Received: 7 February 2023 | Revised: 25 April 2023 | Accepted: 2 May 2023

DOI: 10.1111/mec.16983

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

MHC class II genes mediate susceptibility and resistance to coronavirus infections in bats

Dominik W. Schmid[1] | Magdalena Meyer[1] | Kerstin Wilhelm[1] | Tatiana Tilley[1] | Thomas Link-Hessing[1] | Ramona Fleischer[1] | Ebenezer K. Badu[2] | Evans Ewald Nkrumah[2] | Samuel Kingsley Oppong[2] | Nina Schwensow[1] | Marco Tschapka[1] | Heather J. Baldwin[1,3] | Peter Vallo[1,4] | Victor M. Corman[5,6] | Christian Drosten[5,6] | Simone Sommer[1]

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

[2]Department of Wildlife and Range Management, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[3]Department of Biological Sciences, Macquarie University, Sydney, New South Wales, Australia

[4]Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czech Republic

[5]Charité – Universitätsmedizin Berlin Institute of Virology, German Centre for Infection Research (DZIF), Berlin, Germany

[6]German Centre for Infection Research (DZIF), Berlin, Germany

Correspondence
Dominik W. Schmid, Institute of Evolutionary Ecology and Conservation Genomics, Ulm University, Ulm, Germany.
Email: dominikwerner.schmid@uni-ulm.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: DFG SO 428/17-1, DR 772/3-1, DR 772/7-1, KA1241/18-1 and TH 1420/1-1

Handling Editor: Camille Bonneaud

Abstract

Understanding the immunogenetic basis of coronavirus (CoV) susceptibility in major pathogen reservoirs, such as bats, is central to inferring their zoonotic potential. Members of the cryptic *Hipposideros* bat species complex differ in CoV susceptibility, but the underlying mechanisms remain unclear. The genes of the major histocompatibility complex (MHC) are the best understood genetic basis of pathogen resistance, and differences in MHC diversity are one possible reason for asymmetrical infection patterns among closely related species. Here, we aimed to link asymmetries in observed CoV (CoV-229E, CoV-2B and CoV-2Bbasal) susceptibility to immunogenetic differences amongst four *Hipposideros* bat species. From the 2072 bats assigned to their respective species using the mtDNA *cytochrome b* gene, members of the most numerous and ubiquitous species, *Hipposideros caffer D*, were most infected with CoV-229E and SARS-related CoV-2B. Using a subset of 569 bats, we determined that much of the existent allelic and functional (i.e. supertype) MHC DRB class II diversity originated from common ancestry. One MHC supertype shared amongst all species, ST12, was consistently linked to susceptibility with CoV-229E, which is closely related to the common cold agent HCoV-229E, and infected bats and those carrying ST12 had a lower body condition. The same MHC supertype was connected to resistance to CoV-2B, and bats with ST12 were less likely be co-infected with CoV-229E and CoV-2B. Our work suggests a role of immunogenetics in determining CoV susceptibility in bats. We advocate for the preservation of functional genetic and species diversity in reservoirs as a means of mitigating the risk of disease spillover.

KEYWORDS
coronavirus, CoV-229E, cryptic diversity, *Hipposideros* bat species complex, major histocompatibility complex, pathogen resistance

Dominik W. Schmid and Magdalena Meyer: Contributed equally.

1 | INTRODUCTION

Bats are the most notorious vectors and zoonotic origin for many coronaviruses (CoVs) (Banerjee et al., 2019; Corman et al., 2018; Ruiz-Aravena et al., 2021), including both SARS-CoVs (Li et al., 2005; Zhou et al., 2020), MERS-CoV (Ithete et al., 2013) and two common cold agents, HCoV-229E (Corman et al., 2015) and HCoV-NL63 (Huynh et al., 2012). In fact, CoV sequence variants make up more than a third of all known viral infections in bats (Letko et al., 2020), and more speciose bat communities harbour more diverse CoV strains (Anthony et al., 2017). In humans, host genetics substantially impact transmission susceptibility, disease severity and mortality risk from SARS-CoV-2 (Pathak et al., 2021) and other CoVs (Ovsyannikova et al., 2020). In addition to receptors, such as the transmembrane serine protease 2 or angiotensin converting enzyme 2, which are frequently involved in cell entry of CoVs (Hoffmann et al., 2020), several alleles of the human equivalent of the major histocompatibility complex (MHC)—the human leukocyte antigen (HLA)—are putatively connected to adaptive immune reactions against infections with SARS-CoV-2 (Migliorini et al., 2021) and other CoVs (Chu et al., 2014; Loyal et al., 2021). However, in contrast with humans, very little is known about the relationship between the MHC and resistance to CoVs in their natural reservoirs.

The multigene cluster of the MHC is the best characterised genetic basis of pathogen resistance in jawed vertebrates (Kaufman, 2018a; Sommer, 2005). The genomic region encodes a suite of structurally related yet distinct cell surface glycoproteins—MHC class I and II—which present self- and non-self-peptides in their peptide-binding regions to $CD8^+$ and $CD4^+$ T cells. Foreign peptides then trigger pathogen-specific immune responses. MHC class I and II molecules present peptides derived from intracellular or extracellular pathogens, respectively (Neefjes et al., 2011). Yet, intracellular peptides may be degraded via autophagy and presented by MHC class II molecules instead (Dengjel et al., 2005; Harman et al., 2009; Laing et al., 2019). Aside from cross-presentation, host control over commensal bacteria, which modulate host immunity (Fleischer et al., 2022; Han et al., 2022; Kubinak et al., 2015), is mediated via the MHC class II and associated T-cell responses (Cao et al., 2012; Roland et al., 2020).

Several mutually non-exclusive hypotheses were put forward as drivers of the coevolutionary arms race between host and pathogen and to explain the exceptional polymorphism observed at the MHC (Radwan et al., 2020; Spurgin & Richardson, 2010). At their core, each hypothesis explains how the identity, diversity and frequency of MHC alleles and supertypes (STs; i.e. MHC alleles grouped together based on shared antigen binding properties, Doytchinova et al., 2004; Lighten et al., 2017) relate to host resistance: individuals with functionally heterogeneous MHC alleles/STs, for instance, are able to fend off a greater variety of pathogens (called heterozygote/divergent allele advantage; Lenz et al., 2009; Takahata & Nei, 1990; Wegner et al., 2003). Since pathogens evolve to evade common MHC alleles/STs, advantageous (often rare) MHC alleles/STs may rapidly increase in frequency in the host population, resulting in cyclic negative frequency-dependent selection (Eizaguirre et al., 2012b; Meyer-Lucht & Sommer, 2005; Phillips et al., 2018), and distinct pathogen communities result in fluctuating selection across space and time (Eizaguirre et al., 2012a; Hill et al., 1991). The same mechanisms seem to govern resistance to CoVs in humans: HLA (MHC class I and II) variants are linked to COVID-19 symptoms and disease severity (Bruchez et al., 2020; Castelli et al., 2021; Vietzen et al., 2021), the frequency of two HLA haplotypes correlates negatively and positively with country-wide infection gradients in Italy (Pisanti et al., 2020), and HLA-SARS-CoV-2 associations vary globally (Migliorini et al., 2021).

Bat immunity is frequently treated as a black box owing to its peculiarities (Wang et al., 2021). For example, bats employ antibodies in defence against CoV infections (Müller et al., 2007), but their immune system seems rather to tolerate than purge viral infections (Banerjee et al., 2020), often leading to prolonged infectious periods, despite appearing physically healthy (Irving et al., 2021; Munster et al., 2016; Subudhi et al., 2017). In addition, bats are highly gregarious and often share roosting caves with different, sometimes phylogenetically closely related bat species, further facilitating cross-species viral spillover events and sustaining shared pathogen-mediated selection. Pathogen-mediated selection is likely at the root of the exceptional allelic and functional diversity of the MHC class I and II region observed in bats (e.g. Qurkhuli et al., 2019; Salmier et al., 2016; Schad et al., 2011). Yet, few studies to date draw connections between any of the many viruses, let alone CoVs, infecting bats and host MHC genetics (e.g. Astroviridae-MHC class II (Fleischer et al., 2022); Influenza A viruses-MHC class II (Giotis et al., 2019; Karakus et al., 2019)). Moreover, the consequence of co-habitation of bats with shared functional MHC diversity has never been investigated, even though it represents an essential step towards understanding CoV prevalence and persistence in natural host populations, and its potential risk to public health.

Members of the particularly speciose, palaeotropical leaf-nosed bat family Hipposideridae are host to a number of corona-, Ebola- und paramyxoviruses with zoonotic potential (Annan et al., 2013; Drexler et al., 2012; Hayman et al., 2012; Pfefferle et al., 2009; Suu-Ire et al., 2022). Sub-Saharan hipposiderids harbour an ancestral form of the common cold agent, the alpha-CoV-229E, and two SARS-related beta-CoVs (Corman et al., 2015; Pfefferle et al., 2009). Recent molecular, ecological and behavioural evidence was used to differentiate three morphologically indistinguishable species of the *Hipposideros caffer* complex (Baldwin et al., 2014, 2021; Vallo et al., 2008), and it was suspected that the species also differ in CoV susceptibility (Baldwin, 2015). In the present study, we aim to understand how MHC class II genes mediate susceptibility and resistance to CoV infections in hipposiderids. Whereas MHC class I traditionally is associated with an anti-viral immune cascade, cross-presentation of, for example, autophagically derived viral peptides at MHC class II molecules might be an important anti-viral mechanism in bats (Laing et al., 2019). Additionally, CoVs replicate enterically in bats (Drexler et al., 2014). Resistance to enterically replicating viruses

was previously found to be linked indirectly to MHC class II via host microbiota (Fleischer et al., 2022). We therefore hypothesise if differences in infection prevalence exist among members of the *Hipposideros caffer* complex, infection likelihood might be associated with allelic and functional diversity of the MHC DRB class II region. Our study found differences in infection likelihood between species and identifies consistent associations between CoV infection likelihood and functional MHC supertypes.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Bats were live-trapped at five locations with one to three roosting sites (Buoyem—caves: BUO1 and BUO2; Forikrom—cave: FO; Kwamang—caves: KW1, KW2 and open site: KW3; Akpafu Todzi—mine: AT; Likpe Todome—caves: LT1 and LT2) in 12 two-month long capture periods between September 2010 and August 2012 in Central Ghana, West Africa (Figure 1a). The sampling procedure, environmental and climatic conditions are detailed in Baldwin (2015) and Meyer et al. (in review). If possible, all bats were classified to species level using morphological characteristics. Morphometric details such as forearm length or weight were described elsewhere (Baldwin et al., 2021). Two minimally invasive wing punches (3 mm) were taken from each bat and stored in molecular-grade ethanol at −20°C for DNA extraction. Additionally, faecal samples were collected and stored in RNAlater at −80°C for virus and microbiome screening. Research (A04957) and ethics permit (CHRPE49/09/CITES) were granted by the Wildlife Division of the Forestry Commission of the Ministry of Lands, Forestry and Mines.

2.2 | DNA extraction, cytochrome b sequencing and lineage assignment

DNA extraction was performed using wing punch tissue from 2072 bats of the 6654 bats assigned to the *H. caffer* complex or *H. abae*. The extraction followed an ammonium acetate protocol (Nicholls et al., 2000) with slight modifications from T. Halczok, University of Greifswald. Building on previous primer designs for the *Hipposideros* species complex (Vallo et al., 2008), the mtDNA *cytochrome b* gene (*cytb*) was amplified by polymerase chain reaction (PCR) using adapted primers suitable for high-throughput Illumina sequencing. After sequencing, the *cytb* gene was confirmed by homology analysis using the NCBI BLAST search. Subsequently, all sequences were analysed in Geneious 11.1.5 (https://www.geneious.com) and assigned to the lineages *B, C or D* of the *H. caffer* species complex (henceforth called species, Baldwin et al., 2014, 2021; Vallo et al., 2008) or the sympatric species *H. abae* using the MAFFT alignment tool (Katoh & Standley, 2013; for more detail see Appendix S1; Table S1).

2.3 | Virus screening: RNA purification and CoV characterisation

RNA was purified from approximately 20 mg of faecal material suspended in 500 µL RNAlater stabilising solution using the MagNA Pure 96 system (Roche) with elution volumes set at 100 µL. We used a real-time reverse transcription-PCR assay designed to detect several alpha- and beta-CoVs and genetically related bat CoVs using the SSIII RT-PCR kit (Life Technologies) and a cycling protocol in a LightCycler 480 (Roche) as described previously (Corman et al., 2015; Drexler et al., 2009; Pfefferle et al., 2009; see Appendix S1; Table S2). Bats were categorised as positive for a specific CoV if the CT value was equal or smaller than 38.0 (Pfefferle et al., 2009).

2.4 | Sequencing of MHC class II DRB exon 2 loci

A 171 bp fragment within the MHC class II DRB exon 2 loci of 575 *Hipposideros* samples was amplified using primers modified from Schad et al. (2011) and quality checked in Geneious. The JSN primers are widely used for chiropteran MHC class II DRB as they have an exceptional ability to amplify the region in a variety of species. *Hipposideros* samples were MHC genotyped using an Illumina

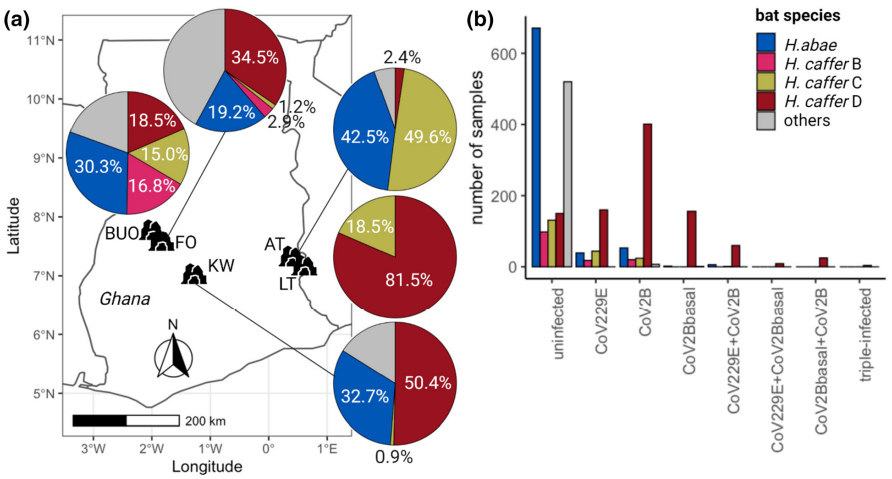

FIGURE 1 (a) Community composition of *Hipposideros* bat species captured at five cave locations in Ghana and (b) number of individuals uninfected, single or co-infected with several alpha- and beta-CoVs.

platform (see Appendix S1). The samples covered all locations, lineages and CoV infections (Table S3 and S4) and, collectively, reached beyond the threshold sample size (>200) suggested for wild populations (Gaigher et al., 2019). We ran duplicates for 53 samples to assess repeatability.

2.5 | MHC class II allele characterisation using ACACIA

The MHC class II DRB exon 2 sequences were analysed using the genotyping pipeline ACACIA (Allele CAlling proCedure for Illumina Amplicon sequencing data; (Gillingham et al., 2021); https://gitlab.com/psc_santos/ACACIA), most recently employed to analyse sequences from a neotropical bat species (Fleischer et al., 2022). ACACIA first filters sequence quality, before confirming MHC DRB sequences by homology analysis using NCBI BLAST search based on other chiropteran MHC DRB sequences. Forward and reverse reads were merged with a minimum overlap of 50 base pairs (bp) and a maximum overlap of 250 bp. Quality filtering removed sequences with a Phred quality score value <30 and a *q*-value <90. For alleles to be retained, the minimum number of reads per allele was set to 10, and the lowest percentage of the total reads of an individual was 1%. The ACACIA workflow and post hoc elimination of singletons and alleles with low reliability preserved reliable MHC allele information for a total of 569 bats (Appendix S1; Figure S1 and Table S3).

2.6 | MHC supertype assignment

Allelic MHC diversity was then grouped into functional supertypes based on shared amino acid motifs at positively selected sites (PSSs), following the assumption that PSSs likely belong to, or are closely linked to, functionally important antigen-binding sites (Roved et al., 2022; Schwensow et al., 2019; Sepil et al., 2013). PSSs were identified for each species separately using CODEML integrated in the program PAML4 (Phylogenetic Analysis by Maximum Likelihood; Yang, 2007) and run in the graphical interface PAML-X GUI (Xu & Yang, 2013). Four PAML models were tested: M1a (nearly neutral), M2a (positive selection), M7 (beta) and M8 (beta and ω). M2a and M8 performed equally well, evidencing selection acting on specific sites. For subsequent supertype assignment across species, a total of 14 'consensus' PSS sites were selected based on being (a) identified by both M2a and M8 with a posterior probability of at least 95% and (b) selected in at least 3 of the 4 species (Figure S2; Table S5).

Antigen-binding specificity can be quantified by z-values describing the physiochemical properties of amino acids encoded by the codon present at PSSs (Sandberg et al., 1998). A matrix containing the *z* values of each allele's PSS amino acid was used in the functions *find.clusters* and *dapc* (i.e. discriminant analysis of principal components) of the 'adegenet' R package (Jombart et al., 2010) to cluster alleles into groups (i.e. MHC supertypes or STs) with similar binding functionality (see Appendix S1, Figure S3).

2.7 | Statistical analyses

2.7.1 | Community composition and CoV infection patterns

Differences in community and abundance between the 2072 sampled hipposiderids were assessed using a permutational analysis of variance (PERMANOVA). A x^2-test was run to compare proportional differences in single and co-infections with any of the three CoVs between species.

2.7.2 | MHC diversity

Individual MHC allelic and ST diversity were compared among roosting locations, species and their interaction using generalised linear models (GLMs) with Poisson distribution. To assess whether MHC allelic and supertype diversity present was captured with the sampling effort across locations and for each species, we used the *specaccum* function, and, to confirm whether an asymptote was reached, the *specpool* function of the 'vegan' R package.

2.7.3 | Evidence for balancing selection and/or parallel evolution

Owing to common ancestry, sites under positive selection should be characterised by fewer synonymous codons if balancing selection maintained trans-species polymorphism (TSP) rather than convergence based on independent parallel evolution (Lundberg et al., 1992). To differentiate between these mechanisms, we compared the proportion of identical codons encoding the same amino acid at PSSs between species pairs (Lenz et al., 2013). Under balancing selection, positively selected amino acid sites should have fewer synonymous codons than under parallel evolution, where we assume identical amino acids are coded by the same proportion of identical codons irrespective of whether the site is under positive selection or not. Following this rationale and R code provided by Gillingham et al. (2016), we drew codons with replacement from all possible codons at the sequence to simulate new sequences synonymous in function to those observed at peptide level. The codon similarity simulation was reiterated 9999 times to generate a distribution of similarity measurements. This distribution represents our expectation under parallel evolution and the contrast with the observed codon similarity allows to statistically distinguish between parallel evolution or balancing selection as mechanism underlying TSP (albeit we cannot rule out introgression as confounding factor; Fijarczyk & Babik, 2015).

2.7.4 | Associations between CoV infection and MHC diversity or supertype identity

We identified associations between the presence of an MHC ST and CoV infections using the probabilistic model of co-occurrence (Veech, 2013) programmed in the 'cooccur' R package (Griffith et al., 2016). A positive association between an MHC ST and CoV infection is assumed when the observed co-occurrence is significantly higher than the expected co-occurrence and vice versa. In other words, a higher co-occurrence implies higher probability of individuals with the particular MHC ST harbouring an infection and, thus, suggests susceptibility. By contrast, a significantly lower co-occurrence suggests resistance. The co-occurrence model was run separately for each species due to differences in MHC ST profiles, but concurrently for all CoV infections present in each species. We only included MHC STs represented in at least 10 individuals to guarantee a reasonable sample size.

The results from the co-occurrence analysis were confirmed with generalised linear mixed effects models (GLMMs) using CoV infections as binomial response variable, and number of STs and its quadratic term as well as the specific STs identified by the co-occurrence analyses as explanatory variables. Number of STs is a proxy for functional MHC diversity given its correlative link to other measures of MHC diversity (Appendix S1; Figure S4). Capture period was set as random effect. Final models retained only explanatory variables suggested by an information theoretic approach using the *dredge* function from the 'MuMIn' R package (Bartoń, 2009). False discovery rate correction was applied when co-occurrence analysis had identified multiple STs and, thus, multiple GLMMs were run on the same response variable (Benjamini & Hochberg, 1995).

3 | RESULTS

3.1 | Community composition and CoV infection pattern in Hipposideros bat species

The community composition of *Hipposideros* bat species differed in the five sampling locations in Ghana (PERMANOVA: $p = .001$; Figure 1a). *H. caffer C* and *D* were present among all locations even though their frequency differed. *H. abae* was only found in four locations, and *H. caffer B* was rarest comprising 18.8% in Buoyem and 2.9% in Forikrom.

The alpha-CoV-229E and the beta-CoV-2B infected all *Hipposideros* species, whereas the more basal beta-CoV-2Bbasal was only found in *H. abae* and *H. caffer D* (Figure 1b). The prevalence of CoVs differed between *Hipposideros* species (prevalence: $x^2_{df=21, n=2072} = 1019.10$, $p < .001$). Co-infections between CoV-229E and CoV-2B as well as CoV-2B and CoV2Bbasal were mainly recorded in *H. caffer D*. The MHC-typed subset reflects this pattern (Table S4).

3.2 | MHC allelic and supertype diversity in the Hipposideros species complex

We detected a total of 165 MHC class II alleles from the 569 *Hipposideros bats* sampled across five different locations in Sub-Saharan Ghana (repeatability: 94.3%). The 171 bp long fragments mapped inside the DRB exon 2 and 8 PSSs were congruent with antigen binding sites of the human HLA-DRB*0101, while the others were within one amino acid position to human antigen binding sites (Table S5). With the exception of W61, conserved sites in the hipposiderid sequences coincided with conserved sites of the human reference sequence (Brown et al., 1993), which is consistent with MHC class II DRB exon 2 sequence variation previously found in bats (Schad et al., 2011).

With a total of 79 alleles, *H. caffer C* displayed the highest allelic diversity, followed by *H. caffer B*, *D* and *H. abae* with 30, 29 and 26, respectively (Figure S5). Individual allelic MHC diversity ranged from 2 to 7 (median: 4) in *H. abae*, 1 to 12 (median: 5) in *H. caffer B*, 1 to 11 (median: 5) in *H. caffer C* and 1 to 12 (median: 5) in *H. caffer D*, implying at least four loci encoding MHC class II information in *H. abae*, but as many as six among members of the *H. caffer* complex. The mean number of alleles per individual did not statistically differ but varied between 4.32 (±1.65 SD) and 5.78 (±2.53 SD) among location (Table S6a) and between 3.80 (±1.14 SD) and 5.25 (±2.09 SD) among *Hipposideros* species (Table S6b). *H. caffer D* in the Forikrom cave showed exceptionally high allelic diversity (mean 6.97 ± 2.13 SD; Table S6c). Overall, we likely captured the present allelic diversity of the four species (Figure S6A,B) and reach levels of allelic diversity similar to that of other Chiroptera (Table S7).

The unambiguously identified alleles were assigned 12 functionally distinct MHC STs based on their shared physicochemical properties of the amino acids at 14 PSSs (Figure 2a). Individual MHC ST diversity ranged from 2 to 5 (median: 3) in *H. abae*, 1 to 6 (median: 4) in *H. caffer B*, 1 to 7 (median: 4) in *H. caffer C* and 1 to 6 (median: 3) in *H. caffer D*. Functional diversity was also similar between locations, species and for each species per location (Table S6a–c) with a mean individual ST diversity of 3.52 (±1.17 SD). The highest allelic diversity at 5.78 was recorded in Forikrom, though the same location showed the lowest ST diversity at 3.10, possibly implying functional redundancy among MHC alleles. Similar to allelic diversity, sampling effort was sufficient to cover ST diversity among the four species (Figure S6C,D).

3.3 | Codon usage analysis

We found identical amino acid sequences for a total of 34 of the 165 MHC class IIB DRB exon 2 alleles (Table 1). Three alleles that grouped within ST12 were shared among all species and another three also within ST12 were shared among all lineages from the *H. caffer* species complex. This encourages the argument for functional redundancy among MHC amino acid alleles, and suggests balancing selection to maintain the observed TSP.

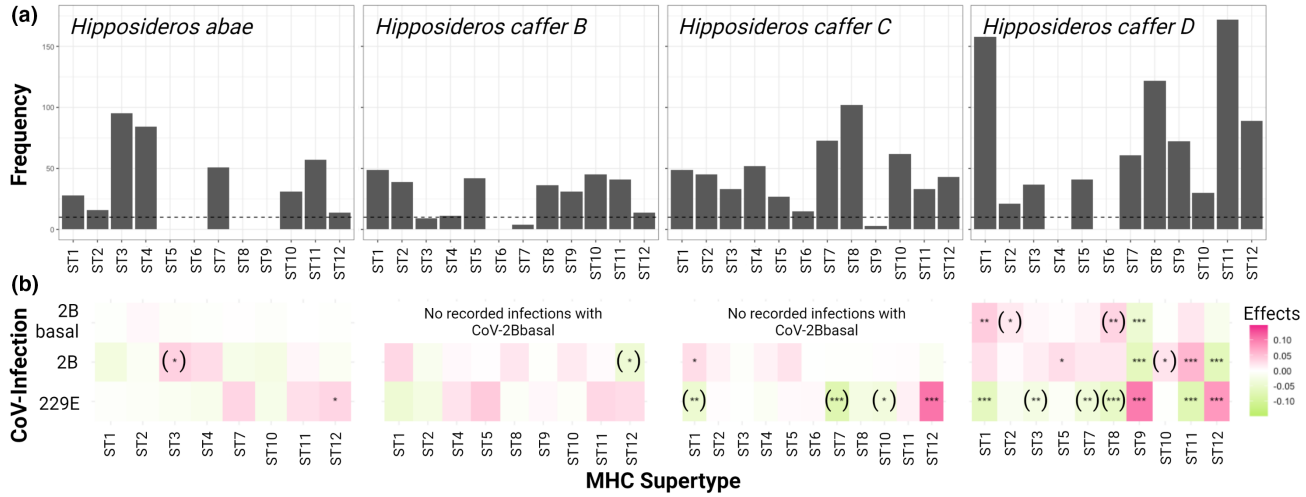

FIGURE 2 (a) Major histocompatibility complex (MHC) supertype frequencies and (b) associations with three common CoV-infections in four Hipposideridae species from Ghana. Dashed line marks the minimum ST frequency ($n = 10$) at which ST was still included in the co-occurrence analysis. The significance of the co-occurrence analysis is presented as: * < .05; ** < .01; *** < .001. 'effects' represent standardized effect sizes and indicate positive (implying susceptibility) and negative (implying resistance) association between MHC ST and CoV infection in pink and green, respectively. Brackets around asterisk imply that the results were not supported by the confirmatory GLMMs.

TABLE 1 Proportion of identical amino acids usage and number of identical sequences (in brackets) in alleles of the major histocompatibility complex class IIB DRB exon 2 among four *hipposiderids*.

	H. abae	*H. caffer B*	*H. caffer C*	*H. caffer D*
H. abae		92.26 (3)	93.07 (3)	91.52 (3)
H. caffer B			92.26 (12)	92.48 (6)
H. caffer C				92.68 (7)
H. caffer D				

Following these results, we estimated the frequency of nonsynonymous to synonymous codons used at PSSs in the hipposiderids to formally test whether the pattern was more likely to arise from parallel evolution or balancing selection. We found an average codon similarity of 92.38% (±0.52 SD) between species (Table 1). Hence, fewer synonymous codon changes were observed than expected from simulated data (all p-values < .001; Figure S7).

3.4 | CoV-MHC diversity and supertype associations

The co-occurrence model identified and subsequent GLMMs confirmed a total of four MHC STs to be associated with CoV-229E infections (Figure 2b, all p-values and effect sizes are reported in Table S8; GLMM results are reported in Table S9): MHC ST12 was consistently, even though not always significantly, positively associated with CoV-229E infections, suggesting higher susceptibility in individuals with MHC ST12. The only other significant positive association was for ST9 in CoV-229E-infected *H. caffer D*. A consistent, albeit not always significant, negative association with CoV-229E, and thus implying a protective function, was found for ST1. Lastly, for *H. caffer D* the likelihood of infection with CoV-229E decreased with in increasing number of MHC STs in five out of seven models (Table S9; Figure S8).

Another five MHC STs were associated with CoV-2B infections and two were found linked to CoV-2Bbasal prevalence (Figure 2b; Tables S8 and S9): MHC ST12 was consistently, although not always significantly, negatively associated with CoV-2B infections, suggesting a resistance advantage of ST12. Such distinct MHC ST effects of susceptibility towards CoV-229E but resistance to CoV-2B suggest pleiotropism. MHC ST9 was negatively associated with CoV-2B and CoV-2Bbasal in *H. caffer D*, while ST11 and ST5 in *H. caffer D* and ST1 in *H. caffer* C were positively associated with CoV-2B.

3.5 | Follow-up: Co-infections, quantitative resistance/susceptibility and host fitness

Motivated by our findings of consistently pleiotropic effects of MHC ST12, which is shared among all species and contains highly conserved alleles, three hypotheses emerged. First, if the ST is indeed functionally divergent, one might expect fewer individuals with CoV-229E-CoV-2B co-infections among those carrying MHC ST12. To test this, we used two x^2-Test of Independence comparing the number of uninfected, singly infected and co-infected individuals with or without MHC ST12 and visualised the results using the *assoc* function of the 'vcd' R package (Zeileis et al., 2007). As hypothesised, significantly fewer individuals carrying MHC ST12 were co-infected with CoV-229E and CoV-2B (Figure 3a, Table S10).

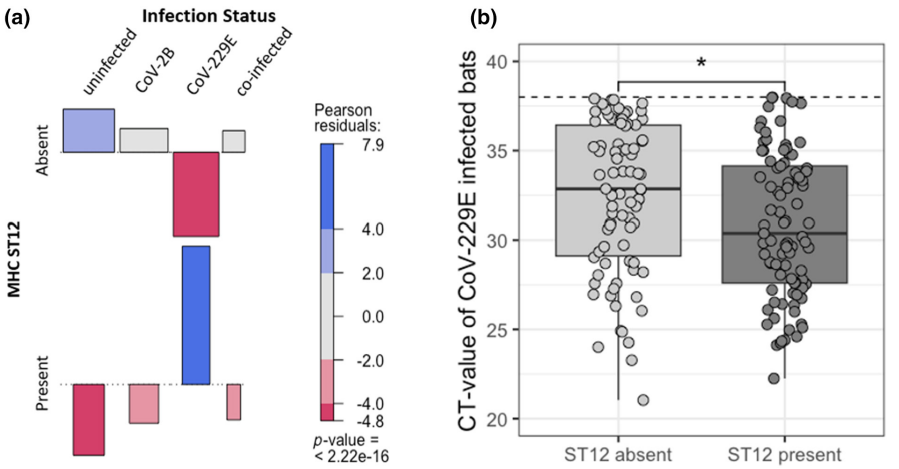

FIGURE 3 (a) Association plot depicts a table of Pearson residuals (i.e. a measure of discrepancy between observed and expected values) highlighting a lower likelihood of co-infection with CoV-229E and CoV-2B for individuals carrying MHC ST12, and (b) shows the CT value (as measure of viral infection intensity) was lower in bats carrying MHC ST12, implying quantitative susceptibility.

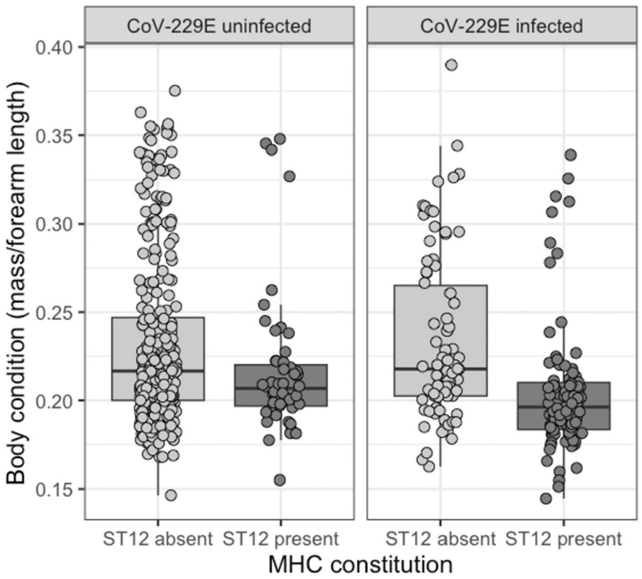

FIGURE 4 Body condition between uninfected and CoV-229E-infected bats and its variation according to whether major histocompatibility complex (MHC) ST12 is part of an individual's MHC constitution.

Second, while MHC-prevalence associations can only suggest qualitative resistance/susceptibility, measures of infection intensity may be used to detect quantitative resistance/susceptibility (Westerdahl et al., 2012). Hence, we compared CT value (as measure of viral infection intensity) between infected individuals with and without MHC ST12 using a Wilcoxon rank sum test. Indeed, CT values were significantly lower for CoV-229E-infected individuals carrying MHC ST12 (Figure 3b; $p = .017$), implying a higher infection intensity and thus quantitative as well as qualitative susceptibility against CoV-229E. By comparison, CT values of CoV-2B-infected bats with MHC ST12 were similar to bats without MHC ST12 ($p = .109$), which suggests that MHC ST12 provides only qualitative resistance against CoV-2B.

Lastly, we suspected MHC ST12-mediated resistance or susceptibility could translate into differences in host condition infected with either CoV-229E or CoV-2B. To test this, we first calculated body condition from body mass divided by forearm length and removed pregnant bats from further analysis. We modelled log-transformed body condition as response variable in a linear mixed-effect model with species, quadratic and non-quadratic number of MHC STs as well as an interaction between presence/absence of MHC ST12 and presence/absence of either an infection with CoV-229E or CoV-2B as explanatory variable. Capture period was set as random effect. The best model showed a higher body condition for members of *H. abae* and *H. caffer* B, but a lower body condition in bats infected with CoV-229E or carrying MHC ST12 (Figure 4; Table S11). The CoV-2B infection status, number of STs and the interaction were not retained in the top models (Table S12).

4 | DISCUSSION

Bats are hosts, reservoirs and vectors for a variety of viruses and the principal source of CoVs with zoonotic potential (Anthony et al., 2017; Letko et al., 2020; Ruiz-Aravena et al., 2021). Yet, few studies aimed to understand the genetic basis of virus resistance and susceptibility in bats (e.g. Fleischer et al., 2022), and none in relation to CoVs. Here we report asymmetrical CoV infection patterns and shared allelic and functional MHC class II diversity among four *Hipposideros* bat species co-inhabiting several roosting caves in Ghana. We found several positive and negative associations between CoV infections and host MHC STs. Specifically, we observe consistently higher susceptibility to the alpha-CoV-229E and reduced body condition in infected individuals or those carrying MHC ST12. The same MHC ST, however, provides resistance towards the SARS-related CoV-2B.

Infections with both alpha- and beta-CoVs were found among the four *Hipposideros* bats studied, although beta-CoV-2Bbasal was only detected in two out of the four species, and infection prevalence with all three CoVs was highest in the most numerous host species, *H. caffer D*, with 84.5% of our samples estimated to be infected with one or multiple CoVs. Asymmetrical infection patterns with 229E-related and SARS-related CoVs are known from

Hipposideros bat species in Ghana (Pfefferle et al., 2009), Gabon (Maganga et al., 2014) and Zimbabwe (Bourgarel et al., 2018). Globally, hipposiderids and their sister family Rhinolophidae are considered major CoV reservoirs (Annan et al., 2013; Corman et al., 2015; Gouilh et al., 2011; Quan et al., 2010). Speciose assemblages also often show high rates of CoV evolution, suggesting links between bat and CoV diversity (Anthony et al., 2017). At the same time, CoV infection prevalence declines in more diverse roosting communities in Ghana (Meyer et al., in review), supporting the idea that more diverse host assemblages alter transmission dynamics (Civitello et al., 2015). In such systems, cross-species spillover events are likely frequent (Latinne et al., 2020), and CoVs may adapt rapidly and extensively (e.g. <10%–40% amino acid divergence in spike protein) to novel host (Boni et al., 2020; Crossley et al., 2012; Lau et al., 2010, 2012). This raises the possibility that episodic diversifying selection in CoV strains facilitates spillover between animal hosts, similar to the mechanism driving variant evolution in SARS-CoV-2 (Tay et al., 2022). If competent CoV-hosts dominate a diverse species community, novel CoV strains may readily evolve and possibly switch host to become a wildlife and public health concern.

The allelic MHC class II diversity of four hipposiderid bats was modest in comparison with other Chiroptera. Though the 79 alleles characterised in *H. caffer C* alone rallies diversity typically only found in birds (Gillingham et al., 2016; Whittingham et al., 2018) and few exceptionally diverse bats with large geographical ranges, such as *Carollia perspicillata* (Qurkhuli et al., 2019). Interestingly, *H. caffer C*'s geographical range is much smaller than that of *C. perspicillata* but instead overlaps with several other hipposiderids (Vallo et al., 2008) and co-habitation is common (Baldwin, 2015). Sociality, with its many opportunities for cross-species transmission of shared pathogens, rather than migration and its associated need to adapt to geographically distinct pathogens, might thus select for high MHC diversity, as also suggested for some colony forming birds (Minias et al., 2017). In contrast, the allelic diversity of *H. abae* and *H. caffer B* and *D* ranged from 27 to 30 alleles. Intra-individual MHC class II diversity with a maximum of 7 alleles (i.e. 4 loci) in *H. abae* and 11/12 (i.e. 6 loci) in *H. caffer B-D* was higher than in many other bat species, but similar or lower than that of *Rhinolophus* spp. as the other important CoV reservoir (Li et al., 2021; Zhao et al., 2014). In terms of functional MHC class II diversity, hipposiderids shared between 8 and 11 of the 12 identified STs, which is comparable with the number of STs detected in *Artibeus jamaicensis* (Fleischer et al., 2022).

Hipposiderids also shared several MHC alleles. Such high degree of retained functional and allelic diversity in the estimated 5.5–4.4 million years since their divergence (Baldwin, 2015) could be an argument for balancing selection driven by common pathogen pressure and frequent cross-species transmission in shared roosting caves. However, introgression of MHC alleles via hybridisation of sympatric species could be a confounding factor (Nadachowska-Brzyska et al., 2012). Balancing selection and hybridisation, for example, explain MHC diversity among Eurasian newts (Dudek et al., 2019; Gaczorek et al., 2022) and green lizards (Sagonas et al., 2018) and suggest an adaptive role of introgression in maintaining genetic diversity in habitats with shared selection pressure (Marques et al., 2019).

Several MHC class II STs were positively or negatively associated with CoV-229E, CoV-2B and, to a lesser degree, with the more ancestral CoV-2Bbasal strain. The associations were most pronounced in the heavily infected *H. caffer D*. Despite the notion that MHC class I plays a more significant role in viral resistance than MHC class II (Kaufman, 2018b), shared immunological pathways (e.g. MHC class II transactivator (CIITA) induces presentation of MHC class I molecules (Bruchez et al., 2020)) and antigen cross-presentation (Neefjes et al., 2011) suggest coordinated anti-pathogen responses. In fact, antigens presented by MHC class II molecules can itself trigger antiviral immune responses. When antigen-presenting cells detect intracellular pathogens and present antigens, the Th1 group of $CD4^+$ T cells become activated and stimulate cytotoxic T cells, macrophages and anti-viral antibody production by B cells. Additionally, CoVs replicate in the enteric tract of many wildlife species, including bats (Drexler et al., 2014). At the mucosal interface such as the intestinal lumen, Th17 cells produce the anti-microbial peptide generating cytokine interleukin 22 (Liang et al., 2006), and coordinate the secretion of IgA antibodies by B cells (Cao et al., 2012). Both, but IgAs, in particular, play important roles in the cross-talk between commensal gut bacteria and host immunity (Huus et al., 2021; Roland et al., 2020), and several studies have confirmed a link between gut microbial diversity and host MHC diversity and constitution (Bolnick et al., 2014; Davies et al., 2022; Silverman et al., 2017). This means competition and production of immunomodulatory molecules by MHC-class II-regulated commensals might feasibly aid anti-viral responses in bats, and was previously proposed to explain infection patterns with an enterically replicating Astrovirus in a neotropical bat (Fleischer et al., 2022) and Adenovirus-infected lemurs (Montero et al., 2021). Another possibility is that certain viruses exploit MHC class II receptor proteins for cell entry (Giotis et al., 2019; Karakus et al., 2019). Regardless, our study is not the first to detect associations between specific MHC alleles/STs and viral infections (e.g. H1N1 influenza (Luckey et al., 2019) hepatitis C virus (McKiernan et al., 2004); CoVs (Migliorini et al., 2021)). Nevertheless, our study adds to the growing body of research that associates MHC class II diversity and composition with viral resistance, though the exact mechanism is still speculative.

Major histocompatibility complex ST12 contained many alleles shared among the four *Hipposideros* species and showed consistently positive (i.e. susceptibility) and negative (i.e. resistance) associations with CoV-229E and CoV-2B, respectively. Furthermore, our findings suggest that CoV-229E infections are more severe in bats with MHC ST12. While evidence for quantitative resistance or susceptibility to ectoparasites linked to bat MHC genetics exists (Schad et al., 2012), we are the first to provide such evidence for viral infections. In addition, individuals carrying MHC class II ST12 had fewer co-infections between CoV-2B and CoV-229E. This reinforces the idea that MHC ST12 has pleiotropic effects, as it confers protection against CoV-2B but makes individuals susceptible to CoV-229E. Pleiotropy in the context of MHC research

is common. MHC diversity, for example, shapes pathogen resistance and mate choice in many vertebrates (e.g. fish (Milinski et al., 2005); birds (Buchholz et al., 2004); mammals (Schwensow et al., 2008)). Finally, we examined whether CoV infections or MHC ST12 had varying impacts on individual body condition. Although we did not find an interaction, we observed that bats infected with CoV-229E had a reduced body condition, and these individuals frequently possessed MHC ST12. Few studies report such measurable costs of infections in bats (e.g. fungal white-nose syndrome negatively impacts fat reserves; Frick et al., 2010); nutritional stress and reproductive decline associated with Hendra virus infection (Plowright et al., 2008); poor body condition associated with seropositivity to Nipah virus (Epstein et al., 2020)), but CoV infection status was previously associated with reduced body condition in Lyle's flying foxes (*Pteropus lylei*; Wacharapluesadee et al., 2018). Infection 'tolerance' is often hailed as unique feature of bat immunity (Banerjee et al., 2019; Letko et al., 2020; Moreno Santillán et al., 2021; Munster et al., 2016), though variation based on host immunogenetics suggests this is not a universal feature. Mark–recapture studies that incorporate data on infection intensity and host immunogenetics could potentially be effective in distinguishing the fitness costs over the course of an infection and between host genotypes.

With our work we were able to illustrate asymmetrical CoV infection patterns, shared MHC class II diversity and CoV susceptibility related to host immunogenetics in hipposiderid bats from Ghana. The diversity of MHC and receptor proteins, such as angiotensin-converting enzyme 2, are candidate host traits most informative to evaluate cross-species transmission potential (Frank et al., 2022), and likely explain mechanistically how greater host (genetic) diversity keeps pathogen prevalence in check (Civitello et al., 2015). Although spillover risks likely depend on a variety of ecological factors (e.g. Eby et al., 2022; Gibb et al., 2020), the importance of immunogenetic diversity cannot be overstated. Population (immuno-)genetic diversity is an essential aspect of biological diversity, determines host competence to harbour and transmit pathogens and, hence, safeguards against future pandemics. Yet, in spite of the presence of CoVs in Ghanaian bats, locals regularly enter the bat caves (Anti et al., 2015; Leroy et al., 2009; Monadjem et al., 2007; Nkrumah et al., 2021). For policymakers and conservationists, this means that in places where stark dependencies between the local community and wildlife still exist, alternative nutritional, cultural and economic incentives need to be created to sustainably curb human–wildlife contact.

AUTHOR CONTRIBUTIONS

SS, DWS and MM developed and conceived the idea behind the present study based on the existing data. MT and PV organised the field work. EEN, EKB, SKO, PV, MT and HJB collected the field data and archived the biological samples. TLH, TT, KW and DWS completed the laboratory work for lineage assignment based on previous work from HJB and PV, and completed MHC typing. HJB, VMC and CD generated the infection data. SS, NS and CD acquired funding. DWS, MM, TT, TLH, RF and KW analysed the data. DWS, MM, TT, TLH and SS wrote the first manuscript draft. All authors contributed to the final version of the manuscript.

ACKNOWLEDGEMENTS

We would like to thank Tobias Lenz and Mark Gillingham for feedback on the codon usage analysis. Special thanks to Alexandre Courtiol, who was imperative to implementing the R code for the codon usage analysis. We thank the Ghanaian chiefs and community leaders for their support during sampling. Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

The research was funded by the German Research Foundation (DFG SO 428/17-1, with samples and metadata obtained by DR 772/3-1 and 7-1, KA1241/18-1, TH 1420/1-1).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

MHC sequences and data is accessible on GitHub (https://doi.org/10.5061/dryad.h9w0vt4p4).

OPEN RESEARCH BADGES

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The datasets used in this study are available in Dryad Repository (https://doi.org/10.5061/dryad.h9w0vt4p4).

BENEFIT-SHARING STATEMENT

A research collaboration was developed with scientists from Ghana where samples originated. All collaborators were included as co-authors. Knowledge gathered from the research will be shared with both the scientific community and general public. Our group is committed to international scientific partnerships, as well as institutional capacity building. We also provide free access to data and code.

ORCID

Dominik W. Schmid https://orcid.org/0000-0001-8908-3882
Magdalena Meyer https://orcid.org/0000-0002-5388-3850
Ramona Fleischer https://orcid.org/0000-0003-1657-9347
Ebenezer K. Badu https://orcid.org/0000-0002-6135-2857
Evans Ewald Nkrumah https://orcid.org/0000-0001-6032-7775
Nina Schwensow https://orcid.org/0000-0003-3453-5823
Marco Tschapka https://orcid.org/0000-0001-9511-6775
Heather J. Baldwin https://orcid.org/0000-0001-9991-5419
Peter Vallo https://orcid.org/0000-0003-2145-1129
Victor M. Corman https://orcid.org/0000-0002-3605-0136
Christian Drosten https://orcid.org/0000-0001-7923-0519
Simone Sommer https://orcid.org/0000-0002-5148-8136

REFERENCES

Annan, A., Baldwin, H. J., Corman, V. M., Klose, S. M., Owusu, M., Nkrumah, E. E., Badu, E. K., Anti, P., Agbenyega, O., Meyer, B., Oppong, S., Sarkodie, Y. A., Kalko, E. K. V., Lina, P. H. C., Godlevska, E. V., Reusken, C., Seebens, A., Gloza-Rausch, F., Vallo, P., … Drexler, J. F. (2013). Human betacoronavirus 2c EMC/2012-related viruses in bats, Ghana and Europe. *Emerging Infectious Diseases*, *19*(3), 456–459. https://doi.org/10.3201/eid1903.121503

Anthony, S. J., Johnson, C. K., Greig, D. J., Kramer, S., Che, X., Wells, H., Hicks, A. L., Joly, D. O., Wolfe, N. D., Daszak, P., Karesh, W., Lipkin, W. I., Morse, S. S., Mazet, J. A. K., & Goldstein, T. (2017). Global patterns in coronavirus diversity. *Virus Evolution*, *3*(1), 12. https://doi.org/10.1093/ve/vex012

Anti, P., Owusu, M., Agbenyega, O., Annan, A., Badu, E. K., Nkrumah, E. E., Tschapka, M., Oppong, S., Adu-Sarkodie, Y., & Drosten, C. (2015). Human–bat interactions in rural west Africa. *Emerging Infectious Diseases*, *21*(8), 1418–1421. https://doi.org/10.3201/eid2108.142015

Baldwin, H. J. (2015). *Epidemiology and ecology of virus and host: Bats and coronaviruses in Ghana, West Africa*. (Thesis). Macquarie University. p. 194.

Baldwin, H. J., Vallo, P., Gardner, M. G., Drosten, C., Tschapka, M., & Stow, A. J. (2014). Isolation and characterization of 11 novel microsatellite loci in a West African leaf-nosed bat, Hipposideros aff. ruber. *BMC Research Notes*, *7*(1), 1–4. https://doi.org/10.1186/1756-0500-7-607

Baldwin, H. J., Vallo, P., Ruiz, A. T., Anti, P., Nkrumah, E. E., Badu, E. K., Oppong, S. K., Kalko, E. K. V., Tschapka, M., & Stow, A. J. (2021). Concordant patterns of genetic, acoustic, and morphological divergence in the West African Old World leaf-nosed bats of the Hipposideros caffer complex. *Journal of Zoological Systematics and Evolutionary Research*, *59*(6), 1390–1407. https://doi.org/10.1111/JZS.12506

Banerjee, A., Baker, M. L., Kulcsar, K., Misra, V., Plowright, R., & Mossman, K. (2020). Novel insights into immune systems of bats. *Frontiers in Immunology*, *1*, 26. https://doi.org/10.3389/fimmu.2020.00026

Banerjee, A., Kulcsar, K., Misra, V., Frieman, M., & Mossman, K. (2019). Bats and coronaviruses. *Viruses*, *11*(1), 41. https://doi.org/10.3390/v11010041

Bartoń, K. (2009). *MuMIn: Multi-model inference*.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B: Methodological*, *57*(1), 289–300.

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

Boni, M. F., Lemey, P., Jiang, X., Tsan-Yuk Lam, T., Perry, B. W., Castoe, T. A., Rambaut, A., & Robertson, D. L. (2020). Evolutionary origins of the SARS-CoV-2 sarbecovirus lineage responsible for the COVID-19 pandemic. *Nature Microbiology*, *5*, 1408–1417. https://doi.org/10.1038/s41564-020-0771-4

Bourgarel, M., Pfukenyi, D. M., Boué, V., Talignani, L., Chiweshe, N., Diop, F., Caron, A., Matope, G., Missé, D., & Liégeois, F. (2018). Circulation of Alphacoronavirus, Betacoronavirus and Paramyxovirus in Hipposideros bat species in Zimbabwe. *Infection, Genetics and Evolution*, *58*, 253–257. https://doi.org/10.1016/J.MEEGID.2018.01.007

Brown, J. H., Jardetzky, T. S., Gorga, J. C., Stern, L. J., Urban, R. G., Strominger, J. L., & Wiley, D. C. (1993). Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature*, *364*(6432), 33–39. https://doi.org/10.1038/364033a0

Bruchez, A., Sha, K., Johnson, J., Chen, L., Stefani, C., McConnell, H., Gaucherand, L., Prins, R., Matreyek, K. A., Hume, A. J., Mühlberger, E., Schmidt, E. V., Olinger, G. G., Stuart, L. M., & Lacy-Hulbert, A. (2020). MHC class II transactivator CIITA induces cell resistance to Ebola virus and SARS-like coronaviruses. *Science*, *370*(6513), 241–247. https://doi.org/10.1126/SCIENCE.ABB3753

Buchholz, R., Jones Dukes, M., Hecht, S., & Findley, A. M. (2004). Investigating the Turkey's "snood" as a morphological marker of heritable disease resistance. *Journal of Animal Breeding and Genetics*, *121*, 176–185.

Cao, A. T., Yao, S., Gong, B., Elson, C. O., & Cong, Y. (2012). Th17 cells upregulate polymeric Ig receptor and intestinal IgA and contribute to intestinal homeostasis. *The Journal of Immunology*, *189*(9), 4666–4673. https://doi.org/10.4049/jimmunol.1200955

Castelli, E. C., de Castro, M. V., Naslavsky, M. S., Scliar, M. O., Silva, N. S. B., Andrade, H. S., Souza, A. S., Pereira, R. N., Castro, C. F. B., Mendes-Junior, C. T., Meyer, D., Nunes, K., Matos, L. R. B., Silva, M. V. R., Wang, J. Y. T., Esposito, J., Coria, V. R., Bortolin, R. H., Hirata, M. H., … Zatz, M. (2021). MHC variants associated with symptomatic versus asymptomatic SARS-CoV-2 infection in highly exposed individuals. *Frontiers in Immunology*, *12*, 742881. https://doi.org/10.3389/fimmu.2021.742881

Chu, H., Zhou, J., Ho-Yin Wong, B., Li, C., Cheng, Z. S., Lin, X., Kwok-Man Poon, V., Sun, T., Choi-Yi Lau, C., Fuk-Woo Chan, J., Kai-Wang To, K., Chan, K. H., Lu, L., Zheng, B. J., & Yuen, K. Y. (2014). Productive replication of Middle East respiratory syndrome coronavirus in monocyte-derived dendritic cells modulates innate immune response. *Virology*, *454–455*(1), 197–205. https://doi.org/10.1016/J.VIROL.2014.02.018

Civitello, D. J., Cohen, J., Fatima, H., Halstead, N. T., Liriano, J., McMahon, T. A., Ortega, C. N., Sauer, E. L., Sehgal, T., Young, S., & Rohr, J. R. (2015). Biodiversity inhibits parasites: Broad evidence for the dilution effect. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(28), 8667–8671. https://doi.org/10.1073/pnas.1506279112

Corman, V. M., Baldwin, H. J., Tateno, A. F., Zerbinati, R. M., Annan, A., Owusu, M., Nkrumah, E. E., Maganga, G. D., Oppong, S., Adu-Sarkodie, Y., Vallo, P., da Silva Filho, L. V., Leroy, E. M., Thiel, V., van der Hoek, L., Poon, L. L., Tschapka, M., Drosten, C., & Drexler, J. F. (2015). Evidence for an ancestral association of human coronavirus 229E with bats. *Journal of Virology*, *89*(23), 11858–11870. https://doi.org/10.1128/JVI.01755-15

Corman, V. M., Muth, D., Niemeyer, D., & Drosten, C. (2018). Hosts and sources of endemic human coronaviruses. *Advances in Virus Research*, *100*, 163–188. https://doi.org/10.1016/BS.AIVIR.2018.01.001

Crossley, B. M., Mock, R. E., Callison, S. A., & Hietala, S. K. (2012). Identification and characterization of a novel Alpaca respiratory coronavirus most closely related to the human coronavirus 229E. *Viruses*, *4*(12), 3689–3700. https://doi.org/10.3390/V4123689

Davies, C. S., Worsley, S. F., Maher, K. H., Komdeur, J., Burke, T., Dugdale, H. L., & Richardson, D. S. (2022). Immunogenetic variation shapes the gut microbiome in a natural vertebrate population. *Microbiome*, *10*(1), 1–22. https://doi.org/10.1186/S40168-022-01233-Y/FIGURES/5

Dengjel, J., Schoor, O., Fischer, R., Reich, M., Kraus, M., Müller, M., Kreymborg, K., Altenberend, F., Brandenburg, J., Kalbacher, H., Brock, R., Driessen, C., Rammensee, H.-G., & Stevanovic, S. (2005). *Autophagy promotes MHC class II presentation of peptides from intracellular source proteins*. www.ncbi.nih.govgeo

Doytchinova, I. A., Guan, P., & Flower, D. R. (2004). Identifiying human MHC supertypes using bioinformatic methods. *Journal of Immunology*, *172*, 4314–4323. https://doi.org/10.4049/jimmunol.172.7.4314

Drexler, J. F., Corman, V. M., & Drosten, C. (2014). Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral Research*, *101*, 45–56.

Drexler, J. F., Corman, V. M., Müller, M. A., Maganga, G. D., Vallo, P., Binger, T., Gloza-Rausch, F., Rasche, A., Yordanov, S., Seebens, A.,

Oppong, S., Sarkodie, Y. A., Pongombo, C., Lukashev, A. N., Schmidt-Chanasit, J., Stöcker, A., Carneiro, A. J. B., Erbar, S., Maisner, A., … Drosten, C. (2012). Bats host major mammalian paramyxoviruses. *Nature Communications*, *3*, 796. https://doi.org/10.1038/ncomms1796

Drexler, J. F., Kupfer, B., Petersen, N., Grotto, R. M. T., Rodrigues, S. M. C., Grywna, K., Panning, M., Annan, A., Silva, G. F., Douglas, J., Koay, E. C., Smuts, H., Netto, E. M., Simmonds, P., De Moura Campos Pardini, M. I., Roth, W. K., & Drosten, C. (2009). A novel diagnostic target in the hepatitis C virus genome. *PLoS Medicine*, *6*(2), e1000031. https://doi.org/10.1371/JOURNAL.PMED.1000031

Dudek, K., Gaczorek, T. S., Zieliński, P., & Babik, W. (2019). Massive introgression of major histocompatibility complex (MHC) genes in newt hybrid zones. *Molecular Ecology*, *28*(21), 4798–4810. https://doi.org/10.1111/mec.15254

Eby, P., Peel, A. J., Hoegh, A., Madden, W., Giles, J. R., Hudson, P. J., & Plowright, R. K. (2022). Pathogen spillover driven by rapid changes in bat ecology. *Nature*, *613*(7943), 340–344. https://doi.org/10.1038/s41586-022-05506-2

Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012a). Divergent selection on locally adapted major histocompatibility complex immune genes experimentally proven in the field. *Ecology Letters*, *15*(7), 723–731. https://doi.org/10.1111/j.1461-0248.2012.01791.x

Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012b). Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. *Nature Communications*, *3*, 621. https://doi.org/10.1038/ncomms1632

Epstein, J. H., Anthony, S. J., Islam, A., Marm Kilpatrick, A., Khan, S. A., Balkey, M. D., Ross, N., Smith, I., Zambrana-Torrelio, C., Tao, Y., Islam, A., Quan, P. L., Olival, K. J., Salah Uddin Khan, M., Gurley, E. S., Jahangir Hossein, M., Field, H. E., Fielder, M. D., Briese, T., … Daszak, P. (2020). Nipah virus dynamics in bats and implications for spillover to humans. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(46), 29190–29201. https://doi.org/10.1073/pnas.2000429117

Fijarczyk, A., & Babik, W. (2015). Detecting balancing selection in genomes: Limits and prospects. *Molecular Ecology*, *24*(14), 3529–3545. https://doi.org/10.1111/mec.13226

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

Frank, H. K., Enard, D., & Boyd, S. D. (2022). Exceptional diversity and selection pressure on coronavirus host receptors in bats compared to other mammals. *Proceedings of the Royal Society B: Biological Sciences*, *289*(1979), 20220193. https://doi.org/10.1098/RSPB.2022.0193

Frick, W. F., Pollock, J. F., Hicks, A. C., Langwig, K. E., Reynolds, D. S., Turner, G. G., Butchkoski, C. M., & Kunz, T. H. (2010). An emerging disease causes regional population collapse of a common North American bat species. *Science*, *329*(5992), 679–682. https://doi.org/10.1126/science.1188594

Gaczorek, T. S., Marszałek, M., Dudek, K., Arntzen, J. W., Wielstra, B., & Babik, W. (2022). Interspecific introgression of MHC genes in Triturus newts: Evidence from multiple contact zones. *Molecular Ecology*, *32*(4), 867–880. https://doi.org/10.1111/mec.16804

Gaigher, A., Burri, R., San-Jose, L. M., Roulin, A., & Fumagalli, L. (2019). Lack of statistical power as a major limitation in understanding MHC-mediated immunocompetence in wild vertebrate populations. *Molecular Ecology*, *28*(23), 5115–5132. https://doi.org/10.1111/mec.15276

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*, 396–402. https://doi.org/10.1038/s41586-020-2562-8

Gillingham, M. A. F., Courtiol, A., Teixeira, M., Galan, M., Bechet, A., & Cezilly, F. (2016). Evidence of gene orthology and trans-species polymorphism, but not of parallel evolution, despite high levels of concerted evolution in the major histocompatibility complex of flamingo species. *Journal of Evolutionary Biology*, *29*(2), 438–454. https://doi.org/10.1111/jeb.12798

Gillingham, M. A. F., Montero, B. K., Wihelm, K., Grudzus, K., Sommer, S., & Santos, P. S. C. (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

Giotis, E. S., Carnell, G., Young, E. F., Ghanny, S., Soteropoulos, P., Wang, L. F., Barclay, W. S., Skinner, M. A., & Temperton, N. (2019). Entry of the bat influenza H17N10 virus into mammalian cells is enabled by the MHC class II HLA-DR receptor. *Nature Microbiology*, *4*(12), 2035–2038 https://doi.org/10.1038/s41564-019-0517-3

Gouilh, M. A., Puechmaille, S. J., Gonzalez, J. P., Teeling, E., Kittayapong, P., & Manuguerra, J. C. (2011). SARS-Coronavirus ancestor's footprints in South-East Asian bat colonies and the refuge theory. *Infection, Genetics and Evolution*, *11*(7), 1690–1702. https://doi.org/10.1016/j.meegid.2011.06.021

Griffith, D. M., Veech, J. A., & Marsh, C. J. (2016). cooccur: Probabilistic species co-occurrence analysis in R. *Journal of Statistical Software*, *69*(1), 1–17. https://doi.org/10.18637/JSS.V069.C02

Han, G., Luong, H., & Vaishnava, S. (2022). Low abundance members of the gut microbiome exhibit high immunogenicity. *Gut Microbes*, *14*(1), 2104086. https://doi.org/10.1080/19490976.2022.2104086

Harman, A. N., Kraus, M., Bye, C. R., Byth, K., Turville, S. G., Tang, O., Mercier, S. K., Nasr, N., Stern, J. L., Slobedman, B., Driessen, C., & Cunningham, A. L. (2009). HIV-1-infected dendritic cells show 2 phases of gene expression changes, with lysosomal enzyme activity decreased during the second phase. *Blood*, *114*(1), 85–94. https://doi.org/10.1182/blood-2008

Hayman, D. T. S., Yu, M., Crameri, G., Wang, L. F., Suu-Ire, R., Wood, J. L. N., & Cunningham, A. A. (2012). Ebola virus antibodies in fruit bats, Ghana, West Africa. *Emerging Infectious Diseases*, *18*(7), 1207–1209. https://doi.org/10.3201/eid1807.111654

Hill, A. V., Allsopp, C. E., Kwiatkowski, D., Anstey, N. M., Twumasi, P., Rowe, P. A., Bennett, S., Brewster, D., McMichael, A. J., & Greenwood, B. M. (1991). Common west African HLA antigens are associated with protection from severe malaria. *Nature*, *352*(6336), 595–600. https://doi.org/10.1038/352595a0

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*, *181*(2), 271–280.e8. https://doi.org/10.1016/J.CELL.2020.02.052

Huus, K. E., Petersen, C., & Finlay, B. B. (2021). Diversity and dynamism of IgA–microbiota interactions. *Nature Reviews Immunology*, *21*(8), 514–525. https://doi.org/10.1038/s41577-021-00506-1

Huynh, J., Li, S., Yount, B., Smith, A., Sturges, L., Olsen, J. C., Nagel, J., Johnson, J. B., Agnihothram, S., Gates, J. E., Frieman, M. B., Baric, R. S., & Donaldson, E. F. (2012). Evidence supporting a zoonotic origin of human coronavirus strain NL63. *Journal of Virology*, *86*(23), 12816–12825. https://doi.org/10.1128/jvi.00906-12

Irving, A. T., Ahn, M., Goh, G., Anderson, D. E., & Wang, L. F. (2021). Lessons from the host defences of bats, a unique viral reservoir. *Nature*, *589*(7842), 363–370. https://doi.org/10.1038/s41586-020-03128-0

Ithete, N. L., Stoffberg, S., Corman, V. M., Cottontail, V. M., Richards, L. R., Schoeman, M. C., Drosten, C., Drexler, J. F., & Preiser, W. (2013). Close relative of human Middle East respiratory syndrome

coronavirus in bat, South Africa. *Emerging Infectious Diseases*, *19*(10), 1697–1699.

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), 1–15. https://doi.org/10.1186/1471-2156-11-94/FIGURES/9

Karakus, U., Thamamongood, 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*, 109–112. https://doi.org/10.1038/s41586-019-0955-3

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010

Kaufman, J. (2018a). Unfinished business: Evolution of the MHC and the adaptive immune system of jawed vertebrates. *Annual Review of Immunology*, *36*(1), 383–409. https://doi.org/10.1146/annurev-immunol-051116-052450

Kaufman, J. (2018b). Generalists and specialists: A new view of how MHC class I molecules fight infectious pathogens. *Trends in Immunology*, *39*(5), 367–379. https://doi.org/10.1016/j.it.2018.01.001

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*, 8642. https://doi.org/10.1038/ncomms9642

Laing, E. D., Sterling, S. L., Weir, D. L., Beauregard, C. R., Smith, I. L., Larsen, S. E., Wang, L. F., Snow, A. L., Schaefer, B. C., & Broder, C. C. (2019). Enhanced autophagy contributes to reduced viral infection in black flying fox cells. *Viruses*, *11*(3), 260. https://doi.org/10.3390/v11030260

Latinne, A., Hu, B., Olival, K. J., Zhu, G., Zhang, L., Li, H., Chmura, A. A., Field, H. E., Zambrana-Torrelio, C., Epstein, J. H., Li, B., Zhang, W., Wang, L.-F., Shi, Z.-L., & Daszak, P. (2020). Origin and cross-species transmission of bat coronaviruses in China. *Nature Communications*, *11*(7), 4235. https://doi.org/10.1038/s41467-020-17687-3

Lau, S. K. P., Li, K. S. M., Huang, Y., Shek, C.-T., Tse, H., Wang, M., Choi, G. K. Y., Xu, H., Lam, C. S. F., Guo, R., Chan, K.-H., Zheng, B.-J., Woo, P. C. Y., & Yuen, K.-Y. (2010). Ecoepidemiology and complete genome comparison of different strains of severe acute respiratory syndrome-related Rhinolophus bat coronavirus in China reveal bats as a reservoir for acute, self-limiting infection that allows recombination events. *Journal of Virology*, *84*(6), 2808–2819. https://doi.org/10.1128/JVI.02219-09

Lau, S. K. P., Li, K. S. M., Tsang, A. K. L., Shek, C.-T., Wang, M., Choi, G. K. Y., Guo, R., Wong, B. H. L., Poon, R. W. S., Lam, C. S. F., Wang, S. Y. H., Fan, R. Y. Y., Chan, K.-H., Zheng, B.-J., Woo, P. C. Y., & Yuen, K.-Y. (2012). Recent transmission of a novel alphacoronavirus, bat coronavirus HKU10, from Leschenault's rousettes to pomona leaf-nosed bats: first evidence of interspecies transmission of coronavirus between bats of different suborders. *Journal of Virology*, *86*(21), 11906–11918. https://doi.org/10.1128/jvi.01305-12

Lenz, T. L., Eizaguirre, C., Kalbe, M., & Milinski, M. (2013). Evaluating patterns of convergent evolution and trans-species polymorphism at mhc immunogenes in two sympatric stickleback species. *Evolution*, *67*(8), 2400–2412. https://doi.org/10.1111/evo.12124

Lenz, T. L., Wells, K., Pfeiffer, M., & Sommer, S. (2009). Diverse MHC IIB allele repertoire increases parasite resistance and body condition in the Long-tailed giant rat (*Leopoldamys sabanus*). *BMC Evolutionary Biology*, *9*, 269. https://doi.org/10.1186/1471-2148-9-269

Leroy, E. M., Epelboin, A., Mondonge, V., Pourrut, X., Gonzalez, J. P., Muyembe-Tamfum, J. J., & Formenty, P. (2009). Human ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. *Vector Borne and Zoonotic Diseases*, *9*(6), 723–728. https://doi.org/10.1089/vbz.2008.0167

Letko, M., Seifert, S. N., Olival, K. J., Plowright, R. K., & Munster, V. J. (2020). Bat-borne virus diversity, spillover and emergence. *Nature Reviews Microbiology*, *18*(8), 461–471. https://doi.org/10.1038/s41579-020-0394-z

Li, W., Shi, Z., Yu, M., Ren, W., Smith, C., Epstein, J. H., Wang, H., Crameri, G., Hu, Z., Zhang, H., Zhang, J., McEachern, J., Field, H., Daszak, P., Eaton, B. T., Zhang, S., & Wang, L. F. (2005). Bats are natural reservoirs of SARS-like coronaviruses. *Science*, *310*(5748), 676–679. https://doi.org/10.1126/science.1118391

Li, X., Liu, T., Li, A., Zhang, L., Dai, W., Jin, L., Sun, K., & Feng, J. (2021). Genetic polymorphisms and the independent evolution of major histocompatibility complex class II-DRB in sibling bat species *Rhinolophus episcopus* and *Rhinolophus siamensis*. *Journal of Zoological Systematics and Evolutionary Research*, *59*(4), 887–901. https://doi.org/10.1111/jzs.12462

Liang, S. C., Tan, X. Y., Luxenberg, D. P., Karim, R., Dunussi-Joannopoulos, K., Collins, M., & Fouser, L. A. (2006). Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *Journal of Experimental Medicine*, *203*(10), 2271–2279. https://doi.org/10.1084/jem.20061308

Lighten, J., Papadopulos, A. S. T., Mohammed, R. S., Ward, B. J., Paterson, I. G., 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), 1294. https://doi.org/10.1038/s41467-017-01183-2

Loyal, L., Braun, J., Henze, L., Kruse, B., Dingeldey, M., Reimer, U., Kern, F., Schwarz, T., Mangold, M., Unger, C., Dörfler, F., Kadler, S., Rosowski, J., Gürcan, K., Uyar-Aydin, Z., Frentsch, M., Kurth, F., Schnatbaum, K., Eckey, M., … Giesecke-Thiel, C. (2021). Cross-reactive CD4+ T cells enhance SARS-CoV-2 immune responses upon infection and vaccination. *Science*, *374*(6564), eabh1823. https://doi.org/10.1126/science.abh1823

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), 19061. https://doi.org/10.1038/s41598-019-55503-1

Lundberg, A. S., & McDevitt, H. O. (1992). Evolution of major histocompatibility complex class II allelic diversity: Direct descent in mice and humans. *Immunology*, *89*, 6545–6549.

Maganga, G. D., Bourgarel, M., Vallo, P., Dallo, T. D., Ngoagouni, C., Drexler, J. F., Drosten, C., Nakouné, E. R., Leroy, E. M., & Morand, S. (2014). Bat distribution size or shape as determinant of viral richness in African bats. *PLoS One*, *9*(6), e100172. https://doi.org/10.1371/journal.pone.0100172

Marques, D. A., Lucek, K., Sousa, V. C., Excoffier, L., & Seehausen, O. (2019). Admixture between old lineages facilitated contemporary ecological speciation in Lake Constance stickleback. *Nature Communications*, *10*, 4240. https://doi.org/10.1038/s41467-019-12182-w

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

Meyer, M., Schmid, D. W., Baldwin, H. J., Wilhelm, K., Nkrumah, E. E., Ebenezer, K. B., Oppong, S. K., Schwensow, N., Vallo, P., Corman, V. M., Tschapka, M., Drosten, C., & Sommer, S. (in review). Bat species assemblage predicts coronavirus prevalence.

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

Migliorini, F., Torsiello, E., Spiezia, F., Oliva, F., Tingart, M., & Maffulli, N. (2021). Association between HLA genotypes and COVID-19 susceptibility, severity and progression: a comprehensive review of the literature. *European Journal of Medical Research*, *26*(1), 84. https://doi.org/10.1186/s40001-021-00563-1

Milinski, M., Griffiths, S., Wegner, K. M., Reusch, T. B. H., Haas-Assenbaum, A., & Boehm, T. (2005). Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. *Proceedings of the National Academy of Sciences*, *102*(12), 4414–4418. https://doi.org/10.1073/pnas.0408264102

Minias, P., Whittingham, L. A., & Dunn, P. O. (2017). Coloniality and migration are related to selection on MHC genes in birds. *Evolution*, *71*(2), 432–441. https://doi.org/10.1111/EVO.13142

Monadjem, A., Taylor, P., Cotteri, F. P. D. W., Kityo, R., & Fahr, J. (2007). Conservation status of bats in sub-Saharan Africa. *Bat Research News*, *48*(4), 267.

Montero, B. K., Wasimuddin Schwensow, N., Gillingham, M. A. F., Ratovonamana, Y. R., Rakotondranary, S. J., Corman, V. M., 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 non – human primate. *PLoS One*, *17*(11), e1009675. https://doi.org/10.1371/journal.ppat.1009675

Moreno Santillán, D. D., Lama, T. M., Gutierrez Guerrero, Y. T., Brown, A. M., Donat, P., Zhao, H., Rossiter, S. J., Yohe, L. R., Potter, J. H., Teeling, E. C., Vernes, S. C., Davies, K. T. J., Myers, E., Hughes, G. M., Huang, Z., Hoffmann, F., Corthals, A. P., Ray, D. A., & Dávalos, L. M. (2021). Large-scale genome sampling reveals unique immunity and metabolic adaptations in Bats. *Molecular Ecology*, *30*(23), 6449–6467. https://doi.org/10.1111/mec.16027

Müller, M. A., Paweska, J. T., Leman, P. A., Drosten, C., Grywna, K., Kemp, A., Braack, L., Sonnenberg, K., Niedrig, M., & Swanepoel, R. (2007). Coronavirus antibodies in African bat species. *Emerging Infectious Diseases*, *13*(9), 1367. https://doi.org/10.3201/EID1309.070342

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*, 21878. https://doi.org/10.1038/SREP21878

Nadachowska-Brzyska, K., Zieliński, P., Radwan, J., & Babik, W. (2012). Interspecific hybridization increases MHC class II diversity in two sister species of newts. *Molecular Ecology*, *21*(4), 887–906. https://doi.org/10.1111/j.1365-294X.2011.05347.x

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

Nicholls, J. A., Double, M. C., Rowell, D. M., & Magrath, R. D. (2000). The evolution of cooperative and pair breeding in thornbills *Acanthiza* (Pardalotidae). *Journal of Avian Biology*, *31*(2), 165–176.

Nkrumah, E. E., Baldwin, H. J., Kofi Badu, E., Anti, P., Vallo, P., Klose, S., Klara, E., Kalko, V., Oppong, S. K., & Tschapka, M. (2021). Diversity and conservation of cave-roosting bats in central Ghana. *Tropical Conservation Science*, *14*, 1–10. https://doi.org/10.1177/19400829211034671

Ovsyannikova, I. G., Haralambieva, I. H., Crooke, S. N., Poland, G. A., Kennedy, R. B., & Richard Kennedy, C. B. (2020). The role of host genetics in the immune response to SARS-CoV-2 and COVID-19 susceptibility and severity. *Immunological Reviews*, *296*, 205–219. https://doi.org/10.1111/imr.12897

Pathak, G. A., Singh, K., Miller-Fleming, T. W., Wendt, F. R., Ehsan, N., Hou, K., Johnson, R., Lu, Z., Gopalan, S., Yengo, L., Mohammadi, P., Pasaniuc, B., Polimanti, R., Davis, L. K., & Mancuso, N. (2021). Integrative genomic analyses identify susceptibility genes underlying COVID-19 hospitalization. *Nature Communications*, *12*(1), 4569. https://doi.org/10.1038/s41467-021-24824-z

Pfefferle, S., Oppong, S., Drexler, J. F., Gloza-Rausch, F., Ipsen, A., Seebens, A., Müller, M. A., Annan, A., Vallo, P., Adu-Sarkodie, Y., Kruppa, T. F., & Drosten, C. (2009). Distant relatives of severe acute respiratory syndrome coronavirus and close relatives of human coronavirus 229E in bats, Ghana. *Emerging Infectious Diseases*, *15*(9), 1377–1384. https://doi.org/10.3201/eid1509.090224

Phillips, K. P., Cable, J., Mohammed, R. S., Herdegen-Radwan, M., Raubic, J., Przesmycka, K. J., van Oosterhout, C., & Radwan, J. (2018). Immunogenetic novelty confers a selective advantage in host–pathogen coevolution. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(7), 1552–1557. https://doi.org/10.1073/pnas.1708597115

Pisanti, S., Deelen, J., Gallina, A. M., Caputo, M., Citro, M., Abate, M., Sacchi, N., Vecchione, C., & Martinelli, R. (2020). Correlation of the two most frequent HLA haplotypes in the Italian population to the differential regional incidence of Covid-19. *Journal of Translational Medicine*, *18*(1), 1–16. https://doi.org/10.1186/S12967-020-02515-5/TABLES/7

Plowright, R. K., Field, H. E., Smith, C., Divljan, A., Palmer, C., Tabor, G., Daszak, P., & Foley, J. E. (2008). Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (*Pteropus scapulatus*). *Proceedings of the Royal Society B: Biological Sciences*, *275*(1636), 861–869. https://doi.org/10.1098/rspb.2007.1260

Quan, P. L., Firth, C., Street, C., Henriquez, J. A., Petrosov, A., Tashmukhamedova, A., Hutchison, S. K., Egholm, M., Osinubi, M. O. V., Niezgoda, M., Ogunkoya, A. B., Briese, T., Rupprecht, C. E., & Ian Lipkin, W. (2010). Identification of a severe acute respiratory syndrome coronavirus-like virus in a leaf-nosed bat in Nigeria. *MBio*, *1*(4), 1–9. https://doi.org/10.1128/mBio.00208-10

Qurkhuli, T., Schwensow, N., Brändel, S. D., Tschapka, M., & Sommer, S. (2019). Can extreme MHC class I diversity be a feature of a wide geographic range? The example of Seba's short-tailed bat (*Carollia perspicillata*). *Immunogenetics*, *71*(8–9), 575–587. https://doi.org/10.1007/s00251-019-01128-7

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

Roland, M. M., Mohammed, A. D., & Kubinak, J. L. (2020). How MHCII signaling promotes benign host-microbiota interactions. *PLOS Pathogens*, *16*(6), e1008558. https://doi.org/10.1371/journal.ppat.1008558

Roved, J., Hansson, B., Stervander, M., Hasselquist, D., & Westerdahl, H. (2022). MHCtools – An R package for MHC high-throughput sequencing data: genotyping, haplotype and supertype inference, and downstream genetic analyses in non-model organisms. *Molecular Ecology Resources*, *22*, 2775–2792. https://doi.org/10.1111/1755-0998.13645

Ruiz-Aravena, M., McKee, C., Gamble, A., Lunn, T., Morris, A., Snedden, C. E., Yinda, C. K., Port, J. R., Buchholz, D. W., Yeo, Y. Y., Faust, C., Jax, E., Dee, L., Jones, D. N., Kessler, M. K., Falvo, C., Crowley, D., Bharti, N., Brook, C. E., … Plowright, R. K. (2021). Ecology, evolution and spillover of coronaviruses from bats. *Nature Reviews Microbiology*, *20*, 299–314. https://doi.org/10.1038/s41579-021-00652-2

Sagonas, K., Runemark, A., Antoniou, A., Lymberakis, P., Pafilis, P., Valakos, E. D., Poulakakis, N., & Hansson, B. (2018). Selection, drift, and introgression shape MHC polymorphism in lizards. *Heredity*, *122*(4), 468–484. https://doi.org/10.1038/s41437-018-0146-2

Salmier, A., De Thoisy, B., Crouau-Roy, B., Lacoste, V., & Lavergne, A. (2016). Spatial pattern of genetic diversity and selection in the MHC class II DRB of three Neotropical bat species. *BMC Evolutionary Biology*, *16*(1), 229. https://doi.org/10.1186/s12862-016-0802-1

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.

Schad, J., Dechmann, D., 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*, 115–126. https://doi.org/10.1038/hdy.2010.173

Schad, J., Dechmann, D. K. N., Voigt, C. C., & Sommer, S. (2012). Evidence for the "Good Genes" model: Association of MHC class II DRB alleles with ectoparasitism and reproductive state in the neotropical lesser bulldog bat, *Noctilio albiventris*. *PLoS One*, *7*(5), e37101. https://doi.org/10.1371/journal.pone.0037101

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*, 65–80. https://doi.org/10.1007/s10592-019-01143-x

Schwensow, N., Eberle, M., & Sommer, S. (2008). Compatibility counts: MHC-associated mate choice in a wild promiscuous primate. *Proceedings of the Royal Society B*, *275*(1634), 555–564. https://doi.org/10.1098/rspb.2007.1433

Sepil, I., Lachish, S., & Sheldon, B. C. (2013). Mhc-linked survival and lifetime reproductive success in a wild population of great tits. *Molecular Ecology*, *22*(2), 384–396. https://doi.org/10.1111/mec.12123

Silverman, M., Kua, L., Tanca, A., Pala, M., Palomba, A., Tanes, C., Bittinger, K., Uzzau, S., Benoist, 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*, 16. https://doi.org/10.1186/1742-9994-2-16

Spurgin, L. G., & Richardson, D. S. (2010). How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1684), 979–988. https://doi.org/10.1098/rspb.2009.2084

Subudhi, S., Rapin, N., Bollinger, T. K., Hill, J. E., Donaldson, M. E., Davy, C. M., Warnecke, L., Turner, J. M., Kyle, C. J., Willis, C. K. R., & Misra, V. (2017). A persistently infecting coronavirus in hibernating *Myotis lucifugus*, the North American little brown bat. *Journal of General Virology*, *98*, 2297–2309. https://doi.org/10.1099/jgv.0.000898

Suu-Ire, R., Obodai, E., Bel-Nono, S. O., Ampofo, W. K., Mazet, J. A. K., Goldstein, T., Johnson, C. K., Smith, B., Boaatema, L., Asigbee, T. W., Awuni, J., Opoku, E., & Kelly, T. R. (2022). Surveillance for potentially zoonotic viruses in rodent and bat populations and behavioral risk in an agricultural settlement in Ghana. *One Health Outlook*, *4*, 6. https://doi.org/10.1186/S42522-022-00061-2

Takahata, N., & Nei, M. (1990). Allelic genealogy under overdominant and frequency-dependent selection and polymorphism of major histocompatibility complex loci. *Genetics*, *124*(4), 967–978.

Tay, J. H., Porter, A. F., Wirth, W., & Duchene, S. (2022). The emergence of SARS-CoV-2 variants of concern is driven by acceleration of the substitution rate. *Molecular Biology and Evolution*, *39*(2), 1–9. https://doi.org/10.1093/molbev/msac013

Vallo, P., Guillén-Servent, A., Benda, P., Pires, D. B., & Koubek, P. (2008). Variation of mitochondrial DNA in the *Hipposideros caffer* complex (Chiroptera: Hipposideridae) and its taxonomic implications. *Acta Chiropterologica*, *10*(2), 193–206. https://doi.org/10.3161/150811008X414782

Veech, J. A. (2013). A probabilistic model for analysing species co-occurrence. *Global Ecology and Biogeography*, *22*, 252–260. https://doi.org/10.1111/j.1466-8238.2012.00789.x

Vietzen, H., Zoufaly, A., Traugott, M., Aberle, J., Aberle, S. W., & Puchhammer-Stöckl, E. (2021). Deletion of the NKG2C receptor encoding KLRC2 gene and HLA-E variants are risk factors for severe COVID-19. *Genetics in Medicine*, *23*(5), 963–967. https://doi.org/10.1038/s41436-020-01077-7

Wacharapluesadee, S., Duengkae, P., Chaiyes, A., Kaewpom, T., Rodpan, A., Yingsakmongkon, S., Petcharat, S., Phengsakul, P., Maneeorn, P., & Hemachudha, T. (2018). Longitudinal study of age-specific pattern of coronavirus infection in Lyle's flying fox (*Pteropus lylei*) in Thailand. *Virology Journal*, *15*, 38. https://doi.org/10.1186/s12985-018-0950-6

Wang, L. F., Gamage, A. M., Chan, W. O. Y., Hiller, M., & Teeling, E. C. (2021). Decoding bat immunity: The need for a coordinated research approach. *Nature Reviews Immunology*, *21*(5), 269–271. https://doi.org/10.1038/s41577-021-00523-0

Wegner, K. M., Kalbe, M., Kurtz, J., Reusch, T., & Milinski, M. (2003). Parasite selection for immunogenetic optimality. *Science*, *301*(5638), 1343. https://doi.org/10.1126/science.1088293

Westerdahl, H., Asghar, M., Hasselquist, D., & Bensch, S. (2012). Quantitative disease resistance: To better understand parasite-mediated selection on major histocompatibility complex. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1728), 577–584. https://doi.org/10.1098/rspb.2011.0917

Whittingham, L. A., Dunn, P. O., Freeman-Gallant, C. R., Taff, C. C., & Johnson, J. A. (2018). Major histocompatibility complex variation and blood parasites in resident and migratory populations of the common yellowthroat. *Journal of Evolutionary Biology*, *31*(10), 1544–1557. https://doi.org/10.1111/JEB.13349

Xu, B., & Yang, Z. (2013). PamlX: A graphical user interface for PAML. *Molecular Biology and Evolution*, *13*(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

Zeileis, A., Meyer, D., & Hornik, K. (2007). Residual-based shadings for visualizing (conditional) independence. *Journal of Computational and Graphical Statistics*, *16*(3), 507–525. https://doi.org/10.1198/106186007X237856

Zhao, Y., Li, D., Xu, Y., & Zhu, Y. (2014). The genetic diversity and evolution of MHC classII-DRB genes in *Rhinolophus sinicus*. *Acta Theriologica Sinica*, *34*(3), 262.

Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., Si, H. R., Zhu, Y., Li, B., Huang, C. L., Chen, H. D., Chen, J., Luo, Y., Guo, H., Di Jiang, R., Liu, M. Q., Chen, Y., Shen, X. R., Wang, X., … Shi, Z. L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, *579*(7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7

SUPPORTING INFORMATION

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

How to cite this article: Schmid, D. W., Meyer, M., Wilhelm, K., Tilley, T., Link-Hessing, T., Fleischer, R., Badu, E. K., Nkrumah, E. E., Oppong, S. K., Schwensow, N., Tschapka, M., Baldwin, H. J., Vallo, P., Corman, V. M., Drosten, C., & Sommer, S. (2023). MHC class II genes mediate susceptibility and resistance to coronavirus infections in bats. *Molecular Ecology*, *00*, 1–14. https://doi.org/10.1111/mec.16983