

ORIGINAL ARTICLE

Shared evolutionary origin of major histocompatibility complex polymorphism in sympatric lemurs

Eva Kaesler¹  | Peter M. Kappeler^{1,2} | Markus Brameier³ | Janina Demeler⁴ | Cornelia Kraus^{1,2} | Josué H. Rakotoniaina² | Anni. M. Hämäläinen² | Elise Huchard⁵

¹Deutsches Primatenzentrum GmbH - Leibniz-Institut für Primatenforschung, Verhaltensökologie & Soziobiologie, Göttingen, Germany

²Johann Friedrich Blumenbach Institut für Zoologie & Anthropologie, Georg-August-Universität Göttingen, Göttingen, Germany

³Deutsches Primatenzentrum GmbH - Leibniz-Institut für Primatenforschung, Göttingen, Germany

⁴Institut für Parasitologie und Tropenveterinärmedizin, Berlin, Germany

⁵Institute for Evolutionary Biology, Montpellier (ISEM, UMR 5554), CNRS, Université Montpellier, Montpellier Cedex 5, France

Correspondence

Eva Kaesler (born Pechouskova), Deutsches Primatenzentrum GmbH - Leibniz-Institut für Primatenforschung, Verhaltensökologie & Soziobiologie, Göttingen, Germany. Email: roneffrit@gmail.com

Funding information

Deutsche Forschungsgemeinschaft, Grant/Award Number: Ka1082/10-1&2; Wissenschaftsgemeinschaft Gottfried Wilhelm Leibniz e.V., Grant/Award Number: SAW-2011-DPZ-4; Deutsches Primatenzentrum GmbH - Leibniz-Institut für Primatenforschung; DFG, Grant/Award Number: Ka1082/10-1&2; ISEM, Grant/Award Number: ISEM 2017-146

Abstract

Genes of the major histocompatibility complex (MHC) play a central role in adaptive immune responses of vertebrates. They exhibit remarkable polymorphism, often crossing species boundaries with similar alleles or allelic motifs shared across species. This pattern may reflect parallel parasite-mediated selective pressures, either favouring the long maintenance of ancestral MHC allelic lineages across successive speciation events by balancing selection (“trans-species polymorphism”), or alternatively favouring the independent emergence of functionally similar alleles post-speciation via convergent evolution. Here, we investigate the origins of MHC similarity across several species of dwarf and mouse lemurs (*Cheirogaleidae*). We examined MHC class II variation in two highly polymorphic loci (DRB, DQB) and evaluated the overlap of gut-parasite communities in four sympatric lemurs. We tested for parasite-MHC associations across species to determine whether similar parasite pressures may select for similar MHC alleles in different species. Next, we integrated our MHC data with those previously obtained from other *Cheirogaleidae* to investigate the relative contribution of convergent evolution and co-ancestry to shared MHC polymorphism by contrasting patterns of codon usage at functional vs. neutral sites. Our results indicate that parasites shared across species may select for functionally similar MHC alleles, implying that the dynamics of MHC-parasite co-evolution should be envisaged at the community level. We further show that balancing selection maintaining trans-species polymorphism, rather than convergent evolution, is the primary mechanism explaining shared MHC sequence motifs between species that diverged up to 30 million years ago.

KEYWORDS

Cheirogaleidae, common ancestry, convergent evolution, host-parasites interactions, molecular evolution, trans-species polymorphism

1 | INTRODUCTION

The Major Histocompatibility Complex (MHC) is a multigene cluster characterized by remarkable polymorphism and a complex evolutionary history. MHC genes encode MHC molecules, cell surface

glycoproteins that trigger the initial phase of an immune response against intracellular (mainly MHC class I) or extracellular pathogens (mainly MHC class II) by binding antigenic peptides at peptide-binding regions (PBR). Antigens are presented to T lymphocytes that initiate an antigen-specific immune response (Trowsdale, 1993). Amino

acid polymorphism lining the peptide-binding groove (Hughes & Nei, 1988) determines the binding specificity of each encoded MHC molecule and consequently the spectrum of antigens recognized (Rammensee, Friede, & Stevanović, 1995). The individual immune repertoire, that is, which pathogens can or cannot be recognized by an individual's adaptive immune response, is thus determined by MHC genotype.

Major histocompatibility complex polymorphism is thought to be largely maintained by balancing selection mediated by co-evolving parasites where specific alleles providing protection against single or multiple pathogens are favoured (Bernatchez & Landry, 2003; Pirotte & Oliver, 2006). Three nonmutually exclusive evolutionary mechanisms have been proposed to explain this polymorphism. First, the negative frequency-dependent selection hypothesis proposes that rare alleles may confer selective advantages compared to more frequent alleles, because co-evolving pathogens may evolve evasive mechanisms against frequently encountered alleles. This co-evolutionary arms race between hosts and pathogens ultimately leads to cyclical changes in allelic frequencies (Snell, 1968; Bodmer, 1972; Slade & McCallum, 1992). Second, the overdominance hypothesis posits that heterozygous individuals have higher fitness than homozygotes due to their ability to mount a specific immune response against a broader spectrum of successive or simultaneous infections (Doherty and Zinkernagel 1975, McClelland, Penn, & Potts, 2003). The benefits of heterozygosity depend on the degree of overlap of binding specificities of individual alleles, and decrease if MHC alleles are largely redundant (e.g., Jeffery & Bangham, 2000). Third, the fluctuating selection hypothesis predicts spatio-temporal variation of pathogen communities, which creates different selective landscapes in different host populations (see Spurgin & Richardson, 2010). Support for a pathogen-mediated selection on MHC stems for instance from associations observed between certain MHC alleles and pathogen prevalence at the population or community level (e.g., Hill et al., 1991; Wegner, Reusch, & Kalbe, 2003; Prugnolle et al., 2005; Schwensow, Fietz, Dausmann, & Sommer, 2007; Schwensow, Eberle, & Sommer, 2010; Schwensow, Dausmann, Eberle, Fietz, & Sommer, 2010; Froeschke & Sommer, 2012; Sepil, Lachish, Hinks, & Sheldon, 2013; Sommer, Rakotondranary, & Ganzhorn, 2014; Pilosof et al., 2014), or from positive correlations between MHC polymorphism and parasite richness across species (Goüy de Bellocq, Charbonnel, & Morand, 2008; Garamszegi & Nunn, 2011).

Host-parasite co-evolution can be seen as a dynamic interplay between multiple hosts and their parasites (Pilosof et al., 2014). For example, under the "negative frequency-dependent hypothesis," alterations in parasite resistance in one host species can influence parasite prevalence in the environment, which can have cascading effect on other host species sharing the same parasite by altering selection imposed by this parasite on their MHC diversity. Thus, it may be important to consider the whole community rather than single species to comprehend the effects of parasite-mediated selection on the evolution of MHC polymorphism (Pilosof et al., 2014).

The extensive MHC polymorphism often crosses species boundaries, with similar alleles or sequence motifs shared among species.

MHC allelic similarity across taxa may originate from their co-ancestry if allelic lineages are maintained across speciation events, which is referred to as trans-species polymorphism; Klein, 1987; Figueroa, Günther, & Klein, 1988; McConnell, Talbot, McIndoe, & Wakeland, 1988; . Here, allelic lineages are thought to be maintained over long evolutionary time scales by parasite-mediated balancing selection through mechanisms such as overdominance or negative frequency-dependent selection (Klein, Satta, O'Huigin, & Takahata, 1993; Spurgin & Richardson, 2010). Alternatively, the occurrence of shared allelic lineages between species may evolve independently post-speciation by convergent evolution. Here, similar pathogens would select for similar antigen-binding properties in the host's MHC (Gustafsson & Andersson, 1994; Yeager & Hughes, 1999).

If co-ancestry is responsible for allelic similarity at coding regions (i.e. PBR), adjacent regions that are not directly involved in antigen recognition (e.g., non-PBR, or flanking noncoding introns) would also show higher similarity than expected by chance. In contrast, convergent evolution would promote amino acid (but not necessarily codon) similarity limited to coding regions but should not affect the structural (nucleotide) composition of noncoding regions that are expected to segregate according to the species phylogeny (Klein, Sato, & Nikolaidis, 2007; Lenz, Eizaguirre, Kalbe, & Milinski, 2013). Instances of shared MHC polymorphism supportive of either scenario have been reported (convergent evolution: Andersson, Sigurdardottir, Borsch, & Gustafsson, 1991; Trtkova, Mayer, O'Huigin, & Klein, 1995; Yeager & Hughes, 1999; Kriener, O'Huigin, Tichy, & Klein, 2000; Kriener, O'Huigin, & Klein, 2001; Srithayakumar, Castillo, Mainguy, & Kyle, 2012; and co-ancestry: Lundberg & McDevitt, 1992; Klein et al., 1993; Graser, Oh'Uigin, Vincek, Meyer, & Klein, 1996; Garrigan & Hedrick, 2003; Lenz et al., 2013; Tobler et al., 2014; Eimes, Townsend, Sepil, Nishiumi, & Satta, 2015; Gillingham et al., 2016; reviewed in Azevedo, Serrano, Amorim, & Cooper, 2015; Těšický & Vinkler, 2015). Identification of evolutionary trajectories leading to shared MHC polymorphism would benefit from simultaneous examination of MHC constitution, including contrasts between coding and noncoding regions (Zhang & Kumar, 1997; Kriener et al., 2000), and MHC-parasite interactions, which remains challenging in natural populations and nonmodel organisms (Lenz et al., 2013).

Additional insights into interactions between pathogens and host immune response can be achieved by reaching beyond nucleotide or amino acid similarity. The binding specificity of each encoded MHC molecule is determined by physico-chemical properties of amino acids positioned at the PBR. Consequently, MHC alleles encoding MHC molecules with similar antigen-binding affinities can be classified into functionally equivalent groups, referred to as "MHC super-types" (Sette & Sidney, 1999; Southwood et al., 1998; Trachtenberg et al., 2003; Lund et al., 2004). Supertype classification has proven valuable to investigate associations between the binding specificity of MHC alleles possessed by a given host, and host susceptibility to pathogens within and across species (e.g., Trachtenberg et al., 2003; Schwensow et al., 2007; Schwensow, Dausmann et al., 2010; Sepil et al., 2013; Pilosof et al., 2014). Functional similarity across species

(i.e. alleles from different species belonging to a same supertype) would suggest the operation of convergent evolution if allelic structural similarity is absent from non-PBR regions. Alternatively, if shared polymorphism at PBR is a consequence of co-ancestry, we expect the similarity of amino acid sequences to reflect the similarity of nucleotide sequences, and so, similar patterns of codon usage (Lundberg & McDevitt, 1992; Lenz et al., 2013).

Here, we aim to address the contribution of convergent evolution and balancing selection maintaining trans-species polymorphism to MHC allelic similarity by simultaneous examination of MHC constitution and parallel selective pressures in a community of four sympatric lemur species—*Microcebus berthae*, *Microcebus murinus*, *Mirza coquereli* and *Cheirogaleus medius* (Primates, *Cheirogaleidae*). This lemur community presents three key advantages for this study. First, they vary in phylogenetic relatedness, allowing us to test the relative contribution of the two evolutionary scenarios across pairs of species that have diverged more or less recently (i.e., within *Microcebus* sp. (7–11 Mya); *M. coquereli* and *Microcebus* sp. (12–18 Mya); *C. medius* and other *Cheirogaleidae* (19–26 Mya); Thiele, Razafimahatratra, & Hapke, 2013). We may expect their contribution to vary with the degree of species relatedness, which may provide insights into the potential longevity of MHC alleles. Second, these species are reproductively isolated and the absence of ongoing hybridization minimizes the risk of confounding factors, such as allelic introgression (see also Lenz et al., 2013; Klein et al., 2007). Third, all these species are small-bodied (<500 g), nocturnal omnivores facing broadly similar environmental conditions. This community includes *M. berthae* and *M. coquereli*, which are solitary and spatially dispersed, more densely populated, pair-living *C. medius* and *M. murinus*, which is socially more cohesive and exhibits the highest population densities (Dammhahn & Kappeler, 2005; Kappeler, Wimmer, Zinner, & Tautz, 2002; Markolf, Roos, & Kappeler, 2008; Fietz, 1999a,b; Eberle & Kappeler, 2002, 2006). These four species exhibit different levels of ecological overlap in their diet and in their demography that is incongruent with their phylogeny (Dammhahn & Kappeler, 2014; Thiele et al., 2013). This constellation may therefore provide an opportunity to assess the importance of ecological overlap in explaining patterns of MHC variation in these species, given that diet, host population density and spatial proximity have all been indicated to affect the level of parasitism (e.g., Vitone, Altizer, & Nunn, 2004; Hughes & Page, 2007; Chen et al., 2008; Godfrey, 2013; Morand, 2015). A considerable overlap in helminth communities, dietary ranges and functional proximity of MHC alleles reported between *M. murinus* and *C. medius* (Schwensow, Dausmann et al., 2010; Dammhahn & Kappeler, 2014) suggest that their ecological similarities are potentially relevant to explain their MHC similarities. While the most common helminth genera identified in these hosts presumably show mild pathogenicity (Raharivololona & Ganzhorn, 2010; Irwin & Raharison, 2009), helminth infections appear to be associated with reduced survival in *M. murinus* (Hämäläinen, Raharivololona, Ravoniarimbina, & Kraus, 2015).

Here, we first assess the extent of overlap in parasites communities—to assess the similarity of pathogen-mediated balancing

selection acting on MHC genes - across these four lemurs and compare patterns of MHC class II allelic variation in the two highly polymorphic loci (DQB and DRB) derived from this and a previous study (*M. berthae*, Pechouskova et al., 2015).

Second, to evaluate the impact of pathogen-mediated selection on shaping MHC allelic variation across species, we evaluate the functional overlap between MHC alleles using supertype classification. Next, we integrate these data with parasite prevalence to test whether shared parasite pressures may select for similar frequency distribution of functionally equivalent MHC alleles across host species. We further examine two distinct scenarios of parasite-driven selection. First, if each MHC supertype protects against one or few parasite species within and across species, we expect to detect a correlation between the frequency of particular MHC superotypes and the prevalence of particular parasites within and across species. In contrast, multiple infestations can impose additional energetic demands causing detrimental effect on host fitness and survival and thereby reduced reproductive output (reviewed e.g., in Morand, 2015). Thus alternatively, if some MHC superotypes have a broader and more generalistic effect than others and may protect against a diverse array of pathogens within and across species, we expect to detect a correlation between the frequency of particular MHC superotypes and parasite richness within and across species.

Third, to determine whether MHC functional similarity stems from their co-ancestry or from convergent evolution, we evaluate the potential co-ancestry of (i) orthologous alleles that are functionally similar and belong to a same supertype and (ii) orthologous peptide motifs at the PBR in species with various degrees of phylogenetic proximity by integrating our MHC data with those previously acquired for other *Cheirogaleidae*. Under the co-ancestry hypothesis, we predict that (i) MHC alleles belonging to the same supertype show higher structural similarity at non-PBR than alleles belonging to different superotypes, and (ii) amino acids at PBR shared across species are expected to show high codon identities. In other words, we expect that shared amino acids are encoded by the same codon if they share a common ancestry, knowing that degeneracy is a salient feature of the genetic code and that they will likely be encoded by different codons if they have evolved independently. Accordingly, under the operation of convergent evolution, selection for similar amino acids, but not necessarily codons, is expected across species. Moreover, allelic similarity should be predominantly limited to PBR (Lenz et al., 2013), especially in more distantly related species. Lastly, we expect the relative contribution of co-ancestry and convergent evolution to vary with the degree of phylogenetic relatedness of species, with co-ancestry having stronger effects among the most closely related species.

2 | MATERIALS AND METHODS

2.1 | Study site, DNA collection and MHC genotyping

The four lemur species live in sympatry within 12,500-ha Kirindy Forest/CNFEREF (Centre National de Formation, d'Etude et de

Recherche en Environnement et Foresterie) in western Madagascar (Kappeler & Fichtel, 2012). Members of each species have been regularly captured between the years 1993 and 2013 at four study sites, using Sherman and Tomahawk live traps. Further details on sampling design and sample size distribution of each species are presented in Appendix S1.

The capture and DNA sampling and extraction as well as PCR amplification targeting the two highly polymorphic loci of the MHC class II region, DRB and DQB followed by MHC genotyping (454 pyrosequencing; Roche, France) were conducted as described in Pechouskova et al. (2015); see Appendix S1 for a brief summary.

All animal handling and sample treatments were in compliance with animal care regulations and applicable national laws of Germany and Madagascar (CITES: 429C-EA10/MG07, 430C-EA10/MG07), approved by the appropriate Animal Use and Care committees of Germany (Bundesministerium für Naturschutz, BfN) and the legal requirements of Madagascar (Ministère de l'Environnement et des Eaux et Forêts, MINEEF).

2.2 | Parasite communities screening

Coprosopic samples used in this study were collected during animal handling and from clean traps between September and December 2012. Samples were immediately homogenized in 10% formalin and stored for further examination. Helminth eggs were extracted using two different techniques: (i) standard FLOTAC protocol (Cringoli, Rinaldi, Maurelli, & Utzinger, 2010) and (ii) Ritchie's formol-ether concentration method (Ritchie, 1948). Parasite presence was determined microscopically following criteria for egg-morphotype classification up to the genus level (Raharivololona & Ganzhorn 2009; Irwin & Raharison, 2009). Only morphotypes that could be identified reliably (eight genera, Table S4: Appendix S1) were retained for subsequent analyses. Further details on the host species-specific sample sizes, discovered parasite genera and analyses of repeatability of helminth egg detection are given in Appendix S1.

We evaluated the extent of overlap among helminth communities in the four host species by testing whether two random samples collected from the same species are more similar than two random samples from different species. A higher proportion of individuals of *M. murinus* (42%) and *C. medius* (41%) were sampled only once, compared to *M. coquereli* (13%) and *M. berthae* (7%). Thus, we homogenized sampling effort across species by including only those individuals that were sampled at least twice throughout two different sampling months. To control for seasonal variability, we performed the same test including a subset of individuals that were sampled on the same trapping session (1–3 days apart). The number of individuals included in each test is provided in Appendix S1 (Table S2). For all tests, Jaccard dissimilarity index was calculated for each pair of samples, using the function “vegdist” implemented in the R package “VEGAN” (Oksanen et al., 2015), and averaged across groups (e.g., “same” vs. “different” species). The resulting average was compared using Mann–Whitney *U* tests.

Next, we created a host–parasite matrix based on helminth prevalence (the proportion of individuals within each species infected by a given parasite) within each host species, following Pilo-sof et al. (2014). The host–parasite matrix (see Figure 1) was transformed into a dissimilarity matrix using *Růžička index* (RI), a quantitative version of the Jaccard index that allows direct comparisons between hosts (Speed, Cooper, Jónsdóttir, Van Der Wal, & Woodin, 2010; Tamás, Podani, & Csontos, 2001), implemented in the R package “VEGAN”. RI value of 1 indicates maximum dissimilarity, where no parasites are shared between a pair of hosts and RI value of 0 indicates that parasites infect hosts with a similar prevalence. All analyses were conducted in R v.3.2.1. (R Core Team, 2014).

Finally, we examined whether the extent of overlap in host parasite communities reflected host phylogenetic proximity. To this end, we constructed a phylogenetic tree based on cytochrome b mitochondrial gene sequences (1,140 bp; NCBI Genebank, *M. coquereli* EU835932; *C. medius* EU825326 in Groeneveld, Rasoloarison, Weisrock, & Kappeler, 2008; Groeneveld, Weisrock, Rasoloarison, Yoder, & Kappeler, 2009; *M. berthae* GU327166, *M. murinus* GU327178 in Weisrock et al., 2010); using a ML algorithm (GTR substitution model, 1000 bootstrap replications; Tavaré, 1986) implemented in PHYL 3.0 (Guindon et al., 2010); Figure 1. We then inferred phylogenetic distances using the function “cophenetic.phylo” implemented in the R package “APE” (Paradis, Claude, & Strimmer, 2004). A Mantel test was used to correlate parasite dissimilarity matrix with phylogenetic distances between host species.

2.3 | MHC variation, patterns of molecular selection and supertype classification

Allelic richness for each locus was compared across the four species, while controlling for sampling effort (number of individuals trapped per species), using a simple resampling set to evaluate the number of alleles present in the population based on the number of alleles retrieved for a given sampling effort (Huchard et al., 2012).

The presence of positive selection operating on nucleotide sequences was tested following a method developed by Yang, Nielsen, Goldman, and Pedersen (2000), Yang, Wong, and Nielsen (2005), using the program CodeML implemented in PAML v.4.7 software (Yang, 2007). The implementation of the method is summarized in Appendix S1.

Classification of MHC alleles into “superotypes” entails characterizing MHC alleles by the binding specificity of amino acid residues lining the peptide-binding groove (here represented by positively selected sites “PSS” identified in a previous step of the analysis) to assess their functional proximity within and across species. At first, we generated a sequence alignment for each locus that was restricted to PSS. Given that the PSS distribution was not homologous across species for either locus, we created a conservative consensus alignment that would enable the design of superotypes across species. We included all PSS detected in any species (20 DRB and 12 DQB) to ensure that any functionally important site was retained in the analysis. Each amino acid from the DRB (or DQB) PSS

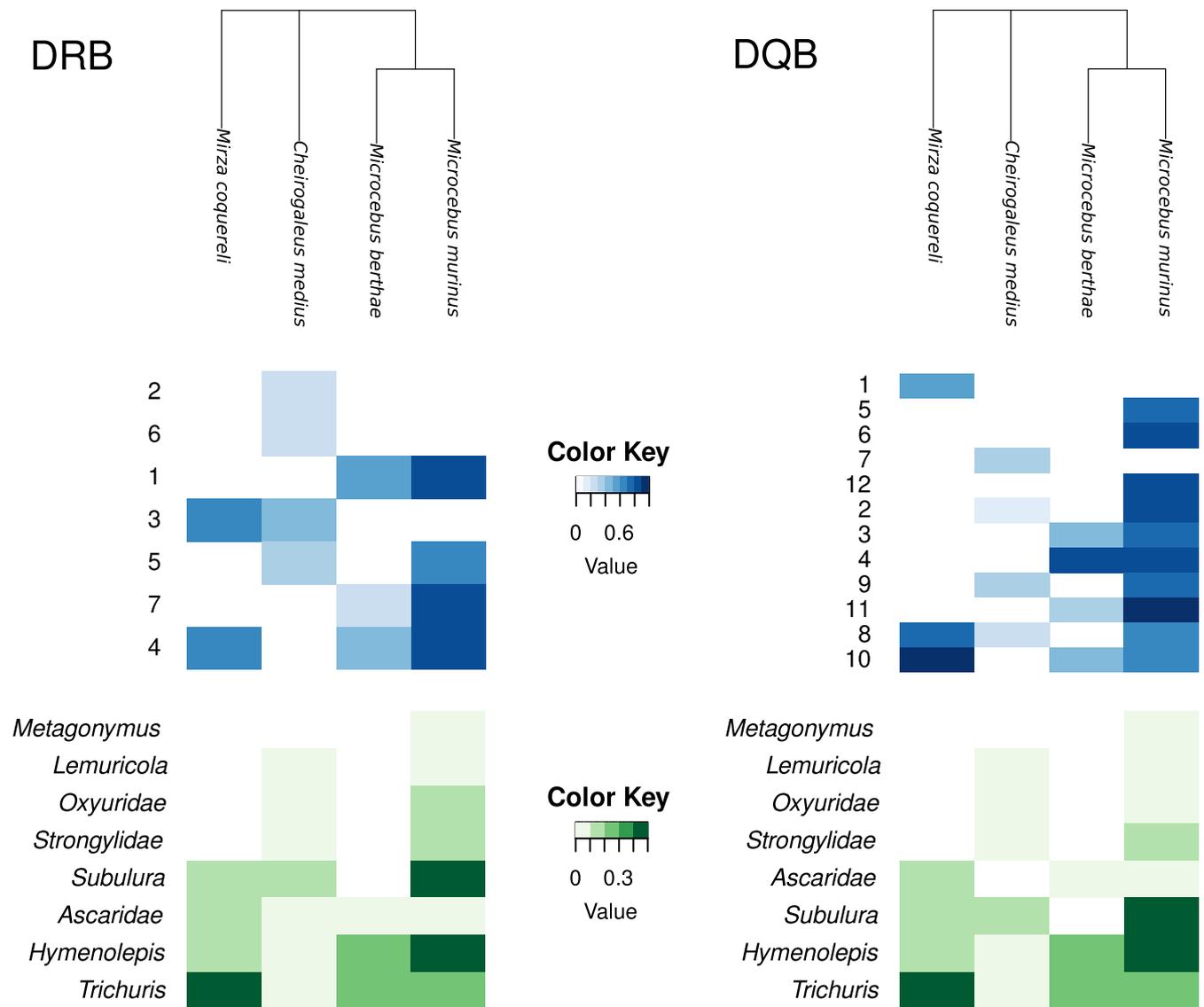


FIGURE 1 Host–parasite matrix (green) based on helminth prevalence defined as the fraction of individuals within host species (columns) infected by a given parasite (rows); and host–MHC supertype matrix (blue) based on supertype frequencies defined as the number of infected individuals of a given host species (columns) possessing a given supertype (rows), divided by the total number of individuals possessing this supertype within the same host species. Phylogenetic relationships among species are shown using ultrametric phylogenetic tree based on 1,140 bp of cytochrome b mitochondrial gene sequences

alignment was characterized by a vector of five physico-chemical descriptor variables that are thought to play a key role in determining antigen-binding specificities (Sandberg, Eriksson, Jonsson, Sjöström, & Wold, 1998) following Doytchinova and Flower (2005).

The resulting matrix was then subjected to a K-means clustering algorithm with an increasing number of groups using the function “find.clusters” implemented in “adegetnet” R package (Jombart, Devillard, & Balloux, 2010) following Sepil, Moghadam, Huchard, and Sheldon (2012). Here, different clustering solutions were compared using the Bayesian Information Criterion (BIC) to identify the optimal number of clusters. Finally, the supertype classification was validated using an automated clustering criterion as proposed by Pilosof et al. (2014); see Appendix S1 for detailed description of the method and its implementation.

2.4 | Parasite–host supertype associations

To examine whether parasite-driven selection is likely to contribute to the distribution of MHC supertypes across species, we investigated associations between helminth infestations and supertype frequency using two different approaches. A total of 187 individuals (*M. berthae* $n = 14$; *M. murinus* $n = 128$; *M. coquereli* $n = 8$; *C. medius* $n = 37$) for which both MHC and data on helminth prevalence were available were included in these analyses.

First, we tested whether species with similar helminth communities may also show similar MHC supertype distributions. Following the procedure described by Pilosof et al. (2014), we created a matrix describing the frequency of MHC supertypes in different host species and then transformed it into distance matrix using *Růžicka index*

(RI) in the same manner as described above for the host–parasite matrix (see Figure 1). We performed a partial Mantel test to evaluate the significance of the correlation between the host–supertype and host–parasite matrices, while controlling for a potential phylogenetic signal among the host species using the phylogenetic distances generated above. To examine whether uneven sample sizes available for each species could influence the supertype–parasite association patterns we repeated the partial Mantel test controlling for host abundance.

Second, if each MHC supertype inhibits or favours infection by a limited array of parasites, we expected to detect a correlation between the frequency of some MHC superotypes and the prevalence of some parasites within and across species. We therefore examined the strength of supertype–helminth associations across samples and individuals from different species, using multivariate generalized linear mixed models (GLMMs) with helminth presence/absence in a given sample as a response variable (binomial error distribution, logit-link function). Alternatively, if some MHC superotypes may have a broader, more generalistic effect than others and inhibit or favour infection by a diverse array of parasites, we expected to detect a correlation between the frequency of some MHC superotypes and parasite richness within and across species. To assess this possibility, we examined whether some superotypes may influence parasite richness measured as the number of helminth genera present in a given sample, using multivariate GLMMs (Poisson-error distribution, log-link function).

For building our models examining the effect of superotypes on one helminth genus across host species, we considered only those helminth genera that were found in multiple host species (Appendix S1: Table S4), as we aimed at testing whether the possession of a given supertype may be linked with the presence of a given parasite in multiple host species. For the same reason, MHC superotypes that occurred in one host species only (Appendix S1: Table S6) were excluded from further analysis. Host species for which infestation by a particular parasite was present in less than two individuals or two observations were not considered in given models, to ensure reliable calculation of model estimates. For models on parasite richness, all helminth genera and all host species were included. We accounted for monthly variation in parasite prevalence and repeated observations of the same individual, by including month of data collection and host individual identity as random effects. Host species and MHC supertype were fitted as fixed effects. Given that pairwise correlations among DRB (labelled “SR”) and among DQB (“SQ”) superotypes showed relatively low level of co-linearity (DRB $r < .23$; DQB $r < .26$), all relevant SR and SQ superotypes were considered in a single model. In addition, variance inflation factors were calculated for each explanatory variable within each model. The significance of fixed effects was evaluated by comparing a model with and a model without the supertype of interest using a likelihood ratio test and Bonferroni’s correction. Significant effects detected across species (“multihost models”) were tested in each species separately (“single-host models”) to ensure that such effects were not driven by the most abundant host (*M. murinus*) only. Statistical significance of

variables was tested using the full model to avoid problems associated with stepwise model selection (Whittingham, Stephens, Bradbury, & Freckleton, 2006; Mundry & Nunn, 2009). All models were conducted using the function “GLMER” implemented in the R package “lme4” (Bates, Maechler, Bolker, & Walker, 2015).

2.5 | Co-ancestry vs. convergent evolution maintaining MHC functional similarity

To assess the relative contribution of the two scenarios, we investigated the origin of sequence similarity at sites that are either neutral or under purifying selection (non-PSS) and at sites that are under positive selection (PSS).

We first tested whether the functional similarity of alleles belonging to the same supertype is likely to arise from their co-ancestry, or independently by convergent evolution. To do this, we compared nucleotide similarity at non-PSS between alleles belonging to a same vs. different supertype, by computing average pairwise nucleotide distances of non-PSS (number of differences) within and between superotypes for each locus separately in MEGA 6 (Tamura, Stecher, Peterson, Filipiński, & Kumar, 2013). Under the co-ancestry scenario, we expect alleles belonging to the same supertype to be more similar at both PSS and non-PSS compared to alleles belonging to different superotypes. Under the convergent evolution scenario, we expect alleles belonging to the same supertype to be more similar at PSS, but not at non-PSS, compared to alleles belonging to different superotypes.

Second, we examined codon usage patterns at PSS following a procedure described by Lenz et al. (2013). We assumed that if amino acids at PSS are preserved across speciation events, it should be reflected by high codon similarity among species. In contrast, if amino acid similarity results from independent convergent evolution, the codon similarity should follow the species-specific codon frequency distribution instead (Lundberg & McDevitt, 1992). Here, we also included MHC alleles described earlier in Kirindy forest and at distant study sites for *C. medius* and *Microcebus* sp.—*M. murinus*, *M. griseorufus* and *M. rufus* (see Appendix S2). For these additional sequences, the positioning of PSS was extrapolated.

First, we performed a Monte Carlo simulation (hereafter referred to as MC sampling) to generate a distribution of the proportions of identical codons expected to occur between species (and populations) at PSS under convergent evolution. To do so, we calculated the number of identical amino acids at PSS in a pairwise fashion between alleles of all pairs of species and recorded whether each pair of shared amino acid was coded by an identical codon generating the proportions of identical codons observed at PSS between each pair of species. Then, we created a drawing pool used for the MC sampling that consists of species-specific codon frequencies derived from the actual codon frequencies present across the whole exon alignment. For MC sampling, the amino acid-specific number of potential codons was randomly drawn 1000 times, and cases of identical codons between species were recorded. As a result, we obtained a simulated probability distribution of the proportion of identical codons expected under convergent evolution (CE_d).

Next, the observed codon similarity between species was compared with the codon similarity expected within species, approximating a scenario of allelic co-ancestry. Here, we performed pairwise comparisons of all alleles within each species and recorded how often the same amino acid was coded by the same or by a different codon. This amino acid-specific identity count served as a second drawing pool, from which the number of identical amino acids observed between species at PSS (as above) was randomly drawn 1,000 times to obtain a probability distribution of the expected proportion of identical codons within species (CA_d). Both procedures were repeated for each species pair within each locus separately. All above simulations were performed using customized Perl scripts available upon request from Lenz et al. (2013).

The proportions of identical codons observed at PSS between species (and populations) were then compared against the simulated distributions (CA_d and CE_d). To compute the relative contribution of convergent evolution vs. co-ancestry in each species pair, we located each species pair on an axis with two ends, where each end corresponds to the value of the median of the simulated distribution of each evolutionary scenario (CE_d , CA_d). This provided a score indicative of the relative contribution of co-ancestry (hereafter “co-ancestry scores”) for each species pair as: (proportion of identical codons between species–median CE_d)/(median CA_d –median CE_d).

We then tested whether co-ancestry scores obtained for all pairwise comparisons would correlate with phylogenetic distances among the species using simple correlation test. We expected the relative contribution of co-ancestry to be greater in closely related species. Phylogenetic distances among the species were inferred from a phylogenetic tree based on cytochrome b mitochondrial gene sequences for the Kirindy community (see above) and for allopatric populations of *M. rufus* and *M. griseorufus* (NCBI Genebank, *M. rufus* GU327245; *M. griseorufus* GU327345 in Weisrock et al., 2010).

3 | RESULTS

3.1 | Parasite communities screening

The overview of helminth infestations across four *Cheirogaleidae* is presented in Appendix S1 (Table S4). Four of eight helminth genera (*Ascaris* sp., *Trichuris* sp., *Hymenolepis* sp. and *Subulura* sp.) were present in 3–4 lemur species and accounted for 87% of the total infections. The proportions of infected individuals ranged from 32% in *C. medius* to 82% in *M. murinus*, which also showed the highest parasite richness. Comparison of parasite communities across species revealed that samples collected from the same host species were more similar (d_j , mean \pm SD 0.68 ± 0.29) than samples from different species (d_j , mean \pm SD 0.91 ± 0.19 ; Mann-Whitney test, $W = 3914187$, $p < .001$). This result held when comparing samples collected during the same trapping session (same host species: d_j , mean \pm SD 0.82 ± 0.28 vs. different host species: d_j , mean \pm SD 0.94 ± 0.19 ; Mann-Whitney test, $W = 1582817$, $p < .001$), although parasite communities were largely overlapping across host species.

TABLE 1 Dissimilarity matrix of helminth prevalences between each pair of species calculated using *Růžicka index* (RI)

	<i>C. medius</i>	<i>M. berthae</i>	<i>M. murinus</i>
<i>M. berthae</i>	0.831		
<i>M. murinus</i>	0.763	0.719	
<i>M. coquereli</i>	0.752	0.574	0.706

An RI value of 1 indicates maximum dissimilarity, with no parasites shared between pair of hosts, and RI value of 0 indicates that parasites infect hosts with a similar prevalence.

The dissimilarity matrix shows considerable overlap in helminth prevalence for each pair of host species (Table 1). *M. coquereli* and *M. berthae* appear the most similar hosts concerning helminth prevalence. Additionally, seven of eight genera were shared among *C. medius* and *M. murinus*. We found no correlation between overlap in helminth prevalence and phylogenetic distances among host species (Mantel test; $r = .22$; $p = .29$).

3.2 | MHC variation, patterns of molecular selection and supertype classification

The overview of genotyped individuals per species is presented in Appendix S1 (Table S3). We present a total of 22 new DRB and 41 new DQB exon 2 sequences (Appendix S2).

The estimated allelic richness (number of distinct alleles) was highest for *M. murinus* in DQB and comparably high for *M. murinus* and *C. medius* in DRB when controlling for sampling effort. *M. berthae* followed by *M. coquereli* displayed the lowest allelic richness in both loci (Figure 2).

The presence of positively selected sites “PSS” was supported in all species and loci (Appendix S1; Table S5). The distribution of PSS showed different patterns across species (Figure 3) especially at DQB between *M. berthae* ($n_{PSS} = 11$) and the other three *Cheirogaleidae* ($n_{PSS} = 1$ in *C. medius* and 2 in *M. murinus* and *M. coquereli*).

The optimal supertype clustering solutions (with lowest BIC values) suggested seven clusters in DRB and 12 in DQB, which concurred with the number of clusters obtained by averaging 500 automated classifications (Appendix S1: Fig. S6).

3.3 | Parasite–host supertype associations

The analysis relating cross-species similarities in helminth prevalence and in the frequency distribution of MHC superotypes (Figure 1) lacked significance in both loci (Partial Mantel test controlling for phylogeny, DRB $r = .76$, $p = .13$; DQB, $r = .77$, $p = .17$; Appendix S1, Fig. S7). Controlling for host abundance did not alter the outcome (DRB $r = .53$, $p = .13$; DQB $r = .67$, $p = .17$).

Next, we investigated whether the possession of a given supertype affects individual infestation by helminth genera that were shared across multiple host species (Appendix S1: Table S4). In DRB, a significant link between the possession of SR4 and the *Hymenolepis* sp. infestation was detected in the multihost model including the

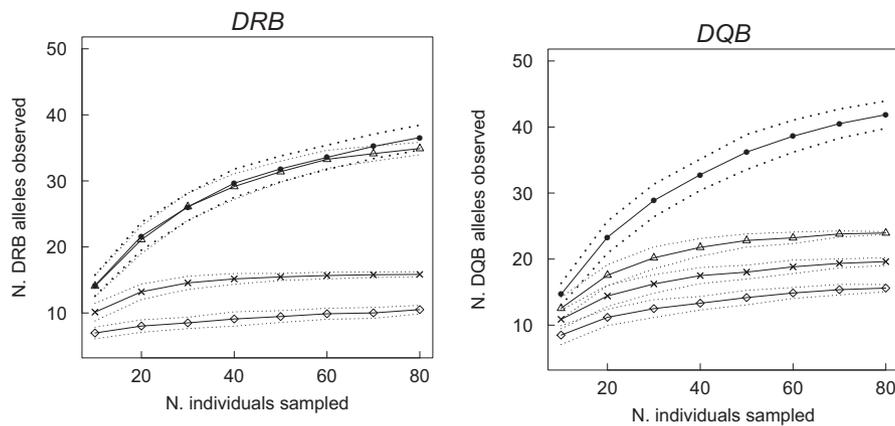


FIGURE 2 Allelic richness for a given sampling effort estimated through a resampling procedure, showing the number of distinct alleles detected at DRB and DQB when randomly drawing an increasing number of individuals from the study population for *M. murinus* (full circle), *C. medius* (triangle), *M. coquereli* (diamond) and *M. berthae* (cross). The dotted lines (highlighted for *M. murinus*) indicate the standard deviation around the estimated mean (solid line)

two *Microcebus* sp. (Table 2, $\chi^2 = 12.46$, $p > .001$) and remained consistent in both hosts when tested in single-host models.

Analysis of the congruence of supertype effects on the risk of multiple infestations (parasite richness) across species revealed a link between the possession of SQ4, shared by the two *Microcebus* sp., and increasing parasite richness in the multihost model ($\chi^2 = 8.09$, $p < .005$) that remained significant when tested in single-host models (Table 2).

Other associations detected using multihost models were not confirmed in single-host models, possibly due to a lack of power, and were thus not considered further.

3.4 | Co-ancestry vs. convergent evolution maintaining MHC functional similarity

The nucleotide similarity of non-PSS was marginally or significantly higher among alleles belonging to the same vs. different superotypes in both loci (mean \pm SD for same vs. different supertype, Student's *t* test, DRB 2.21 ± 0.79 vs. 2.87 ± 0.97 , $p = .08$; DQB 5.00 ± 1.35 vs. 8.05 ± 1.85 , $p < .001$), suggesting that at least in DQB, co-ancestry is partially responsible for the functional similarity of alleles belonging to the same supertype.

Second, we analysed codon usage patterns at PSS by calculating the proportions of shared codons at PSS in a pairwise fashion between all pairs of species and evaluated its relative distances from the expected codon similarity distributions (obtained by MC sampling) under the two evolutionary scenarios (CE_d , CA_d). The proportions of shared codons at PSS, co-ancestry scores, phylogenetic distances and median of the CE_d , CA_d obtained for all pairwise comparisons are summarized in Table 3. For all pairwise comparisons in both loci, the proportion of identical codons was significantly higher than expected under CE_d , and notably closer but still significantly lower than expected under CA_d (for all comparisons $p < .001$, one-tailed *t*-test). In DRB, we found a significant negative correlation between phylogenetic distances and co-ancestry scores (Pearson product-moment correlation coefficient; $r = -.48$, $p < .03$; Figure 4).

In DQB, the direction of the correlation was the same, but weakened probably due to lack of statistical power compared to DRB (6 vs. 21 comparisons) ($r = -0.58$, $p = .23$). This indicates that functional similarity of MHC alleles in closely related species stems largely from their co-ancestry, rather than convergent evolution.

4 | DISCUSSION

Our study addressed the relative contribution of convergent evolution and balancing selection maintaining trans-species polymorphism in shaping functional MHC class II variation within a lemur community (*Cheirogaleidae*). We examined how parallel parasite-mediated selection may shape MHC variation across species by integrating MHC data with data on helminth prevalence. We show that particular MHC superotypes are associated with infection by particular parasites as well as the risk of multiple infections across host species. Comparison of MHC sequences among lemur species of varying phylogenetic relatedness suggests that interspecific MHC allelic similarity primarily stems from allelic co-ancestry, inferred from both nucleotide and codon similarity at sites directly involved in interactions with pathogens vs. other sites.

4.1 | Parasite community overlap, MHC variation and selection patterns

We evaluated the potential of parallel parasite-driven selection pressures across hosts, expected under both convergent evolution and balancing selection maintaining trans-species polymorphism, by assessing the extent of overlap in gut helminth communities. Although its composition differed across species, four of eight helminth genera were present in more than two host species, and parasite communities substantially overlapped across all species. *Microcebus berthae* and *M. coquereli* appeared most similar based on helminth prevalence. They shared three of four helminth genera with both direct and indirect transmission routes, which may reflect the substantial overlap in their

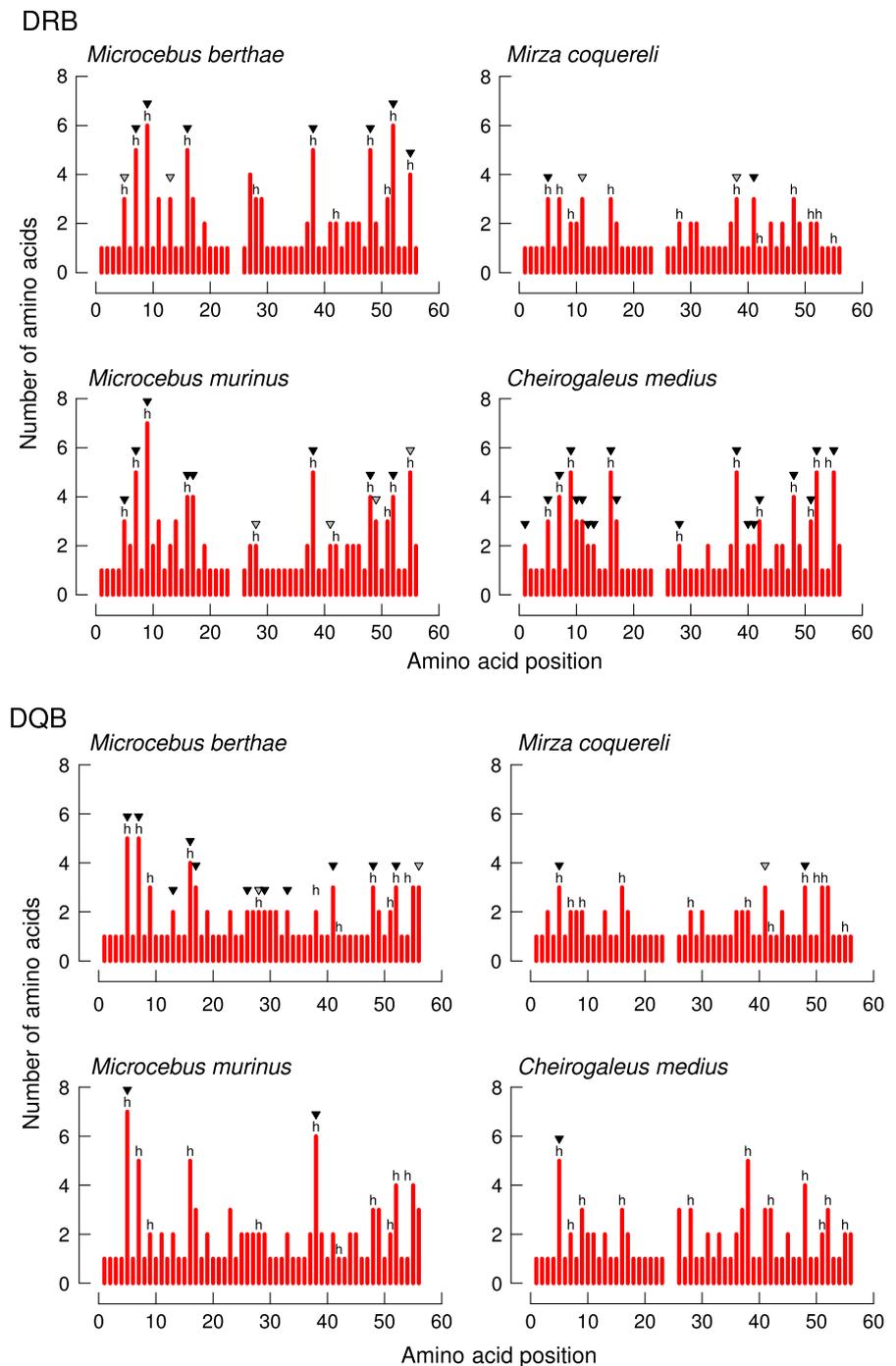


FIGURE 3 Amino acid variation plots for MHC-DRB and DQB in four sympatric lemurs. Human Antigen-Binding Sites (ABS) are indicated by the letter 'h' and positively selected sites are indicated by black ($P > 99\%$) and grey triangles ($P > 95\%$) [Colour figure can be viewed at wileyonlinelibrary.com]

diets, that is the amount of fruit/animal matter (Dammhahn & Kappeler, 2014). Similarly, overlaps in helminth prevalence were previously described in *C. medius* and *M. murinus* (Schwensow, Dausmann et al., 2010), and largely confirmed here, with seven of eight helminth genera shared between species.

Divergent patterns of PSS distribution were observed across the four *Cheirogaleidae* at both loci. In *C. medius*, a striking difference between the two loci, with 19 positively selected sites (PSS) detected in DRB and only one in DQB, was also reflected by a slightly lower allelic richness in DQB (24) vs. DRB (36). In other sympatric lemurs, allelic richness was higher in DQB than in DRB

but was not accompanied by a high PSS density, except in *M. berthae*. Divergent patterns of past selection at different loci may indicate a different function of these loci, as was previously suggested in *M. murinus*, where DRB was under stronger diversifying selection and more influential on MHC-based mate choice than DQB (Huchard et al., 2012; Huchard, Baniel, Schliehe-Diecks, & Kappeler, 2013). Past and current demographic fluctuations most likely contribute substantially to the overall variation in MHC polymorphism across species. Accordingly, allelic richness was higher in the two species with greatest population sizes (*M. murinus* and *C. medius*).

TABLE 2 Associations between MHC supertypes and (a) *Hymenolepis* sp. prevalence (yes/no) and (b) infestation by multiple helminth genera “parasite richness” as response variables

Model	Fixed effect	Estimate	SE	z value	Pr(> z)	CI 95%		VIF ^a
						2.5%	97.5%	
(a) Effect of DRB supertypes on infection by <i>Hymenolepis</i> sp.								
Multihost model								
Including <i>M. berthae</i> & <i>M. murinus</i> (ref category: <i>M. berthae</i>)	(Intercept)	−3.38	1.03	−3.28	0.001	−5.401	−1.360	
	<i>M. murinus</i>	1.13	0.72	1.56	0.119	−0.288	2.540	1.08
	SR1	0.47	0.44	1.05	0.293	−0.404	1.339	1.38
	SR4	1.62	0.49	3.31	0.001	0.662	2.583	1.39
	SR7	0.22	0.40	0.54	0.591	−0.573	1.007	1.16
Single-host models								
<i>M. berthae</i>	(Intercept)	−3.53	1.13	−3.13	0.002	−5.747	−1.322	
	SR4	3.13	1.33	2.35	0.019	0.514	5.744	
<i>M. murinus</i>	(Intercept)	−1.71	0.64	−2.69	0.007	−2.958	−0.465	
	SR4	1.22	0.44	2.76	0.006	0.354	2.081	
(b) Effect of DQB supertypes on parasite richness								
Multihost model								
Including all four <i>Cheirogaleidae</i> species (ref category: <i>C. medius</i>)	(Intercept)	−1.22	0.28	−4.29	<0.001	−1.777	−0.663	
	<i>M. berthae</i>	−0.74	0.44	−1.66	0.096	−1.612	0.132	2.15
	<i>M. coquereli</i>	1.91	0.52	3.65	0.000	0.885	2.943	2.93
	<i>M. murinus</i>	1.41	0.28	4.96	0.000	0.855	1.971	2.69
	SQ1	−2.24	0.62	−3.64	0.000	−3.445	−1.035	2.07
	SQ2	−0.23	0.16	−1.41	0.159	−0.541	0.088	1.26
	SQ3	−0.39	0.16	−2.47	0.014	−0.702	−0.081	1.20
	SQ4	0.49	0.17	2.91	0.004	0.161	0.821	1.31
	SQ5	−0.46	0.26	−1.74	0.082	−0.979	0.059	1.11
	SQ8	−0.11	0.23	−0.47	0.640	−0.550	0.338	1.22
	SQ9	−0.36	0.23	−1.61	0.107	−0.808	0.079	1.10
	SQ10	−0.22	0.19	−1.20	0.230	−0.591	0.142	1.35
	SQ11	0.24	0.16	1.49	0.137	−0.075	0.546	1.37
SQ12	−0.15	0.15	−1.06	0.291	−0.438	0.131	1.27	
Single host models								
<i>M. murinus</i>	(Intercept)	−0.08	0.00	−33.16	<0.001	0.083	−0.074	
	SQ4	0.49	0.00	206.04	<0.001	0.481	0.490	
<i>M. berthae</i>	(Intercept)	−3.45	1.07	−3.23	0.001	−5.545	−1.360	
	SQ4	2.21	1.12	1.96	0.0495	0.005	4.409	

All models were analysed using Generalized Linear Mixed Models. For each approach, we present multihost models including all relevant species and supertypes and then single-host models testing only those predictors found to be significant in multihost models to confirm that effects observed in multihost models are not driven by a single-host species. Host species and the possession of several MHC supertypes (yes/no) were fitted as fixed effects; individual identity and month of collection as random effects. Only models for which significant associations were found in multi- and single-host models, respectively, are shown here.

^aVariance inflation factors.

4.2 | MHC functional similarity through convergent evolution or co-ancestry?

First, we examined the extent of functional overlap among MHC alleles by classifying them by their binding specificity into seven DRB and 12 DQB supertypes across species. Except for two

supertypes in either locus, all were shared by two to four host species. We subsequently detected an association between species similarity in the distribution of MHC supertypes and helminth prevalence (DRB $r = .76$, DQB: $r = .77$), which was comparable in strength to the association previously found in a multihost-parasite ecological network of 11 rodent species and 26 helminth taxa (DRB $r = .62$;

TABLE 3 Results of the codon usage analysis presenting the proportion of identical codons at PSS, the median of the proportion of shared codons expected under a scenario of convergent evolution (CE_d), and of common ancestry (CA_d) obtained from simulated distributions, co-ancestry scores and phylogenetic distance between all pairs of species in DRB and DQB

Species pair		Proportion of identical codons	Median CE	Median CA	Co-ancestry scores	Phylogenetic distance
DRB						
<i>M. murinus</i> (distant)	<i>M. murinus</i> (Kirindy)	0.964	0.919	0.986	0.683	-
<i>M. rufus</i>	<i>M. berthae</i>	0.975	0.929	0.989	0.772	0.043
<i>M. griseorufus</i>	<i>M. murinus</i> (Kirindy)	0.969	0.918	0.986	0.754	0.167
<i>M. murinus</i> (distant)	<i>M. griseorufus</i>	0.967	0.906	0.991	0.717	0.167
<i>M. griseorufus</i>	<i>M. berthae</i>	0.975	0.922	0.994	0.737	0.199
<i>M. rufus</i>	<i>M. griseorufus</i>	0.983	0.918	0.992	0.882	0.202
<i>M. murinus</i> (Kirindy)	<i>M. berthae</i>	0.966	0.932	0.985	0.633	0.232
<i>M. murinus</i> (distant)	<i>M. berthae</i>	0.968	0.928	0.992	0.623	0.232
<i>M. rufus</i>	<i>M. murinus</i> (Kirindy)	0.972	0.925	0.984	0.792	0.235
<i>M. murinus</i> (distant)	<i>M. rufus</i>	0.970	0.922	0.989	0.721	0.235
<i>M. griseorufus</i>	<i>M. coquereli</i>	0.975	0.934	0.998	0.629	0.367
<i>M. murinus</i> (Kirindy)	<i>M. coquereli</i>	0.979	0.939	0.987	0.830	0.388
<i>M. murinus</i> (distant)	<i>M. coquereli</i>	0.978	0.936	0.993	0.738	0.388
<i>M. griseorufus</i>	<i>C. medius</i>	0.954	0.902	0.992	0.578	0.419
<i>M. berthae</i>	<i>M. coquereli</i>	0.966	0.936	0.991	0.537	0.432
<i>M. rufus</i>	<i>M. coquereli</i>	0.982	0.939	0.992	0.801	0.435
<i>C. medius</i>	<i>M. coquereli</i>	0.959	0.904	0.988	0.655	0.436
<i>M. murinus</i> (Kirindy)	<i>C. medius</i>	0.960	0.907	0.985	0.671	0.440
<i>M. murinus</i> (distant)	<i>C. medius</i>	0.959	0.903	0.990	0.651	0.440
<i>M. berthae</i>	<i>C. medius</i>	0.940	0.908	0.989	0.397	0.483
<i>M. rufus</i>	<i>C. medius</i>	0.947	0.906	0.989	0.495	0.486
DQB						
<i>M. murinus</i> (Kirindy)	<i>M. berthae</i>	0.962	0.777	0.98	0.909	0.232
<i>M. murinus</i> (Kirindy)	<i>M. coquereli</i>	0.943	0.664	0.972	0.907	0.388
<i>M. murinus</i> (Kirindy)	<i>C. medius</i>	0.917	0.762	0.977	0.719	0.440
<i>M. berthae</i>	<i>M. coquereli</i>	0.939	0.661	0.978	0.878	0.432
<i>M. berthae</i>	<i>C. medius</i>	0.907	0.761	0.982	0.662	0.483
<i>C. medius</i>	<i>M. coquereli</i>	0.942	0.681	0.969	0.906	0.436

Pilosof et al., 2014), suggesting that MHC-parasite associations may often occur beyond the host species level. However, with only four lemur species, we lacked statistical power to reach significance. In addition to our small sample size, our noninvasive gut-parasite screening only captured a subset of host parasite communities, which encompass a variety of other extracellular parasites and pathogens.

The possession of a particular supertype (SR4) appeared to be positively associated with infestation by a common helminth (*Hymenolepis* sp., Cestoda) in the two *Microcebus* sp. Such MHC-parasite associations, shared by two closely related species (diverged less than ca 9 Mya, Thiele et al., 2013), might reflect the long-term maintenance of particular allelic motifs, rather than convergent evolution occurring post-speciation. Association of particular allelic motifs with infestation by particular helminths has already been described in

more distantly related *M. murinus* and *C. medius*, where *Mimu*-DRB*28 was associated with increased risk of *Ascaris* sp. infection and showed a functional overlap with a particular *Chme*-DRB sequence (Schwensow, Dausmann et al., 2010). In our study, *Mimu*-DRB*28 was grouped into SR4 which appeared to be associated with an increased risk of infestation by *Hymenolepis* sp. in the two *Microcebus* sp. Although the mechanism underlying such positive associations between a MHC supertype and a particular species is unknown, it may also reflect the outcome of parasite-mediated selection. Here, individuals carrying other superotypes may show an excess parasite-induced mortality than individuals carrying SR4, generating the observed association at the time of sampling. Indeed the higher frequency of SR4 in the population of the most abundant *M. murinus* may be an indication of higher survival rate of individuals carrying this allele. Moreover, the possession of a particular DQB

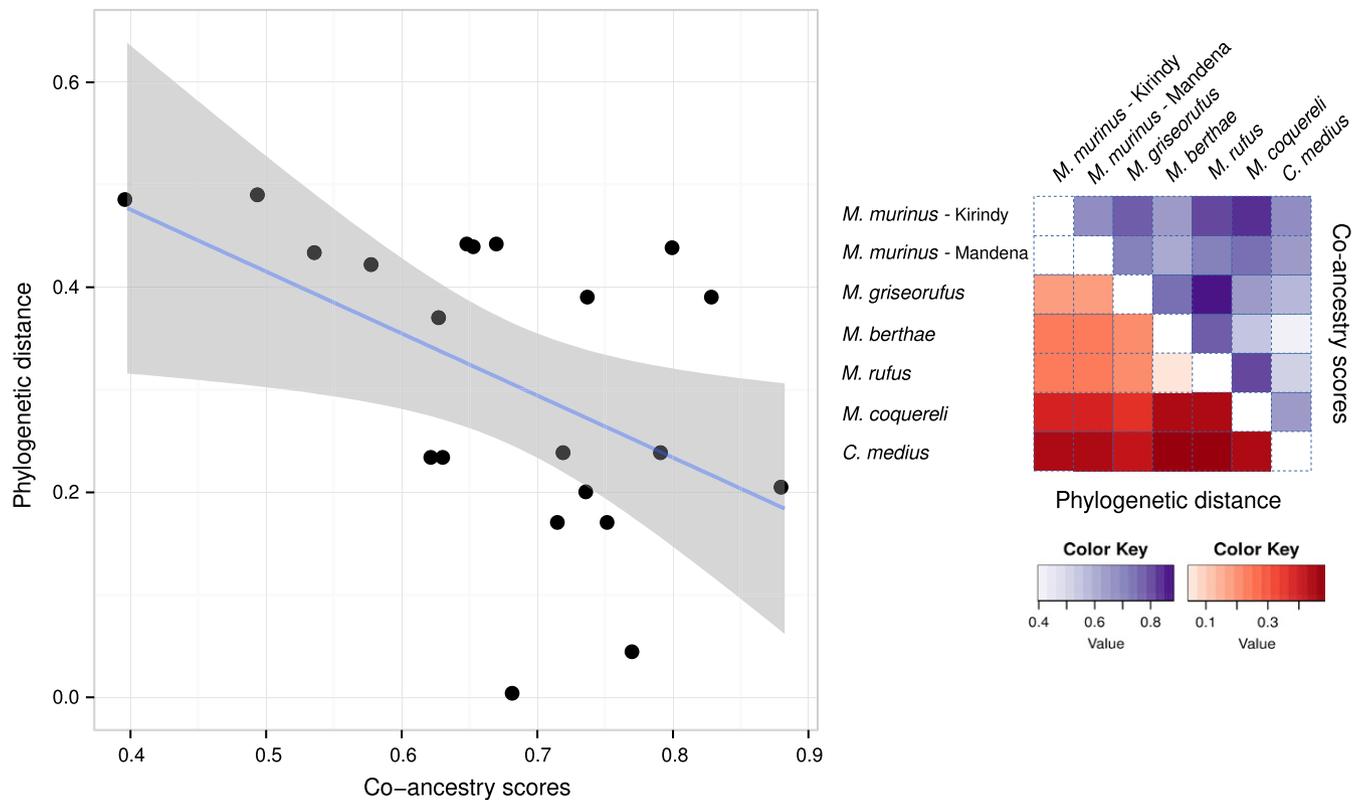


FIGURE 4 The relative contribution of convergent evolution and co-ancestry to MHC-DRB allelic functional similarity in six lemur species (*Cheirogaleidae*) that exhibit various levels of phylogenetic relatedness (left). Co-ancestry scores were derived from the proportions of identical codons at positively selected sites ‘PSS’, calculated in a pairwise fashion among sequences of all pairs of species, in relation to the simulated distributions of codon usage expected under convergent evolution and co-ancestry. Phylogenetic relationships among species were derived from a phylogenetic tree based on 1,140 bp of cytochrome b mitochondrial gene sequences. Heatmap (right) illustrates co-ancestry scores and phylogenetic distances obtained for each species pair

supertype was associated with multiple infestations in the two *Microcebus* sp., indicating that certain alleles or allelic motifs may be associated with multiple parasites. The disadvantageous character of some MHC alleles in connection to parasite prevalence (e.g., Hendel et al., 1999; Segal & Hill, 2003; Schad, Ganzhorn, & Sommer, 2005; Bonneaud, Pérez-Tris, Federici, Chastel, & Sorci, 2006; Westerdahl, Stjernman, Råberg, Lannefors, & Nilsson, 2013) has been interpreted as support for the “rare allele advantage hypothesis,” further enhanced by antagonistic effects of some alleles that can explain their persistence and frequency shifts in the population (Apanius, Penn, Slev, Ruff, Potts, 1997; Loiseau et al., 2008; 2011; Froeschke & Sommer, 2012).

While intestinal helminths seem to possess a relatively high diversity of protein-encoding genes (Pearce & Tarlton, 2002), the immune responses of mammalian hosts inducing a CD4⁺ T helper cell type 2 (Th2) cytokine response (Finkelman et al., 2004; Perigou, Marshall, & Artis, 2008; Allen & Maizels, 2011) seem to vary very little. Host immune systems may therefore only have a limited ability to distinguish among different helminth species (Finkelman et al., 2004). Some alleles (or supertypes) may have a generalist profile (see also Wegner et al., 2003; Tollenaere et al., 2008; Oliver, Telfer, & Piertney, 2009; Froeschke & Sommer, 2012; Pilosof et al.,

2014; Tobler et al., 2014) by interacting directly with “ubiquitous” antigenic peptides, which may be part of molecules underlying the basic architecture or physiology of many parasites, in a similar way as large-spectrum antibiotic or antiparasitic agents may inhibit or kill organisms belonging to broad taxonomic groups. Such “broad-spectrum” supertypes may then confer protection against several parasites from any parasite community and would consequently likely be under stabilizing selection. They may rarely undergo the drastic drops in frequency expected under frequency-dependent selection and may easily be retained during speciation events.

More generally, envisioning MHC–parasite interactions at the community level may have profound implications for our conceptual understanding of MHC–parasite dynamics. For example, the lack of host-specificity of many parasites, together with the sharing of MHC supertypes among several species, may largely disrupt or inhibit the co-evolutionary cycles (Pilosof et al., 2014; Tobler et al., 2014) that have long been assumed to occur under negative frequency-dependent selection (Slade & McCallum, 1992). Indeed, theory proposes that parasites may often evolve resistance against MHC alleles (or supertypes) that rise in frequency in the host population, and these alleles may subsequently drop in frequency after losing their advantage. Empirical data supporting such theory are difficult to gather

given that such cycles occur at the population level over evolutionary scales so that relevant tests would involve extensive sampling over multiple generations. As a result, this scenario remains essentially theoretical. As envisaged by Pilosof et al. (2014), a multihost perspective may however cast doubts on its generality, because both MHC alleles and parasites that are common in one host may be rare in others, so changes in MHC–parasite interactions may commonly occur at the community, rather than at the single population scale. It could therefore be beneficial to move from the population level framework prevailing in the evolutionary literature on MHC towards more integrative perspectives envisaging MHC–host–parasite dynamics at the community level.

Finally, we explored functional similarity among MHC alleles in species with gradual phylogenetic relatedness. A higher similarity at non-PSS of MHC alleles belonging to the same vs. different super-types indicated that their similarity arose from co-ancestry. Further support for the co-ancestry of shared alleles comes from the codon usage analysis. This analysis showed that when different species exhibited shared amino acids in the anticipated antigen-binding groove of their MHC molecules, these amino acids showed high codon similarity congruent with the levels expected under the co-ancestry scenario, suggesting that sequences of orthologous alleles show signs of co-ancestry even at PSS. This implies that functional similarity is largely inherited, and arises from the long-term retention of alleles or allelic motifs across multiple speciation events, rather than from independent evolutionary pathways. This pattern echoes previous observations in two stickleback species that diverged at least 7 million years ago (Mya) (Bell & Foster, 1994; Lenz et al., 2013) and six flamingo species that diverged ca. 3–6 Mya (Torres, Ogawa, Gillingham, Ferrari, & Van Tuinen, 2014; Gillingham et al., 2016). Moreover, co-ancestry scores for DRB were significantly correlated with phylogenetic distances among species, suggesting that functionally similar alleles are progressively lost as species diverge.

It has been argued that alternative explanations to co-ancestry and convergent evolution may generate the observed patterns. For example, allelic introgression through hybridization, or gene conversion and recombination, may produce structurally and functionally similar alleles across species (Wegner & Eizaguirre, 2012). However, allelic introgression is rather unlikely to affect patterns observed in *Cheirogaleidae* because no recent hybridization is known to occur in the clade, except for the distant populations of *M. murinus* and *M. griseorufus* (Gligor et al., 2009; Sommer et al., 2014). We did not detect cross-specific sharing of identical DRB alleles among the four sympatric members of the *Cheirogaleidae* studied here, nor with sequences published previously, in line with other studies focusing on wild lemur populations (e.g., Schad et al., 2005; Schwensow, Eberle et al., 2010; Schwensow, Dausmann et al., 2010; Huchard et al., 2012; Grogan, McGinnis, Sauther, Cuozzo, & Drea, 2016), except for one instance where hybridization is known to occur between two *Microcebus* sp. (Sommer et al., 2014). In contrast, a study based largely on a captive colony of lemurs described identical DRB alleles, both within genus and across families that diverged up to 40 Mya, and attributed this finding to trans-species polymorphism

(Go et al. 2002). The evolutionary mechanism through which entire allelic fragments (i.e., 202 bp in Go et al. 2002) could remain strictly identical across multiple speciation events is unclear, as our results show that only short sequence motives were preserved across comparable time scales. Alternative explanations for the findings of Go et al. (2002), such as hybridization, cannot be ruled out, especially given that in most cases only 1–5 individuals were genotyped per species and that nearly all sampled specimens (22 of 24 species) came from the same zoo.

The operation of convergent evolution has rarely been shown and may predominantly explain allelic similarity limited to coding regions among species that split at least 30 Mya (e.g., human and bovine: Andersson et al., 1991; primates and rodent: Yeager, Kumar, & Hughes, 1997; humans and New-world monkeys: Kriener et al., 2001; skunks and raccoons: Srithayakumar et al., 2012). Although the split between *C. medius* and other *Cheirogaleidae* is close to 30 Mya (19–26 Mya), in contrast to divergence times estimated within *Microcebus* sp. (7–11 Mya) and between *M. coquereli* and *Microcebus* sp. (12–18 Mya) (Thiele et al., 2013), we did not find strong support for allelic similarity by convergent evolution in response to shared parasite pressures. Additionally, balancing selection has generally been found to homogenize, rather than diversify, MHC alleles in sympatric species after speciation, despite differences in parasite communities and an erosion of background genomic diversity (e.g., Fraser & Neff, 2010; Tobler et al., 2014). Our study adds to an emerging body of evidence showing that trans-species polymorphism primarily reflects the long-term maintenance of MHC alleles, even among species that diverged about 30 Mya ago, suggesting that some polymorphisms may survive over million years and across multiple speciation events.

In conclusion, our results show that parasite communities are largely shared by sympatric lemurs. Functionally similar MHC alleles (supertypes), or allelic motifs, may affect susceptibility to similar parasites, and some supertypes are associated with a higher risk of multiple parasitic infections across several host species. Moreover, we show that allelic similarity at coding, as well as noncoding, sites resulted from the long-term maintenance of ancestral sequence motifs or allelic lineages across speciation events, for periods of time encompassing up to 20–30 million years. These results have at least two important implications. First, they contribute to a growing body of evidence suggesting that the evolutionary ecology of MHC should proceed from the population level framework towards a more integrative, multihost and multi-infection perspective. This permits us to revisit some of the fundamental mechanisms envisioned to explain the exceptional MHC polymorphism. Second, they imply that MHC polymorphism is a cumulative capital, gathered through the interactions between multiple hosts and diverse parasite communities over evolutionary times and passed on during speciation events, a richness which may only be slowly recovered when eroded, and should become a priority focus for conservation.

ACKNOWLEDGEMENTS

We would like to thank all people who have contributed to the collection and processing of DNA samples, especially to the Kirindy

field assistants, Melanie Dammhahn, Claudia Fichtel and Christina Glaschke. We also thank the Département de Biologie Animale, Université d'Antananarivo, the CAFF of the Direction des Eaux et Forêts and the CNFEREF Morondava for granting a research permit. Special thanks go to Prof. Lutz Walter, Dr. Christian Roos and Nico Westphal for the help with sequencing. This research was funded by a SAW project of Leibniz Graduate School for the Foundations of Primate Social Behaviour issued by Wissenschaftsgemeinschaft Gottfried Wilhelm Leibniz e.V. (SAW-2011-DPZ-4) and field work was supported by the Deutsches Primatenzentrum GmbH - Leibniz-Institut für Primatenforschung and DFG grant (Ka1082/10-1&2). ISEM contribution n°ISEM 2017-146.

DATA ACCESSIBILITY

DNA sequences: Genbank accessions LT627130-LT627190. Full sequence assembly used in this study is presented in Appendix S2. Raw genotypes and parasite counts are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.dv415>.

AUTHOR CONTRIBUTIONS

DNA and parasitological samples were collected, MHC genotyping, parasitological screening and data analysis were performed, and manuscript written by E.K. Processing of raw NGS data was helped by M.B. Parasitological screening was performed by J.D. and J.H.R. Contribution to the acquisition of parasitological data and DNA samples were carried out by J.H.R., A.M.H. & C.K. Contribution to the funding, field logistics and research design was carried out by P.M.K. and contribution to the sample collection, field logistics, MHC genotyping, data analysis and research design was carried out by E.H. All authors contributed to the manuscript drafting.

ORCID

Eva Kaesler  <http://orcid.org/0000-0002-4348-7459>

REFERENCES

- Allen, J. E., & Maizels, R. M. (2011). Diversity and dialogue in immunity to helminths. *Nature Reviews Immunology*, *11*, 375–388.
- Andersson, L., Sigurdardottir, S., Borsch, C., & Gustafsson, K. (1991). Evolution of MHC polymorphism—extensive sharing of polymorphic sequence motifs between human and bovine DRB alleles. *Immunogenetics*, *33*, 188–193.
- Apanius, V., Penn, D., Slev, P., Ruff, L. R., & Potts, W. K. (1997). The nature of selection on the major histocompatibility complex. *Critical Review of Immunology*, *17*, 179–224.
- Azevedo, L., Serrano, C., Amorim, A., & Cooper, D. N. (2015). Trans-species polymorphism in humans and the great apes is generally maintained by balancing selection that modulates the host immune response. *Human Genomics*, *9*, 21.
- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-9, <https://CRAN.R-project.org/package=lme4>.
- Bell, M. A., & Foster, S. A. (1994). *The evolutionary biology of the three-spine stickleback*. Oxford, UK: Oxford University Press.
- Bernatchez, L., & Landry, C. (2003). MHC studies in nonmodel vertebrates: What have we learned about natural selection in 15 years? *Journal of Evolutionary Biology*, *16*, 363–377.
- Bodmer, W. F. (1972). Evolutionary significance of the HL-A system. *Nature*, *237*, 139–183.
- 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*, 383–389.
- Chen, H. W., Liu, W. C., Davis, A. J., Jordán, F., Hwang, M. J., & Shao, K. T. (2008). Network position of hosts in food webs and their parasite diversity. *Oikos*, *117*, 1847–1855.
- Cringoli, G., Rinaldi, L., Maurelli, M. P., & Utzinger, J. (2010). FLOTAC: New multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nature protocols*, *5*, 503–515.
- Dammhahn, M., & Kappeler, P. M. (2005). Social system of *Microcebus berthae*, the world's smallest primate. *International Journal of Primatology*, *26*, 407–435.
- Dammhahn, M., & Kappeler, P. M. (2014). Stable isotope analyses reveal dense trophic species packing and clear niche differentiation in a Malagasy primate community. *American Journal of Physical Anthropology*, *153*, 249–259.
- Danon, L., Diaz-Guilera, A., Duch, J., & Arenas, A. (2005). Comparing community structure identification. *Journal of Statistical Mechanics: Theory and Experiment*, *9*, P09008.
- Doherty, P. C., & Zinkernagel, R. M. (1975). Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature*, *256*, 50–52.
- Doytchinova, I. A., & Flower, D. R. (2005). In silico identification of super-types for class II MHCs. *Journal of Immunology*, *174*, 7085–7095.
- Eberle, M., & Kappeler, P. (2002). Mouse lemurs in space and time: A test of the socioecological model. *Behavioral Ecology and Sociobiology*, *51*, 131–139.
- Eberle, M., & Kappeler, P. M. (2006). Family insurance: Kin selection and cooperative breeding in a solitary primate (*Microcebus murinus*). *Behavioral Ecology and Sociobiology*, *60*, 582–588.
- Eimes, J. A., Townsend, A. K., Sepil, I., Nishiumi, I., & Satta, Y. (2015). Patterns of evolution of MHC class II genes of crows (*Corvus*) suggest trans-species polymorphism. *PeerJ*, *3*, e853.
- Fietz, J. (1999a). Monogamy as a rule rather than exception in nocturnal lemurs: The case of the fat tailed dwarf lemur, *Cheirogaleus medius*. *Ethology*, *105*, 255–272.
- Fietz, J. (1999b). Demography and floating males in a population of *Cheirogaleus medius*. In B. Rakotosamimanana, H. Rasaminanana, & J. U. Ganzhorn (Eds.), *New directions in lemur studies* (pp. 159–172). New York, NY: Kluwer Academic, Plenum Publishers.
- Figuerola, F., Günther, E., & Klein, J. (1988). MHC polymorphism pre-dating speciation. *Nature*, *335*, 265–267.
- Finkelman, F. D., Shea-Donohue, T., Morris, S. C., Gildea, L., Strait, R., Madden, K. B., ... Urban, J. F. (2004). Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. *Immunological Reviews*, *201*, 139–155.
- Fraser, B. A., & Neff, B. D. (2010). Parasite mediated homogenizing selection at the MHC in guppies. *Genetica*, *138*, 273–278.
- Froeschke, G., & Sommer, S. (2005). MHC class II DRB variability and parasite load in the striped mouse (*Rhabdomys pumilio*) in the Southern Kalahari. *Molecular Biology and Evolution*, *22*, 1254–1259.
- 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*, e31820.
- Froeschke, G., & Sommer, S. (2014). Role of selection versus neutral processes determining genetic variation in a small mammal along a

- climatic gradient in southern Africa. *Evolutionary Ecology*, 28, 1169–1190.
- Garamszegi, L. Z., & Nunn, C. L. (2011). Parasite-mediated evolution of the functional part of the MHC in primates. *Journal of Evolutionary Biology*, 24, 184–195.
- Garrigan, D., & Hedrick, P. W. (2003). Perspective: Detecting adaptive molecular polymorphism: Lessons from the mhc. *Evolution*, 57, 1707–1722.
- Gillingham, M. A., 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, 438–454.
- Gligor, M., Ganzhorn, J. U., Rakotondravony, D., Ramilijaona, O. R., Razafimahatratra, E., Zischler, H., & Hapke, A. (2009). Hybridization between mouse lemurs in an ecological transition zone in southern Madagascar. *Molecular Ecology*, 18, 520–533.
- Go, Y., Satta, Y., Kawamoto, Y., Rakotoarisoa, G., Randrianjafy, A., Koyama, N., & Hirai, H. (2002). Mhc-DRB genes evolution in lemurs. *Immunogenetics*, 54, 403–417.
- Godfrey, S. S. (2013). Networks and the ecology of parasite transmission: a framework for wildlife parasitology. *International Journal for Parasitology: Parasites and Wildlife*, 2, 235–245.
- Goüy de Bellocq, J., Charbonnel, N., & Morand, S. (2008). Coevolutionary relationship between helminth diversity and MHC class II polymorphism in rodents. *Journal of Evolutionary Biology*, 21, 1144–1150.
- Graser, R., Oh'Uigin, C., Vincek, V., Meyer, A., & Klein, J. (1996). Trans-species polymorphism of class II MHC loci in Danio fishes. *Immunogenetics*, 44, 36–48.
- Groeneveld, L. F., Rasoloarison, R. M., Weisrock, D., & Kappeler, P. M. (2008). Dwarf lemurs in space and time: Phylogeography of the genus *Cheirogaleus*. In L. Groeneveld (Ed.), *Species delimitation, phylogeography and population genetics of the endemic Malagasy dwarf lemurs (genus Cheirogaleus)*. Göttingen, Göttingen: PhD thesis, Georg-August Universität.
- Groeneveld, L. F., Weisrock, D. W., Rasoloarison, R. M., Yoder, A. D., & Kappeler, P. M. (2009). Species delimitation in lemurs: Multiple genetic loci reveal low levels of species diversity in the genus *Cheirogaleus*. *BMC Evolutionary Biology*, 9, 30.
- Grogan, K. E., McGinnis, G. J., Sauther, M. L., Cuozzo, F. P., & Drea, C. M. (2016). Next-generation genotyping of hypervariable loci in many individuals of a non-model species: Technical and theoretical implications. *BMC Genomics*, 17, 204.
- Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PHYLX 3.0. *Systematic Biology*, 59, 307–321.
- Gustafsson, K., & Andersson, L. (1994). Structure and polymorphism of horse MHC class-II DRB genes: Convergent evolution in the antigen-binding site. *Immunogenetics*, 39, 355–358.
- Hämäläinen, A., Raharivololona, B., Ravoniarimbinina, P., & Kraus, C. (2015). Host sex and age influence endoparasite burdens in the gray mouse lemur. *Frontiers in Zoology*, 12, 25.
- Hendel, H., Caillat-Zucman, S., Lebuane, H., Carrington, M., O'Brien, S., Andrieu, J. M., ... Zagury, J. F. (1999). New class I and II HLA alleles strongly associated with opposite patterns of progression to AIDS. *Journal of Immunology*, 162, 6942–6946.
- Hill, A. V. S., Allsopp, C. E. M., Kwiatkowski, D., Anstey, N. M., Twumasi, P., Rowe, P. A., ... Greenwood, B. M. (1991). Common West African HLA antigens are associated with protection from severe malaria. *Nature*, 352, 595–600.
- Huchard, E., Albrecht, C., Schliehe-Diecks, S., Baniel, A., Roos, C., Peter, P. M., & Brameier, M. (2012). Large-scale MHC class II genotyping of a wild lemur population by next generation sequencing. *Immunogenetics*, 64, 895–913.
- Huchard, E., Baniel, A., Schliehe-Diecks, S., & Kappeler, P. M. (2013). MHC-disassortative mate choice and inbreeding avoidance in a solitary primate. *Molecular Ecology*, 22, 4071–4086.
- Hughes, A. L., & Nei, M. (1988). Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. *Nature*, 335, 167–170.
- Hughes, J., & Page, R. D. (2007). Comparative tests of ectoparasite species richness in seabirds. *BMC Evolutionary Biology*, 7, 227.
- Irwin, M. T., & Raharison, J. L. (2009). A review of the endoparasites of the lemurs of Madagascar. *Malagasy Nature*, 2, 66–93.
- Jeffery, K. J. M., & Bangham, C. R. M. (2000). Do infectious diseases drive MHC diversity? *Microbes and Infection*, 2, 1335–1341.
- 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, 94.
- Kappeler, P. M., & Fichtel, C. (2012). A 15-year perspective on the social organization and life history of sifaka in Kirindy Forest. In P. M. Kappeler, D. P. Watts (Eds.), *Long term field studies of primates* (pp. 101–121). Berlin, Heidelberg: Springer-Verlag.
- Kappeler, P. M., Wimmer, B., Zinner, D., & Tautz, D. (2002). The hidden matrilineal structure of a solitary lemur: Implications for primate social evolution. *Proceedings of the Royal Society of London B: Biological Sciences*, 269, 1755–1763.
- Klein, J. (1987). Origin of major histocompatibility complex polymorphism: The trans-species hypothesis. *Human Immunology*, 19, 155–162.
- Klein, D., Ono, H., O'Huigin, C., Vincek, V., Goldschmidt, T., & Klein, J. (1993). Extensive MHC variability in cichlid fishes of Lake Malawi. *Nature*, 364, 330–334.
- Klein, J., Sato, A., & Nikolaidis, N. (2007). MHC, TSP, and the origin of species: From immunogenetics to evolutionary genetics. *Annual Review of Genetics*, 41, 281–304.
- Klein, J., Satta, Y., O'Huigin, C., & Takahata, N. (1993a). The molecular descent of the major histocompatibility complex. *Annual Review of Immunology*, 11, 269–295.
- Kriener, K., O'Huigin, C., & Klein, J. (2001). Independent origin of functional MHC class II genes in humans and New World monkeys. *Human Immunology*, 62, 1–14.
- Kriener, K., O'Huigin, C., Tichy, H., & Klein, J. (2000). Convergent evolution of major histocompatibility complex molecules in humans and New World monkeys. *Immunogenetics*, 51, 169–178.
- 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, 2400–2412.
- Loiseau, C., Zoorob, R., Garnier, S., Birard, J., Federici, P., Julliard, R., & Sorci, G. (2008). Antagonistic effects of a MHC class I allele on malaria-infected house sparrows. *Ecology Letters*, 11, 258–265.
- Lund, O., Nielsen, M., Kesmir, C., Petersen, A. G., Lundegaard, C., Wornig, P., ... Brunak, S. (2004). Definition of supertypes for HLA molecules using clustering of specificity matrices. *Immunogenetics*, 55, 797–810.
- Lundberg, A. S., & McDevitt, H. O. (1992). Evolution of major histocompatibility complex class II allelic diversity: Direct descent in mice and humans. *Proceedings of the National Academy of Sciences of the United States of America*, 89, 6545–6549.
- Markolf, M., Roos, C., & Kappeler, P. (2008). Genetic and demographic consequences of a rapid reduction in population size in a solitary lemur (*Mirza coquereli*). *The Open Conservation Biology Journal*, 2, 21–29.
- McClelland, E. E., Penn, D. J., & Potts, W. K. (2003). Major histocompatibility complex heterozygote superiority during coinfection. *Infection and Immunity*, 71, 2079–2086.

- McConnell, T. J., Talbot, W. S., McIndoe, R. A., & Wakeland, E. K. (1988). The origin of MHC class II gene polymorphism within the genus *Mus*. *Nature*, *332*, 651–654.
- Morand, S. (2015). (macro-) Evolutionary ecology of parasite diversity: From determinants of parasite species richness to host diversification. *International Journal for Parasitology: Parasites and Wildlife*, *4*, 80–87.
- Mundry, R., & Nunn, C. L. (2009). Stepwise model fitting and statistical inference: Turning noise into signal pollution. *American Naturalist*, *173*, 119–123.
- Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., ... Wagner, H. (2015) VEGAN: community ecology package. R package version 2.3-0. <http://CRAN.R-project.org/package=vegan>
- Oliver, M. K., Telfer, S., & Piertney, S. B. (2009). Major histocompatibility complex (MHC) heterozygote superiority to natural multi-parasite infections in the water vole (*Arvicola terrestris*). *Proceedings of the Royal Society of London B: Biological Sciences*, *276*, 1119–1128.
- Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*, 289–290.
- Pearce, E. J., & Tarlton, R. L. (2002). Overview of the parasitic pathogens. In S. H. E. Kaufmann, A. Sher, & R. Ahmed (Eds.), *Immunology of infectious diseases* (pp. 39–52). Washington, DC: ASM Press.
- Pechouskova, E., Dammhahn, M., Brameier, M., Fichtel, C., Kappeler, P. M., & Huchard, E. (2015). MHC class II variation in a rare and ecological specialist mouse lemur reveals lower allelic richness and contrasting selection patterns compared to a generalist and widespread sympatric congener. *Immunogenetics*, *67*, 229–245.
- Perrigou, J. G., Marshall, F. A., & Artis, D. (2008). On the hunt for helminths: Innate immune cells in the recognition and response to helminth parasites. *Cellular Microbiology*, *10*, 1757–1764.
- Piertney, S. B., & Oliver, M. K. (2006). The evolutionary ecology of the major histocompatibility complex. *Heredity*, *96*, 7–21.
- Pilosof, S., Fortuna, M. A., Cosson, J. F., Galan, M., Kittipong, C., Ribas, A., ... Bascompte, J. (2014). Host-parasite network structure is associated with community-level immunogenetic diversity. *Nature Communications*, *5*, 5172.
- Prugnolle, F., Manica, A., Charpentier, M., Guégan, J. F., Guernier, V., & Balloux, F. (2005). Pathogen-driven selection and worldwide HLA class I diversity. *Current Biology*, *15*, 1022–1027.
- R Core Team. (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Raharivololona, B., & Ganzhorn, J. (2009). Gastrointestinal parasite infection of the Gray mouse lemur (*Microcebus murinus*) in the littoral forest of Mandena, Madagascar: Effects of forest fragmentation and degradation. *Madagascar Conservation & Development*, *4*, 103–112.
- Raharivololona, B., & Ganzhorn, J. (2010). Seasonal variations in gastrointestinal parasites excreted by the gray mouse lemur *Microcebus murinus* in Madagascar. *Endangered Species Research*, *11*, 113–122.
- Rammensee, H. G., Friede, T., & Stevanović, S. (1995). MHC ligands and peptide motifs: First listing. *Immunogenetics*, *41*, 178–228.
- Ritchie, L. S. (1948). An ether sedimentation technique for routine stool examinations. *Bulletin of the 639 US Army Medical Department United States Army Medical Department*, *8*, 326.
- 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*, 2481–2491.
- 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*, e37101.
- Schad, A. J., Ganzhorn, J. U., & Sommer, S. (2005). Parasite burden and constitution of major histocompatibility complex in the malagasy mouse lemur, *Microcebus murinus*. *Evolution*, *59*, 439–450.
- Schwensow, N., Axtner, J., & Sommer, S. (2011). Are associations of immune gene expression, body condition and parasite burden detectable in nature? A case study in an endemic rodent from the Brazilian Atlantic forest. *Infection, Genetics and Evolution*, *11*, 23–30.
- Schwensow, N., Dausmann, K., Eberle, M., Fietz, J., & Sommer, S. (2010). Functional associations of similar MHC alleles and shared parasite species in two sympatric lemurs. *Infection, Genetics and Evolution*, *10*, 662–668.
- Schwensow, N., Eberle, M., & Sommer, S. (2010). Are there ubiquitous parasite-driven major histocompatibility complex selection mechanisms in gray mouse lemurs? *International Journal of Primatology*, *31*, 519–537.
- Schwensow, N., Fietz, J., Dausmann, K. H., & Sommer, S. (2007). Neutral versus adaptive genetic variation in parasite resistance: Importance of major histocompatibility complex supertypes in a free-ranging primate. *Heredity*, *99*, 265–277.
- Segal, S., & Hill, A. V. S. (2003). Genetic susceptibility to infectious disease. *Trends in Microbiology*, *11*, 445–448.
- Sepil, I., Lachish, S., Hinks, A. E., & Sheldon, B. C. (2013). MHC superotypes confer both qualitative and quantitative resistance to avian malaria infections in a wild bird population. *Proceedings of the Royal Society of London B: Biological Sciences*, *280*, 20130134.
- Sepil, I., Moghadam, H. K., Huchard, E., & Sheldon, B. C. (2012). Characterization and 454 pyrosequencing of major histocompatibility complex class I genes in the great tit reveal complexity in a passerine system. *BMC Evolutionary Biology*, *12*, 68.
- Sette, A., & Sidney, J. (1999). Nine major HLA class I supertypes account for the vast preponderance of HLA-A and -B polymorphism. *Immunogenetics*, *50*, 201–212.
- Slade, R. W., & McCallum, H. I. (1992). Overdominant vs. frequency-dependent selection at MHC loci. *Genetics*, *132*, 861–862.
- Snell, G. D. (1968). The H-2 locus of the mouse: Observations and speculations concerning its comparative genetics and its polymorphism. *Folia Biologica (Praha)*, *14*, 335–358.
- Sommer, S., Rakotondranary, S. J., & Ganzhorn, J. U. (2014). Maintaining microendemic primate species along an environmental gradient—parasites as drivers for species differentiation. *Ecology and Evolution*, *4*, 4751–4765.
- Southwood, S., Sidney, J., Kondo, A., del Guercio, M. F., Appella, E., Hoffman, S., ... Sette, A. (1998). Several common HLA-DR types share largely overlapping peptide binding repertoires. *The Journal of Immunology*, *160*, 3363–3373.
- Speed, J. D. M., Cooper, E. J., Jónsdóttir, I. S., Van Der Wal, R., & Woodin, S. J. (2010). Plant community properties predict vegetation resilience to herbivore disturbance in the Arctic. *The Journal of Ecology*, *98*, 1002–1013.
- Spurgin, L. G., & Richardson, D. S. (2010). How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. *Proceedings of the Royal Society of London B: Biological Sciences*, *277*, 979–988.
- Srithayakumar, V., Castillo, S., Mainguy, J., & Kyle, C. J. (2012). Evidence for evolutionary convergence at MHC in two broadly distributed mesocarnivores. *Immunogenetics*, *64*, 289–301.
- Tamás, J., Podani, J., & Csontos, P. (2001). An extension of presence/absence coefficients to abundance data: A new look at absence. *Journal of Vegetation Science*, *12*, 401–410.
- Tamura, K., Stecher, G., Peterson, D., Filipiński, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*, 2725–2729.
- Tavaré, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences*, *17*, 57–86.

- Těšický, M., & Vinkler, M. (2015). Trans-species polymorphism in immune genes: General pattern or MHC-restricted phenomenon? *Journal of Immunology Research*, 2015, 1–10.
- Thiele, D., Razafimahatratra, E., & Hapke, A. (2013). Discrepant partitioning of genetic diversity in mouse lemurs and dwarf lemurs—Biological reality or taxonomic bias? *Molecular Phylogenetics and Evolution*, 69, 593–609.
- Tobler, M., Plath, M., Riesch, R., Schlupp, I., Grasse, A., Munimanda, G. K., ... Moodley, Y. (2014). Selection from parasites favours immunogenetic diversity but not divergence among locally adapted host populations. *Journal of Evolutionary Biology*, 27, 960–974.
- Tollenaere, C., Bryja, J., Galan, M., Cadet, P., Deter, J., Chaval, Y., ... Charbonnel, N. (2008). Multiple parasites mediate balancing selection at two MHC class II genes in the fossorial water vole: Insights from multivariate analyses and population genetics. *Journal of Evolutionary Biology*, 21, 1307–1320.
- Torres, C. R., Ogawa, L. M., Gillingham, M. A., Ferrari, B., & Van Tuinen, M. (2014). A multi-locus inference of the evolutionary diversification of extant flamingos (*Phoenicopteridae*). *BMC Evolutionary Biology*, 14, 1.
- Trachtenberg, E., Korber, B., Sollars, C., Kepler, T. B., Hraber, P. T., Hayes, E., ... Wolinsky, S. (2003). Advantage of rare HLA supertype in HIV disease progression. *Nature Medicine*, 9, 928–935.
- Trowsdale, J. (1993). Genomic structure and function in the MHC. *Trends in Genetics*, 9, 117–122.
- Trtkova, K., Mayer, W. E., O'Huigin, C., & Klein, J. (1995). MHC-DRB genes and the origin of New World monkeys. *Molecular Phylogenetics and Evolution*, 4, 408–419.
- Vitone, N. D., Altizer, S., & Nunn, C. L. (2004). Body size, diet and sociality influence the species richness of parasitic worms in anthropoid primates. *Evolutionary Ecology Research*, 6, 183–199.
- Wegner, K. M., & Eizaguirre, C. (2012). New(t)s and views from hybridizing MHC genes: Introgression rather than trans-species polymorphism may shape allelic repertoires. *Molecular Ecology*, 21, 779–781.
- Wegner, K. M., Reusch, T. B. H., & Kalbe, M. (2003). Multiple parasites are driving major histocompatibility complex polymorphism in the wild. *Journal of Evolutionary Biology*, 16, 224–232.
- Weisrock, D. W., Rasoloarison, R. M., Fiorentino, I., Ralison, J. M., Goodman, S. M., Kappeler, P. M., & Yoder, A. D. (2010). Delimiting species without nuclear monophyly in Madagascar's mouse lemurs. *PLoS ONE*, 5, e9883.
- Westerdahl, H., Stjernman, M., Råberg, L., Lannefors, M., & Nilsson, J.-Å. (2013). MHC-I affects infection intensity but not infection status with a frequent avian malaria parasite in blue tits. *PLoS ONE*, 8, e72647.
- Whittingham, M. J., Stephens, P. A., Bradbury, R. B., & Freckleton, R. P. (2006). Why do we still use stepwise modelling in ecology and behaviour? *Journal of Animal Ecology*, 75, 1182–1189.
- Yang, Z. (2007). PAML 4: Phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution*, 24, 1586–1591.
- Yang, Z., Nielsen, R., Goldman, N., & Pedersen, A. M. (2000). Codon-substitution models for heterogeneous selection pressure at amino acid sites. *Genetics*, 155, 431–449.
- Yang, Z., Wong, W. S. W., & Nielsen, R. (2005). Bayes empirical bayes inference of amino acid sites under positive selection. *Molecular Biology and Evolution*, 22, 1107–1118.
- Yeager, M., & Hughes, A. L. (1999). Evolution of the mammalian MHC: Natural selection, recombination, and convergent evolution. *Immunological Reviews*, 167, 45–58.
- Yeager, M., Kumar, S., & Hughes, A. L. (1997). Sequence convergence in the peptide-binding region of primate and rodent MHC class Ib molecules. *Molecular Biology and Evolution*, 14, 1035–1041.
- Zhang, J., & Kumar, S. (1997). Detection of convergent and parallel evolution at the amino acid sequence level. *Molecular Biology and Evolution*, 14, 527–536.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Kaesler E, Kappeler PM, Brameier M, et al. Shared evolutionary origin of major histocompatibility complex polymorphism in sympatric lemurs. *Mol Ecol*. 2017;26:5629–5645. <https://doi.org/10.1111/mec.14336>