et al., 2012), depending on the type of plastic response and its underlying mechanism.

An important question, not least in the context of rapid global change, is whether and how fast adaptive responses can enable organisms to cope with environmental fluctuations and variability (Fox et al., 2019). In particular, species with low population genetic diversity have limited capacity for genetic adaptations when challenged by environmental change (Ørsted et al., 2019; Sgrò et al., 2011; Willi et al., 2006). This raises the question of whether this evolutionary constraint can be compensated for by nongenetic mechanisms with the potential to shape phenotypes (Donelson et al., 2019; Lande, 2009; Sgrò et al., 2016). Advancing our understanding of phenotypic responses shaped by nongenetic mechanisms is important, as they may play a key role in modulating adaptive responses to environmental change in species with low genetic diversity.

Local phenotypic responses can occur via mechanisms other than genetic adaptations, including epigenetic marks (e.g., histone modifications and DNA methylation) that may regulate gene function, and may be mitotically and/or meiotically heritable (Cavalli, 2006; Heckwolf et al., 2019; Henikoff et al., 2004; Holliday, 1987; Wu & Morris, 2001). Epigenetic changes in, for example, DNA methylation profiles can alter the phenotype of the individual (Cubas et al., 1999; Heckwolf et al., 2019; Jablonka, 2017). The various functions of DNA methylation as an epigenetic feature are only partially understood, but a role in relation to phenotypic change, such as by regulation of gene function, has been suggested in several species (Gatzmann et al., 2018; Keller et al., 2016; Liu, Aagaard, et al., 2019; Sarda et al., 2012; Varriale, 2014; Xu et al., 2021). Within invertebrates, methylation is enriched in gene bodies, but the function of this pattern remains unclear (Duncan et al., 2022). The highly structured distribution of DNA methylation across the genome suggests a functional role, and various hypotheses have been proposed, such as regulating gene expression, either directly (*cis*) or by modifying histone acetylation (*trans*), alternative splicing and stabilizing gene expression (Choi et al., 2020; Duncan et al., 2022; Gatzmann et al., 2018; Kvist et al., 2018; Lev Maor et al., 2015; Liu, Ma, et al., 2019; Neri et al., 2017; Xu et al., 2021). Several studies have demonstrated that DNA methylation profiles can change as a function of environmental stressors in common garden experiments, in species such as corals, sticklebacks, cockroaches and dandelions (Dimond & Roberts, 2020; Metzger & Schulte, 2017; Peña et al., 2021; Verhoeven et al., 2010). This is consistent with the idea that variation in phenotypes may be mediated by environmentally induced changes in DNA methylation profiles, which may facilitate the ability of populations to cope with changes in local climatic conditions on a shorter timescale than that of adaptive genetic changes. Furthermore, studies have shown that epigenetic changes can be heritable and may persist across generations (Harney et al., 2022; Nätt et al., 2012; Riddle & Richards, 2002; Sutherland et al., 2000). Studies on how DNA methylation variation is structured across geographical locations and combining this with variation in environmental factors such as, for example, temperature and precipitation, can inform and substantiate hypotheses on the role of DNA methylation in generating locally advantageous phenotypes. Environmental association studies have revealed strong relationships between epigenetic variants and climatic or environmental parameters (Fischer et al., 2013; Gugger et al., 2016; Rico et al., 2014; Verhoeven et al., 2010). However, it is often unclear if such relationships reflect epigenetic variants, or geographical variation in genetic control over epigenetic variants (Dubin et al., 2015).

It is also possible that local responses can be mediated by host–symbiont interactions. All organisms engage in interactions with microbes, and the microbiome represents a source of variation. Symbiotic interactions have huge potential to modulate host phenotype. Indeed, there is ample evidence to suggest that symbiotic interactions with the bacterial microbiome can shape numerous physiological, reproductive and behavioural functions of the host (Bang et al., 2018; Dunbar et al., 2007; Moran et al., 2019; Mueller et al., 2020). Responses in host phenotype mediated by changes in microbiome composition may contribute to improved performance of individuals in their local environment (Henry et al., 2019; Mueller et al., 2020), through new and potentially locally beneficial functions such as improved nutrition, energy production, temperature resistance or pathogen protection (Lynch & Hsiao, 2019; McFall-Ngai et al., 2013; Raza et al., 2020). Adjustments of the microbiome that provide beneficial local adjustments in host phenotype will naturally depend on the specific context, and can vary from changes in overall microbiome composition, to presence/absence and abundance of specific microbes or strains of microbes, and/or changes in strain composition of specific microbial species (Rennison et al., 2019; Rudman et al., 2019; Shigenobu & Wilson, 2011; Wernegreen, 2012). In pea aphids, populations harbour different strains of the obligate symbiont *Buchnera*, which differ in the expression of a heat-shock gene caused by a deletion in the promoter sequence. Aphid populations harbouring low-expression *Buchnera* perform better in colder environments, while populations harbouring high-expression *Buchnera* perform better in warmer environments (Dunbar et al., 2007). In reef-corals it was recently shown that their symbiont composition is shaped by environmental temperature and potentially mediates adaptive host phenotypes (Herrera et al., 2021). Association studies between microbiome composition and environmental variation are, however, relatively scarce (Busck et al., 2020; Suzuki et al., 2019; Walters et al., 2020), and only a few studies have revealed differences in host phenotypes as a function of the environmental context and its microbiome (Walters et al., 2020).

Social spiders of the genus *Stegodyphus* harbour very low species-wide genetic diversity, and *S. dumicola* is known to have one of the lowest genetic diversities recorded in any animal species

(Leffler et al., 2012; Settepani et al., 2017). It has been suggested that the lack of genetic diversity in this species may reduce its evolutionary potential (Settepani et al., 2017), but nevertheless they persist across broad climatic gradients in southern Africa, spanning several climate zones (Majer et al., 2015; Ngaira, 2007). We therefore hypothesize that responses to local environmental factors caused by nongenetic variants, such as DNA methylation and microbiome composition, may facilitate adaptive responses to local conditions. Although some level of heritable variation conferring local adaptation may be present (e.g., in response to temperature challenges; Malmos et al., 2021), the high level of genetic similarity of populations provides an excellent opportunity to evaluate the role of epigenetic and microbiome variation in population differentiation.

A first step towards revealing the molecular mechanisms underlying population-specific responses can be taken through environmental association studies (Morgan et al., 2018; Rellstab et al., 2015; Thomas, 2010; Ungerer et al., 2008). If there are population-specific evolutionary adaptations to climate, we expect to see associations between environmental parameters and genetic variants. Similarly, associations between epigenetic and microbial variants and environmental parameters are predicted if these features, either as induced or as inherited variants, have a role in phenotypic responses to local environmental factors. We use an environmental association approach in which we examine the relationship between genetic, epigenetic and bacterial microbiome diversity with a set of climatic parameters in populations of social spiders. Our aim is to characterize the mechanisms that may govern phenotypic responses of the social spider *S. dumicola* to different climatic variables within their natural habitats. Given low levels of genetic variation in the *S. dumicola* system (populations and species-wide), we hypothesize that DNA methylation and microbiome composition contribute to *S. dumicola* population differentiation, and are associated with environmental and climatic variation across populations.

2 | MATERIALS AND METHODS

2.1 | Study species and sampling

Stegodyphus dumicola is one of three independently evolved social spider species from the genus *Stegodyphus* (Settepani et al., 2016). *S. dumicola* live in family groups in nests of hundreds to thousands of individuals that live their entire life in and around their natal nest, resulting in extremely high levels of inbreeding (Lubin & Bilde, 2007). Additionally, sex ratios are highly female-biased and only a small proportion of females participate in reproduction. Genetic drift is consequently a strong evolutionary force in this species (Settepani et al., 2014, 2017). *S. dumicola* is distributed in the southern part of Africa (Majer et al., 2015), across a range of climatic conditions (Figure 1).

We sampled one female from each of 15 *S. dumicola* nests in each of six different populations, 90 females (from 90 different nests) in total, during December 2015. Five populations are located on a north–south gradient in Namibia and one population is located in South Africa (Figure 1; Table S1). Individual spiders were cut in half and placed directly in DNA extraction buffer (ATL buffer, DNeasy Blood & Tissue; Qiagen) in the field and transported to the laboratory at Aarhus University at ambient temperature. Cutting the spiders in half ensures proper penetration of buffer into the samples. One spider from each nest (i.e., a total of 90 spiders) was used for sequencing.

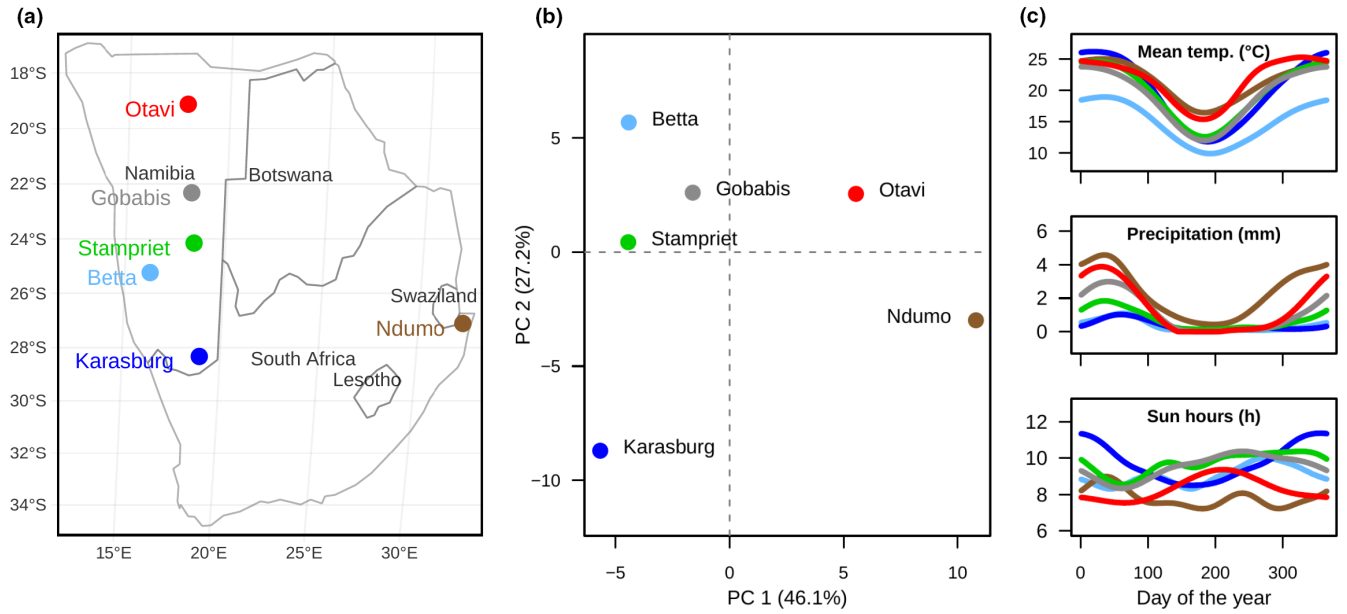

FIGURE 1 (a) Map of southern Africa showing the locations of social spider populations. (b) Climatic separation of the geographical locations on the two main environmental axes from the PCA (see details in Figures S1–S3 and Table S2). (c) Yearly variation in three climatic variables; top: Mean temperature, Centre: Precipitation, bottom: Daily hours of direct sun.

2.2 | DNA extraction, sequencing and quantitative PCR

DNA was extracted from all samples using the DNeasy Blood and Tissue kit from Qiagen following the animal tissue protocol. Prior to extraction, samples were homogenized using a pellet pestle. For each round of DNA extraction, one extraction blank (i.e., no sample was added to the tube) was included. The resulting DNA extracts were used for either (i) whole-genome (WG) resequencing, (ii) whole-genome bisulphite (WGB) resequencing or (iii) bacterial 16S rRNA gene amplicon sequencing and quantitative polymerase chain reaction (qPCR).

For WG and WGB sequencing, we pooled the DNA from each population in equimolar ratios before construction of WG and WGB libraries, and sequenced on a HiSeq2500 platform. While individuals may not be equally represented in pooled sequencing, it enables us to find population-specific differences, while still being cost efficient. After first WG sequencing, another set of libraries were made from the same DNA samples to obtain high enough coverage, and a total of 12 WG libraries were sequenced. For bacterial 16S rRNA gene amplicon sequencing, the primers Bac341F and Bac 805R (Herlemann et al., 2011) were used to amplify the V3–V4 region and libraries were prepared according to Illumina's 16S Metagenomic Sequencing Library Preparation guide. Paired-end (2×301 bp) sequencing was done on a MiSeq desktop sequencer (Illumina). Both DNA extraction blanks and PCR negatives were included for amplicon sequencing. Samples were run in two independent sequencing runs.

Quantitative polymerase chain reaction was used to estimate the number of bacteria in individual spiders as described previously (Busck et al., 2022). To compensate for differences in spider body size, we normalized the bacterial 16S rRNA gene copy number to a spider gene copy number (gene 5F, Settepani et al., 2016), and this ratio is referred to as bacterial load (number of bacterial 16S rRNA gene copies/number of spider gene copies). Highly similar coverage of the gene 5F relative to the entire genome indicates a single copy in all populations. All qPCRs were run in triplicate. For details see Busck et al. (2022).

2.3 | Whole genome mapping and variant calling

Whole-genome sequencing of the 12 libraries resulted in 622 Gb of raw data (paired-end reads, each of 150 bp and insert size of 300 or 500 bp). WGB sequencing of the six libraries resulted in 274 Gb of raw data (paired-end reads with each read 100 bp and insert size of 169–225 bp). The raw data were filtered using TRIM GALORE version 0.4.1 by allowing "--trim1." After the filtering, 264 Gb remained.

We mapped the WG resequencing reads to the *S. dumicola* genome (Liu, Aagaard, et al., 2019) using BWA (version 0.7.15) "aln" (Li & Durbin, 2009) allowing a maximum of two mismatches and converted them to bam files using SAMTOOLS (version 1.2) (Li et al., 2009). We extracted single nucleotide polymorphisms (SNPs) using SAMTOOLS mpileup with minimum mapping quality of 20 (Li, 2011) and the POPOOLATION2 (version 1.201) script snp-frequency-diff.pl (--min-count 1 --min-coverage 1 --max-coverage 50,50,50,50,50,40) (Kofler, Pandey, & Schlötterer, 2011). We estimated nucleotide diversities (π) for each population using a Variance-sliding.pl script (--window-size 10,000 --step-size 10,000 --min-count 5 --min-coverage 10 --max-coverage 400 --min-qual 20 --pool-size 15) from POPOOLATION2 (Kofler, Orozco-terWengel, et al., 2011), after converting bam files to pileup files using the mpileup function in SAMTOOLS (Li et al., 2009). To obtain a single estimate of genetic diversity for all samples, we downsampled population bam files to the same size and merged them using the view and merge functions in SAMTOOLS (Li et al., 2009) before converting to pileup format and Variance-sliding.pl. To construct the phylogenetic relationship among the studied populations, WG resequencing data from all individuals from each location were mapped to the *S. dumicola* genome (Liu, Aagaard, et al., 2019) using BWA (version 0.7.15) "aln" (Li & Durbin, 2009) allowing a maximum of two mismatches and converted to location-specific bam files using SAMTOOLS (version 1.2) (Li et al., 2009). We called variants into vcf files using BCFTOOLS version 1.5 ("mpileup" without indel calling [-I] and "call") (Li, 2011). We extracted coding positions using SAMTOOLS "faidx" (Li et al., 2009), and we called consensus sequences using BCFTOOLS version 1.5 "consensus" (Danecek & McCarthy, 2017). We joined consensus sequences into a single concatenated sequence per location and aligned them. We reconstructed a neighbour-joining phylogeny using MEGA X (Kumar et al., 2018). In total, 1000 bootstraps were used to add support to the topology. Genewise F_{ST} estimates were calculated using POPOOLATION2 scripts Create-genewise-sync.pl and fst-sliding.pl (--min-count 3 --min-coverage 20 --max-coverage 100 --pool-size 30 --min-covered-fraction 0.0 --window-size 1,000,000 --step-size 1,000,000).

We mapped WGB sequencing reads with BISMARK (version 0.19.9) (Krueger & Andrews, 2011) using --bowtie1. Methylation status of all C sites was called using Bismark_methylation_extractor and coverage was extracted using the bismark2bedgraph script. DNA methylations were filtered to only include sites with a depth above 10 and below 30, and proportions of C sites methylated in CpG, CHG and CHH (where H = A, T, or C) context were calculated. The methylation level for each cytosine in CpG context was determined as the ratio of reads indicating methylation over the total number of reads for that position, a level referred to as site methylation level (SML) (Schultz et al., 2012). DNA methylations within gene bodies were extracted using the genome annotation and BEDTOOLS INTERSECT version 2.29.2 (Quinlan & Hall, 2010), and the weighted methylation level (WML) of all CpG sites in each gene separately was estimated (mean of proportions of mapped reads being methylated in all CpG sites) (Schultz et al., 2012). Nei's F_{ST} (Nei & Kumar, 2000) was calculated for each gene and between each population pair.

2.4 | 16S rRNA gene amplicon analysis

We obtained 16S rRNA amplicon sequences from individuals from 78 nests (between 11 and 15 per population). From four nests (Otavi)

two individuals were sequenced per nest, the duplicate individuals were merged in PHYLOSEQ (McMurdie & Holmes, 2013) prior to calculating relative abundances of the amplicon sequence variants (ASVs). qPCR data were obtained from 69 of the individuals (between nine and 14 per population). A sample summary is given in Table S1.

CUTADAPT (Martin, 2011) was used for barcode and primer removal and sequence quality trimming. Using R version 3.6.1 (R Core Team, 2019) the two independent sequencing runs were processed separately using DADA2 version 1.12.1 (Callahan et al., 2016) for quality filtering, denoising and merging of paired-end reads. Filtering was set to maxEE = (2, 2), trncQ = 2 and truncLen = 280 and 200 bp for forward and reverse reads, respectively, in order to identify ASVs. Data from the two sequencing runs were merged prior to chimera finding and classification using DADA2 and Silva small subunit (SSU) reference database nr132 (Quast et al., 2013). ASVs were filtered to a minimum length of 400 bp, and nonbacterial ASVs, chloroplasts and mitochondrial ASVs were excluded. Samples with fewer than 8000 reads were removed from further analysis.

Using the R package PHYLOSEQ version 1.28.0 (McMurdie & Holmes, 2013), all samples were subsampled to the same read depth of 8227 reads (the smallest sample size, seed = 42). Both relative ASV abundances and absolute ASV abundances (based on bacterial load from qPCR analyses) were estimated. ASVs were filtered to only contain ASVs with a prevalence above 25% in at least one population. This retained 57 ASVs for absolute abundance, and 60 ASVs for relative abundance. Bray–Curtis dissimilarity matrices were obtained using the vegdist function in VEGAN version 2.5-6 (Oksanen et al., 2019). Bray–Curtis dissimilarities were calculated for all ASVs across all nests between and within populations, as well as for single ASVs and genera across each population using population-wise abundance means.

2.5 | Environmental variables

Thirty years (1961–1990) of climate data were downloaded using the application NEW_LOCCLIM_1.10 (Grieser et al., 2006), which interpolates climate station measurements (FAOCLIM database) to the input GPS positions from the six populations, and outputs daily climate estimations of selected variables. Three to seven nest GPS points (Table S1) were used to represent each population to create a mean climate estimate for each of the populations. Downloaded climatic variables included 30-year mean daily estimates of mean, maximum and minimum temperature (°C), precipitation (mm), potential evapotranspiration (mm), sun fraction (%), day length (hr), sun hours (hr), water vapour pressure (hPa) and wind speed ($km\,h^{-1}$). Shepard's Interpolation method was used for temperature data, while a thin-plate-spline was used for the remaining variables. For each of these variables, monthly and yearly mean, maximum, minimum and variation was calculated, along with the number of days where temperatures exceeded or went below set thresholds. Longitude, latitude and altitude were also included. The means of all monthly estimates were calculated, to obtain a monthly based yearly mean. All in all, 99 climate variable factors were calculated for the six populations.

To reduce the number of environmental variables, a principal component analysis (PCA) was run on 96 out of 99 variables (those solely containing zeros were excluded), using scaled and centred prcomp in R. A summary can be seen in Figures S1–S3 and Table S2. Based on these analyses, the first five PC axes were applied as composite environmental variables explaining a substantial amount of variation of the initial 96 environmental variables. The distance between populations in PC axes was calculated using dist() in the R stats package. Because previous research has indicated that temperature and precipitation are particularly important drivers for local phenotypic responses in arthropod species (Gefen et al., 2015; Malmos et al., 2021; Toolson, 1982) we selected 51 aspects of temperature and precipitation (see *x*-axis Figure 6) (many of which may be correlated), and directly calculated population distances using dist() from the R stats package. For an explanation on how these parameters were calculated, see Table S3.

2.6 | Environmental association analyses

Genetic (F_{ST}), DNA methylation (F_{ST}) and microbiome (Bray–Curtis dissimilarity) distances among the six populations were correlated to the distance in environmental PC axes through a set of analyses. (i) Partial Mantel tests were run for each gene separately to test for correlations between environmental axes and genetic divergence (F_{ST}) based on gene-body SNPs corrected for neutral population structure based on all SNPs (overall F_{ST}), and between environmental axes and DNA methylation divergence based on gene-body WML (gene-wise F_{ST}), using two different corrections: assumed neutral population structure based on all SNPs (overall F_{ST}); and *cis*-genetic variation based on the SNP variation in the given gene (gene-wise F_{ST}). (ii) Partial Mantel tests were run to test for correlations between microbiome ASVs and environmental axes, while correcting for neutral population structure based on all SNPs (overall F_{ST}). (iii) Multiple regressions were run on distance matrices (MRM function from the ECODIST R package; Goslee & Urban, 2007) featuring F_{ST} distance measures for gene-body SNPs and WML, and Bray–Curtis dissimilarity of microbiome variables as a function of the distance in each environmental axis. Genes with no variation between populations were removed before analyses (all $F_{ST} = 0$). For gene-body WML corrected for *cis* genetic variation, associations using simple Mantel tests were used in cases with no SNPs within the given gene. To assess temperature and precipitation associations explicitly, we ran the above-mentioned Mantel and Partial Mantel tests, exchanging distance in PC axes with distances in 51 individual temperature and precipitation aspects. *p*-Values are not particularly informative in this type of analysis where sample sizes can be extremely large and numerous closely related correlations are run. To identify biologically significant relationships we compared two distributions: (i) a distribution of actual correlation coefficients stemming from the above-mentioned partial Mantel analyses, and (ii) an expected distribution of the same correlations as in (i), but where the environmental axis or climate parameter were permuted (hereafter

termed the null distribution). To discern which climatic parameter or environmental axes showed stronger correlations than expected based on the null distribution, we took the number of genes (both nucleotide and methylation) in the actual distribution exceeding the 99.99th percentile of the null distribution. This arbitrary threshold represents a conservative approach to identifying correlations that may represent adaptive variants in response to climate, and that are unlikely to occur only by chance. For the microbiome, a similar approach was used, but because we only analyse 61 ASVs, the 99.99th percentile is not meaningful. Instead, we used the highest correlation coefficient from the null distribution as a threshold and included those that exceeded the highest correlation coefficient of the null distribution.

Gene ontology (GO) enrichment analyses were run for genes strongly correlated (above 99.99% threshold) to every environmental axis for gene-wise SNPs corrected for population structure, WML corrected for population structure and *cis*-genetic variation. Gene functional annotation was performed using EGGNOG orthology data and EGGNOG MAPPER (emapper-2.1.9) using DIAMOND SEARCH version 0.9.21 (Buchfink et al., 2021; Cantalapiedra et al., 2021; Huerta-Cepas et al., 2019), while ontology enrichment analysis was done using the R packages GOSTATS version 2.52.0 and GSEABASE version 1.58.0. (Falcon & Gentleman, 2007; Morgan et al., 2022).

For data handling and analyses run in R, we used the following main packages: USEDIST version 0.4.0 (Bittinger, 2020) and DPLYR version 1.0.6 (Wickham et al., 2021), while graphics were performed using base R (R Core Team, 2019), VENNDIAGRAM version 1.6.20 (Chen, 2018) and TMAP version 2.3.2 (Tennekes, 2018).

3 | RESULTS

3.1 | Whole genome sequencing and bisulphite sequencing

The WG sequencing mapping rates of the six spider populations varied between 71.9% and 80.8% when mapped to the reference genome, with coverage depth ranging from 21 to 29 (Table S4). The total number of SNPs called in each population varied from 655,000 to 1,119,000 (Table S4). For WGB sequencing (WGBS) λDNA was used as a control for a bisulphite conversion rate, and 99% of the unmethylated cytosines were converted. WGBS mapping rates of the six populations varied between 40.5% and 46.6%. The total number of sites that were at least partially methylated (>0 reads suggesting methylation) in each population varied from 3,441,377 to 6,218,880, while the number of sites that were methylated across all reads in each population varied from 398,114 to 580,440, about 9% on average (Table S4). Most methylations were found in CpG sequence context, and of all cytosines in CpG context, between 9.4% and 11.3% were at least partially methylated in the six populations. Methylations in CHG and CHH sequence context comprised less than 1% in all populations. Mapping and SNP/methylation statistics are summarized in Table S4.

3.2 | Bacterial microbiome

On average 30,321 quality filtered reads were obtained from 82 samples and the minimum and maximum number of reads were 8227 and 49,237, respectively (Table S1). A total of 3378 bacterial ASVs were identified, but the 10 most abundant ASVs accounted for more than 80% of all reads (Table S1, ASV table). The bacterial load (calculated as the number of bacterial 16S rRNA gene copies divided by the number of spider gene copies) was determined in 73 out of the 82 samples used for amplicon sequencing (Table S1, sample list). The average bacterial load was 5.7, ranging from 0.02 to 61.6, and the load did not differ significantly between populations (ANOVA, $p = .0825$, Figure S4).

The spider microbiome was dominated by *Mycoplasma*, *Diplorickettsia*, *Borrelia* and *Weeksellaceae* (Figure S5), corroborating a previous study in *Stegodyphus dumicola* (Busck et al., 2020, 2022). Bray–Curtis dissimilarity between individuals from different nests differed between populations, with the highest dissimilarity index found in Otavi (Figure S6). Overall, individuals from the same population had more similar microbiome composition compared with individuals from different populations. Significant differences in Bray–Curtis dissimilarities between populations were not driven by any single population (Figure S6). We recovered similar results whether analyses were based on relative or absolute abundances (Figure S6).

3.3 | Population phylogeny and population genetic diversity

A phylogenetic reconstruction of populations is shown in Figure 2. Phylogenetic relationships among populations cannot be predicted directly from geographical locations; for example, we show that Betta is phylogenetically closest to Otavi, while geographically closest to Stampriet. Genome-wide nucleotide diversity varied from 0.00021 in Betta to 0.00071 in Gobabis, while nucleotide diversity for all samples pooled was 0.00091.

3.4 | Population divergences—Genetic, DNA methylation and microbiome

Pairwise molecular distances among populations, estimated as F_{ST} values, when averaging over all genes, were between 0.04 and 0.15 based on SNPs, and between 0.004 and 0.01 for WML. Pairwise microbiome ASV distances among populations, estimated as Bray–Curtis dissimilarities, ranged from 0.21 to 0.87 for relative abundances and 0.19 to 0.80 for absolute abundances (Figure S7c,d). We found significant isolation-by-distance when considering nucleotide variation distance ($r = .75$, $p = .048$), and this correlation was predominantly driven by the Ndumo population, which is a long way to the east of the Namibian populations (Figure 3). No isolation-by-distance was found when analysing WML and microbiome distances

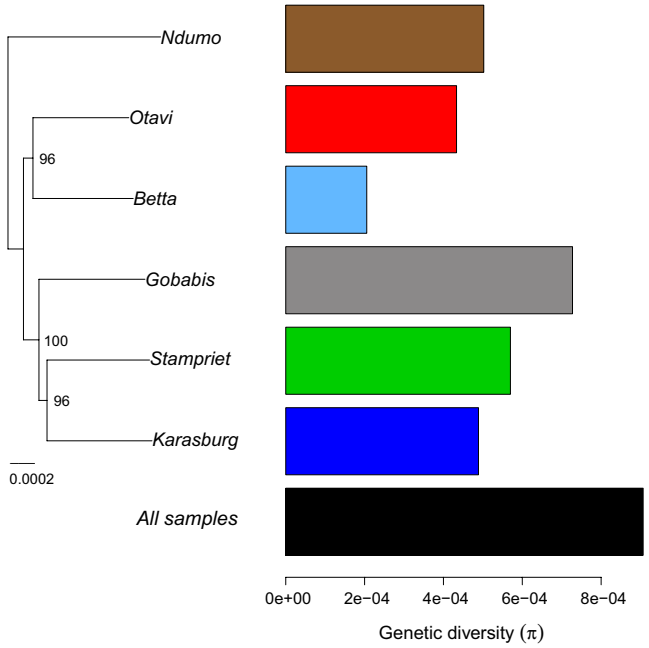

FIGURE 2 Phylogenetic relationships among social spider populations (location names), bars show nucleotide diversities (π) for populations, and the black bar shows genetic diversity across all populations joined. Bootstrap values above 60% are shown.

(Figure 3). The distribution of F_{ST} values estimated per gene across the entire genome (Figure S7a,b) revealed that population differentiation based on DNA methylation (gene-body WML) is generally lower than population differentiation based on genetic variants (SNPs). However, for DNA methylation data, there is a long tail (Figure S7b), indicating that some genes are strongly differentiated among populations.

3.5 | Environmental parameters

The six populations differ substantially in local climate, for example in mean and seasonal fluctuations in temperature and precipitation (see Figures 1 and S8). An overview of all investigated climate variables and their population patterns is provided in Figure S8. PCA on the environmental factors resulted in five axes each explaining a substantial amount of variance in the data (Figures S2 and S3). The first three axes explained 92% of the total variance. The populations are relatively well separated on PC1 and PC2 (Figure 1), with Karasburg and Ndumo being more different compared to the other populations (see Figure S9 for plots of the remaining PC axes). The 20 main loadings driving each PC axis were extracted (Table S2), and this revealed that PC1 is driven mainly by precipitation, minimum temperature, sunshine fraction and sunshine hours, PC2 is mainly driven by maximum temperature and a mixture of other variables, while PC3 is driven mainly by wind, day length and mean temperature. PC4 is driven by temperature, potential evapotranspiration and water vapour pressure, while PC5 does not reveal any clear patterns regarding climate variables (see Table S2 for details).

3.6 | Environmental association analyses

When averaging across all loci or symbionts, we found no isolation by environment (Wang & Bradburd, 2014) across any of the PC axes (Figure 4 and Figure S10). The lack of an overall isolation by environment makes it possible to identify individual variants potentially involved in responses to local environments, and we subsequently analysed each gene/symbiont separately.

For most correlations between variation in climate axes and variation in genetic, DNA methylation and microbiome features, the histograms of correlation coefficients for the null distributions appear normal (Figure 5a, grey distributions), while the distribution of actual correlation coefficients are right skewed in most cases (Figure 5a, coloured distributions; Figure S11). The peak of the observed correlation coefficient distribution mainly falls within the negative correlation coefficients, an observation that is difficult to interpret as climate-related responses, while the right-hand tail represents the most strongly positively correlated genes/microbiome features, which represent candidates for local adaptive variants. In the right-hand tail, we generally see an excess of genes/microbiome features compared to the null distribution (Figures 5a and S11).

When correcting for neutral population structure, partial Mantel correlations between the distance in climatic axes and the genetic distance among populations showed substantially more genes correlating strongly than expected based on the null distribution obtained by permuting the environmental axes, especially PC2 and PC4 (Figure 5a,b). For DNA methylation, partial Mantel analyses revealed more genes strongly correlating to PC axes than expected based on the null distribution (most clearly PC3 and PC4, Figure 5a,c). The same overall pattern was revealed, regardless of whether we corrected for population structure (Figure 5c, dark bars) or *cis* nucleotide variation (light bars). GO term enrichment analysis revealed that various broad categories of biological processes and molecular functions were enriched in genes strongly correlated to environment (Table S5), but none of them were clearly related to climate. We note that 2413 genes could not be functionally annotated, a common issue for nonmodel organisms. To further investigate whether the genes that show strong correlations to the climate axes were shared between nucleotide variants and DNA methylation variants, we used Venn diagrams (Figure 5e).

A very low number of genes showed a strong correlation with both DNA methylation variants and nucleotide variants (Figure 5e and Table S6, overlap). This is in contrast to the large number of genes co-occurring in both DNA methylation variants corrected for population structure and *cis* genetic variants within each gene (between 60% and 95%, Figure 5d and Table S6, overlap). The large overlap among gene-wise methylation variants corrected for population structure or corrected for *cis* genetic structure indicates that variation in DNA methylation is not a function of *cis*-nucleotide variation. This suggests that DNA methylation is either a function of *trans*-nucleotide variation or arises independently of nucleotide variation.

Correlation analyses of microbiome Bray–Curtis dissimilarity across all ASVs and genera and divergence in climate axes revealed

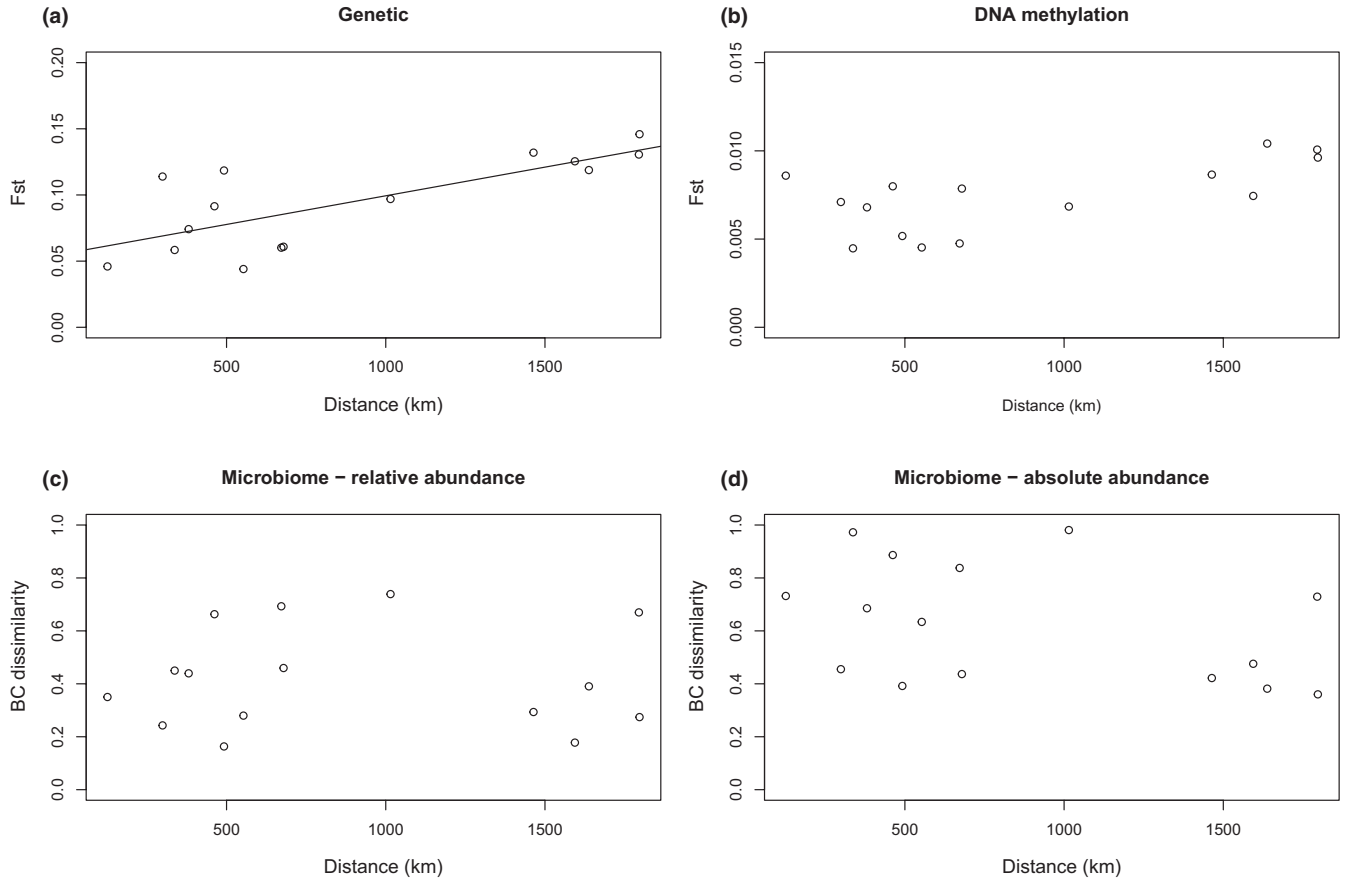

FIGURE 3 Isolation-by-distance plots of (a) genetic divergence (F_{ST}), (b) DNA methylation divergence (F_{ST}), and (c,d) microbiome divergence (Bray–Curtis [BC] dissimilarity); BC dissimilarities were estimated as a function of both (c) relative and (d) absolute abundance. Isolation-by-distance was only significant when considering genetic divergence, but this was driven by the Ndumo population, and no isolation-by-distance was observed within Namibia.

more ASVs/genera correlating strongly to climate axes than expected based on the null distribution (Figure 5a,d). Examples of the strongest correlations between climate and gene-wise SNP, WML and microbiome ASVs are shown in Figure S12, while distribution plots of correlation coefficients are presented in Figures 5a and S11.

To verify the results based on Mantel tests, we also analysed the associations using multiple regression tests on distance matrices (Castellano & Balletto, 2002; Guillot & Rousset, 2013; Legendre, 2000; Legendre et al., 2015; Raufaste & Rousset, 2001). These analyses yielded results very similar to the Mantel tests (Figure S14), suggesting that the revealed patterns are robust.

Genetic variation at individual genes correlated most closely especially with specific mean temperature parameters, as well as yearly minimum precipitation (Figure 6 top, blue bars). DNA methylation at individual genes often correlated with parameters related to minimum temperature as well as yearly minimum precipitation (Figure 6 top, orange bars). Both genetic and methylation variation within genes seem to correlate strongly with specific aspects of maximum temperature in a large number of genes (Figure 6 top). For the microbiome presented as ASVs or genera, many specific aspects of both mean temperature and precipitation were found to correlate more strongly with microbiome than the strongest correlation from the null distribution (Figure 6 bottom). Distribution plots of correlation coefficients for the correlations between genetic, DNA methylation, microbiome variation, and temperature and precipitation parameters can be seen in Figure S15.

Heatmaps of the microbiome data (Figures S17 and S18) show that significant and very strong correlations are not generally driven by one or a few ASVs. Most ASVs correlate strongly and/or significantly with few climatic parameters. An exception is *Enhydrobactor* (ASV 27, absolute abundance Figure S17a), which correlates with multiple climate parameters (Figure S17). A clear clustering of ASVs is evident around the precipitation parameters, mainly driven by *Mycoplasma* (ASV 4) and *Proteus* (ASV 26) (relative abundance, Figure S17b), but many ASVs contribute to the cluster. When sorting the ASVs according to abundance, however, no clear clustering was seen (Figure S18a,b).

4 | DISCUSSION

Populations of the social spider species Stegodyphus *dumicola* inhabit wide climatic gradients across southern Africa, raising the question of how they respond to variation in local conditions in the face of extremely low species-wide genetic diversity (Settepani et al., 2017). We investigated sources of variation that potentially

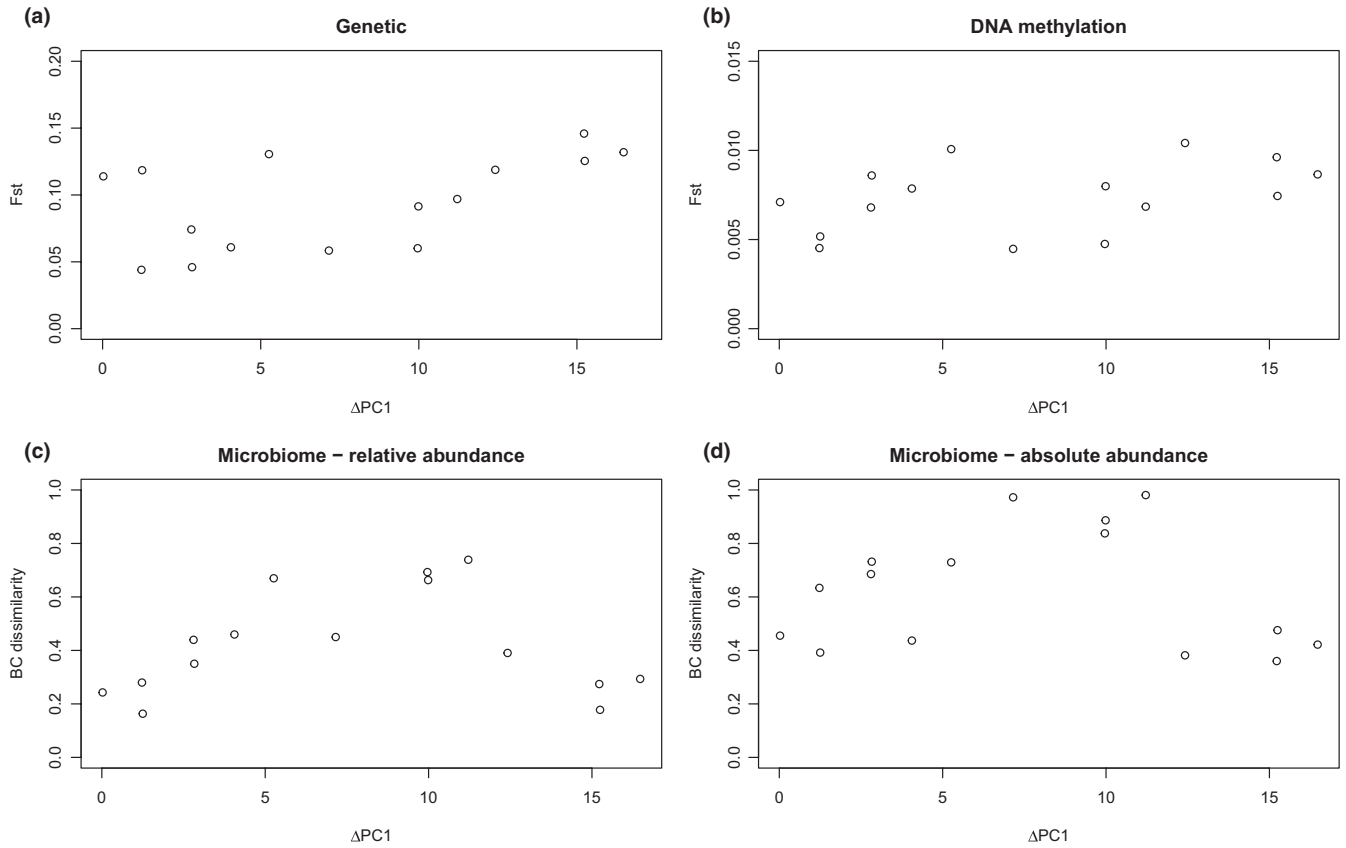

FIGURE 4 Isolation-by-environment plots after averaging (a) genetic, (b) DNA methylation and (c,d) microbiome divergence across all loci and symbionts. The environmental divergence presented here is distance on PC axis 1. Isolation-by-environment plots with PC axes 2–5 are shown in Figure S10.

mediate local responses, by correlating environmental variation with genetic, epigenetic and microbiome variation. Here we highlight three main conclusions that we discuss in more detail below. (i) In *S. dumicola*, despite low species-wide genetic diversity, we find genetic variants associated with environmental variation, consistent with patterns of local adaptation to environmental conditions, particularly in relation to mean temperatures. (ii) DNA methylation variation is associated with environmental variation, as expected if there is an epigenetic role in response to local climatic conditions. DNA methylations show different environmental association patterns than those of genetic variants, and show strong associations across temperature aspects. (iii) The bacterial microbiome correlates with environmental variation; most notably we detect the strongest associations with mean temperature and humidity-related climatic factors. These results suggest that both genetic adaptation and responses mediated by nongenetic mechanisms might contribute to population differentiation in *S. dumicola*.

4.1 | Population genetics

Population genetic structure was characterized by weak but significant isolation-by-distance, mainly driven by the South African Ndumo population, which is distant from the Namibian populations (>1500 km) (Figure 3a). When assessing only the five Namibian populations, it is clear that geographical and genetic distances do not match, suggesting that populations do not differentiate due to geographical distance. The genomic differentiation among populations (F_{ST} estimates of 9.4%) could be the result of neutral evolution, especially as a result of recurrent extinction and colonization events and genetic drift, or the differentiation could be caused by selection. Despite large census population sizes of *S. dumicola* (Settepani et al., 2017), we estimated extremely low genome-wide population-specific genetic diversities (on average $\pi = 0.00048$) (Figure 2), corroborating similar findings of a RAD sequencing study (Settepani et al., 2017). In small populations characterized by inbreeding and lack of gene flow, such as seen in the social spiders, we expect to detect strong population genetic structure caused by lineage divergence. However, high population extinction/colonization rates can act to homogenize genetic structure (Settepani et al., 2016, 2017), and indeed, species-wide genetic diversity was very low (across populations: $\pi = 0.00091$). This genetic pattern is expected to impede the efficacy of selection and local adaptation (Jensen & Bachtrog, 2011; Settepani et al., 2017), since the probability of segregating variants that are advantageous in a changing environment is lower when genetic diversity is low (Barrett & Schluter, 2008; Lande & Shannon, 1996; Rousselle et al., 2020). Nevertheless, we identified associations between genetic and

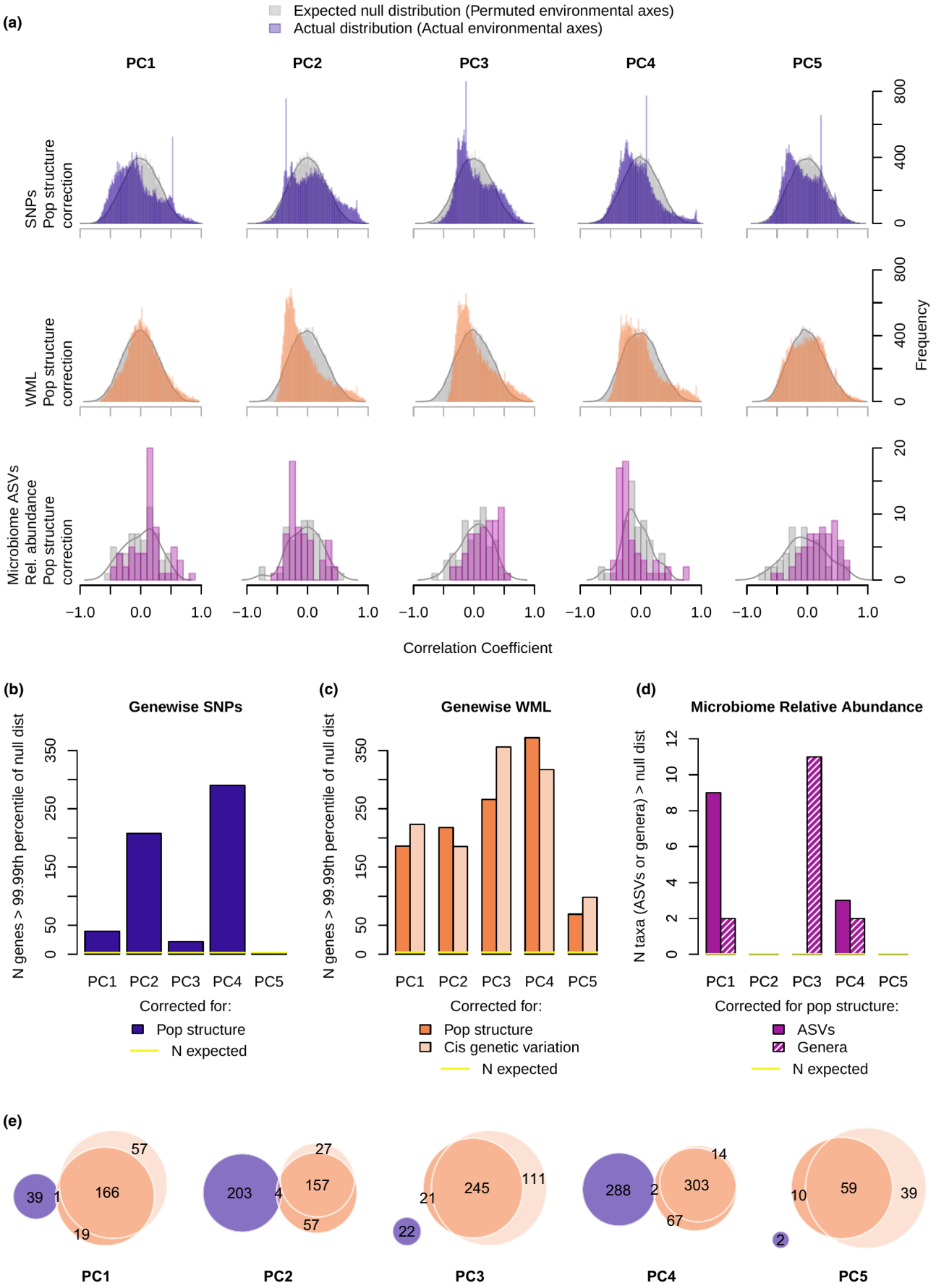
(a)
Expected null distribution (Permuted environmental axes)
Actual distribution (Actual environmental axes)
PC1
PC2
PC3
PC4
PC5
SNPs Pop structure correction
WML Pop structure correction
Microbiome ASVs Rel. abundance Pop structure correction
Frequency
Correlation Coefficient
(b)
Genewise SNPs
N genes > 99.99th percentile of null dist
Corrected for:
Pop structure
N expected
(c)
Genewise WML
N genes > 99.99th percentile of null dist
Corrected for:
Pop structure
Cis genetic variation
N expected
(d)
Microbiome Relative Abundance
N taxa (ASVs or genera) > null dist
Corrected for pop structure:
ASVs
Genera
N expected
(e)
39
1
166
57
19
PC1
203
4
157
27
57
PC2
22
21
245
111
PC3
288
2
303
14
67
PC4
2
10
59
39
PC5

FIGURE 5 Correlation results when running partial Mantel tests correlating gene-wise genetic distance, gene-wise methylation distance and distance in microbiome ASVs/genera with environmental distances. (a) Distribution plots of correlation coefficients for the expected null distribution (grey), made by permuting the environmental axes, and the actual distribution (coloured), made with the actual environmental axes. For the distribution plots of the remaining data sets see Figure S11. (b,c) Number of genes exceeding the 99.99th percentile of the null distribution. (b) Gene-wise nucleotide variation (blue). (c) Gene-wise DNA methylation variation corrected for population structure (dark orange) and *cis* genetic variation (light orange). (d) Number of microbiome features with a correlation coefficient exceeding the highest correlation coefficient in the null distribution. The microbiome is represented as relative abundance of ASVs (purple) and genera (hashed). Absolute abundance is given in Figure S13. The horizontal yellow lines indicate the theoretically expected number of genes/microbiome features (0.01 percent of correlations). (e) Venn diagrams showing the number of genes co-occurring between nucleotide variation (blue), DNA methylation corrected for population structure (dark orange) and DNA methylation corrected for *cis* genetic variation (light orange) (Table S6).

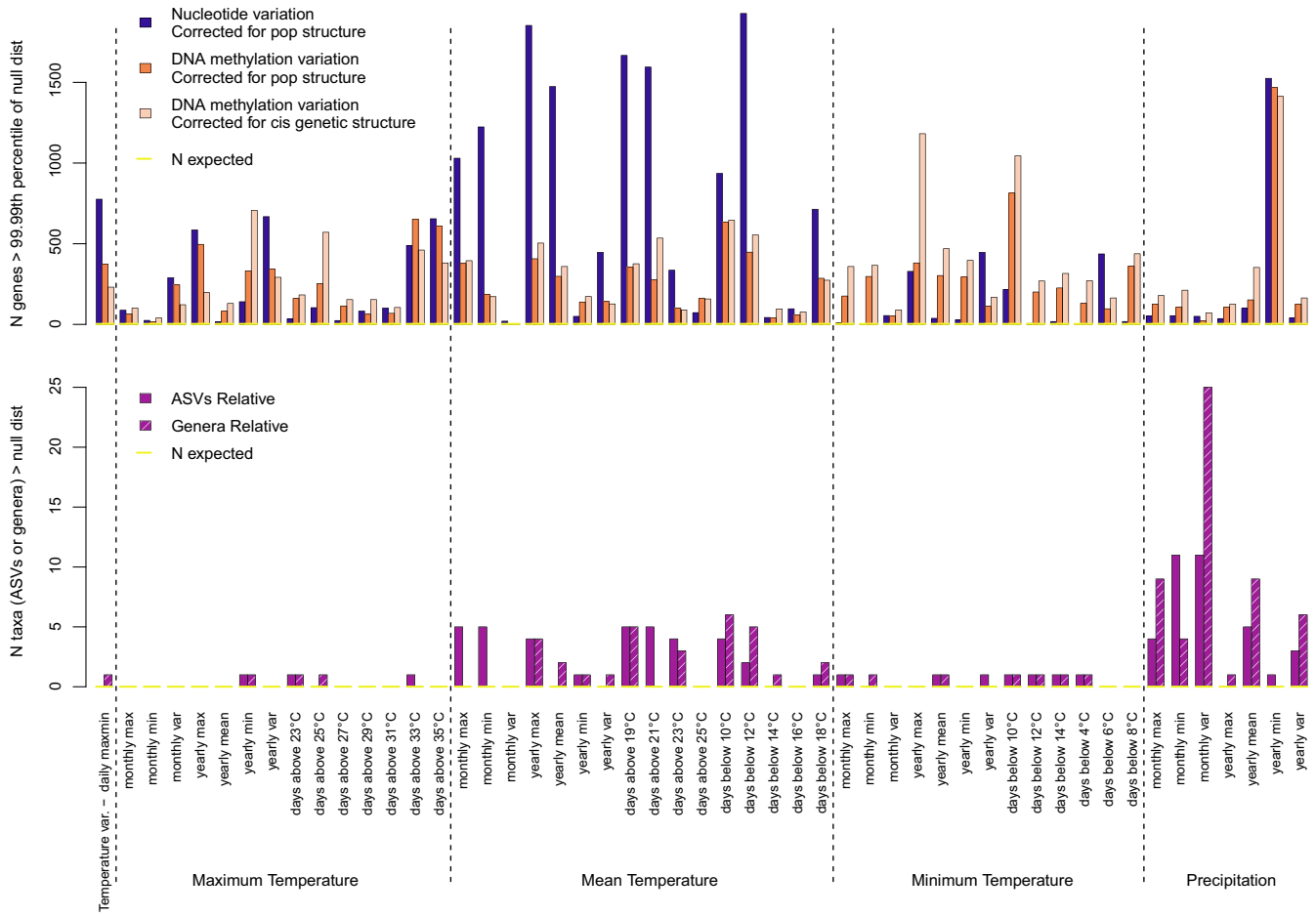

FIGURE 6 Correlations, presented as the number of genes or microbiome components correlating very strongly with distance in specific temperature and precipitation parameters. Upper graph: Number of genes correlating more strongly than the 99.99th percentile of the null distribution. Blue bars: Gene-wise nucleotide variation, orange bars: DNA Methylation variation, dark orange bars: Corrected for population structure, light orange bars: Corrected for *cis* genetic variation. Bottom graph: Number of microbiome features represented as relative abundance correlating more strongly with environmental parameters than the highest correlation coefficient of the null distribution. Purple bars: Single ASVs, hashed bars: Genera. A similar representation of results for absolute abundance is presented in Figure S16.

environmental variants (Figure 5a), consistent with the existence of adaptive genetic diversity. In support of this scenario, the distribution of genetic differentiation among populations of individual genes is relatively even (Figure S7a), contrasting with the expected left-skewed bell shape for neutral loci (Schwartz et al., 2007), suggesting that selection may have affected individual genes differently.

4.2 | Association patterns between climate variables and nucleotide variation

We identified hundreds of strong associations between nucleotide variants averaged across genes and climate axes (Figure 5b), contradicting our hypothesis that populations with strong drift and low efficacy of selection harbour low amounts of adaptive genetic variation.

Focusing on associations between different aspects of temperature and precipitation and nucleotide variation within genes suggests that aspects of mean temperature (Figure 6) are the strongest drivers of local adaptation to climate. In populations with high genetic drift, strong selection is required to maintain adaptive variation and local adaptation. Exposure to high temperatures is known to exert selection for phenotypic responses to avoid heat stress, leading to local adaptation in temperature responses in natural populations of arthropods (Sørensen & Loeschcke, 2002; Tregenza et al., 2021; Williams et al., 2012). In addition, our study species, *S. dumicola*, shows population-specific variation in behavioural and physiological responses to high temperatures (Barton, 2011; Malmos et al., 2021; Sandfeld et al., 2022), substantiating a role for genetic variation in temperature adaptation. However, relationships between genes that are differentiated between climatically divergent populations and specific phenotypic adaptations have yet to be established.

4.3 | DNA methylation variation

We found relatively high CpG methylation in *S. dumicola* (about 10%) (Table S4) as compared with most invertebrates (see overview by Bewick et al., 2017; de Mendoza et al., 2020). This finding corroborates previous findings in social spiders (Liu, Aagaard, et al., 2019). With no indication of genome-wide isolation-by-environment (Figure 4), the overall pattern of DNA methylation is seemingly not shaped by climate. However, analyses of DNA methylation of single genes separately revealed a long tail of genes that show strong differentiation between populations (Figure S7b). It is possible that methylation in these genes is responsible for aspects of phenotype that relate to temperature tolerance (Agwunobi et al., 2021), for example by potentially regulating gene expression or being involved in alternative splicing (Flores et al., 2013; Lev Maor et al., 2015; Liu, Aagaard, et al., 2019; Xu et al., 2021). The average level of DNA methylation divergence among populations was considerably lower than that of nucleotide divergence (Figure S7a,b, on average $F_{ST(WML)} = 0.007$). However, because these F_{ST} estimates are based on different types of data and analyses, they are not easily comparable (Coates et al., 2009). One plausible reason for lower divergence in DNA methylation compared with nucleotide divergence is that a large proportion of DNA methylation may be constrained in its variation, for example due to roles in development (Gao et al., 2012), differences between tissues or individual differences (Marshall et al., 2019). This implies that only a subset of methylation variants may differentiate among populations. DNA methylations can be induced by environmental variance as shown in fish, mammals, plants, birds and invertebrates (Bossdorf et al., 2008; Cropley et al., 2012; Harney et al., 2022; Heckwolf et al., 2019; Nätt et al., 2012), and are proposed to have the potential to modulate phenotypes in a plastic manner (Duncan et al., 2022; Flores et al., 2012; Gatzmann et al., 2018; Marshall et al., 2019). Furthermore, inducible DNA methylations can be transmitted across generations, which exposes them to evolutionary forces at least on short timescales (Cubas et al., 1999; Nätt et al., 2012; Riddle & Richards, 2002; Sutherland et al., 2000).

4.4 | Association patterns between climate variables and DNA methylation variation

Currently, we do not know whether DNA methylations can mediate local responses to ecological factors in spiders. However, we identified an excess of strong associations between variants in DNA methylation and divergence across climate axes (Figure 5c). Some genes differed substantially in their patterns of methylation between populations, a pattern consistent with a role in mediating responses to local climate differences. Methylations mediating responses to the local climate have been found in other species; for example, gene body methylation of a coral changed in response to transplantation, and corals with methylation patterns more similar to the local specimens had higher fitness (Dixon et al., 2018). Methylation patterns are influenced by environmental factors such as salinity in *Daphnia* (Asselman et al., 2015), and both salinity and temperature in the ascidian *Ciona* (Hawes et al., 2018). In a cockroach, *Diploptera punctata*, methylation patterns in heat shock protein 70 respond to temperature, potentially providing a fast-response mechanism to regulate expression of heat shock proteins (Peña et al., 2021). We found that associations between DNA methylation level and climatic variables were largely independent of *cis*-genetic variation and of overall genetic population structure (Figure 5e, overlap between dark/light orange). This indicates that DNA methylation is not solely a function of the local DNA sequence itself, but we cannot rule out that DNA methylation is regulated by *trans*-acting loci or influenced by SNPs further upstream of the gene region. Very few genes (seven in total) showed evidence of strong associations between both nucleotide and DNA methylation variants and climate axes (Figure 5e, overlap blue/orange), suggesting that nucleotide variants and DNA methylation to a large extent are independent. These few genes present interesting candidates to investigate functional relationships in more detail, for example by using experimental molecular methods combined with analyses of gene expression associated with phenotypic changes. Speculative explanations for the pattern in these genes include: (i) locally adapted genes with DNA methylation variants fine-tuning the local response; (ii) genetically based differences in gene expression cause differences in DNA methylation patterns (Secco et al., 2015); and (iii) a plastic gene that has become locally adapted (plasticity first hypothesis, Perry et al., 2018).

More genes showed a strong association to climate in their DNA methylation than nucleotide variants (Figure 5b,c). This may be surprising considering the lower number of genes that show significant differentiation among populations in DNA methylation compared to nucleotides (Figure S7a,b). Drift and limited gene flow act to increase differentiation of genetic variants, which may not be the case for DNA methylation variants if they are plastically induced. This could lead to more genes that are divergent with respect to nucleotide variation than to DNA methylation. However, if DNA methylations are generally transmitted across generations, drift will also lead to differentiation

of DNA methylation among populations. Despite the lower number of genes diverging in DNA methylation, many more genes are both strongly and significantly associated with climate in DNA methylation. This suggests that genes that are differentially methylated among populations are important in mediating local responses to climate.

The scrutiny of selected temperature and precipitation variables and their associations to DNA methylation variation within genes suggests that DNA methylation may be particularly involved in responses to differences in minimum temperature, but many genes associate strongly across environmental parameters (Figure 6, orange bars). Associations between DNA methylation and temperature have previously been identified. For example, DNA methylation level in 43 RAD loci was highly associated with maximum temperature in the oak species *Quercus lobata* in California (Gugger et al., 2016). Methylation level in Hsp70 responds to heat in the mollusc *Biomphalaria glabratahe* (Ittiprasert et al., 2015). However, our results suggest that minimum temperature may be a driver for differential DNA methylation, potentially by responding to low temperature through fast plastic responses. This was found in an alpine Brassicaceae that respond to chilling with an alteration of DNA methylation, suggesting that methylations mediate fast responses to cold stress (Song et al., 2015). In the goldenrod gall moth, low temperatures cause an increase in expression of DNA methyltransferases (Williamson et al., 2021). A study on ticks showed the importance of DNA methyltransferases in regulating the cold response (Agwunobi et al., 2021); they knocked out the DNA methyltransferases and ticks subsequently exposed to sublethal temperatures died.

Our results also indicate that local responses to precipitation may be mediated primarily by DNA methylation variation (Figure 6). In plants it has been shown that epigenetic signals may guide development of stomatal cells in response to relative humidity in the environment (Tricker et al., 2012), and in humans associations between blood cell methylation patterns and ambient relative humidity were identified, which furthermore interacts with temperature (Bind et al., 2014).

4.5 | Bacterial microbiome composition

Analyses of *S. dumicola* microbiome composition revealed no pattern of isolation by distance. Within the same location, we recovered substantial variation in microbiome composition between nests, with only little additional variation found between different geographical locations (Figure S6). This finding corroborates previous microbiome studies of *S. dumicola* populations across Southern Africa (Busck et al., 2020, 2022). The actual symbionts identified in the *S. dumicola* microbiome also overlap substantially. We know from preliminary data that bacteria are not vertically but rather socially transmitted among nest mates in *S. dumicola* (our unpublished data). Within nests, the microbiome composition does not change much across generations, suggesting relatively high transmission fidelity within nests (Busck et al., 2022), but nests within a population often carry substantially different microbiome compositions (Busck et al., 2020, 2022).

4.6 | Association patterns between climate variables and microbiome variation

Correlation analyses revealed an excess of strong associations between microbiome composition and the majority of environmental axes (Figure 5d). In some cases, the microbiome composition or presence of certain strains was found to associate with the ambient environment of the host, revealing a host phenotype better fitted to a particular environment (Dunbar et al., 2007; Herrera et al., 2021). Such changes in microbiome composition can be caused by mutualistic relationships with the host, or differential survival within hosts across a climate gradient. Several aspects of precipitation associated more than expected with relative abundance of bacterial symbionts (Figure 6). A previous study showed an association between high precipitation and microbiome composition (Busck et al., 2022), and together these studies indicate that the microbiome composition of *S. dumicola* is shaped by aspects of humidity. This association is driven by 10 ASVs from different taxonomic groups that correlate strongly and/or significantly with aspects of precipitation (Figure S17). An effect of precipitation-related variables has also been found in mosquitoes, where the gut microbiome changes along a landscape–moisture gradient (Medeiros et al., 2021), and exposure to altered humidity has been shown to change the microbiome in mice (Yin et al., 2022). However, our association study cannot discern whether symbiont abundance is shaped directly by humidity irrespective of the host or indirectly by the host as a response to humidity. Further steps are required to disentangle these processes and investigate a potential functional relationship with the host. In contrast to the patterns recovered for genetic and epigenetic variation, we detected a more scattered pattern of strong associations between microbiome composition and aspects of mean temperature (Figure 5d), a pattern driven primarily by five ASVs from different genera (Figure S17). A relationship between temperature and microbiome was found in other species, for example for the insect *Wolbachia* in relation to mean temperature (Woodhams et al., 2020), or for the *Drosophila* gut microbiome (Mazzucco & Schlötterer, 2021; Walters et al., 2020).

We find that the most abundant symbionts are not necessarily those showing the strongest correlations with the environment (Figure S18), indicating that strict abundance filters on microbiome data may remove functionally important symbionts. Under the assumption of a mutualistic relationship between host and symbiont, this implies that symbionts that govern host phenotypic responses may be found among the less abundant symbionts in the microbiome community. An example of a low-abundance taxon that can contribute valuable functions for the host is found in the human gut: Christensenellaceae are associated with health and longevity despite mostly being present with a relative abundance well below 0.1% (Kong et al., 2016; Waters & Ley, 2019). In addition to abundance and presence/absence, strain variation may be important in a mutualistic relationship between host and symbiont. Strain variation

caused by indel polymorphism was shown to be important in providing the host with different functions in the aphid symbiont *Buchnera* (Dunbar et al., 2007).

4.7 | Concluding remarks

We aimed to take a step towards understanding varied sources of variation that allow a species to occupy a range of habitats, by examining associations between environmental variation and genetic, epigenetic and microbiome variation. We identified gene-wise genetic variants that are associated strongly with environmental variation, particularly in mean temperature, a result which is consistent with local genetic adaptation. DNA methylations show different environmental association patterns compared with genetic variants, by having strong correlations to all climate axes and across aspects of temperature and precipitation. This pattern follows the expectation of an epigenetic role in responses to local climatic conditions. The microbiome also correlated with environmental variation, but also showed an independent pattern of association with most strong associations being with mean temperature and humidity-related climatic factors. We hypothesize that nongenetic sources of variation underlying adaptive responses to environmental change may be important in species with low standing variation. The next steps would be to assess functional relationships and determine whether molecular variants associated with phenotypic change are inducible and/or transmitted across generations. Common garden studies designed to substantiate links between the environment, phenotypic change and underlying molecular mechanism may be useful for establishing specific functional relationships, while causal relationships may require molecular experiments and analyses of gene expression connected with phenotypic change.

AUTHOR CONTRIBUTIONS

AA, TB, JB and AS designed the research and obtained funding; AA, SL, JB and MBL performed research and data analysis; KJFV, TB, TT, AS and MBL aided by suggesting new analysis methods; all authors wrote the manuscript, with substantial contributions from AA, JB, TB and TT.

ACKNOWLEDGEMENTS

We thank Virginia Settepani, Anja Junghanns and Tharina Bird for collecting spiders from the field. We also owe thanks to Marie Rosenstand Hansen, Susanne Nielsen, Britta Poulsen and Lykke Beinta Bjærge Bamdali for spider care and technical assistance in the laboratory, and Mette Marie Busck for establishing the microbiome data analysis pipeline. We are indebted to GenomeDK and Aarhus University for providing computational resources for the computing of data (GenomeDK cluster, genome.au.dk). The study was supported the Danish Council for Independent Research (grant no. DFF - 6108-00565) and by a Novo Nordisk Foundation Interdisciplinary Synergy Grant (NNF16OC0021110).

Permits for exporting the spiders for research purposes were obtained from the Ministry of Environment and Tourism, Windhoek, Namibia, permit no. 2110/2016, Ezemvelo KZN Wildlife, Pietermaritzburg, KwaZulu-Natal, South Africa, permit no. OP 5074/2014 and Department of Environment and Nature Conservation, Kimberly, South Africa, permit no. FAUNA 1450/2015.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

All data have been uploaded to GenBank: Bioproject PRJNA754001. Previously published genetic data are available at GenBank: Bioproject PRJNA510316. A document with information about which files are available at which bioproject has been uploaded as Supporting Information for this article.

ORCID

Anne Aagaard https://orcid.org/0000-0001-7044-5177
Tom Tregenza https://orcid.org/0000-0003-4182-2222
Marie Braad Lund https://orcid.org/0000-0003-1543-1009
Andreas Schramm https://orcid.org/0000-0002-7614-9616
Koen J. F. Verhoeven https://orcid.org/0000-0003-3002-4102
Jesper Bechsgaard https://orcid.org/0000-0003-3273-0174
Trine Bilde https://orcid.org/0000-0002-0341-161X

REFERENCES

Agwunobi, D. O., Zhang, M., Shi, X., Zhang, S., Zhang, M., Wang, T., Masoudi, A., Yu, Z., & Liu, J. (2021). DNA methyltransferases contribute to cold tolerance in ticks *Dermacentor silvarum* and *Haemaphysalis longicornis* (Acari: Ixodidae). *Frontiers in Veterinary Science*, *8*, 1–17. https://doi.org/10.3389/fvets.2021.726731

Asselman, J., de Coninck, D. I., Vandegehuchte, M. B., Jansen, M., Decaestecker, E., de Meester, L., vanden Bussche, J., Vanhaecke, L., Janssen, C. R., & de Schamphelaere, K. A. (2015). Global cytosine methylation in *Daphnia magna* depends on genotype, environment, and their interaction. *Environmental Toxicology and Chemistry*, *34*(5), 1056–1061. https://doi.org/10.1002/ETC.2887

Bang, C., Dagan, T., Deines, P., Dubilier, N., Duschl, W. J., Fraune, S., Hentschel, U., Hirt, H., Hülter, N., Lachnit, T., Picazo, D., Pita, L., Pogoreutz, C., Rädecker, N., Saad, M. M., Schmitz, R. A., Schulenburg, H., Voolstra, C. R., Weiland-Bräuer, N., … Bosch, T. C. G. (2018). Metaorganisms in extreme environments: Do microbes play a role in organismal adaptation? *Zoology*, *127*, 1–19. https://doi.org/10.1016/j.zool.2018.02.004

Barrett, R. D. H., & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution*, *23*(1), 38–44. https://doi.org/10.1016/j.tree.2007.09.008

Barton, B. T. (2011). Local adaptation to temperature conserves top-down control in a grassland food web. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1721), 3102–3107. https://doi.org/10.1098/rspb.2011.0030

Bewick, A. J., Vogel, K. J., Moore, A. J., & Schmitz, R. J. (2017). Evolution of DNA methylation across insects. *Molecular Biology and Evolution*, *34*(3), 654–665. https://doi.org/10.1093/molbev/msw264

Bind, M.-A., Zanobetti, A., Gasparrini, A., Peters, A., Coull, B., Baccarelli, A., Tarantini, L., Koutrakis, P., Vokonas, P., Schwartz, J. (2014). Effects of temperature and relative humidity on DNA methylation. *Epidemiology (Cambridge, Mass.)*, *25*(4), 561. https://doi.org/10.1097/EDE.0000000000000120

Bittinger, K. (2020). *usedist: Distance matrix utilities*. Retrieved from https://cran.r-project.org/package=usedist

Bossdorf, O., Richards, C. L., & Pigliucci, M. (2008). Epigenetics for ecologists. *Ecology Letters*, *11*(2), 106–115. https://doi.org/10.1111/j.1461-0248.2007.01130.x

Buchfink, B., Reuter, K., & Drost, H. G. (2021). Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nature Methods*, *18*(4), 366–368. https://doi.org/10.1038/s41592-021-01101-x

Busck, M. M., Lund, M. B., Bird, T. L., Bechsgaard, J. S., Bilde, T., & Schramm, A. (2022). Temporal and spatial microbiome dynamics across natural populations of the social spider *Stegodyphus dumicola*. *FEMS Microbiology Ecology*, *98*, 1–11. https://doi.org/10.1093/femsec/fiac015

Busck, M. M., Settepani, V., Bechsgaard, J., Lund, M. B., Bilde, T., & Schramm, A. (2020). Microbiomes and specific symbionts of social spiders: Compositional patterns in host species, populations, and nests. *Frontiers in Microbiology*, 1–14. https://doi.org/10.3389/FMICB.2020.01845

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

Cantalapiedra, C. P., Herɲandez-Plaza, A., Letunic, I., Bork, P., & Huerta-Cepas, J. (2021). eggNOG-mapper v2: Functional annotation, Orthology assignments, and domain prediction at the metagenomic scale. *Molecular Biology and Evolution*, *38*(12), 5825–5829. https://doi.org/10.1093/MOLBEV/MSAB293

Castellano, S., & Balletto, E. (2002). Is the partial mantel test inadequate? *Evolution*, *56*(9), 1871–1873. https://doi.org/10.1111/j.0014-3820.2002.tb00203.x

Cavalli, G. (2006). Chromatin and epigenetics in development: Blending cellular memory with cell fate plasticity. *Development*, *133*(11), 2089–2094. https://doi.org/10.1242/dev.02402

Charlesworth, D., Barton, N. H., & Charlesworth, B. (2017). The sources of adaptive variation. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1855), 1–12. https://doi.org/10.1098/rspb.2016.2864

Chen, H. (2018). *VennDiagram: Generate High-Resolution Venn and Euler Plots*. Retrieved from https://cran.r-project.org/package=VennDiagram%0A

Choi, J., Lyons, D. B., Kim, M. Y., Moore, J. D., & Zilberman, D. (2020). DNA methylation and histone H1 jointly repress transposable elements and aberrant intragenic transcripts. *Molecular Cell*, *77*(2), 310–323.e7. https://doi.org/10.1016/J.MOLCEL.2019.10.011

Coates, B. S., Sumerford, D. V., Miller, N. J., Kim, K. S., Sappington, T. W., Siegfried, B. D., & Lewis, L. C. (2009). Comparative performance of single nucleotide polymorphism and microsatellite markers for population genetic analysis. *Journal of Heredity*, *100*(5), 556–564. https://doi.org/10.1093/jhered/esp028

Cropley, J. E., Dang, T. H. Y., Martin, D. I. K., & Suter, C. M. (2012). The penetrance of an epigenetic trait in mice is progressively yet reversibly increased by selection and environment. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1737), 2347–2353. https://doi.org/10.1098/rspb.2011.2646

Cubas, P., Vincent, C., & Coen, E. (1999). An epigenetic mutation responsible for natural variation in floral symmetry. *Nature*, *401*(6749), 157–161. https://doi.org/10.1038/43657

Dahlgaard, J., Loeschcke, V., Michalak, P., & Justesen, J. (1998). Induced thermotolerance and associated expression of the heat-shock protein Hsp70 in adult *Drosophila melanogaster*. *Functional Ecology*, *12*(5), 786–793. https://doi.org/10.1046/j.1365-2435.1998.00246.x

Danecek, P., & McCarthy, S. A. (2017). BCFtools/csq: Haplotype-aware variant consequences. *Bioinformatics*, *33*(13), 2037–2039. https://doi.org/10.1093/bioinformatics/btx100

de Mendoza, A., Lister, R., & Bogdanovic, O. (2020). Evolution of DNA methylome diversity in eukaryotes. *Journal of Molecular Biology*, *432*(6), 1687–1705. https://doi.org/10.1016/j.jmb.2019.11.003

Dimond, J. L., & Roberts, S. B. (2020). Convergence of DNA methylation profiles of the reef coral *Porites astreoides* in a novel environment. *Frontiers in Marine Science*, *6*, 792. https://doi.org/10.3389/fmars.2019.00792

Dixon, G., Liao, Y., Bay, L. K., & Matz, M. V. (2018). Role of gene body methylation in acclimatization and adaptation in a basal metazoan. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(52), 13342–13346. https://doi.org/10.1073/pnas.1813749115

Donelson, J. M., Sunday, J. M., Figueira, W. F., Gaitán-Espitia, J. D., Hobday, A. J., Johnson, C. R., … Munday, P. L. (2019). Understanding interactions between plasticity, adaptation and range shifts in response to marine environmental change. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *374*(1768), 20180186. https://doi.org/10.1098/rstb.2018.0186

Dubin, M. J., Zhang, P., Meng, D., Remigereau, M. S., Osborne, E. J., Paolo Casale, F., Drewe, P., Kahles, A., Jean, G., Vilhjálmsson, B., Jagoda, J., Irez, S., Voronin, V., Song, Q., Long, Q., Rätsch, G., Stegle, O., Clark, R. M., & Nordborg, M. (2015). DNA methylation in *Arabidopsis* has a genetic basis and shows evidence of local adaptation. *eLife*, *4*, 1–23. https://doi.org/10.7554/eLife.05255

Dunbar, H. E., Wilson, A. C. C., Ferguson, N. R., & Moran, N. A. (2007). Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. *PLoS Biology*, *5*(5), 1006–1015. https://doi.org/10.1371/journal.pbio.0050096

Duncan, E. J., Cunningham, C. B., & Dearden, P. K. (2022). Phenotypic plasticity: What has DNA methylation got to do with it? *13*(2), 110. Retrieved from https://www.mdpi.com/2075-4450/13/2/110/htm

Falcon, S., & Gentleman, R. (2007). Using GOstats to test gene lists for GO term association. *Bioinformatics*, *23*(2), 257–258. https://doi.org/10.1093/BIOINFORMATICS/BTL567

Fischer, M. C., Rellstab, C., Tedder, A., Zoller, S., Gugerli, F., Shimizu, K. K., … Widmer, A. (2013). Population genomic footprints of selection and associations with climate in natural populations of *Arabidopsis halleri* from the Alps. *Molecular Ecology*, *22*(22), 5594–5607. https://doi.org/10.1111/mec.12521

Flores, K. B., Wolschin, F., & Amdam, G. V. (2013). The role of methylation of DNA in environmental adaptation. *Integrative and Comparative Biology*, *53*(2), 359–372. https://doi.org/10.1093/icb/ict019

Flores, K. B., Wolschin, F., Corneveaux, J. J., Allen, A. N., Huentelman, M. J., & Amdam, G. V. (2012). Genome-wide association between DNA methylation and alternative splicing in an invertebrate. *BMC Genomics*, *13*(1), 1–9. https://doi.org/10.1186/1471-2164-13-480

Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitán-Espitia, J. D. (2019). Beyond buying time: The role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *374*(1768), 1–9. https://doi.org/10.1098/rstb.2018.0174

Gao, F., Liu, X., Wu, X. P., Wang, X. L., Gong, D., Lu, H., Xia, Y., Song, Y., Wang, J., Du, J., Liu, S., Han, X., Tang, Y., Yang, H., Jin, Q., Zhang, X., & Liu, M. (2012). Differential DNA methylation in discrete developmental stages of the parasitic nematode *trichinella spiralis*. *Genome Biology*, *13*(10), R100. https://doi.org/10.1186/gb-2012-13-10-r100

Gatzmann, F., Falckenhayn, C., Gutekunst, J., Hanna, K., Raddatz, G., Carneiro, V. C., & Lyko, F. (2018). The methylome of the marbled crayfish links gene body methylation to stable expression of poorly accessible genes. *Epigenetics & Chromatin*, *11*(1), 1–12. https://doi.org/10.1186/s13072-018-0229-6

Gefen, E., Talal, S., Brendzel, O., Dror, A., & Fishman, A. (2015). Variation in quantity and composition of cuticular hydrocarbons in the scorpion *Buthus occitanus* (Buthidae) in response to acute exposure to desiccation stress. *Comparative Biochemistry and Physiology. Part A*, *182*, 58–63. https://doi.org/10.1016/J.CBPA.2014.12.004

Ghalambor, C. K., Hoke, K. L., Ruell, E. W., Fischer, E. K., Reznick, D. N., & Hughes, K. A. (2015). Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature*, *525*(7569), 372–375. https://doi.org/10.1038/nature15256

Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus nonadaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, *21*(3), 394–407. https://doi.org/10.1111/j.1365-2435.2007.01283.x

Gong, T. W., Fairfield, D. A., Fullarton, L., Dolan, D. F., Altschuler, R. A., Kohrman, D. C., & Lomax, M. I. (2012). Induction of heat shock proteins by hyperthermia and noise overstimulation in Hsf1−/− mice. *JARO – Journal of the Association for Research in Otolaryngology*, *13*(1), 29–37. https://doi.org/10.1007/s10162-011-0289-9

Gonzalo-Turpin, H., & Hazard, L. (2009). Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *Journal of Ecology*, *97*(4), 742–751. https://doi.org/10.1111/j.1365-2745.2009.01509.x

Goslee, S. C., & Urban, D. L. (2007). The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, *22*(7), 1–19. https://doi.org/10.18637/jss.v022.i07

Grieser, J., Gommes, R., & Bernardi, M. (2006). New LocClim – the local climate estimator of FAO. *Geophysical Research Abstracts*, *8*(1), 8305.

Gugger, P. F., Fitz-Gibbon, S., Pellegrini, M., & Sork, V. L. (2016). Species-wide patterns of DNA methylation variation in *Quercus lobata* and their association with climate gradients. *Molecular Ecology*, *25*(8), 1665–1680. https://doi.org/10.1111/mec.13563

Guillot, G., & Rousset, F. (2013). Dismantling the mantel tests. *Methods in Ecology and Evolution*, *4*(4), 336–344. https://doi.org/10.1111/2041-210x.12018

Hanson, D., Hu, J., Hendry, A. P., & Barrett, R. D. H. (2017). Heritable gene expression differences between lake and stream stickleback include both parallel and antiparallel components. *Heredity*, *119*(5), 339–348. https://doi.org/10.1038/hdy.2017.50

Harney, E., Paterson, S., Collin, H., Chan, B. H. K., Bennett, D., & Plaistow, S. J. (2022). Pollution induces epigenetic effects that are stably transmitted across multiple generations. *Evolution Letters*, *6*(2), 118–135. https://doi.org/10.1002/EVL3.273

Hawes, N. A., Tremblay, L. A., Pochon, X., Dunphy, B., Fidler, A. E., & Smith, K. F. (2018). Effects of temperature and salinity stress on DNA methylation in a highly invasive marine invertebrate, the colonial ascidian *Didemnum vexillum*. *PeerJ*, *2018*(6), 1–18. https://doi.org/10.7717/PEERJ.5003/SUPP-4

Heckwolf, M. J., Meyer, B. S., Häsler, R., Höppner, M. P., Eizaguirre, C., & Reusch, T. B. H. (2019). Two different epigenetic pathways detected in wild three-spined sticklebacks are involved in salinity adaptation. BioRxiv, 649574. doi: https://doi.org/10.1101/649574

Henikoff, S., Furuyama, T., & Ahmad, K. (2004). Histone variants, nucleosome assembly and epigenetic inheritance. *Trends in Genetics*, *20*(7), 320–326. https://doi.org/10.1016/j.tig.2004.05.004

Henry, L. P., Bruijning, M., Forsberg, S. K. G., & Ayroles, J. F. (2019). Can the microbiome influence host evolutionary trajectories? BioRxiv, 700237. doi: https://doi.org/10.1101/700237

Herlemann, D. P. R., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., & Andersson, A. F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME Journal*, *5*(10), 1571–1579. https://doi.org/10.1038/ismej.2011.41

Herrera, M., Klein, S. G., Campana, S., Chen, J. E., Prasanna, A., Duarte, C. M., & Aranda, M. (2021). Temperature transcends partner specificity in the symbiosis establishment of a cnidarian. *ISME Journal*, *15*(1), 141–153. https://doi.org/10.1038/s41396-020-00768-y

Holliday, R. (1987). The inheritance of epigenetic defects. *Science, New Series*, *238*(4824), 163–170.

Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S. K., Cook, H., Mende, D. R., Letunic, I., Rattei, T., Jensen, L. J., von Mering, C., & Bork, P. (2019). eggNOG 5.0: A hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Research*, *47*(D1), D309–D314. https://doi.org/10.1093/NAR/GKY1085

Ittiprasert, W., Miller, A., Knight, M., Tucker, M., & Hsieh, M. H. (2015). Evaluation of cytosine DNA methylation of the *Biomphalaria glabratahe* at shock protein 70 locus after biological and physiological stresses. *Journal of Parasitology and Vector Biology*, *7*(10), 182–193. https://doi.org/10.5897/JPVB2015.0207

Jablonka, E. (2017). The evolutionary implications of epigenetic inheritance. *Interface Focus*, *7*(5), 1–46. https://doi.org/10.1098/rsfs.2016.0135

Jensen, J. D., & Bachtrog, D. (2011). Characterizing the influence of effective population size on the rate of adaptation: Gillespie's Darwin domain. *Genome Biology and Evolution*, *3*(1), 687–701. https://doi.org/10.1093/gbe/evr063

Keller, T. E., Han, P., & Yi, S. V. (2016). Evolutionary transition of promoter and gene body DNA methylation across invertebrate-vertebrate boundary. *Molecular Biology and Evolution*, *33*(4), 1019–1028. https://doi.org/10.1093/molbev/msv345

Kofler, R., Orozco-terWengel, P., de Maio, N., Pandey, R. V., Nolte, V., Futschik, A., Kosiol, C., & Schlötterer, C. (2011). Popoolation: A toolbox for population genetic analysis of next generation sequencing data from pooled individuals. *PLoS One*, *6*(1), e15925. https://doi.org/10.1371/journal.pone.0015925

Kofler, R., Pandey, R. V., & Schlötterer, C. (2011). PoPoolation2: Identifying differentiation between populations using sequencing of pooled DNA samples (Pool-seq). *Bioinformatics*, *27*(24), 3435–3436. https://doi.org/10.1093/bioinformatics/btr589

Kong, F., Hua, Y., Zeng, B., Ning, R., Li, Y., & Zhao, J. (2016). Gut microbiota signatures of longevity. *Current Biology*, *26*(18), R832–R833. https://doi.org/10.1016/j.cub.2016.08.015

Krueger, F., & Andrews, S. R. (2011). Bismark: A flexible aligner and methylation caller for bisulfite-seq applications. *Bioinformatics*, *27*(11), 1571–1572. https://doi.org/10.1093/bioinformatics/btr167

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Kvist, J., Gonçalves Athanàsio, C., Shams Solari, O., Brown, J. B., Colbourne, J. K., Pfrender, M. E., & Mirbahai, L. (2018). Pattern of DNA methylation in *daphnia*: Evolutionary perspective. *Genome Biology and Evolution*, *10*(8), 1988–2007. https://doi.org/10.1093/gbe/evy155

Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology*, *22*(7), 1435–1446. https://doi.org/10.1111/j.1420-9101.2009.01754.x

Lande, R., & Shannon, S. (1996). The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution*, *50*(1), 434–437. https://doi.org/10.1111/j.1558-5646.1996.tb04504.x

Leffler, E. M., Bullaughey, K., Matute, D. R., Meyer, W. K., Ségurel, L., Venkat, A., Andolfatto, P., & Przeworski, M. (2012). Revisiting an old Riddle: What determines genetic diversity levels within species? *PLoS Biology*, *10*(9), e1001388. https://doi.org/10.1371/journal.pbio.1001388

Legendre, P. (2000). Comparison of permutation methods for the partial correlation and partial mantel tests. *Journal of Statistical Computation*

and Simulation, *67*(1), 37–73. https://doi.org/10.1080/00949650008812035

Legendre, P., Fortin, M. J., & Borcard, D. (2015). Should the mantel test be used in spatial analysis? *Methods in Ecology and Evolution*, *6*(11), 1239–1247. https://doi.org/10.1111/2041-210X.12425

Lev Maor, G., Yearim, A., & Ast, G. (2015). The alternative role of DNA methylation in splicing regulation. *Trends in Genetics*, *31*(5), 274–280. https://doi.org/10.1016/J.TIG.2015.03.002

Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*, *27*(21), 2987–2993. https://doi.org/10.1093/bioinformatics/btr509

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics*, *25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Liu, S., Aagaard, A., Bechsgaard, J., & Bilde, T. (2019). DNA methylation patterns in the social spider, Stegodyphus dumicola. *Genes*, *10*(2), 1–17. https://doi.org/10.3390/genes10020137

Liu, Y., Ma, S., Chang, J., Zhang, T., Chen, X., Liang, Y., & Xia, Q. (2019). Programmable targeted epigenetic editing using CRISPR system in *Bombyx mori*. *Insect Biochemistry and Molecular Biology*, *110*, 105–111. https://doi.org/10.1016/J.IBMB.2019.04.013

Lubin, Y., & Bilde, T. (2007). The evolution of sociality in spiders. *Advances in the Study of Behavior*, *37*, 83–145. https://doi.org/10.1016/S0065-3454(07)37003-4

Lynch, J. B., & Hsiao, E. Y. (2019). Microbiomes as sources of emergent host phenotypes. *Science*, *365*(6460), 1405–1409. https://doi.org/10.1126/science.aay0240

Majer, M., Svenning, J. C., & Bilde, T. (2015). Habitat productivity predicts the global distribution of social spiders. *Frontiers in Ecology and Evolution*, *3*(101), 1–10. https://doi.org/10.3389/fevo.2015.00101

Malmos, K. G., Lüdeking, A. H., Vosegaard, T., Aagaard, A., Bechsgaard, J., Sørensen, J. G., & Bilde, T. (2021). Behavioural and physiological responses to thermal stress in a social spider. *Functional Ecology*, *35*, 2728–2742. https://doi.org/10.1111/1365-2435.13921

Marshall, H., Lonsdale, Z. N., & Mallon, E. B. (2019). Methylation and gene expression differences between reproductive and sterile bumblebee workers. *Evolution Letters*, *3*(5), 485–499. https://doi.org/10.1002/evl3.129

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10. https://doi.org/10.14806/ej.17.1.200

Mazzucco, R., & Schlötterer, C. (2021). Long-term gut microbiome dynamics in *Drosophila melanogaster* reveal environment-specific associations between bacterial taxa at the family level. *Proceedings of the Royal Society B*, *288*(1965), 20212193. https://doi.org/10.1098/RSPB.2021.2193

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C. G., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S. F., Hentschel, U., King, N., Kjelleberg, S., Knoll, A. H., Kremer, N., Mazmanian, S. K., Metcalf, J. L., Nealson, K., Pierce, N. E., … Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(9), 3229–3236. https://doi.org/10.1073/pnas.1218525110

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*(4), 1–11. https://doi.org/10.1371/journal.pone.0061217

Medeiros, M. C. I., Seabourn, P. S., Rollins, R. L., & Yoneishi, N. M. (2021). Mosquito microbiome diversity varies along a landscape-scale moisture gradient. *Microbial Ecology*, *1*, 1–8. https://doi.org/10.1007/s00248-021-01865-x

Metzger, D. C. H., & Schulte, P. M. (2017). Persistent and plastic effects of temperature on dna methylation across the genome of threespine stickleback (*gasterosteus aculeatus*). *Proceedings of the Royal Society B: Biological Sciences*, *284*(1864), 1–7. https://doi.org/10.1098/rspb.2017.1667

Moran, N. A., Ochman, H., & Hammer, T. J. (2019). Evolutionary and ecological consequences of gut microbial communities. *Annual Review of Ecology, Evolution, and Systematics*, *50*, 451–475. https://doi.org/10.1146/annurev-ecolsys-110617-062453

Morgan, M., Falcon, S., & Gentleman, R. (2022). GSEABase: Gene set enrichment data structures and methods.

Morgan, T. J., Herman, M. A., Johnson, L. C., Olson, B. J. C. S., & Ungerer, M. C. (2018). Ecological genomics: Genes in ecology and ecology in genes, *61*(4), v–vii. https://doi.org/10.1139/Gen-2018-0022

Mueller, E. A., Wisnoski, N. I., Peralta, A. L., & Lennon, J. T. (2020). Microbial rescue effects: How microbiomes can save hosts from extinction. *Functional Ecology*, *34*(10), 2055–2064. https://doi.org/10.1111/1365-2435.13493

Nätt, D., Rubin, C. J., Wright, D., Johnsson, M., Beltéky, J., Andersson, L., & Jensen, P. (2012). Heritable genome-wide variation of gene expression and promoter methylation between wild and domesticated chickens. *BMC Genomics*, *13*(1), 1–12. https://doi.org/10.1186/1471-2164-13-59

Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics (first edit)*. Oxford University Press, USA.

Neri, F., Rapelli, S., Krepelova, A., Incarnato, D., Parlato, C., Basile, G., Maldotti, M., Anselmi, F., & Oliviero, S. (2017). Intragenic DNA methylation prevents spurious transcription initiation. *Nature*, *543*(7643), 72–77. https://doi.org/10.1038/nature21373

Ngaira, K. J. W. (2007). Impact of climate change on agriculture in Africa by 2030. *Scientific Research and Essays*, *2*(7), 238–243. https://doi.org/10.5897/SRE.9000567

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2019). Vegan: Community ecology pack. Retrieved from https://cran.r-project.org/package=vegan

Ørsted, M., Hoffmann, A. A., Sverrisdóttir, E., Nielsen, K. L., & Kristensen, T. N. (2019). Genomic variation predicts adaptive evolutionary responses better than population bottleneck history. *PLoS Genetics*, *15*(6), 1–18. https://doi.org/10.1371/journal.pgen.1008205

Peña, M. V., Piskobulu, V., Murgatroyd, C., & Hager, R. (2021). DNA methylation patterns respond to thermal stress in the viviparous cockroach *Diploptera punctata*. *Epigenetics*, *16*(3), 313–326. https://doi.org/10.1080/15592294.2020.1795603

Perry, B. W., Schield, D. R., & Castoe, T. A. (2018). Evolution: Plasticity versus selection, or plasticity and selection? *Current Biology*, *28*(18), R1104–R1106. https://doi.org/10.1016/J.CUB.2018.07.050

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, 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

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics*, *26*(6), 841–842. https://doi.org/10.1093/bioinformatics/btq033

R Core Team. (2019). *R: A language and environment for computing (R Foundation for Statistical Computing, ed.)*. R Foundation for Statistical Computing Retrieved from https://www.r-project.org/

Raufaste, N., & Rousset, F. (2001). Are partial Mantel test adequate? *Evolution*, *55*(8), 1703–1705. https://doi.org/10.1554/0014-3820(2001)055[1703:APMTA]2.0.CO;2

Raza, M. F., Wang, Y., Cai, Z., Bai, S., Yao, Z., Awan, U. A., … Zhang, H. (2020). Gut microbiota promotes host resistance to low-temperature stress by stimulating its arginine and proline metabolism pathway in adult *Bactrocera dorsalis*. *PLoS Pathogens*, *16*(4), e1008441. https://doi.org/10.1371/journal.ppat.1008441

Rellstab, C., Gugerli, F., Eckert, A. J., Hancock, A. M., & Holderegger, R. (2015). A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology*, *24*(17), 4348–4370. https://doi.org/10.1111/mec.13322

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), 1–9. https://doi.org/10.1098/rspb.2019.1911

Rico, L., Ogaya, R., Barbeta, A., & Peñuelas, J. (2014). Changes in DNA methylation fingerprint of *Quercus ilex* trees in response to experimental field drought simulating projected climate change. *Plant Biology*, *16*(2), 419–427. https://doi.org/10.1111/plb.12049

Riddle, N. C., & Richards, E. J. (2002). The control of natural variation in cytosine methylation in *Arabidopsis*. *Genetics*, *162*(1), 355–363. https://doi.org/10.1093/genetics/162.1.355

Rousselle, M., Simion, P., Tilak, M. K., Figuet, E., Nabholz, B., & Galtier, N. (2020). Is adaptation limited by mutation? A timescale-dependent effect of genetic diversity on the adaptive substitution rate in animals. *PLoS Genetics*, *16*(4), 1–24. https://doi.org/10.1371/journal.pgen.1008668

Rudman, S. M., Greenblum, S., Hughes, R. C., Rajpurohit, S., Kiratli, O., Lowder, D. B., … Schmidt, P. (2019). Microbiome composition shapes rapid genomic adaptation of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(40), 20025–20032. https://doi.org/10.1073/pnas.1907787116

Sandfeld, T., Malmos, K. G., Nielsen, C. B., Lund, M. B., Aagaard, A., Bechsgaard, J., … Schramm, A. (2022). Metabolite profiling of the social spider *Stegodyphus dumicola* along a climate gradient. *Frontiers in Ecology and Evolution*, *10*, 1–11. https://doi.org/10.3389/fevo.2022.841490

Sarda, S., Zeng, J., Hunt, B. G., & Yi, S. V. (2012). The evolution of invertebrate gene body methylation. *Molecular Biology and Evolution*, *29*(8), 1907–1916. https://doi.org/10.1093/molbev/mss062

Schultz, M. D., Schmitz, R. J., & Ecker, J. R. (2012). 'Leveling' the playing field for analyses of single-base resolution DNA methylomes. *Trends in Genetics*, *28*(12), 583–585. https://doi.org/10.1016/j.tig.2012.10.012

Schwartz, M. K., Luikart, G., & Waples, R. S. (2007). Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution*, *22*(1), 25–33. https://doi.org/10.1016/j.tree.2006.08.009

Secco, D., Wang, C., Shou, H., Schultz, M. D., Chiarenza, S., Nussaume, L., … Lister, R. (2015). Stress induced gene expression drives transient DNA methylation changes at adjacent repetitive elements. *eLife*, *4*, 1–26. https://doi.org/10.7554/ELIFE.09343.001

Settepani, V., Bechsgaard, J., & Bilde, T. (2014). Low genetic diversity and strong but shallow population differentiation suggests genetic homogenization by metapopulation dynamics in a social spider. *Journal of Evolutionary Biology*, *27*(12), 2850–2855. https://doi.org/10.1111/jeb.12520

Settepani, V., Bechsgaard, J., & Bilde, T. (2016). Phylogenetic analysis suggests that sociality is associated with reduced effectiveness of selection. *Ecology and Evolution*, *6*(2), 469–477. https://doi.org/10.1002/ece3.1886

Settepani, V., Schou, M. F., Greve, M., Grinsted, L., Bechsgaard, J., & Bilde, T. (2017). Evolution of sociality in spiders leads to depleted genomic diversity at both population and species levels. *Molecular Ecology*, *26*(16), 4197–4210. https://doi.org/10.1111/mec.14196

Sgrò, C. M., Lowe, A. J., & Hoffmann, A. A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications*, *4*(2), 326–337. https://doi.org/10.1111/j.1752-4571.2010.00157.x

Sgrò, C. M., Terblanche, J. S., & Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annual Review of Entomology*, *61*, 433–451. https://doi.org/10.1146/annurev-ento-010715-023859

Shigenobu, S., & Wilson, A. C. C. (2011). Genomic revelations of a mutualism: The pea aphid and its obligate bacterial symbiont. *Cellular and Molecular Life Sciences*, *68*(8), 1297–1309. https://doi.org/10.1007/s00018-011-0645-2

Song, Y., Liu, L., Feng, Y., Wei, Y., Yue, X., He, W., … An, L. (2015). Chilling- and freezing- induced alterations in cytosine methylation and its association with the cold tolerance of an alpine Subnival plant, *Chorispora bungeana*. *PLoS One*, *10*(8), e0135485. https://doi.org/10.1371/JOURNAL.PONE.0135485

Sørensen, J. G., & Loeschcke, V. (2002). Natural adaptation to environmental stress via physiological clock-regulation of stress resistance in *drosophila*. *Ecology Letters*, *5*(1), 16–19. https://doi.org/10.1046/J.1461-0248.2002.00296.X

Sutherland, H. G. E., Kearns, M., Morgan, H. D., Headley, A. P., Morris, C., Martin, D. I. K., & Whitelaw, E. (2000). Reactivation of heritably silenced gene expression in mice. *Mammalian Genome*, *11*(5), 347–355. https://doi.org/10.1007/s003350010066

Suzuki, T. A., Martins, F. M., & Nachman, M. W. (2019). Altitudinal variation of the gut microbiota in wild house mice. *Molecular Ecology*, *28*(9), 2378. https://doi.org/10.1111/MEC.14905

Tennekes, M. (2018). Tmap: Thematic maps in R. *Journal of Statistical Software*, *84*(1), 1–39. https://doi.org/10.18637/JSS.V084.I06

Thomas, D. (2010). Gene–environment-wide association studies: Emerging approaches. *Nature Reviews Genetics*, *11*(4), 259–272. https://doi.org/10.1038/nrg2764

Toolson, E. C. (1982). Effects of rearing temperature on cuticle permeability and epicuticular lipid composition in *Drosophila pseudoobscura*. *Journal of Experimental Zoology*, *222*(3), 249–253. https://doi.org/10.1002/JEZ.1402220307

Tregenza, T., Rodríguez-Muñoz, R., Boonekamp, J. J., Hopwood, P. E., Sørensen, J. G., Bechsgaard, J., Settepani, V., Hegde, V., Waldie, C., May, E., Peters, C., Pennington, Z., Leone, P., Munk, E. M., Greenrod, S. T. E., Gosling, J., Coles, H., Gruffydd, R., Capria, L., … Bilde, T. (2021). Evidence for genetic isolation and local adaptation in the field cricket *Gryllus campestris*. *Journal of Evolutionary Biology*, *34*(10), 1624–1636. https://doi.org/10.1111/JEB.13911

Tricker, P. J., Gibbings, J. G., Rodríguez López, C. M., Hadley, P., & Wilkinson, M. J. (2012). Low relative humidity triggers RNA-directed de novo DNA methylation and suppression of genes controlling stomatal development. *Journal of Experimental Botany*, *63*(10), 3799–3813. https://doi.org/10.1093/JXB/ERS076

Ungerer, M. C., Johnson, L. C., & Herman, M. A. (2008). Ecological genomics: Understanding gene and genome function in the natural environment. *Heredity*, *100*(2), 178–183. https://doi.org/10.1038/sj.hdy.6800992

Varriale, A. (2014). DNA methylation, epigenetics, and evolution in vertebrates: Facts and challenges. *International Journal of Evolutionary Biology*, *2014*, 1–7. https://doi.org/10.1155/2014/475981

Verhoeven, K. J. F., Jansen, J. J., van Dijk, P. J., & Biere, A. (2010). Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytologist*, *185*(4), 1108–1118. https://doi.org/10.1111/j.1469-8137.2009.03121.x

Walters, A. W., Hughes, R. C., Call, T. B., Walker, C. J., Wilcox, H., Petersen, S. C., Rudman, S. M., Newell, P. D., Douglas, A. E., Schmidt, P. S., & Chaston, J. M. (2020). The microbiota influences the *Drosophila melanogaster* life history strategy. *Molecular Ecology*, *29*(3), 639–653. https://doi.org/10.1111/MEC.15344

Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. *Molecular Ecology*, *23*(23), 5649–5662. https://doi.org/10.1111/MEC.12938

Waters, J. L., & Ley, R. E. (2019). The human gut bacteria Christensenellaceae are widespread, heritable, and associated with health. *BMC Biology*, *17*(1), 1–11. https://doi.org/10.1186/s12915-019-0699-4

Wernegreen, J. J. (2012). Mutualism meltdown in insects: Bacteria constrain thermal adaptation. *Current Opinion in Microbiology*, *15*(3), 255–262. https://doi.org/10.1016/j.mib.2012.02.001

Wickham, H., François, R., Henry, L., & Müller, K. (2021). Dplyr: A grammar of data manipulation. Retrieved from https://cran.r-project.org/package=dplyr

Willi, Y., Van Buskirk, J., & Hoffmann, A. A. (2006). Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics*, *37*, 433–458. https://doi.org/10.1146/annurev.ecolsys.37.091305.110145

Williams, B. R., Van Heerwaarden, B., Dowling, D. K., & Sgrò, C. M. (2012). A multivariate test of evolutionary constraints for thermal tolerance in *Drosophila melanogaster*. *Journal of Evolutionary Biology*, *25*(7), 1415–1426. https://doi.org/10.1111/j.1420-9101.2012.02536.x

Williamson, S. M., Ingelson-Filpula, W. A., Hadj-Moussa, H., & Storey, K. B. (2021). Epigenetic underpinnings of freeze avoidance in the goldenrod gall moth, *Epiblema scudderiana*. *Journal of Insect Physiology*, *134*, 104298. https://doi.org/10.1016/J.JINSPHYS.2021.104298

Woodhams, D. C., Bletz, M. C., Becker, C. G., Bender, H. A., Buitrago-Rosas, D., Diebboll, H., Huynh, R., Kearns, P. J., Kueneman, J., Kurosawa, E., LaBumbard, B., Lyons, C., McNally, K., Schliep, K., Shankar, N., Tokash-Peters, A. G., Vences, M., & Whetstone, R. (2020). Host-associated microbiomes are predicted by immune system complexity and climate. *Genome Biology*, *21*(1), 1–20. https://doi.org/10.1186/S13059-019-1908-8

Wu, C. T., & Morris, J. R. (2001). Genes, genetics, and epigenetics: A correspondence. *Science*, *293*(5532), 1103–1105. https://doi.org/10.1126/SCIENCE.293.5532.1103

Xu, G., Lyu, H., Yi, Y., Peng, Y., Feng, Q., Song, Q., … Zheng, S. (2021). Intragenic DNA methylation regulates insect gene expression and reproduction through the MBD/Tip60 complex. *IScience*, *24*(2), 102040. https://doi.org/10.1016/J.ISCI.2021.102040

Yin, H., Zhong, Y., Wang, H., Hu, J., Xia, S., Xiao, Y., … Xie, M. (2022). Short-term exposure to high relative humidity increases blood urea and influences colonic urea-nitrogen metabolism by altering the gut microbiota. *Journal of Advanced Research*. https://doi.org/10.1016/J.JARE.2021.03.004

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Aagaard, A., Liu, S., Tregenza, T., Braad Lund, M., Schramm, A., Verhoeven, K. J. F., Bechsgaard, J., & Bilde, T. (2022). Adapting to climate with limited genetic diversity: Nucleotide, DNA methylation and microbiome variation among populations of the social spider *Stegodyphus dumicola*. *Molecular Ecology*, *31*, 5765–5783. https://doi.org/10.1111/mec.16696