

Interaction between MHC diversity and constitution, gut microbiota and Astrovirus infections in a neotropical bat

Ramona Fleischer¹  | Dominik W. Schmid¹ | Wasimuddin²  | Stefan D. Brändel^{1,3} |
 Andrea Rasche^{3,4} | Victor M. Corman^{4,5}  | Christian Drosten^{4,5} | Marco Tschapka^{1,3} |
 Simone Sommer¹ 

¹Institute of Evolutionary Ecology and Conservation Genomics, University of Ulm, Ulm, Germany

²CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India

³Smithsonian Tropical Research Institute, Ancon, Panama

⁴Institute of Virology, Charité-Universitätsmedizin Berlin, Corporate Member of Free University, Humboldt-University and Berlin Institute of Health, Berlin, Germany

⁵German Centre for Infection Research (DZIF), Associated Partner Charité, Berlin, Germany

Correspondence

Ramona Fleischer and Simone Sommer,
 Institute of Evolutionary Ecology and
 Conservation Genomics, University of
 Ulm, Ulm, Germany.

Emails: [\(R.F.\)](mailto:ramona.fleischer@uni-ulm.de); [\(S. S.\)](mailto:simone.sommer@uni-ulm.de)

Funding information

German Science Foundation (DFG), Grant/Award Number: SO 428/9-1, SO 428/9-2, TS 81/7-1, TS 81/7-2 and DR 772/8-1

Handling Editor: Camille Bonneau

Abstract

Astroviruses (AstVs) infect numerous mammalian species including reservoirs such as bats. Peptides encoded by the genes of the highly polymorphic Major Histocompatibility Complex (MHC) form the first line of host defence against pathogens. Aside from direct involvement in mounting adaptive immune responses, MHC class II genes are hypothesized to regulate gut commensal diversity and shape the production of immune-modulatory substances by microbes, indirectly affecting host susceptibility. Despite initial empirical evidence for the link between host MHC and the microbiota, associations among these factors remain largely unknown. To fill this gap, we examined MHC allelic diversity and constitution, the gut bacterial community and abundance pattern of a wild population of a neotropical bat (*Artibeus jamaicensis*) challenged by AstV infections. First, we show an age-dependent relationship between the host MHC class II diversity and constitution and the gut microbiota in AstV-uninfected bats. Crucially, these associations changed in AstV-infected bats. Additionally, we identify changes in the abundance of specific bacterial taxa linked to the presence of certain MHC supertypes and AstV infection. We suggest changes in the microbiota to be either a result of AstV infection or the MHC-mediated modulation of microbial communities. The latter could subsequently affect microbe-mediated immunity and resistance against AstV infection. Our results emphasize that the reciprocal nature of host immune genetics, gut microbial diversity and pathogen infection require attention, which are particularly important given their repercussions for disease susceptibility and severity in wild animal populations with a history of zoonotic spillover and frequent human contact.

KEY WORDS

Artibeus jamaicensis, astrovirus infection, gut microbiome, immunity, Major Histocompatibility Complex (MHC), zoonosis

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs License](https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
 © 2022 The Authors. *Molecular Ecology* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

Bats are well-known reservoirs for zoonotic diseases (Olival et al., 2017; Shipley et al., 2019; Wang & Anderson, 2019). Common bat pathogens include Astroviruses (AstVs), which infect numerous mammalian species and have a very large zoonotic potential (Donato & Vijaykrishna, 2017; Roach & Langlois, 2021; Wohlgemuth et al., 2019). In humans, AstVs are a major cause of infectious diarrhoea in children, elderly and immunocompromised patients (Ma et al., 2011) especially in developing tropical countries (Vu et al., 2017). Besides gastrointestinal symptoms, some AstV strains have also been linked to meningitis and encephalitis (Burbelo et al., 2011; Cordey et al., 2016; Naccache et al., 2015). Crucially, several studies have revealed interactions between the virus and the host gut microbiota even amongst AstV infections that proceed asymptotically (Cortez et al., 2020). The capsid protein of AstV can disrupt the gut barrier integrity and increase epithelial barrier permeability. This, in turn, enables the transit of gut microbes and their compounds beyond the intestinal barrier (Cortez et al., 2019; Ma et al., 2011; Meliopoulos et al., 2016; Moser et al., 2007). As a result, AstV-microbe interactions may alter the gut microbiota composition and threaten gut homeostasis. Indeed, potentially pathogenic bacterial taxa flourished in the gut of asymptomatic AstV⁺ Jamaican fruit bats, *Artibeus jamaicensis*, showing a decrease in commensals especially amongst young bats with a nonmature immune system (Wasimuddin et al., 2018). A diverse and functionally rich community of commensal gut bacteria is vital to maintain gut homeostasis and functions, including microbe-mediated immunity (Barko et al., 2018; Jacobson et al., 2018; Lathrop et al., 2011; Lin & Zhang, 2017). Shifts away from this optimal composition, referred to as "dysbiosis", have been linked to various diseases, and facilitate co-infections (Duvallet et al., 2017; Halfvarson et al., 2017; Kong et al., 2020; Visconti et al., 2019).

In healthy individuals, host immunity plays a key role not only in the defence against pathogenic invaders but also the tolerance of commensal bacteria (Donaldson et al., 2018; Levy et al., 2017; Noguera et al., 2018; Rooks & Garrett, 2016). Genes of the Major Histocompatibility Complex (MHC) encode proteins that bind and present antigenic peptides to CD8⁺ and CD4⁺ T cells to trigger the appropriate pathogen-specific immune response (Belasen et al., 2019; Cheng et al., 2012; Kumánovics et al., 2003; Neefjes et al., 2011; Sommer, 2005). Alleles of the MHC class I were found to code for molecules that display intracellular peptides (e.g., viruses), whereas MHC class II molecules mainly present extracellular peptides (e.g., bacteria) (Neefjes et al., 2011). This relationship is by no means invariable and so MHC class II was found to be involved in the clearance of influenza (Lucky et al., 2019) and both class I and II were linked to the clearance of hepatitis C (McKiernan et al., 2004). The gene-dense MHC region also harbours proteins that do not participate in antigen processing but have other immune and cell signalling functions (including MHC class III genes, complement components factor B, C2 and C4, the cytokines tumour necrosis factor and lymphotoxins) (Gruen & Weissman, 1997), yet the function of many MHC nonclassical and class III genes remain poorly characterized, particularly in nonmodel organisms (D'Souza

et al., 2019; Kelley et al., 2004). Crucially, the exceptionally high polymorphism found amongst MHC genes is maintained by several mutually nonexclusive pathogen-driven selection mechanisms, for example "rare allele advantage" (=frequency-dependent selection) (Bolnick & Stutz, 2017; Eizaguirre et al., 2012) and "divergent allele-advantage" (=heterozygosity advantage) (Fröeschke & Sommer, 2012; Lenz et al., 2013; Lighten et al., 2017). Naturally, research aiming to understand host-pathogen interactions initially focused on linking individual MHC constitution (i.e., presence/absence of specific MHC alleles and supertypes) and diversity (genetic distance between individual MHC alleles and functionally important antigen-binding sites) with pathogen infections in experimental animal models (e.g. Eizaguirre et al., 2012; Wegner et al., 2003) and wild populations (e.g. Belasen et al., 2019; Cheng et al., 2012; Meyer-Lucht & Sommer, 2005; Rivero-de Aguilar et al., 2016; Westerdahl, 2005). Nevertheless, MHC class II allele diversity and constitution also regulate the diversity and composition of commensal gut bacteria (Bolnick et al., 2014; Khan et al., 2019; Silverman et al., 2017). Changes to the bacterial composition alter the balance of immunomodulatory substances, such as proinflammatory cytokines, which has consequences for microbe-mediated immunity (Gaboriau-Routhiau et al., 2009; Hall et al., 2008; Ichinohe et al., 2011; Round & Mazmanian, 2009). In addition, commensal gut bacteria compete with gastroenteric pathogens for resources or produce substances to hinder pathogen establishment or further colonization (Baumler & Sperandio, 2016; Cortez et al., 2020; Pelassey et al., 2014). In turn, MHC-mediated changes to the microbiota may feedback to increase host susceptibility, as demonstrated for a murine model challenged by *Salmonella enterica* (Kubinak et al., 2015). Taken together, two characteristics of the MHC can guard against diverse pathogens (reviewed by Piertney & Oliver, 2006; Sommer, 2005), MHC allele – pathogen/microbe specificity (i.e., determined by the number of individual MHC alleles) and functional MHC diversity (i.e., sequence variation and genetic distance among individual alleles). Both can equally modulate the host's commensal bacterial composition (Atarashi et al., 2011; Bolnick et al., 2014; Donaldson et al., 2018; Mao et al., 2018). However, the reciprocity between MHC variation and gut microbial composition with respect to the host's pathogen resistance is unexplored in wild animal populations.

Here we used a natural host-microbe-virus system to investigate the interconnectedness between three factors: host immune genetics, host gut bacterial composition and diversity, and AstV infection status (Figure 1). We focused on MHC II DRB because recent research showed this locus alters both gut microbiota composition and virus resistance in bats and other mammals (Karakus et al., 2019). The host, the neotropical Jamaican fruit bat (Phyllostomidae: Stenodermatinae), is a generalist species in the neotropics, inhabiting a wide range of natural and human-disturbed habitats. The species is a well-known host for several zoonotic RNA and DNA viruses, including AstV (Calisher et al., 2006; Drexler et al., 2011; Munster et al., 2016). Furthermore, AstV infection induces age-dependent dysbiosis of the gut microbiome (Wasimuddin et al., 2018).

Specifically, we first examined associations between MHC diversity and constitution on AstV infection. According to pathogen-driven

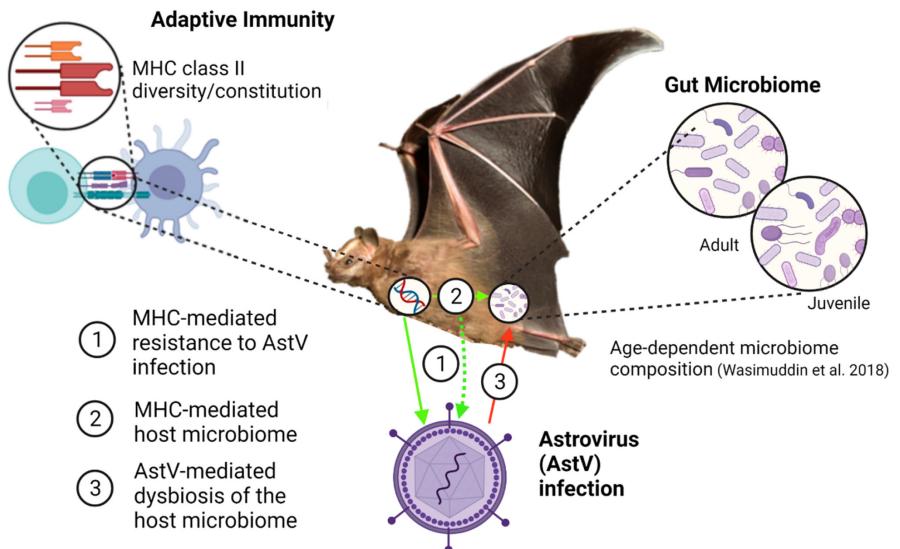


FIGURE 1 Schematic of potential interactions between MHC genetics, gut microbiome composition and AstV infection in the Jamaican fruit bat, *Artibeus jamaicensis* (Phyllostomidae: Stenodermatinae). Individual MHC constitution (i.e., presence/absence of specific alleles or supertypes) and diversity (i.e., the number of individual MHC alleles or supertypes, and sequence variation and genetic distance among individual alleles) can directly (solid green lines) confer infection resistance or susceptibility, and shape the gut microbiome. Individual MHC diversity and constitution may also indirectly (dashed line) contribute to pathogen resistance by regulating the abundance of specific microbes, which, in turn, can confer resistance or susceptibility to infection. Illustration created with BioRender.com [Colour figure can be viewed at wileyonlinelibrary.com]

selection hypotheses, we expected MHC diversity and specific alleles to be linked to AstV infection status. Next, we investigated the direct and indirect effects of MHC diversity and constitution on the diversity and composition of the gut microbiota in AstV-negative bats. Since compounds of gut bacteria and the immune system interact to establish gut homeostasis, we predicted both MHC diversity and constitution shape microbial diversity in AstV-uninfected individuals. In addition, the presence/absence of single MHC alleles should control the abundance of single bacterial taxa. Finally, we tested whether individual MHC diversity and constitution modify the gut microbiota, and indirectly contribute to MHC-mediated pathogen resistance. Along this line, we studied whether MHC-microbiota associations change in AstV-infected bats and predicted that amplicon sequence variants (ASVs) differing in abundance between AstV⁻ and AstV⁺ bats were also associated with MHC constitution. At the same time, pathogenic bacteria may increase in abundance following AstV-mediated dysbiosis. Our results highlight the complex interactions between host immune genetics, gut microbial diversity and pathogen susceptibility, which has far reaching implications in a world of increasing anthropogenic impact affecting wildlife immunogenetic and microbial diversity, and associated zoonotic risks.

2 | MATERIAL AND METHODS

2.1 | Study species and sample collection

We captured Jamaican fruit bats using mist-nets (Ecotone) at 17 locations in three different landscapes in Central Panama between October 2013 and October 2015. The handling of bats, as well as

sample collection, has been described in detail by Brändel et al. (2020) and Wasimuddin et al. (2018). Individuals were sexed and aged based on the ossification of the epiphyses of the fingers (Handley et al., 1991). Only young individuals (<1 year) have gaps between the epiphyses of the fingers and are not sexually active (hereafter termed as "young"). All sexually mature individuals (>1 year old) have closed epiphyses (hereafter termed as "adults"). Small tissue samples were taken from wing punches for MHC characterization. Bat faecal matter was either collected directly from the rectal area using a nylon swab or gathered from the clean, soft cotton bags after having kept individual bats in each for up to 1 h (Wasimuddin et al., 2018). Faecal samples were preserved in Eppendorf vials filled with 500 µl RNAlater (Life Technologies) for subsequent microbiome analysis and AstV screening.

2.2 | AstV infection and gut microbiota data

The AstV infection status and microbiota information were taken from Wasimuddin et al. (2018) and included 169 AstV-negative samples ($N_{AstV^-} = 68$ young, 101 adults) and 82 AstV-positive samples ($N_{AstV^+} = 36$ young, 46 adults; AstV-prevalence in the overall data set: 11.8%). For AstV detection, viral RNA was extracted from the faeces using the MagNAPure 96 DNA and Viral NA Small Volume Kit (Roche) according to the manufacturer's instructions. The target was amplified by a nested reverse transcription polymerase chain reaction (PCR) assay (Chu et al., 2008) and sequenced on an Illumina MiSeq platform.

Sequencing data of the 16S rRNA gene of the hypervariable V4 region are available at the NCBI sequence read archive

under BioProject PRJNA473909 with accession no. SRP149464 (Wasimuddin et al., 2018). For the present study, the microbial sequences of all available 251 individuals were re-analysed with the latest R version 3.6.2 (R Core Development Team, 2019). We removed all sequences that (i) did not belong to the kingdom bacteria, (ii) were not identified to the phylum level, (iii) were of class or order chloroplast or (iv) family mitochondria. Thus, the gut microbiota data only included gut bacteria, explicitly filtering out fungi, archaea or other types of microbes. The filtering steps removed 15.44% of ASVs. Next, we excluded all singletons from the data set, as they are presumably artefacts, and removed eight samples from the data set with a low sequencing depth (i.e., fewer than 11,000 reads). We kept 243 individuals with appropriate sequencing depth and known AstV status for subsequent analyses.

We rarefied the data set to a read number of 11,000 to normalize the data (Weiss et al., 2017). Rarefaction is appropriate prior to beta diversity analysis (Hong et al., 2022; McKnight et al., 2019; Weiss et al., 2017), but it can obscure alpha diversity metrics. Since rarefaction randomly excludes sequences to achieve equal sampling depth, differences in the count of ASVs and loss of rare bacterial taxa are potential drawbacks (McMurdie & Holmes, 2014). However, robust effects can overcome those limitations (McMurdie & Holmes, 2014). To account for this, we checked whether alpha diversity scores calculated on the rarefied data set are congruent with alpha diversity scores calculated on the filtered, but un rarefied, microbiota data set using Pearson correlation (Figure S1). For full transparency, we report results for un rarefied alpha diversity scores in the Appendix S1. MHC diversity was then characterized for 224 of the 243 samples with information on rarefied alpha and beta bacterial diversity, for which we had wing punch samples.

2.3 | MHC characterization and diversity estimates

We used high-throughput amplicon sequencing to characterize the immune gene diversity of the 224 individuals on an Illumina MiSeq platform. The DNA of wing punches was extracted using the NucleoSpin DNA RapidLyse kit according to the manufacturer's instructions. We amplified a 216-bp fragment of the exon 2 region of the MHC class II DRB gene using the primer pair JS2Cape-DRB (5'3')/JSi2Cape-DRB (5'3') (Schad et al., 2011), which also reliably amplify MHC class II in *Carollia perspicillata* (Schad et al., 2012), another sympatric bat species of the family Phyllostomidae. We prepared the Illumina sequencing libraries by performing two consecutive rounds of PCR following the approach for the Fluidigm System (Access Array System for Illumina Sequencing Systems; Fluidigm Corporation) and sequenced the libraries using the Illumina MiSeq platform. Sequence data were processed using the open access ACACIA pipeline (Gillingham et al., 2021; code available under https://gitlab.com/psc_santos/ACACIA). Forward and reverse reads were merged with a minimum overlap of 50 bp and a maximum overlap of 250 bp. Quality filtering removed sequences with a Phred quality score value <30 and a q-value <90 and the remaining

sequences were aligned by FLASH (Magoč & Salzberg, 2011). Chimeras were removed using UCHIME (Edgar et al., 2011). Finally, the remaining sequences were blasted against the MHC-DRB-Database, including MHC-DRB sequences of various mammalian and known *Artibeus jamaicensis* MHC-DRB-sequences in order to eliminate non-MHC-DRB sequences (Del Real-Monroy & Ortega, 2017; Real-Monroy et al., 2014). For all sequences, the minimum number of reads was set to 10 and the lowest percentage of reads per individual to 1% to be retained and to be called a "true" MHC allele (Gillingham et al., 2021). Our final data set included 224 individuals with MHC, microbiome and AstV infection data as well as information on host age, sex and capture landscape ($N_{AstV^-} = 152$ [61 young, 91 adults]; $N_{AstV^+} = 72$ [32 young, 40 adults]).

To assess functional diversity among MHC-DRB alleles, we first identified positively selected sites (PSS) following the assumption that PSS are probably part of, or close to, functionally important antigen-binding-sites (ABS). This approach also assumes that alleles that comprise distinct amino acids at PSS probably bind different antigens, and vice versa that MHC alleles that share amino acid motifs at PSS have a similar antigen-binding specificity (Cohen, 2002; Schwensow et al., 2019; Sepil et al., 2013). The latter can then be grouped into so-called MHC supertypes to emphasize functional similarity (Schwensow et al., 2019; Sidney et al., 1996), an approach confirmed by peptide prediction algorithms and widely used in human vaccine design (Lenz, 2011; Sidney et al., 1996; Wang et al., 2016).

Along this line, we investigated signs of positive selection on codons by using the HYPHY software (Pond et al., 2005) available on the Datamonitor public webserver (Delport et al., 2010; Pond & Frost, 2005). We employed complementary methods: FEL (fixed effects likelihood), FUBAR (fast unconstrained Bayesian approximation), MEME (mixed effects model of evolution) and SLAC (single-likelihood ancestor counting) (Table S1). In addition, we tested for codons under positive selection using CODEML integrated in the program PAML4 (Phylogenetic Analysis by Maximum Likelihood) (Yang, 2007) running in the PAML-X GUI (Xu & Yang, 2013). PAML is based on maximum likelihood procedures of different models of nucleotide sequence evolution to identify species-specific positively selected codon sites ($\omega = dN/dS > 1$) (Table S1). We then transformed the amino acids at the defined PSS into a matrix of five z-variables describing the physiochemical properties of each amino acid (Sandberg et al., 1998). The amino acids were then clustered into groups of alleles, hereafter MHC supertypes, with putatively similar physiochemical characteristics and binding spectra (Schwensow et al., 2019). Lastly, we used the discriminant analysis of principal components (DAPC) in the R package adegenet (Jombart et al., 2010) and grouped the MHC alleles into 10 MHC supertypes (Table S2) identified as the optimal number of clusters determined through visual assessment of the Bayesian information criterion (BIC) curve (Figure S2).

Individual MHC diversity can be assessed based on different parameters. Aside from the number of distinct MHC alleles (N_{Alleles}) and supertypes (N_{ST}), the evolutionary distance among all allele pairs found in an individual can be calculated from the number of

nucleotides or amino acids that differ between two sequences. Using MEGA-x (Kumar et al., 2018), we calculated the mean and sum of p-distances among individual MHC alleles using either the whole sequence, or only the previously identified PSS to account for distinct functional, antigen-binding capabilities (Santos et al., 2016, 2017). We also repeated the procedures with Poisson distances revealing almost identical values (data not shown). Lastly, we calculated Faith's Phylogenetic Diversity (PD), which considers the phylogenetic distance of individual MHC alleles (Pineaux et al., 2020). In summary, we used the following estimates of MHC diversity for each bat:

1. Number of distinct alleles (at amino acid level) observed per individual, N_{Alleles} .
2. Number of distinct supertypes observed per individual, N_{ST} .
3. Mean p-distance among all alleles observed per individual, $\text{Mean}_{\text{pdist}}$.
4. Sum of p-distances among all alleles observed per individual, $\text{Sum}_{\text{pdist}}$.
5. Mean p-distance among the PSS observed per individual, $\text{Mean}_{\text{pdistPSS}}$.
6. Sum of p-distances among the PSS observed per individual, $\text{Sum}_{\text{pdistPSS}}$.
7. Faith's PD among individual alleles, $\text{MHC}_{\text{Faith'sPD}}$

2.4 | Statistical analyses

2.4.1 | Effects of individual MHC diversity and constitution on AstV-infection status

We applied the probabilistic model of co-occurrence (Veech, 2013) implemented in the package *cooccur* (Griffith et al., 2016) to identify associations between the presence of specific MHC alleles/supertypes (i.e., MHC constitution) and AstV infection status. If the observed frequency is significantly higher than expected by chance, a positive association is assumed, whereas a significantly lower observed frequency than expected by chance indicates a negative association. We only included MHC alleles present in at least five individuals (58 out of 131 detected MHC alleles) and MHC ST present in at least 10 individuals (nine out of 10 defined ST) to guarantee a reasonable sample size and improve statistical power.

We further tested if the individual MHC diversity estimates (N_{Alleles} , N_{ST} , $\text{Mean}_{\text{pdist}}$, $\text{Sum}_{\text{pdist}}$, $\text{Mean}_{\text{pdistPSS}}$, $\text{Sum}_{\text{pdistPSS}}$, $\text{MHC}_{\text{Faith'sPD}}$) differed with AstV infection status, age, sex or landscape using Kruskal-Wallis tests. In addition, we fitted generalized linear mixed-effect models (GLMMs) to test for the impact of age and MHC diversity estimate on infection status using landscape and sex as random factors. We used separate models for each MHC diversity estimate because of high collinearity among estimates. Finally, we subdivided the data into "above" and "below" median MHC diversity estimates for each age group and used chi-square tests to ask whether infection status is explained by either "above"/"below" median MHC diversity.

2.4.2 | Effects of MHC diversity and constitution on the gut microbial alpha and beta diversity in AstV^- bats

To decipher the link between individual MHC diversity and constitution and gut microbiota, we first focused on AstV^- bats ($N = 152$) as the null model, because AstV infection is known to affect gut microbial diversity and composition in *A. jamaicensis* (Wasimuddin et al., 2018). This will allow us to disentangle potential microbial shifts induced by AstV infection from those owing to individual MHC diversity and constitution. Moreover, because AstV^- adult and young individuals have distinct microbial composition (Wasimuddin et al., 2018), we examined potential effects of MHC diversity on the gut microbiota separately for each age group. Using Wilcoxon tests, we analysed the impact of individual MHC diversity and constitution estimates on four bacterial alpha diversity indices: number of observed ASVs, Shannon Index, Chao1 Index and Faith's PD. Concerning the unrefined data set, we analysed the effect of MHC diversity and constitution on the gut microbial alpha diversity in linear models controlling for sequencing depth.

To explore the effect of individual MHC diversity and constitution on the gut bacterial beta diversity, we used taxonomic information down to the genus level (retaining 370 taxa for analysis). This allowed us to reduce the number of bacterial taxa without filtering out potentially important ASVs. Then we used canonical correspondence analysis (CCA) implemented in the *vegan* package (Dixon, 2003) on a chord-transformed matrix for adult ($N = 91$) and young ($N = 61$) bats separately, and tested for significance of the overall CCA model with an analysis of variance (ANOVA). The significance of each potential predictor variable was assessed with a permutation test using the function *envfit* and the number of permutations was set to 10,000. Because of the high number of variables describing individual MHC diversity and constitution, we started the CCA modelling by assessing the importance of presence/absence of all MHC supertypes and the MHC diversity estimates N_{ST} and $\text{Mean}_{\text{pdist}}$ as predictors, thereby avoiding collinearity. We repeated the process keeping significant variables only to prevent overparameterization and examined the amount of variation explained.

2.4.3 | Impact of MHC diversity and constitution on the gut microbial alpha and beta diversity of AstV^+ bats

For AstV^+ bats ($N = 72$; $N = 32$ young, $N = 40$ adults) we followed the same analytical procedure as described above. In brief, we investigated the impact of individual MHC diversity and constitution on four alpha diversity indices using Wilcoxon tests. Again, we analysed the effect of MHC on the unrefined gut microbial alpha diversity in linear models controlling for sequencing depth. Next, we explored the contribution of individual MHC diversity and constitution to the gut bacterial beta diversity (at the genus level) using CCA and a subsequent ANOVA.

2.4.4 | Differential abundance of specific ASVs between AstV⁻ and AstV⁺ bats

Lastly, we assessed differential abundance of specific ASVs between AstV⁻ and AstV⁺ bats using an analysis of composition of microbes (ANCOM) (Kaul et al., 2017; Mandal et al., 2015). Since ANCOM includes an internal normalization step we used the unrefined microbiota data and filtered for taxa present in at least 30% of the overall samples prior to analysis. Sex and landscape were set as random factors and the analysis was run separately for young and adult bats. ANCOM performs pairwise tests between noninfected and infected bats for each ASV and calculates how often the null hypothesis that an ASV is equally abundant in both groups is rejected (i.e., $p < .05$) as the W score (=the sum of rejected null hypotheses for each ASV). Therefore, a high W score indicates a difference in the relative abundance of an ASV between AstV⁻ and AstV⁺ bats. Since many ASVs are relatively rare we included only ASVs with at least 30% abundance across the whole data set ($N = 224$ of 753 ASVs in total), which minimizes the weight of very rare taxa. We visualized the results in volcano plots and reported ASVs with a W score above a threshold of 0.6 (=60% of null hypotheses rejected).

Using Wilcoxon tests, we examined whether the ASVs that differ in abundance between AstV⁻ and AstV⁺ bats were also associated with MHC constitution. To normalize data for these comparisons, similar to the ANCOM algorithm, log-scaled abundance +1 was used for all ASVs.

3 | RESULTS

3.1 | Effects of individual MHC diversity and constitution on AstV infection status

In total, we identified 134 MHC nucleotide alleles corresponding to 131 amino acid alleles (GenBank accession no. PRJNA825462). Most of the alleles were rare and found in fewer than five individuals (Figure S3a). Each bat carried between one and six MHC amino acid alleles (mean 3.04 ± 1.12) assigned to one to five distinct MHC supertypes (mean 2.58 ± 0.88) (Figure S3b). MHC diversity did not differ between the sexes or landscapes (Table S3). However, individuals reaching adulthood tended to have a higher genetic diversity than young bats and AstV⁺ individuals tended to carry more STs than AstV⁻ bats (Table S3). The co-occurrence model identified five MHC class II alleles and one supertype to be associated with AstV infection: MHC alleles Arja-DRB*13 ($p = .033$, st.effSize = 0.014), Arja-DRB*31 ($p = .038$, st.effSize = 0.011), Arja-DRB*42 ($p = .038$, st.effSize = 0.011), Arja-DRB*55 ($p = .038$, st.effSize = 0.011) and ST 5 ($p = .028$, st.effSize = 0.032) were positively associated with AstV⁺ bats (Figure 2a,b). The allele Arja-DRB*58 ($p = .042$, st.effSize = 0.012) was negatively associated with AstV infection, suggesting a protective function against AstV infection (Figure 2a). A comparison amongst the amino acid sequences of these alleles

revealed that three PSS at amino acid positions 17, 26 and 64 were identical in all susceptible alleles but different in the protective allele (Figure 2c). Furthermore, the AstV-negatively associated allele Arja-DRB*58, and the AstV-positively associated allele Arja-DRB*13 were both assigned to ST5. Both the Kruskal-Wallis test and GLMM confirmed a relationship between infection status and estimates of MHC diversity: specifically, AstV⁺ bats carried more STs than AstV⁻ individuals (Kruskal-Wallis: $p = .044$, Table S3; GLMM: $p = .021$, Table S4). There was no association between below/above average MHC diversity and AstV infection (Table S5).

3.2 | Effect of MHC diversity and constitution on gut microbial alpha and beta diversity in AstV⁻ bats

In adults, MHC constitution did not affect microbial richness, evenness or phylogenetic diversity, whilst in young bats two MHC STs impacted microbial alpha diversity: ST5 was associated with a reduction in alpha diversity, and ST6 with an increase in all alpha diversity indices except one (Figure 3). This pattern was also supported by the unrefined data, although not for every metric; for example, the number of observed ASVs was no longer significantly associated with ST5 (Figure S4, Table S6), and sequencing depth was a strong predictor of microbial richness (Table S6). Individual MHC diversity weakly impacted microbial alpha diversity: MHC_{Faith'sPD} was associated with microbial richness in AstV⁻ adults, where individuals with below median MHC_{Faith'sPD} harboured a higher number of observed ASVs compared to individuals with above median MHC diversity ($p = .040$; Figure S5)—an effect that is not supported by unrefined data (Table S7), while the other MHC diversity estimates still did not significantly affect microbial alpha diversity.

Beta diversity was shaped by the MHC in young individuals. In adult AstV⁻ bats, five of the 10 MHC supertypes (but none of the MHC diversity estimates) explained 7.37% of the total variation in composition, but the overall model was not significant (ANOVA: $F_{df\ 6} = 1.114$, $p = .156$, $\chi^2 = 0.460$) (Table S8; Figure 4a). Microbial composition was driven by ST5/ST7 and ST2/ST6 largely along the first axis and in opposing directions, whereas ST10 follows a distinctly intermediate direction.

In young bats, four STs, Nr_{ST} and Mean_{pdist} explained 13.21% of the total variation in the gut bacterial composition (ANOVA: $F_{df\ 6} = 1.370$, $p = .027$, $\chi^2 = 0.675$) (Table S9; Figure 4b). Unlike in adults, the gut microbiota composition of young bats was shaped by MHC constitution (i.e., the presence or absence of ST2, ST4, ST9 and ST10) and MHC diversity (i.e., Nr_{ST}, Mean_{pdist}), although each parameter drove the composition in a distinct direction. On the second axis all MHC parameters point in the same direction. Yet, while Mean_{pdist} and ST2 align in their impact on the composition and point in a similar direction, ST4/ST10 show an opposing direction on the first axis, and Nr_{ST} takes an intermediate position. Interestingly, the distinct measures of MHC diversity, Mean_{pdist} and Nr_{ST}, point in different directions of the microbial composition.

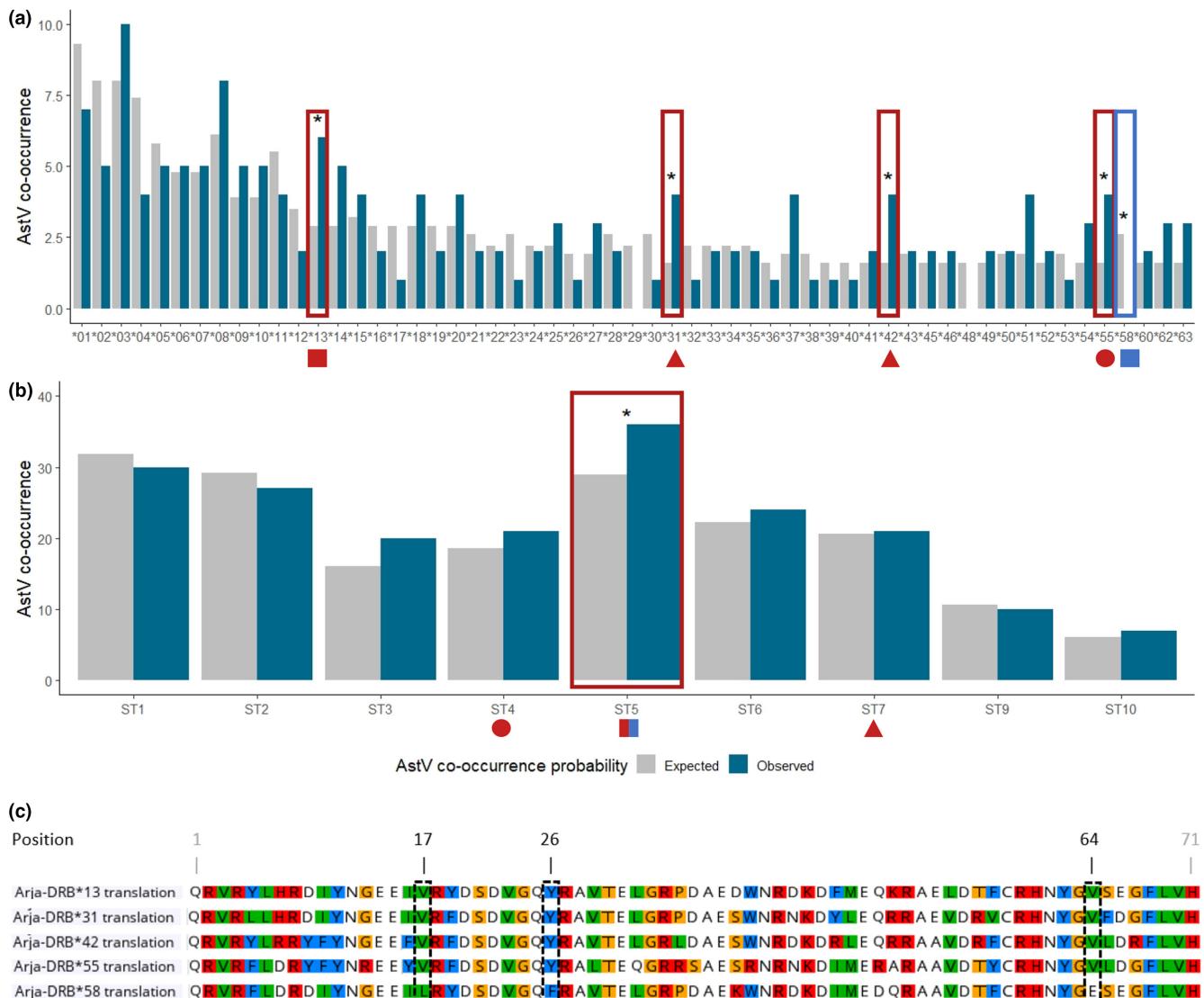


FIGURE 2 Association of MHC constitution and AstV infection. Observed (grey) and expected (teal) co-occurrence of (a) MHC DRB* alleles and (b) MHC supertypes and AstV infection. Symbols (square, triangle, circle) indicate the assigned ST for each allele, and colours of symbols and boxes signal a positive (red) or negative (blue) association with AstV infection. (c) Amino acid sequences of MHC alleles with an association with AstV infection. Note that only MHC alleles and supertypes present in at least five or 10 individuals, respectively, are displayed (see Figure S3 for more details) [Colour figure can be viewed at wileyonlinelibrary.com]

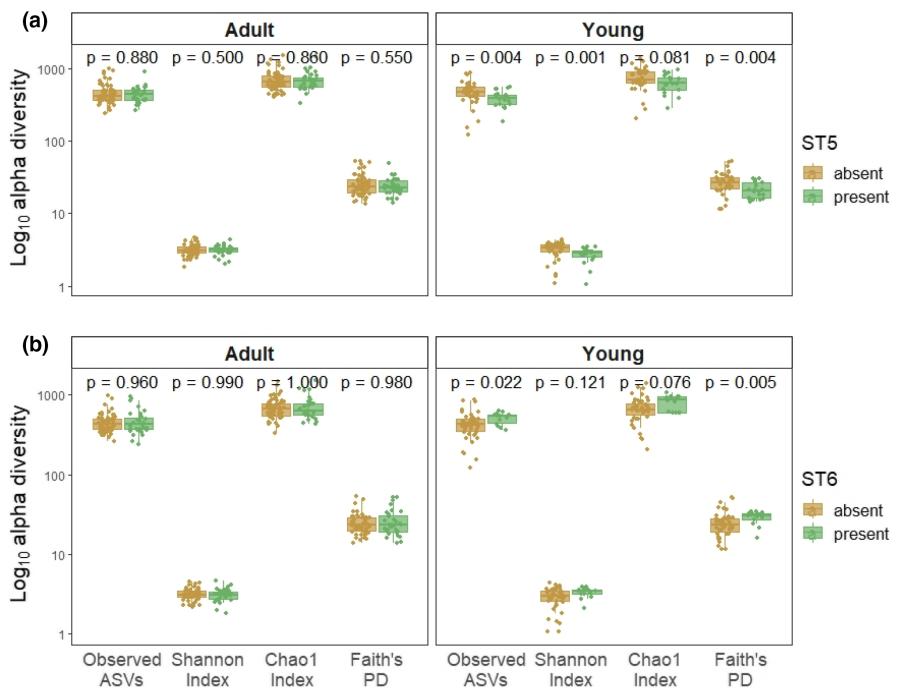
3.3 | Implications of MHC diversity and constitution on gut microbial alpha and beta diversity in AstV⁺ bats

Whereas alpha diversity was unaffected by MHC constitution in AstV⁺ adults, young AstV⁺ bats without ST10 demonstrated higher Chao1 ($p = .040$; Figure S6). This effect, however, was not detected using the metrics calculated from unrefined microbial data (Table S10). Mean_{pdist} and Mean_{pdistPSS} showed age-dependent associations with the number of observed ASVs, Chao1 and Faith's PD (Figure 5): while adult bats with above average MHC diversity had higher Chao1 and Faith's PD and tentatively a higher number of observed ASVs, young bats with above average MHC diversity had a lower number of observed ASVs and a trending decrease for

Shannon and Chao1 (Figure 5). For Mean_{pdist} this pattern was supported by unrefined data, but in adults the number of observed ASVs was also significant, while this was not found in young individuals (Figure S7a, Table S11). Mean_{pdistPSS} suggests a similar pattern but this is solely a tendency in adults and not found in young bats (Figure S7b, Table S11).

In addition to microbial alpha diversity, the gut microbial beta diversity of young AstV-infected bats was influenced by individual MHC diversity and constitution. In adult AstV⁺ bats, ST1 and ST7 explained 6.5% of the total variation in composition (ANOVA: $F_{df\ 2} = 1.281$, $p = .064$, $\chi^2 = 0.275$), while individual MHC diversity estimates had no measurable impact (Figure 6a; Table S12), although the overall model showed a tendency but was not significant. In young bats, ST6, ST10 and Mean_{pdist} explained 14.69%

FIGURE 3 Effects of specific MHC supertypes on rarefied gut microbial alpha diversity in adult and young AstV⁺ bats [Colour figure can be viewed at wileyonlinelibrary.com]



of the total variation in gut bacterial composition (ANOVA: $F_{df\ 3} = 1.607$, $p = .027$, $\chi^2 = 0.683$) (Figure 6b; Table S13). Strikingly, even in AstV⁺ young bats ST10 and Mean_{pdist} drive the composition in distinct directions, similar to effects observed in young AstV⁻ bats. Yet unlike in their uninfected counterparts, ST1 and ST6 become prominent effectors of beta diversity in adult and young infected bats, respectively.

3.4 | Differential abundance of specific ASVs between AstV⁻ and AstV⁺ bats

The abundance of eight ASVs from five families and two phyla differed with AstV infection status in adult bats (Figure 7a; Figure S8a, Table S14). Most of them were more abundant in AstV⁺ bats, among them members of the families Clostridiaceae, Helicobacteraceae, Staphylococcaceae and Streptococcaceae. Only one ASV from the family Pseudomonadaceae was more abundant in AstV⁻ bats (Table S14).

In young bats, nine ASVs belonging to three families and three distinct bacterial phyla differed in abundance between AstV⁻ and AstV⁺ bats (Figure 7b; Figure S8b, Table S15): Mycoplasmataceae were more abundant in AstV⁺ bats, whereas Clostridiaceae were more abundant in AstV⁻ bats. One member of the family Helicobacteraceae occurred in higher abundance in AstV⁻ bats, while another was more abundant in AstV⁺ individuals.

Three STs were linked to the abundance of AstV-associated ASVs in adults (Figure 8a). ASV1141646, a member of the family Streptococcaceae, was more abundant in bats carrying ST6, and ASV730049, belonging to the family Staphylococcaceae, was more abundant in bats with ST1. Both ASVs were also more abundant in AstV⁺ bats. By contrast, bats with ST4 harboured one

Pseudomonadaceae member (ASV 813945) in higher abundance—an ASV more abundant in AstV⁻ bats.

In young bats, four distinct STs were linked to the abundance of AstV-associated ASVs (Figure 8b). Bats with ST2, for instance, harboured fewer members of the family Mycoplasmataceae (ASV298, ASV76821, ASV7477), although this family was generally more abundant in AstV⁺ bats. At the same time, ASV7477 and ASV76821 were abundant in bats carrying ST4. Moreover, bats with ST4 harboured more ASV40, a member of the Helicobacteraceae, which was also more abundant in AstV⁺ bats. ASV112997, another Helicobacteraceae, was less abundant in bats with ST9. Bats with ST2 contained ASV4451477, a Clostridiaceae, in higher abundance, whilst another member of this family (ASV342666) was less abundant in bats with ST5.

In total, ASVs linked to MHC STs in adults always increased in abundance, whilst in young bats having an ST was linked to both higher and lower abundance of specific ASVs. Additionally, only ST4 had an effect in adult and young individuals, but on ASVs of distinct bacterial families.

4 | DISCUSSION

This study aimed to investigate interactions between MHC genetics, gut microbiota composition and AstV infections in a wild population of the neotropical bat *Artibeus jamaicensis*, and expands on earlier findings showing host age-dependent changes in the gut microbiota after AstV infection (Wasimuddin et al., 2018). By adding information on individual MHC constitution and diversity, we aimed to resolve the direct and indirect MHC-mediated impacts on the gut microbiota and pathogen resistance. Apart from initial experimental support (Kubinak et al., 2015), few studies to date have

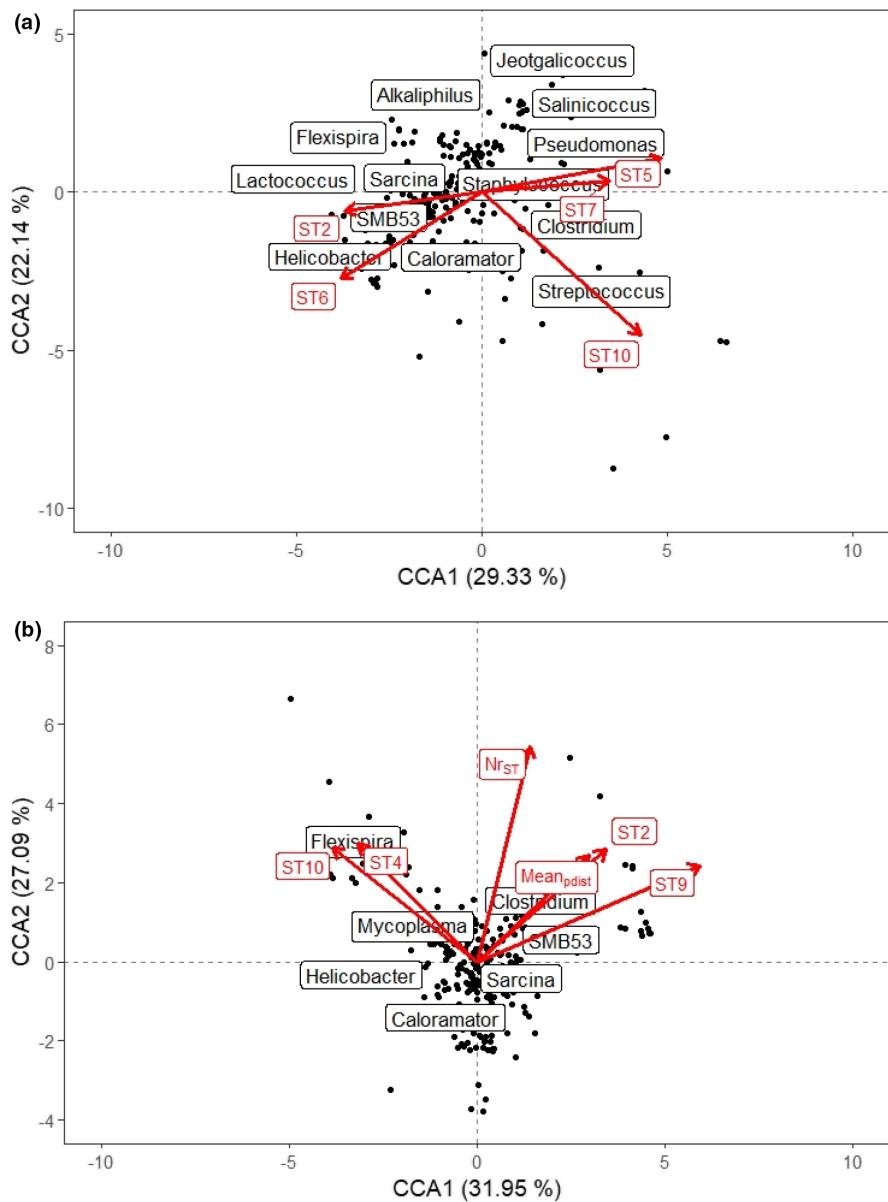


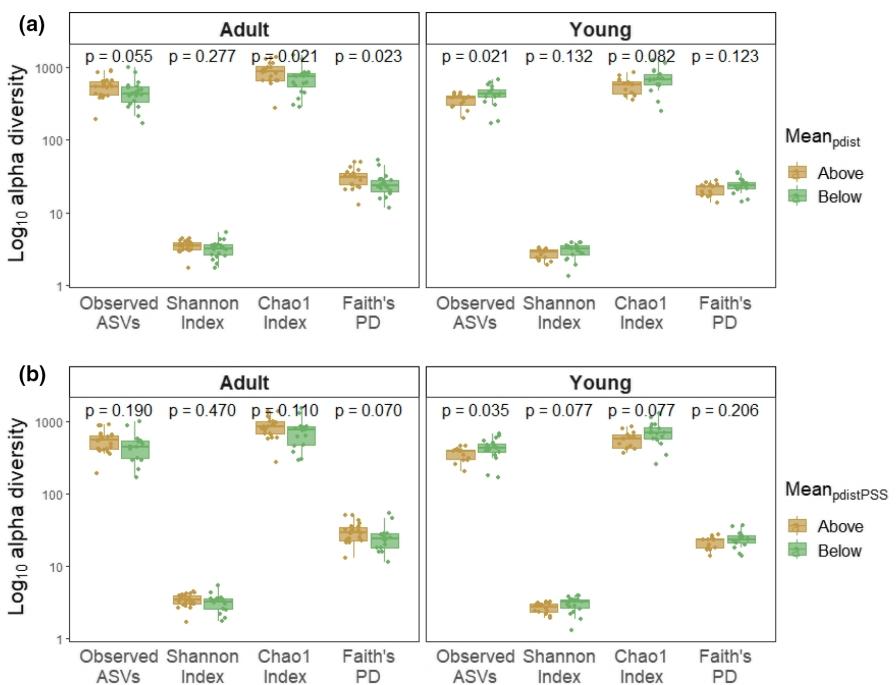
FIGURE 4 Gut microbiota composition CCA plot of (a) adult and (b) young *AstV*⁺ bats. Bacterial genera are shown as dots. MHC variables with significant impact on the gut composition are depicted as arrows and labelled in red. For visual reasons only a subset of bacterial taxa are labelled. Lists of all bacterial taxa found are available in the data files uploaded on GitHub [Colour figure can be viewed at wileyonlinelibrary.com]

explored this relationship. First, we verified an association between host MHC class II diversity and constitution and *AstV* susceptibility. Second, we investigated the effects of host MHC class II diversity and constitution on the gut microbiota in *AstV*⁺ bats. Then we studied whether these MHC-microbiota associations change in *AstV*⁺ bats, suggesting *AstV* infection induced changes. Lastly, we showed age-dependent, joined associations between specific bacterial taxa, MHC STs and *AstV* infection status.

Parasite-mediated selection principally explains variation in the MHC (Apanius et al., 2017; Bonneauaud et al., 2006; Piertney & Oliver, 2006; Radwan et al., 2020; Schwensow et al., 2017). Consistent with this large body of studies, we showed several MHC class II alleles and a single ST are linked to *AstV* infection. Four MHC alleles and one ST were more likely than by chance associated with *AstV* infection, suggesting bats with these alleles are more susceptible to *AstV* infections. One allele was negatively associated with *AstV* infections, suggesting a protective function. At this point it

is important to reiterate that, unlike the link between MHC class I and virus resistance, MHC class II probably modulates resistance via indirect microbiota-mediated mechanisms (e.g. Hall et al., 2008; Kubinak et al., 2015). Interestingly, however, the protective allele Arja-DRB*58 and the susceptible allele Arja-DRB*13 were grouped into the same ST during DAPC clustering. This probably arises from highly similar amino acid properties at PSS, which were used for clustering (Jombart & Collins, 2015; Jombart et al., 2010). However, the size of the peptide repertoire may differ somewhat between MHC alleles, resulting in distinct functions despite similar peptide motifs (Kaufman, 2020) and even though each was assigned to the same ST. In fact, all susceptible alleles shared amino acid sequences at three positions, but differed from those found in the protective allele. They also yielded distinct amino acid products: among susceptibility alleles positions 17, 26 and 64 coded for valine, tyrosine and valine, respectively, while the same positions coded for leucine, phenylalanine and glutamic acid in the resistance allele. Taken together,

FIGURE 5 Associations between individual MHC diversity estimates measured as (a) Mean_{pdist} and (b) Mean_{pdistPSS} and gut microbial alpha diversity in adult and young AstV⁺ bats [Colour figure can be viewed at wileyonlinelibrary.com]



the fitness disadvantage of susceptible alleles may arise from such shared structural features being exploited by the evolving virus as a cellular receptor as recently reported for MHC class II alleles and bat influenza viruses (Karakus et al., 2019).

The relationship between the MHC and commensal microbes is likely to be equally multifaceted as the relationship between MHC and pathogens (Bolnick et al., 2014; Kubinak et al., 2015). Using AstV⁺ bats as a baseline, we found the associations between MHC STs and microbial beta diversity in adult bats to be distinct from those associations found in young bats. The amount of variation explained by the MHC was also higher in young bats (13.2%) compared with adults (7.4%), even though the overall model was not significant in adults, which suggests effects of MHC constitution are negligible. Moreover, young bats with ST5 displayed lower, and those with ST6 higher microbial alpha diversity. No such relationship was found in adult bats. Age-dependent differences in the microbial community were previously reported in this population (Wasimuddin et al., 2018) and are common observations in mammalian taxa with diet shifts during ontogeny—for example, meerkats (Risely et al., 2021), chimpanzees (Reese et al., 2021) and humans (Kurilshikov et al., 2021)—but here we extend previous results to show that MHC-microbiota relationships are age-dependent. Members of the Clostridiaceae, for example, are more abundant in younger, AstV⁺ animals (e.g., humans: Odamaki et al., 2016) where they probably perform immunomodulatory functions (Aleman & Valenzano, 2019). MHC ST2 and ST5 were found to impact the abundance of ASVs from this family only in young bats. By contrast, a single ASV from the family Pseudomonadaceae was more abundant in AstV⁺ adults and bats with MHC ST4. Members of this bacterial family (i.e., *Pseudomonas fluorescens*) were found to inhibit the growth of the fungus *Pseudogymnoascus destructans*—the main cause of white-nose syndrome in North American bats (Cheng et al., 2017; Hoyt

et al., 2015). Collectively, these results indicate age-specific MHC-microbiota relationships and altered immunomodulatory function of an ageing microbiome (Ghosh et al., 2020).

Aside from MHC ST constitution, MHC allelic (i.e., *p*-distance) and ST (i.e., number of STs) diversity impacted beta diversity in young bats alone. Associations with MHC allelic diversity metrics stem from a divergent allele repertoire conferring selective advantages (Pierini & Lenz, 2018; Wakeland et al., 1990), whereas STs are a conglomerate of alleles with shared peptide-binding function based on peptide motifs (Lenz et al., 2011; Schwesow et al., 2019). Hence, the presence or absence of STs is expected to link to the presence or absence of certain bacterial taxa, rather than determine the microbial community as a whole. However, overall, MHC allelic diversity seems to play a subordinate role in shaping gut bacterial diversity, which is supported by previous findings (Bolnick et al., 2014; Khan, Stephens, et al., 2019). Rather, our results suggest that predominantly functionally distinct MHC STs structure microbial communities in uninfected bats. A specific MHC ST may, for example, tolerate certain microbes which, in turn, competitively exclude other microbial taxa. This might lead to an overall decrease in microbial alpha diversity (Bauer et al., 2018). Nevertheless, our results emphasize a stronger link between MHC diversity and the gut microbiota in young rather than adult bats. The link is probably more pronounced in young individuals because the process of establishing homeostasis among competing gut microbes, their compounds and cells of the immune system is still ongoing (Guzman-Bautista et al., 2020; Lathrop et al., 2011; Levy et al., 2017; Mazmanian et al., 2005; Rooks & Garrett, 2016; Zhao & Elson, 2018).

In AstV⁺ bats, however, the associations between MHC and the gut microbiota changed. Fewer and distinct STs shaped the microbial composition in infected individuals, and MHC diversity estimates (Mean_{pdist}, Mean_{pdistPSS}) became more important in shaping microbial

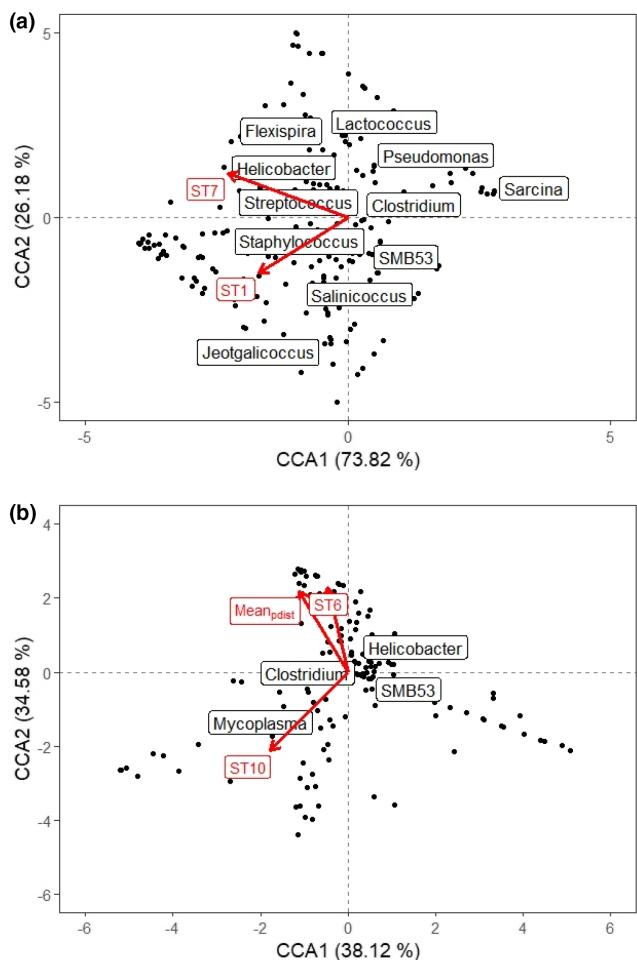


FIGURE 6 Gut microbiota composition CCA plot of (a) adult and (b) young *AstV*⁺ bats. Bacterial genera are shown as dots. MHC variables with significant impact on the gut composition are depicted as arrows and labelled in red. For visual reasons only a subset of bacterial taxa are labelled. Lists of all bacterial taxa found are available in the data files uploaded on GitHub [Colour figure can be viewed at wileyonlinelibrary.com]

alpha and beta diversity from both age groups. This indicates the infection with *AstV* might cause changes in the associations between MHC and microbiota. Such changed associations could result from two nonmutually exclusive processes: infections could directly affect the gut microbiota (Wasimuddin et al., 2018, 2019), changing microbiota-mediated production of immunomodulatory molecules (Hair et al., 2010), and microbe–microbe interactions. Murine *AstV*, for example, promotes mucus and mucus-associated bacteria in the gut (Cortez et al., 2019). Thus, infection directly affects competition among gut microbes, triggering further shifts in composition, potentially leading to gut dysbiosis (Cortez et al., 2017, 2019; Moser et al., 2007). Such trans-kingdom interactions within the gut microbial community, which consists mainly of bacteria, but also archaea, fungi, viruses and helminths, are common and another important factor involved in shaping the microbiota–host immunity interplay (Domínguez-Díaz et al., 2019; Pfeiffer & Virgin, 2016). Thereby, such interactions can improve host defence against pathogen invasion, for example by production of potential antiviral compounds, but also promote infection (e.g., Poliovirus exploits surface polysaccharides of specific microbes to improve receptor binding; Domínguez-Díaz et al., 2019). Alternatively or additionally, the host's MHC-mediated immune reaction against *AstV* infection could alter established commensal interactions between host immune defences and the microbiota, thus affecting microbiota-mediated immunity (Gaboriau-Routhiau et al., 2009; Ichinohe et al., 2011; Round & Mazmanian, 2009), and with implications for infection susceptibility. The latter mechanism is supported by experiments on murine models showing that MHC variation shaped microbiota composition and host susceptibility to *Salmonella enterica* infections (Kubinak et al., 2015).

Host age also impacted the direction of microbiota–MHC associations in *AstV*⁺ individuals. Adults with above average MHC diversity had a higher microbial diversity than those with below average MHC diversity, whereas young bats with above average MHC diversity showed lower microbial diversity compared to those with below average MHC diversity. Young *AstV*⁺ bats also harboured three Mycoplasmataceae members in higher

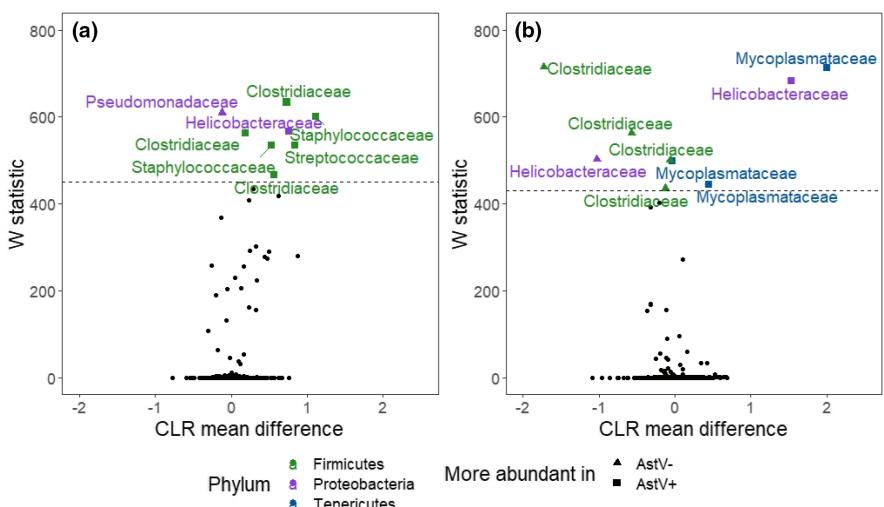
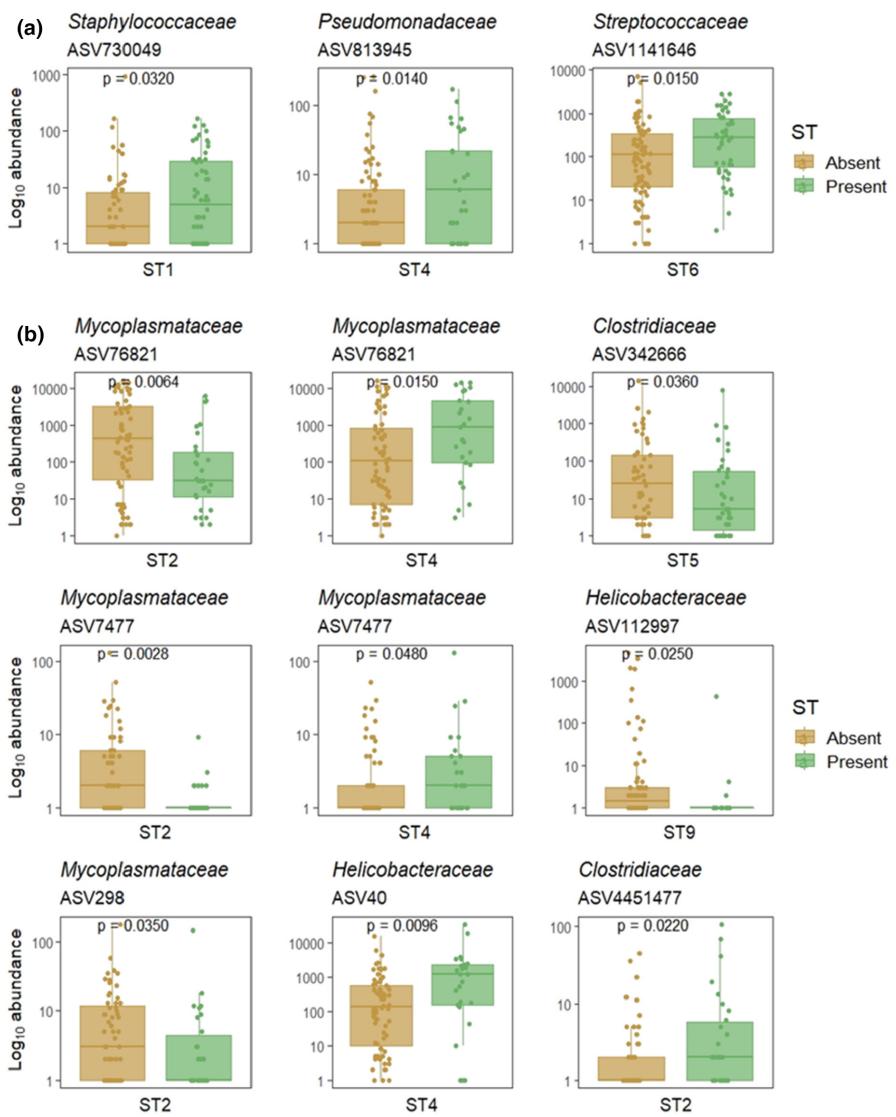


FIGURE 7 Volcano plots illustrating differential abundance of ASVs in (a) adult and (b) young bats distinct in their *AstV* infection status. Points represent single ASVs. ASVs above a threshold of $W + 0.6$ are defined at the family level, coloured by phylum and shaped by infection status. The dashed line indicates a W -threshold of 0.6 [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 8 Effect of specific MHC supertypes on ASV abundance. Log-scaled abundance of ASVs in relation to presence/absence of specific MHC STs in (a) adult and (b) young bats, compared with Wilcoxon tests [Colour figure can be viewed at wileyonlinelibrary.com]



abundance, and four Clostridiaceae in lower abundance than AstV^- bats. By contrast, AstV^+ adults harboured more ASVs of Clostridiaceae than AstV^- individuals. Proteins synthesized by some members of the genus *Mycoplasma* bind pattern-recognition receptors (PRRs), Toll-like receptors (TLRs), and nucleotide-binding and oligomerization domain (NOD)-like receptors, which may cause an inflammatory reaction (Benedetti et al., 2020) and are associated with various infectious diseases (Rottem, 2003). By contrast, Clostridiaceae perform important immunomodulatory functions (Aleman & Valenzano, 2019), particularly in young animals, where AstV infections proceed most symptomatically (Bosch et al., 2014; Ma et al., 2011; Vu et al., 2017). Host age, again, reveals fine-scale changes to infection-induced dysbiotic microbiota (Ghosh et al., 2020). Additionally, mycoplasmal bacteria were less abundant in young individuals that carried MHC ST2, suggesting alleles of ST2 might be involved in the direct suppression of *Mycoplasma* multiplication. Conversely, the same mycoplasmal ASVs were more abundant in young bats with MHC ST4. This supports the hypothesis that specific MHC STs control the abundance of potentially pathogenic bacteria, but tolerate commensals (Khan,

Yurkovetskiy, et al., 2019), since gut microbes educate the immune system to establish gut homeostasis (Lathrop et al., 2011; Zhao & Elson, 2018). However, it also indicates STs may have contrary effects on the microbial composition.

We also hypothesized that MHC-induced changes of microbiota composition and diversity could add to the direct adaptive immune response in providing pathogen resistance. However, even though ST5 was positively associated with AstV infection across all individuals, we found only one AstV -associated ASV differentially abundant in young individuals with ST5. This contradiction might originate from the two MHC alleles (Arja-DRB*13, Arja-DRB*58) that showed opposite associations with AstV infection, but still are similar enough to be grouped within the same ST.

Limitations of the current work emerge from uncertainty concerning the direction of associations among the host's immune response, shifts in gut microbial composition and pathogenic impacts (Hooks & O'Malley, 2017). We cannot rule out that the associations with MHC and pathogen resistance stem from linked genes that, for instance, affect physiology, thus altering conditions in the gut and influence the abundance of specific gut microbes and microbiota-mediated

immunity. A factor heedlessly ignored in this *ménage-à-trois* are coinfections. Particularly in a bat family frequently infected with multiple parasites (Canuti et al., 2011; Finoketti et al., 2019), the immune system and microbiota are probably challenged by other parasites than just AstV, which, in turn, might blur links between the MHC, microbial diversity and AstV infection. Additionally, our nondestructive, low-impact AstV assessment cannot address the prolonged impacts recent infections have on the microbiota of recovering bats. Antibody information from blood samples may be an interesting addition to future work (Bogdanoff et al., 2017).

Lastly, we found some differences using alpha diversity calculated on the unrarefied and rarefied microbiota data set. Since rarefaction randomly excludes sequences to achieve equal sampling depth, differences in the count of ASVs and loss of rare bacterial taxa are potential drawbacks (McMurdie & Holmes, 2014). However, robust effects can overcome those limitations (McMurdie & Holmes, 2014). In fact, the main pattern that MHC constitution and diversity are linked to microbiota diversity and composition differently depending on bat age and AstV infection is consistent, independent of rarefaction. We might, yet, underestimate subtle signals of the MHC on gut microbial diversity.

Since bats act as reservoirs for zoonotic diseases and harbour a high AstV strain diversity (Kohl et al., 2020; Olival et al., 2017; Vu et al., 2017; Wang & Anderson, 2019), their zoonotic potential is high. Encouragingly, our results suggest interactions between the MHC and gut microbiota could indirectly benefit resistance against AstV infection in the neotropical bat *A. jamaicensis*. We show MHC diversity and constitution are associated with differences in the gut microbial composition and diversity in young individuals, while only MHC constitution affects the gut microbiota of adults. MHC diversity, thus, seems to aid the establishment and maintenance of a “healthy” microbial composition—especially in young individuals—which highlights the importance of host age in the MHC–gut microbiota relationship. Given the role of MHC in mediating gut microbial composition and, consequently, host health, one can hypothesize that a loss in MHC diversity owing to anthropogenic disturbances might expose natural populations to higher gut microbial turnover and functional decline, leading to pathogen susceptibility.

5 | CONCLUSIONS

In a time where the zoonotic potential of many ecosystems seems enriched (Fackelmann et al., 2021; Gibb et al., 2020), we emphasize the importance of investigating the reciprocal relationships between MHC genetics, gut microbial composition and pathogen resistance. As the reciprocity between host traits (e.g., MHC genes), parasites and the microbiota as determining factors for disease severity becomes increasingly evident (see also Bernardo-Cravo et al., 2020; Montero et al., 2021; Schmeller et al., 2020), so does a potential mechanistic link that connects anthropogenic disturbance with zoonotic potential in (immuno-)genetically impoverished host populations.

ACKNOWLEDGEMENT

We thank the Smithsonian Tropical Research Institute in Panama for providing the essential infrastructure for our fieldwork. Our thanks are extended to Rachel Page for her logistical support in Panama, to the private landowners in Panama, to Kerstin Wilhelm, Ulrike Stehle, Luca Langianese and Moritz Nusser for laboratory support, to Nadine Müller-Klein and Nina Schwensow for bioinformatic support and discussions, and to Calum Melville for linguistic assistance. We are indebted to all field assistants for their work. This research was funded by the German Science Foundation (DFG) and is part of the DFG Priority Program SPP 1596/2 Ecology and Species Barriers in Emerging Infectious Diseases (SO 428/9-1, SO 428/9-2; TS 81/7-1, TS 81/7-2; DR 772/8-1).

CONFLICT OF INTEREST

The authors declare no conflicts of interest in regard to this paper.

DATA AVAILABILITY STATEMENT

Data and code for this paper are openly available and downloadable at <https://github.com/rfleischer93/Interaction-between-MHC-gut-microbiota-and-Astrovirus-infections>. MHC sequences are available under the BioProject accession no. PRJNA825462 on NCBI. Microbiome sequences are downloadable from the original study (Wasimuddin et al., 2018).

ORCID

- Ramona Fleischer  <https://orcid.org/0000-0003-1657-9347>
Wasimuddin  <https://orcid.org/0000-0002-8314-8160>
Victor M. Corman  <https://orcid.org/0000-0002-3605-0136>
Simone Sommer  <https://orcid.org/0000-0002-5148-8136>

REFERENCES

- Aleman, F. D. D., & Valenzano, D. R. (2019). Microbiome evolution during host aging. *PLoS Path.*, 15(7), e1007727. <https://doi.org/10.1371/JOURNAL.PPAT.1007727>
- Apanius, V., Penn, D., Slev, P. R., Ramelle Ruff, L., & Potts, W. K. (2017). The nature of selection on the major histocompatibility complex. *Critical Reviews in Immunology*, 37(2–6), 75–120. <https://doi.org/10.1615/CritRevImmunol.v37.i2-6.10>
- Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., Taniguchi, T., Takeda, K., Hori, S., Ivanov, I. I., Umesaki, Y., Itoh, K., & Honda, K. (2011). Induction of colonic regulatory T cells by indigenous clostridium species. *Science*, 331(6015), 337–341. <https://doi.org/10.1126/SCIENCE.1198469>
- Barko, P. C., McMichael, M. A., Swanson, K. S., & Williams, D. A. (2018). The gastrointestinal microbiome: A review. *Journal of Veterinary Internal Medicine*, 32(1), 9–25. <https://doi.org/10.1111/JVIM.14875>
- Bauer, M. A., Kainz, K., Carmona-Gutierrez, D., & Madeo, F. (2018). Microbial wars: Competition in ecological niches and within the microbiome. *Microbial Cell*, 5(5), 215–219. <https://doi.org/10.15698/mic2018.05.628>
- Bäumler, A. J., & Sperandio, V. (2016). Interactions between the microbiota and pathogenic bacteria in the gut. *Nature*, 535(7610), 85–93. <https://doi.org/10.1038/nature18849>

- Belasen, A. M., Bletz, M. C., Leite, D. D. S., Toledo, L. F., & James, T. Y. (2019). Long-term habitat fragmentation is associated with reduced MHC IIB diversity and increased infections in amphibian hosts. *Frontiers in Ecology and Evolution*, 6, 236. <https://doi.org/10.3389/fevo.2018.00236>
- Benedetti, F., Curreli, S., & Zella, D. (2020). Mycoplasmas-host interaction: Mechanisms of inflammation and association with cellular transformation. *Microorganisms*, 8(9), 1351. <https://doi.org/10.3390/microorganisms8091351>
- Bernardo-Cravo, A. P., Schmeller, D. S., Chatzinotas, A., Vredenburg, V. T., & Loyau, A. (2020). Environmental factors and host microbiomes shape host-pathogen dynamics. *Trends in Parasitology*, 36(7), 616–633. <https://doi.org/10.1016/J.PT.2020.04.010>
- Bogdanoff, W. A., Campos, J., Perez, E. I., Yin, L., Alexander, D. L., & DuBois, R. M. (2017). Structure of a human astrovirus capsid-antibody complex and mechanistic insights into virus neutralization. *Journal of Virology*, 91(2), e01859-16. <https://doi.org/10.1128/JVI.01859-16>
- Bolnick, D. I., Snowberg, L. K., Caporaso, J. G., Lauber, C., Knight, R., & Stutz, W. E. (2014). Major histocompatibility complex class IIB polymorphism influences gut microbiota composition and diversity. *Molecular Ecology*, 23(19), 4831–4845. <https://doi.org/10.1111/mec.12846>
- Bolnick, D. I., & Stutz, W. E. (2017). Frequency dependence limits divergent evolution by favouring rare immigrants over residents. *Nature*, 546(7657), 285–288. <https://doi.org/10.1038/nature22351>
- Bonneaud, C., Pérez-Tris, J., Federici, P., Chastel, O., & Sorci, G. (2006). Major histocompatibility alleles associated with local resistance to malaria in a passerine. *Evolution*, 60(2), 383. <https://doi.org/10.1554/05-409.1>
- Bosch, A., Pintó, R. M., & Guix, S. (2014). Human astroviruses. *Clinical Microbiology Reviews*, 27(4), 1048–1074. <https://doi.org/10.1128/CMR.00013-14>
- Brändel, S. D., Hiller, T., Halczok, T. K., Kerth, G., Page, R. A., & Tschapka, M. (2020). Consequences of fragmentation for neotropical bats: The importance of the matrix. *Biological Conservation*, 252, 108792. <https://doi.org/10.1016/j.biocon.2020.108792>
- Burbelo, P. D., Ching, K. H., Esper, F., Iadarola, M. J., Delwart, E., Lipkin, W. I., & Kapoor, A. (2011). Serological studies confirm the novel astrovirus HMOAstV-C as a highly prevalent human infectious agent. *PLoS One*, 6(8), e22576. <https://doi.org/10.1371/journal.pone.0022576>
- Calisher, C. H., Childs, J. E., Field, H. E., Holmes, K. V., & Schountz, T. (2006). Bats: Important reservoir hosts of emerging viruses. *Clinical Microbiology Reviews*, 19(3), 531–545. <https://doi.org/10.1128/CMR.00017-06>
- Canuti, M., Eis-Huebinger, A. M., Deijs, M., de Vries, M., Drexler, J. F., Oppong, S. K., Müller, M. A., Klose, S. M., Wellinghausen, N., Cottontail, V. M., Kalko, E. K. V., Drosten, C., & van der Hoek, L. (2011). Two novel parvoviruses in frugivorous new and old world bats. *PLoS One*, 6(12), e29140. <https://doi.org/10.1371/journal.pone.0029140>
- Cheng, T. L., Mayberry, H., McGuire, L. P., Hoyt, J. R., Langwig, K. E., Nguyen, H., Parise, K. L., Foster, J. T., Willis, C. K. R., Kilpatrick, A. M., & Frick, W. F. (2017). Efficacy of a probiotic bacterium to treat bats affected by the disease white-nose syndrome. *Journal of Applied Ecology*, 54(3), 701–708. <https://doi.org/10.1111/1365-2664.12757>
- Cheng, Y., Sanderson, C., Jones, M., & Belov, K. (2012). Low MHC class II diversity in the Tasmanian devil (*Sarcophilus harrisii*). *Immunogenetics*, 64(7), 525–533. <https://doi.org/10.1007/s00251-012-0614-4>
- Chu, D. K. W., Poon, L. L. M., Guan, Y., & Peiris, J. S. M. (2008). Novel astroviruses in insectivorous bats. *Journal of Virology*, 82(18), 9107–9114. <https://doi.org/10.1128/JVI.00857-08>
- Cohen, S. (2002). Strong positive selection and habitat-specific amino acid substitution patterns in Mhc from an estuarine fish under intense pollution stress. *Molecular Biology and Evolution*, 19(11), 1870–1880. <https://doi.org/10.1093/oxfordjournals.molbev.a004011>
- Cordey, S., Vu, D. L., Schibler, M., L'Huillier, A. G., Brito, F., Docquier, M., Posfay-Barbe, K. M., Petty, T. J., Turin, L., Zdobnov, E. M., & Kaiser, L. (2016). Astrovirus MLB2, a new gastroenteric virus associated with meningitis and disseminated infection. *Emerging Infectious Diseases*, 22(5), 846–853. <https://doi.org/10.3201/eid2205.151807>
- Cortez, V., Boyd, D. F., Crawford, J. C., Sharp, B., Livingston, B., Rowe, H. M., Davis, A., Alsallaq, R., Robinson, C. G., Vogel, P., Rosch, J. W., Margolis, E., Thomas, P. G., & Schultz-Cherry, S. (2020). Astrovirus infects actively secreting goblet cells and alters the gut mucus barrier. *Nature Communications*, 11(1), 1–9. <https://doi.org/10.1038/s41467-020-15999-y>
- Cortez, V., Margolis, E., & Schultz-Cherry, S. (2019). Astrovirus and the microbiome. *Current Opinion in Virology*, 37, 10–15. <https://doi.org/10.1016/j.coviro.2019.05.002>
- Cortez, V., Meliopoulos, V. A., Karlsson, E. A., Hargest, V., Johnson, C., & Schultz-Cherry, S. (2017). Astrovirus biology and pathogenesis. *Annual Review of Virology*, 1(1), 327–348. <https://doi.org/10.1146/annurev-virology-101416-041742>
- D'Souza, M. P., Adams, E., Altman, J. D., Birnbaum, M. E., Boggiano, C., Casorati, G., Chien, Y.-H., Conley, A., Eckle, S. B. G., Fröhlich, K., Gondré-Lewis, T., Hassan, N., Huang, H., Jayashankar, L., Kasmar, A. G., Kunwar, N., Lavelle, J., Lewinsohn, D. M., Moody, B., ... Yewdell, J. W. (2019). Casting a wider net: Immunosurveillance by nonclassical MHC molecules. *PLoS Pathogens*, 15(2), e1007567. <https://doi.org/10.1371/journal.ppat.1007567>
- Del Real-Monroy, M., & Ortega, J. (2017). Spatial distribution of microsatellite and MHC-DRB exon 2 gene variability in the Jamaican fruit bat (*Artibeus jamaicensis*) in Mexico. *Mammalian Biology*, 84, 1–11. <https://doi.org/10.1016/j.mambio.2016.12.005>
- Delpont, W., Poon, A. F. Y., Frost, S. D. W., & Kosakovsky Pond, S. L. (2010). Datamonkey 2010: A suite of phylogenetic analysis tools for evolutionary biology. *Bioinformatics*, 26(19), 2455–2457. <https://doi.org/10.1093/bioinformatics/btq429>
- Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, 14(6), 927–930. <https://doi.org/10.1111/j.1654-1103.2003.tb02228.x>
- Domínguez-Díaz, C., García-Orozco, A., Riera-Leal, A., Padilla-Arellano, J. R., & Fafutis-Morris, M. (2019). Microbiota and its role on viral evasion: Is it with us or against us? *Frontiers in Cellular and Infection Microbiology*, 9, 256. <https://doi.org/10.3389/fcimb.2019.00256>
- Donaldson, G. P., Ladinsky, M. S., Yu, K. B., Sanders, J. G., Yoo, B. B., Chou, W. C., Conner, M. E., Earl, A. M., Knight, R., Bjorkman, P. J., & Mazmanian, S. K. (2018). Gut microbiota utilize immunoglobulin A for mucosal colonization. *Science*, 360(6390), 795–800. <https://doi.org/10.1126/science.aaq0926>
- Donato, C., & Vijaykrishna, D. (2017). The broad host range and genetic diversity of mammalian and avian astroviruses. *Viruses*, 9(5), 102. <https://doi.org/10.3390/v9050102>
- Drexler, J. F., Corman, V. M., Wegner, T., Tateno, A. F., Zerbini, R. M., Gloza-Rausch, F., Seebens, A., Müller, M. A., & Drosten, C. (2011). Amplification of emerging viruses in a bat colony. *Emerging Infectious Diseases*, 17(3), 449–456. <https://doi.org/10.3201/eid1703.100526>
- Duvallat, C., Gibbons, S. M., Gurry, T., Irizarry, R. A., & Alm, E. J. (2017). Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. *Nature Communications*, 8(1), 1–10. <https://doi.org/10.1038/s41467-017-01973-8>
- Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimer detection. *Bioinformatics*, 27(16), 2194–2200. <https://doi.org/10.1093/bioinformatics/btr381>
- Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012). Rapid and adaptive evolution of MHC genes under parasite selection in

- experimental vertebrate populations. *Nature Communications*, 3(1), 1–6. <https://doi.org/10.1038/ncomms1632>
- Fackelmann, G., Gillingham, M. A. F., Schmid, J., Heni, A. C., Wilhelm, K., Schwensow, N., & Sommer, S. (2021). Human encroachment into wildlife gut microbiomes. *Communications Biology*, 4(1), 1–11. <https://doi.org/10.1038/s42003-021-02315-7>
- Finoketti, F., dos Santos, R. N., Campos, A. A. S., Zani, A. L. D. S., Barboza, C. M., Fernandes, M. E. S., de Souza, T. D. C. P., dos Santos, D. D., Bortolanza, G. W., Filho, H. O., Roehe, P. M., Franco, A. C., & de Carvalho Ruthner Batista, H. B. (2019). Detection of adenovirus, papillomavirus and parvovirus in Brazilian bats of the species *Artibeus lituratus* and *Sturnira lilium*. *Archives of Virology*, 164, 1015–1025. <https://doi.org/10.1007/s00705-018-04129-1>
- Froeschke, G., & Sommer, S. (2012). Insights into the complex associations between MHC class II DRB polymorphism and multiple gastrointestinal parasite infestations in the striped mouse. *PLoS One*, 7(2), e31820. <https://doi.org/10.1371/journal.pone.0031820>
- Gaboriau-Routhiau, V., Rakotobe, S., Lécuyer, E., Mulder, I., Lan, A., Bridonneau, C., Rochet, V., Pisi, A., De Paepe, M., Brandi, G., Eberl, G., Snel, J., Kelly, D., & Cerf-Bensussan, N. (2009). The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity*, 31(4), 677–689. <https://doi.org/10.1016/j.immuni.2009.08.020>
- Ghosh, T. S., Das, M., Jeffery, I. B., & O'Toole, P. W. (2020). Adjusting for age improves identification of gut microbiome alterations in multiple diseases. *eLife*, 9, e50240. <https://doi.org/10.7554/ELIFE.50240>
- Gibb, R., Redding, D. W., Chin, K. Q., Donnelly, C. A., Blackburn, T. M., Newbold, T., & Jones, K. E. (2020). Zoonotic host diversity increases in human-dominated ecosystems. *Nature*, 584(7821), 398–402. <https://doi.org/10.1038/s41586-020-2562-8>
- Gillingham, M. A. F., Montero, B. K., Wilhelm, K., Grudzus, K., Sommer, S., & Santos, P. S. (2021). A novel workflow to improve genotyping of multigene families in wildlife species: An experimental set-up with a known model system. *Molecular Ecology Resources*, 21(3), 982–998. <https://doi.org/10.1111/1755-0998.13290>
- Griffith, D. M., Veech, J. A., & Marsh, C. J. (2016). Cooccur: Probabilistic species co-occurrence analysis in R. *Journal of Statistical Software*, 69(2), 1–17. <https://doi.org/10.18637/jss.v069.c02>
- Gruen, J. R., & Weissman, S. M. (1997). Evolving views of the major histocompatibility complex. *Blood*, 90(11), 4252–4265. <https://doi.org/10.1182/BLOOD.V90.11.4252>
- Guzman-Bautista, E. R., Suzuki, K., Asami, S., & Fagharasan, S. (2020). Bacteria-immune cells dialog and the homeostasis of the systems. In *Current Opinion in Immunology*, 66, 82–89. <https://doi.org/10.1016/j.coि.2020.05.010>
- Hair, P. S., Gronemus, J. Q., Crawford, K. B., Salvi, V. P., Cunnion, K. M., Thielen, N. M., Arlaud, G. J., Rawal, N., & Krishna, N. K. (2010). Human astrovirus coat protein binds C1q and MBL and inhibits the classical and lectin pathways of complement activation. *Molecular Immunology*, 47(4), 792–798. <https://doi.org/10.1016/j.molimm.2009.10.006>
- Halfvarson, J., Brislawn, C. J., Lamendella, R., Vázquez-Baeza, Y., Walters, W. A., Bramer, L. M., D'Amato, M., Bonfiglio, F., McDonald, D., Gonzalez, A., McClure, E. E., Dunklebarger, M. F., Knight, R., & Jansson, J. K. (2017). Dynamics of the human gut microbiome in inflammatory bowel disease. *Nature Microbiology*, 2(5), 1–7. <https://doi.org/10.1038/nmicrobiol.2017.4>
- Hall, J. A., Bouladoux, N., Sun, C. M., Wohlfert, E. A., Blank, R. B., Zhu, Q., Grigg, M. E., Berzofsky, J. A., & Belkaid, Y. (2008). Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses. *Immunity*, 29(4), 637–649. <https://doi.org/10.1016/j.immuni.2008.08.009>
- Handley, C. O. Jr, Wilson, D. E., & Gardner, A. L. (1991). Demography and natural history of the common fruit bat, *Artibeus jamaicensis*, on Barro Colorado Island, Panama. *Smithsonian Contributions to Zoology*, 511, 19–41.
- Hong, J., Karaoz, U., de Valpine, P., & Fithian, W. (2022). To rarefy or not to rarefy: Robustness and efficiency trade-offs of rarefying microbiome data. *Bioinformatics*, 38(9), 2389–2396. <https://doi.org/10.1093/BIOINFORMATICS/BTAC127>
- Hooks, K. B., & O'Malley, M. A. (2017). Dysbiosis and its discontents. *MBio*, 8(5), e01492-17. <https://doi.org/10.1128/mBio.01492-17>
- Hoyt, J. R., Cheng, T. L., Langwig, K. E., Hee, M. M., Frick, W. F., & Kilpatrick, A. M. (2015). Bacteria isolated from bats inhibit the growth of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome. *PLoS One*, 10(4), e0121329. <https://doi.org/10.1371/JOURNAL.PONE.0121329>
- Ichinohe, T., Pang, I. K., Kumamoto, Y., Peaper, D. R., Ho, J. H., Murray, T. S., & Iwasaki, A. (2011). Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proceedings of the National Academy of Sciences of the United States of America*, 108(13), 5354–5359. <https://doi.org/10.1073/pnas.1019378108>
- Jacobson, A., Lam, L., Rajendram, M., Tamburini, F., Honeycutt, J., Pham, T., Van Treuren, W., Pruss, K., Stabler, S. R., Lugo, K., Bouley, D. M., Vilches-Moure, J. G., Smith, M., Sonnenburg, J. L., Bhatt, A. S., Huang, K. C., & Monack, D. (2018). A gut commensal-produced metabolite mediates colonization resistance to *Salmonella* infection. *Cell Host & Microbe*, 24(2), 296–307.e7. <https://doi.org/10.1016/j.chom.2018.07.002>
- Jombart, T., & Collins, C. (2015). A tutorial for discriminant analysis of principal components (DAPC) using adegenet. *R vignette*. <https://doi.org/10.1038/72708>
- Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, 11(1), 94. <https://doi.org/10.1186/1471-2156-11-94>
- Karakus, U., Thamamgood, T., Ciminski, K., Ran, W., Günther, S. C., Pohl, M. O., Eletto, D., Jeney, C., Hoffmann, D., Reiche, S., Schinköthe, J., Ulrich, R., Wiener, J., Hayes, M. G. B., Chang, M. W., Hunziker, A., Yángüez, E., Aydillo, T., Krammer, F., & Stertz, S. (2019). MHC class II proteins mediate cross-species entry of bat influenza viruses. *Nature*, 567(7746), 109–112. <https://doi.org/10.1038/s41586-019-0955-3>
- Kaufman, J. (2020). From chickens to humans: The importance of peptide repertoires for MHC class I alleles. *Frontiers in Immunology*, 11, 3089. <https://doi.org/10.3389/FIMMU.2020.601089>
- Kaul, A., Mandal, S., Davidov, O., & Peddada, S. D. (2017). Analysis of microbiome data in the presence of excess zeros. *Frontiers in Microbiology*. <https://doi.org/10.3389/fmicb.2017.02114>
- Kelley, J., Walter, L., & Trowsdale, J. (2004). Comparative genomics of major histocompatibility complexes. *Immunogenetics*, 56(10), 683–695. <https://doi.org/10.1007/s00251-004-0717-7>
- Khan, A. A., Yurkovetskiy, L., O'Grady, K., Pickard, J. M., de Pooter, R., Antonopoulos, D. A., Golovkina, T., & Chervonsky, A. (2019). Polymorphic immune mechanisms regulate commensal repertoire. *Cell Reports*, 29(3), 541–550.e4. <https://doi.org/10.1016/j.celrep.2019.09.010>
- Khan, M. W., Stephens, W. Z., Mohammed, A. D., Round, J. L., & Kubinak, J. L. (2019). Does MHC heterozygosity influence microbiota form and function? *PLoS One*, 14(5), e0215946. <https://doi.org/10.1371/journal.pone.0215946>
- Kohl, C., Brinkmann, A., Radonić, A., Dabrowski, P. W., Nitsche, A., Mühlendorfer, K., Kurth, A. (2020). Zwiesel bat banyangvirus, a potentially zoonotic Huaiyangshan banyangvirus (Formerly known as SFTS)-like banyangvirus in Northern bats from Germany. *Scientific Reports*, 10(1), 1–6.
- Kong, G., Cao, K. A. L., Judd, L. M., Li, S. S., Renoir, T., & Hannan, A. J. (2020). Microbiome profiling reveals gut dysbiosis in a transgenic mouse model of Huntington's disease. *Neurobiology of Disease*, 135, 104268. <https://doi.org/10.1016/j.NBD.2018.09.001>

- Kubinak, J. L., Stephens, W. Z., Soto, R., Petersen, C., Chiaro, T., Gogokhia, L., Bell, R., Ajami, N. J., Petrosino, J. F., Morrison, L., Potts, W. K., Jensen, P. E., O'Connell, R. M., & Round, J. L. (2015). MHC variation sculpts individualized microbial communities that control susceptibility to enteric infection. *Nature Communications*, 6(1), 1–3. <https://doi.org/10.1038/ncomms9642>
- Kumánovics, A., Takada, T., & Fischer Lindahl, K. (2003). Genomic organization of the mammalian Mhc. *Annual Review of Immunology*, 21(1), 629–657. <https://doi.org/10.1146/annurev.immunol.21.090501.080116>
- 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>
- Kurilshikov, A., Medina-Gomez, C., Bacigalupe, R., Radjabzadeh, D., Wang, J., Demirkiran, A., Le Roy, C. I., Garay, J. A. R., Finicum, C. T., Liu, X., Zhernakova, D. V., Bonder, M. J., Hansen, T. H., Frost, F., Rühlemann, M. C., Turpin, W., Moon, J.-Y., Kim, H.-N., Lüll, K., & Zhernakova, A. (2021). Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nature Genetics*, 53(2), 156–165. <https://doi.org/10.1038/s41588-020-00763-1>
- Lathrop, S. K., Bloom, S. M., Rao, S. M., Nutsch, K., Lio, C. W., Santacruz, N., Peterson, D. A., Stappenbeck, T. S., & Hsieh, C. S. (2011). Peripheral education of the immune system by colonic commensal microbiota. *Nature*, 478(7368), 250–254. <https://doi.org/10.1038/nature10434>
- Lenz, T. L. (2011). Computational prediction of MHC II-antigen binding supports divergent allele advantage and explains trans-species polymorphism. *Evolution*, 65(8), 2380–2390. <https://doi.org/10.1111/j.1558-5646.2011.01288.x>
- Lenz, T. L., Mueller, B., Trillmich, F., & Wolf, J. B. W. (2013). Divergent allele advantage at MHC-DRB through direct and maternal genotypic effects and its consequences for allele pool composition and mating. *Proceedings of the Royal Society B: Biological Sciences*, 280(1762), 20130714. <https://doi.org/10.1098/rspb.2013.0714>
- Levy, M., Blacher, E., & Elinav, E. (2017). Microbiome, metabolites and host immunity. *Current Opinion in Microbiology*, 35, 8–15. <https://doi.org/10.1016/j.mib.2016.10.003>
- Lighter, J., Papadopoulos, A. S. T., Mohammed, R. S., Ward, B. J., Paterson, G. I., Baillie, L., Bradbury, I. R., Hendry, A. P., Bentzen, P., & Van Oosterhout, C. (2017). Evolutionary genetics of immunological supertypes reveals two faces of the Red Queen. *Nature Communications*, 8(1), 1–11. <https://doi.org/10.1038/s41467-017-01183-2>
- Lin, L., & Zhang, J. (2017). Role of intestinal microbiota and metabolites on gut homeostasis and human diseases. *BMC Immunology*, 18(1), 1–25. <https://doi.org/10.1186/s12865-016-0187-3>
- Luckey, D., Weaver, E. A., Osborne, D. G., Billadeau, D. D., & Taneja, V. (2019). Immunity to influenza is dependent on MHC II polymorphism: study with 2 HLA transgenic strains. *Scientific Reports*, 9(1), 1–10. <https://doi.org/10.1038/s41598-019-55503-1>
- Ma, C., Wu, X., Nawaz, M., Li, J., Yu, P., Moore, J. E., & Xu, J. (2011). Molecular characterization of fecal microbiota in patients with viral diarrhea. *Current Microbiology*, 63(3), 259–266. <https://doi.org/10.1007/s00284-011-9972-7>
- Magoč, T., & Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21), 2957–2963. <https://doi.org/10.1093/bioinformatics/btr507>
- Mandal, S., Van Treuren, W., White, R. A., Eggesbø, M., Knight, R., & Peddada, S. D. (2015). Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microbial Ecology in Health & Disease*, 26(1), 27663. <https://doi.org/10.3402/mehd.v26.27663>
- Mao, K., Baptista, A. P., Tamoutounour, S., Zhuang, L., Bouladoux, N., Martins, A. J., Huang, Y., Gerner, M. Y., Belkaid, Y., & Germain, R. N. (2018). Innate and adaptive lymphocytes sequentially shape the gut microbiota and lipid metabolism. *Nature*, 554(7691), 255–259. <https://doi.org/10.1038/nature25437>
- Mazmanian, S. K., Cui, H. L., Tzianabos, A. O., & Kasper, D. L. (2005). An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell*, 122(1), 107–118. <https://doi.org/10.1016/j.cell.2005.05.007>
- McKiernan, S. M., Hagan, R., Curry, M., McDonald, G. S. A., Kelly, A., Nolan, N., Walsh, A., Hegarty, J., Lawlor, E., & Kelleher, D. (2004). Distinct MHC class I and II alleles are associated with hepatitis C viral clearance, originating from a single source. *Hepatology*, 40(1), 108–114. <https://doi.org/10.1002/HEP.20261>
- McKnight, D. T., Huerlimann, R., Bower, D. S., Schwarzkopf, L., Alford, R. A., & Zenger, K. R. (2019). Methods for normalizing microbiome data: An ecological perspective. *Methods in Ecology and Evolution*, 10(3), 389–400. <https://doi.org/10.1111/2041-210X.13115>
- McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: Why rarefying microbiome data is inadmissible. *PLOS Computational Biology*, 10(4), e1003531. <https://doi.org/10.1371/JOURNAL.PCBI.1003531>
- Meliopoulos, V. A., Marvin, S. A., Freiden, P., Moser, L. A., Nighot, P., Ali, R., Blikslager, A., Reddivari, M., Heath, R. J., Koci, M. D., & Schultz-Cherry, S. (2016). Oral administration of astrovirus capsid protein is sufficient to induce acute diarrhea in vivo. *MBio*, 7(6), e01494-16. <https://doi.org/10.1128/mBio.01494-16>
- Meyer-Lucht, Y., & Sommer, S. (2005). MHC diversity and the association to nematode parasitism in the yellow-necked mouse (*Apodemus flavicollis*). *Molecular Ecology*, 14(7), 2233–2243. <https://doi.org/10.1111/j.1365-294X.2005.02557.x>
- Montero, B. K., Wasimuddin, Schwensow, N., Gillingham, M. A. F., Ratovonamana, Y. R., Rakotondranary, S. J., Corman, V., Drosten, C., Ganzhorn, J. U., & Sommer, S. (2021). Evidence of MHC class I and II influencing viral and helminth infection via the microbiome in a nonhuman primate. *PLoS Path*, 17(11), e1009675. <https://doi.org/10.1371/JOURNAL.PPAT.1009675>
- Moser, L. A., Carter, M., & Schultz-Cherry, S. (2007). Astrovirus increases epithelial barrier permeability independently of viral replication. *Journal of Virology*, 81(21), 11937–11945. <https://doi.org/10.1128/jvi.00942-07>
- Munster, V. J., Adney, D. R., van Doremalen, N., Brown, V. R., Miazgowicz, K. L., Milne-Price, S., Bushmaker, T., Rosenke, R., Scott, D., Hawkinson, A., de Wit, E., Schountz, T., & Bowen, R. A. (2016). Replication and shedding of MERS-CoV in Jamaican fruit bats (*Artibeus jamaicensis*). *Scientific Reports*, 6(1), 21878. <https://doi.org/10.1038/srep21878>
- Naccache, S. N., Peggs, K. S., Mattes, F. M., Phadke, R., Garson, J. A., Grant, P., Samayoa, E., Federman, S., Miller, S., Lunn, M. P., Gant, V., & Chiu, C. Y. (2015). Diagnosis of neuroinvasive astrovirus infection in an immunocompromised adult with encephalitis by unbiased next-generation sequencing. *Clinical Infectious Diseases*, 60(6), 919–923. <https://doi.org/10.1093/cid/ciu912>
- Neefjes, J., Jongsma, M. L. M., Paul, P., & Bakke, O. (2011). Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nature Reviews Immunology*, 11(12), 823–836. <https://doi.org/10.1038/nri3084>
- Noguera, J. C., Aira, M., Pérez-Losada, M., Domínguez, J., & Velando, A. (2018). Glucocorticoids modulate gastrointestinal microbiome in a wild bird. *Royal Society Open Science*, 5(4), 171743. <https://doi.org/10.1098/rsos.171743>
- Odamaki, T., Kato, K., Sugahara, H., Hashikura, N., Takahashi, S., Xiao, J., Abe, F., & Osawa, R. (2016). Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC Microbiology*, 16(1), 1–12. <https://doi.org/10.1186/s12865-016-0708-5>

- Olival, K. J., Hosseini, P. R., Zambrana-Torrelío, C., Ross, N., Bogich, T. L., & Daszak, P. (2017). Host and viral traits predict zoonotic spill-over from mammals. *Nature*, 546(7660), 646–650. <https://doi.org/10.1038/nature22975>
- Pelaseyed, T., Bergström, J. H., Gustafsson, J. K., Ermund, A., Birchenough, G. M. H., Schütte, A., van der Post, S., Svensson, F., Rodríguez-Piñeiro, A. M., Nyström, E. E. L., Wising, C., Johansson, M. E. V., & Hansson, G. C. (2014). The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. *Immunological Reviews*, 260(1), 8–20. <https://doi.org/10.1111/imr.12182>
- Pfeiffer, J. K., & Virgin, H. W. (2016). Viral immunity: Transkingdom control of viral infection and immunity in the mammalian intestine. *Science*, 351(6270), aad5872. <https://doi.org/10.1126/science.aad5872>
- Pierini, F., & Lenz, T. L. (2018). Divergent allele advantage at human MHC genes: Signatures of past and ongoing selection. *Molecular Biology and Evolution*, 35(9), 2145–2158. <https://doi.org/10.1093/molbev/msy116>
- Piertney, S. B., & Oliver, M. K. (2006). The evolutionary ecology of the major histocompatibility complex. *Heredity*, 96(1), 7–21. <https://doi.org/10.1038/sj.hdy.6800724>
- Pineaux, M., Merkling, T., Danchin, E., Hatch, S., Duneau, D., Blanchard, P., & Leclaire, S. (2020). Sex and hatching order modulate the association between MHC-II diversity and fitness in early-life stages of a wild seabird. *Molecular Ecology*, 29(17), 3316–3329. <https://doi.org/10.1111/mec.15551>
- Pond, S. L. K., & Frost, S. D. W. (2005). Datamonitor: Rapid detection of selective pressure on individual sites of codon alignments. *Bioinformatics*, 21(10), 2531–2533. <https://doi.org/10.1093/bioinformatics/bti320>
- Pond, S. L. K., Frost, S. D. W., & Muse, S. V. (2005). HyPhy: Hypothesis testing using phylogenies. *Bioinformatics*, 21(5), 676–679. <https://doi.org/10.1093/bioinformatics/bti079>
- R Core Development Team (2019). *R: A language and environment for statistical computing*.
- Radwan, J., Babik, W., Kaufman, J., Lenz, T. L., & Winternitz, J. (2020). Advances in the evolutionary understanding of MHC polymorphism. *Trends in Genetics*, 36(4), 298–311. <https://doi.org/10.1016/j.tig.2020.01.008>
- Real-Monroy, M. D., Martínez-Méndez, N., & Ortega, J. (2014). MHC-DRB Exon 2 diversity of the jamaican fruit-eating bat (*Artibeus jamaicensis*) from Mexico. *Acta Chiropterologica*, 16(2), 301–314. <https://doi.org/10.3161/150811014x687260>
- Reese, A. T., Phillips, S. R., Owens, L. A., Venable, E. M., Langergraber, K. E., Machanda, Z. P., Mitani, J. C., Muller, M. N., Watts, D. P., Wrangham, R. W., Goldberg, T. L., Thompson, E., Emery Thompson, M., & Carmody, R. N. (2021). Age patterning in wild chimpanzee gut microbiota diversity reveals differences from humans in early life. *Current Biology*, 31(3), 613–620.e3. <https://doi.org/10.1016/j.cub.2020.10.075>
- Risely, A., Wilhelm, K., Clutton-Brock, T., Manser, M. B., & Sommer, S. (2021). Diurnal oscillations in gut bacterial load and composition eclipse seasonal and lifetime dynamics in wild meerkats. *Nature Communications*, 12(1), 1–12. <https://doi.org/10.1038/s41467-021-26298-5>
- Rivero-de Aguilar, J., Westerdahl, H., Martínez-de la Puente, J., Tomás, G., Martínez, J., & Merino, S. (2016). MHC-I provides both quantitative resistance and susceptibility to blood parasites in blue tits in the wild. *Journal of Avian Biology*, 47(5), 669–677. <https://doi.org/10.1111/jav.00830>
- Roach, S. N., & Langlois, R. A. (2021). Intra- and cross-species transmission of astroviruses. *Viruses*, 13(6), 1127. <https://doi.org/10.3390/v13061127>
- Rooks, M. G., & Garrett, W. S. (2016). Gut microbiota, metabolites and host immunity. *Nature Reviews Immunology*, 16(6), 341–352. <https://doi.org/10.1038/nri.2016.42>
- Rottem, S. (2003). Interaction of mycoplasmas with host cells. *Physiological Reviews*, 83(2), 417–432. <https://doi.org/10.1152/physrev.00030.2002>
- Round, J. L., & Mazmanian, S. K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews Immunology*, 9(5), 313–323. <https://doi.org/10.1038/nri2515>
- Sandberg, M., Eriksson, L., Jonsson, J., Sjöström, M., & Wold, S. (1998). New chemical descriptors relevant for the design of biologically active peptides. A multivariate characterization of 87 amino acids. *Journal of Medicinal Chemistry*, 41(14), 2481–2491. <https://doi.org/10.1021/jm9700575>
- Santos, P. S. C., Courtiol, A., Heidel, A. J., Höner, O. P., Heckmann, I., Nagy, M., Mayer, F., Platzer, M., Voigt, C. C., & Sommer, S. (2016). MHC-dependent mate choice is linked to a trace-amine-associated receptor gene in a mammal. *Scientific Reports*, 6(1), 1–9. <https://doi.org/10.1038/srep38490>
- Santos, P. S. C., Michler, F. U., & Sommer, S. (2017). Can MHC-assortative partner choice promote offspring diversity? A new combination of MHC-dependent behaviours among sexes in a highly successful invasive mammal. *Molecular Ecology*, 26(8), 2392–2404. <https://doi.org/10.1111/mec.14035>
- Schad, J., Dechmann, D. K. N., Voigt, C. C., & Sommer, S. (2011). MHC class II DRB diversity, selection pattern and population structure in a neotropical bat species, *Noctilio albiventris*. *Heredity*, 107(2), 115–126. <https://doi.org/10.1038/hdy.2010.173>
- Schad, J., Voigt, C. C., Greiner, S., Dechmann, D. K. N., & Sommer, S. (2012). Independent evolution of functional MHC class II DRB genes in New World bat species. *Immunogenetics*, 64(7), 535–547. <https://doi.org/10.1007/s00251-012-0609-1>
- Schmeller, D. S., Courchamp, F., & Killeen, G. (2020). Biodiversity loss, emerging pathogens and human health risks. *Biodiversity and Conservation*, 29(11–12), 3095–3102. <https://doi.org/10.1007/s10531-020-02021-6>
- Schwensow, N., Castro-Prieto, A., Wachter, B., & Sommer, S. (2019). Immunological MHC supertypes and allelic expression: How low is the functional MHC diversity in free-ranging Namibian cheetahs? *Conservation Genetics*, 20(1), 65–80. <https://doi.org/10.1007/s10592-019-01143-x>
- Schwensow, N., Mazzoni, C. J., Marmesat, E., Fickel, J., Peacock, D., Kovalík, J., Sinclair, R., Cassey, P., Cooke, B., & Sommer, S. (2017). High adaptive variability and virus-driven selection on major histocompatibility complex (MHC) genes in invasive wild rabbits in Australia. *Biological Invasions*, 19(4), 1255–1271. <https://doi.org/10.1007/s10530-016-1329-5>
- Sepil, I., Lachish, S., Hinks, A. E., & Sheldon, B. C. (2013). Mhc supertypes confer both qualitative and quantitative resistance to avian malaria infections in a wild bird population. *Proceedings of the Royal Society B: Biological Sciences*, 280(1759), 20130134. <https://doi.org/10.1098/rspb.2013.0134>
- Shipley, R., Wright, E., Selden, D., Wu, G., Aegeerter, J., Fooks, A. R., & Banyard, A. C. (2019). Bats and viruses: emergence of novel lyssaviruses and association of bats with viral zoonoses in the EU. *Tropical Medicine and Infectious Disease*, 4(1), 31. <https://doi.org/10.3390/tropicalmed4010031>
- Sidney, J., Grey, H. M., Kubo, R. T., & Sette, A. (1996). Practical, biochemical and evolutionary implications of the discovery of HLA class I supermotifs. *Immunology Today*, 17(6), 261–266. [https://doi.org/10.1016/0167-5699\(96\)80542-1](https://doi.org/10.1016/0167-5699(96)80542-1)
- Silverman, M., Kua, L., Tanca, A., Pala, M., Palomba, A., Tanes, C., Bittinger, K., Uzzau, S., Benoit, C., & Mathis, D. (2017). Protective major histocompatibility complex allele prevents type 1 diabetes by shaping the intestinal microbiota early in ontogeny. *Proceedings of the National Academy of Sciences of the United States of America*, 114(36), 9671–9676. <https://doi.org/10.1073/pnas.1712280114>

- Sommer, S. (2005). The importance of immune gene variability (MHC) in evolutionary ecology and conservation. *Frontiers in Zoology*, 2(1), 1–8. <https://doi.org/10.1186/1742-9994-2-16>
- Veech, J. A. (2013). A probabilistic model for analysing species co-occurrence. *Global Ecology and Biogeography*, 22(2), 252–260. <https://doi.org/10.1111/j.1466-8238.2012.00789.x>
- Visconti, A., Le Roy, C. I., Rosa, F., Rossi, N., Martin, T. C., Mohney, R. P., Li, W., de Rinaldis, E., Bell, J. T., Venter, J. C., Nelson, K. E., Spector, T. D., & Falchi, M. (2019). Interplay between the human gut microbiome and host metabolism. *Nature Communications*, 10(1), 1–10. <https://doi.org/10.1038/s41467-019-12476-z>
- Vu, D. L., Bosch, A., Pintó, R. M., & Guix, S. (2017). Epidemiology of classic and novel human astrovirus: Gastroenteritis and beyond. *Viruses*, 9(2), 33. <https://doi.org/10.3390/v9020033>
- Wakeland, E. K., Boehme, S., She, J. X., Lu, C. C., McIndoe, R. A., Cheng, I., Ye, Y., & Potts, W. K. (1990). Ancestral polymorphisms of MHC class II genes: Divergent allele advantage. *Immunologic Research*, 9(2), 115–122. <https://doi.org/10.1007/BF02918202>
- Wang, C., Li, P., Liu, L., Pan, H., Li, H., Cai, L., & Ma, Y. (2016). Self-adjuvanted nanovaccine for cancer immunotherapy: Role of lysosomal rupture-induced ROS in MHC class I antigen presentation. *Biomaterials*, 79, 88–100.
- Wang, L. F., & Anderson, D. E. (2019). Viruses in bats and potential spillover to animals and humans. *Current Opinion in Virology*, 34, 79–89. <https://doi.org/10.1016/J.COVIRO.2018.12.007>
- Wasimuddin, Brändel, S. D., Tschapka, M., Page, R., Rasche, A., Corman, V. M., Drosten, C., & Sommer, S. (2018). Astrovirus infections induce age-dependent dysbiosis in gut microbiomes of bats. *The ISME Journal*, 12(12), 2883–2893. <https://doi.org/10.1038/s4139-018-0239-1>
- Wasimuddin, Corman, V. M., Ganzhorn, J. U., Rakotondranary, J., Ratovonamana, Y. R., Drosten, C., & Sommer, S. (2019). Adenovirus infection is associated with altered gut microbial communities in a non-human primate. *Scientific Reports*, 9(1), 1–12. <https://doi.org/10.1038/s41598-019-49829-z>
- Wegner, K., Kalbe, M., Kurtz, J., Reusch, T., & Milinski, M. (2003). Parasite selection for immunogenetic optimality. *Science*, 301(56388), 1343. <https://doi.org/10.1126/science.1088293>
- Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J. R., Vázquez-Baeza, Y., Birmingham, A., Hyde, E. R., & Knight, R. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, 5(1), 1–18. <https://doi.org/10.1186/s40168-017-0237-y>
- Westerdahl, H., Waldenström, J., Hansson, B., Hasselquist, D., von Schantz, T., & Bensch, S. (2005). Associations between malaria and MHC genes in a migratory songbird. *Proceedings of the Royal Society B: Biological Sciences*, 272(1571), 1511–1518. <https://doi.org/10.1098/rspb.2005.3131>
- Wohlgemuth, N., Honce, R., & Schultz-Cherry, S. (2019). Astrovirus evolution and emergence. *Infection, Genetics and Evolution*, 69, 30–37. <https://doi.org/10.1016/j.meegid.2019.01.009>
- Xu, B., & Yang, Z. (2013). PamlX: A graphical user interface for PAML. *Molecular Biology and Evolution*, 30(12), 2723–2724. <https://doi.org/10.1093/molbev/mst179>
- Yang, Z. (2007). PAML 4: Phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution*, 24(8), 1586–1591. <https://doi.org/10.1093/molbev/msm088>
- Zhao, Q., & Elson, C. O. (2018). Adaptive immune education by gut microbiota antigens. *Immunology*, 154(1), 28–37. <https://doi.org/10.1111/imm.12896>

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Fleischer, R., Schmid, D. W., Wasimuddin, Brändel, S. D., Rasche, A., Corman, V. M., Drosten, C., Tschapka, M., & Sommer, S. (2022). Interaction between MHC diversity and constitution, gut microbiota and Astrovirus infections in a neotropical bat. *Molecular Ecology*, 31, 3342–3359. <https://doi.org/10.1111/mec.16491>