5-2023

Past and Present Patterns of Neutral and Adaptive Genetic Diversity in Wild Mandrills (Mandrillus Sphinx)

Anna Weber
University of New Orleans, annaweber52@gmail.com

Past and Present Patterns of Neutral and Adaptive Genetic Diversity in Wild Mandrills *(Mandrillus Sphinx)*

A Dissertation

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy
in
Integrative Biology

by

Anna Weber

B.S. Alma College, 2014

May, 2023

Acknowledgement

This project would not have been possible without support from many individuals and organizations. First and foremost, I thank my advisor, Nicola Anthony, for her support and guidance over the last seven years. I also thank my collaborators, whose assistance on this research has been invaluable: Amour Guibinga Mickala, Prakhar Gahlot, Stephan Ntie, Patrick Mickala, David Lehmann, Katharine Abernethy, Jackie Lighten, and Cock van Oosterhout. I would also like to express my appreciation for the research staff and park managers at Lopé National Park, in particular Josué Edzang Ndong, Loïc Makaga, and field assistants Bridget, Arthur, and Pacôme, among others, who kept us safe in the forest and helped with sample collection. I also received much-needed assistance in the laboratory from multiple undergraduate assistants: Ibraheem Hachem, Justine Davis, Shyla Irthum, Kaleb Hill, Gina Kissee, Claire Melancon, Patrick Hall and Gabrielle Sehon. Finally, I thank my friends, family, and colleagues for their continued support and encouragement.

Financial support for these research projects was provided by the Freeport McMoran Endowed Chair awarded by the Audubon Nature Institute to Nicola Anthony, and by the University of New Orleans Office of Research. Permits for research and entry to Lopé National Park were granted by the Agence National des Parcs Nationaux (ANPN) and the Centre National de la Recherche Scientifique et Technologique (CENAREST). I sincerely thank these institutions for their support.

Table of Contents

List of Figures

Chapter 1

Chapter 2

Chapter 3

List of Tables

Chapter 1

Chapter 2

Chapter 3

Appendix

Abstract

Although primates have fascinated researchers and the public alike for generations, one species that has remained enigmatic is the mandrill (*Mandrillus sphinx*), a large Cercopithecine monkey endemic to Central Africa. Mandrills are currently in decline due to bushmeat hunting, urbanization, and habitat loss. Neutral and adaptive genetic diversity are important tools for understanding evolutionary history and future viability, since diversity influences a species' ability to adapt to a changing environment. However, thus far, minimal genetic information has been available for wild mandrills. Because of the dense vegetation in their tropical forest habitat, studying wild mandrills has proven to be a challenge, and the majority of research on this species has been performed on semi-captive populations. Here, I present findings from three studies using the first genetic data to be generated from a wild mandrill population, primarily using non-invasive sampling of feces. First, we use demographic history modeling to test for evidence of population bottlenecks in mandrills and three other forest-associated species (blue duiker [*Philantomba monticola*], Peters's duiker [*Cephalophus callipygus*], and western lowland gorillas [*Gorilla gorilla gorilla*]). Despite a severe loss of forest cover in central Africa approximately 2,500 years ago, our results suggest that none of the four species experienced major population declines. Second, we perform next-generation sequencing of the class II major histocompatibility complex (MHC), a gene family involved in adaptive immunity. We test for a difference in replicability between sequences generated from DNA extracted from feces and that extracted from higher quality tissue. We also present a new method of assigning MHC alleles to individuals using degraded samples, and we use that method to characterize the MHC genes in the focal population. Lastly, we test for sex-specific selection on the MHC in male and female mandrills. Male mandrills are thought to be more vulnerable to pathogens than females, which may result in stronger selective pressure on the MHC genes in males. Results from these three studies will contribute to our understanding of mandrill

evolutionary history and conservation by providing insight on the role of demographic processes and selective pressures in shaping their past and present populations.

Keywords: *Mandrillus sphinx*, genetic diversity, major histocompatibility complex, demographic history, Central Africa

Introduction

Central Africa's tropical rainforests are a center of biodiversity (Küper et al. 2014), home to a variety of endemic taxa (MacKinnon et al. 2017). One such species is the mandrill (*Mandrillus sphinx*), a charismatic Cercopithecine monkey found only in Gabon, Cameroon, the Congo, and Equatorial Guinea (Abernethy and Maisels 2019). Although they were originally considered to be a species of baboon, in the mid-1990s, mandrills were determined to be more closely related to mangabeys (genus *Cercocebus*) and therefore were reclassified into a new genus, *Mandrillus*, along with their sole living congener, the drill (*Mandrillus leucophaeus*) (Disotell, Honeycutt, and Ruvolo 1992). Mandrills fill an important role in the forest ecosystem as seed dispersers and as prey for leopards and pythons (Abernethy and Maisels 2019; Lahm 1986). However, like many central African species, mandrills are in decline due to habitat loss and bushmeat hunting (Abernethy et al. 2013; Abernethy and Maisels 2019). As a result, they have been categorized as Vulnerable by the International Union for the Conservation of Nature (IUCN), and international commercial trade in mandrill products is prohibited under Appendix I of the Convention on International Trade in Endangered Species (CITES).

Considerable research has been conducted in semi-captive mandrill populations on their reproductive ecology, social systems, and evolutionary history, but studies in wild populations have been limited. Much of the foundational research was undertaken in captive mandrill hordes at the Centre International de Recherches Medicales de Franceville (CIRMF) in Gabon. Because the captive population is confined, provisioned, and provided with veterinary care, it is unclear whether findings from these studies are equally relevant to wild populations. Additional research has been conducted at Lékédi Park in Gabon, where a total of 65 mandrills from the CIRMF population were translocated to a 1,750 ha fenced area across two releases in 2002 and 2006 (Peignot et al. 2008). This newly-established population is considered to be free-ranging, as the fencing appears to be at least semi-permeable to primates. Wild males were observed to join the group and reproduce, and today, the Lékédi population

comprises a single habituated horde of ~250 individuals (Charpentier et al. 2019; Mandrillus Project n.d.). Although this horde may reflect the ecology and behavior of wild populations more closely, the horde is considerably smaller than has been observed for wild mandrills (Abernethy, White, and Wickings 2002; Guibinga Mickala et al. 2022), and their near-daily interactions with humans may disrupt their natural behavior (Brockmeyer et al. 2015). To date, only a few studies have focused on natural populations of wild mandrills, and many questions relating to mandrill ecology, evolution, and their current conservation status remain unanswered.

One particularly understudied area of mandrill research relates to the genetic diversity of wild populations. In general, genetic variation is described as either neutral or adaptive, and each type contributes to different but complementary information in evolutionary research (Holderegger, Kamm, and Gugerli 2006). Neutral genetic markers, such as microsatellites and mitochondrial haplotypes, are not subject to natural selection, but they are affected by demographic processes such as genetic drift and dispersal. Therefore, they are useful in understanding population connectivity, past and present population size, and relatedness structure within populations. Adaptive or functional diversity, on the other hand, is subject to natural selection and can serve as a tool to study adaptive capacity and local adaptation. Adaptive variation can directly impact fitness, and, in some cases, it can be considered a measure of the conservation "value" of a particular population in terms of its predicted ability to withstand external threats (Selmoni et al. 2020). Genetic diversity for adaptive traits is not necessarily related to neutral diversity (Teixeira and Huber 2021), so both must be studied separately in order to develop a thorough understanding of evolutionary processes and adaptive capacity in a species.

One potential application for neutral genetic data is in the study of a species' demographic response to historical changes in its habitat. Little is known about the ability of mandrills to tolerate changes in forest cover. Environmental conditions in central Africa have been in constant flux throughout the Pleistocene and Holocene, with the forest contacting and expanding in concert with

glacial cycles of temperature and humidity (Maley 1996; Maley and Brenac 1998). Pleistocene-era shifts in climate are thought to have led to historical population fragmentation and/or speciation in some taxa (Anthony et al. 2007; Born et al. 2011; Nicolas et al. 2011). Telfer et al. (2003) hypothesized that mandrills may have retreated into northern and southern forest refugia within Gabon during periods of forest contraction, as evidenced by their genetically distinct populations either side of the Ogooué River. Between three and five thousand years ago, drills, the only other extant member of genus *Mandrillus*, experienced a 15-fold decline in population size, likely due to mid-Holocene environmental change and loss of forest cover (Ting et al. 2012).

Understanding the effects of these past environmental shifts on flora and fauna may aid in predicting the ability of populations to withstand future change in forest cover. In the coming decades, central African rainforests are predicted to be heavily impacted by climate change and anthropogenic development (Abernethy, Maisels, and White 2016; James and Washington 2013). These changes may mean that resource and habitat availability are likely to decline for multiple primate species, especially great apes (Bush et al. 2020; Junker et al. 2012; Walsh et al. 2003). Some of those declining resources, such as fruit, are also used by mandrills, so they are also likely to be vulnerable to loss of habitat (Bush et al. 2020; Lahm 1986). For mandrills, and many other forest-dependent species, understanding the effects of historical habitat loss on population demography may shed light on the conservation implications of future climate change.

In addition to understanding mandrills' ability to tolerate forest loss, past demographic history also has implications for their future adaptability. Genetic diversity is an important component of adaptive capacity, but population bottlenecks typically cause a loss of genetic variation (Holderegger, Kamm, and Gugerli 2006). In a changing environment, the survival of a species is tied to its ability to adapt to new conditions (Reed and Frankham 2003). As central African ecosystems are altered by climate change and anthropogenic influences, it will be crucial to further our understanding of mandrill

genetic diversity so that this information can be effectively incorporated into mitigation and conservation plans. Genetic data can be used to delineate conservation units based on population genetic structure, identify vulnerable populations with small effective sizes, predict the risk of inbreeding or outbreeding depression, and plan for translocations or captive breeding programs (Frankham 1995; Funk et al. 2012; von der Heyden 2017).

Although the study of demographic history relies primarily upon neutral genetic diversity, adaptive markers also play an important role in conservation since they directly impact individual fitness and species' adaptive capacity (Holderegger, Kamm, and Gugerli 2006). One marker of adaptive diversity that has been studied in captive mandrill populations is the major histocompatibility complex (MHC). The MHC is a family of genes encoding proteins that play an integral role in immunity by binding to pathogenic peptides and presenting them to T cells for destruction (Kelley, Walter, and Trowsdale 2005). While the class I MHC proteins provide protection against intracellular pathogens such as viruses, class II molecules bind to peptides from extracellular pathogens. The functional genes encoding the class II MHC are subject to pathogen-mediated balancing selection, and as a result, they are some of the most polymorphic loci in the vertebrate genome (Piertney and Oliver 2006).

The extremely high adaptive diversity found in the MHC makes these loci useful in studying evolutionary history and selective pressure from pathogens. For instance, in multiple taxa such as cichlids (Bracamonte et al. 2022), frogs (Trujillo et al. 2021), and giant pandas (Zhu et al. 2013), MHC sequencing has been used to detect population differentiation due to variation in parasite communities. Parasite-mediated balancing selection on the MHC has also been studied across many primates, since it is thought that selective pressure from pathogens can maintain similar MHC lineages across species in a phenomenon known as trans-species polymorphism (TSP) (Azevedo et al. 2015; Wroblewski et al. 2015). In species exhibiting TSP, MHC alleles can be more similar to sequences found in another species than they are to other alleles within the same species. Some species pairs, such as rhesus and cynomolgous

macaques (*Macaca mulatta* and *M. fascicularis*, respectively), even exhibit identical MHC alleles (Doxiadis et al. 2006). Lastly, MHC genes can be very important in understanding population health, since they are closely tied to immunity. Presence or absence of particular MHC alleles, and MHC heterozygosity, can have major implications for individual fitness and disease resistance, as has been shown in populations of leopard frogs with chytridiomycosis (Savage et al. 2018; Trujillo et al. 2021), in chimpanzees infected with simian immunodeficiency virus (Wroblewski et al. 2015), and in lizards with ectoparasites (Hacking et al. 2018).

In addition to parasite-mediated balancing selection, MHC diversity is also maintained through sexual selection (Milinski 2006). With its important role in immunity and fitness, females of many primate species, including mandrills, use male MHC genotype as a criterion for mate choice (Huchard et al. 2010, 2013; Schwensow, Eberle, and Sommer 2008; Setchell et al. 2010). Mate choice favoring diverse or dissimilar males helps to promote MHC diversity in offspring, which could make them more successful by reducing disease vulnerability (Penn, Damjanovich, and Potts 2002; Worley et al. 2010) or improving reproductive success (Sauermann et al. 2001).

Although MHC variation has been assessed in captive mandrills at CIRMF (Abbott, Wickings, and Knapp 2006; Setchell et al. 2009), it has never been studied in a wild population. The CIRMF mandrills are known to be inbred (Charpentier et al. 2006), so the levels of MHC diversity found in that population likely represent an underestimate of the variation that would be found in a natural population. Characterizing the MHC in a wild mandrill population would provide valuable information on adaptive genetic diversity in this vulnerable species.

However, significant challenges are associated with the study of MHC loci in wild populations. First, the MHC is a notoriously difficult region of the genome to sequence. Most MHC genes have undergone duplication and recombination (Kulski et al. 2002), resulting in copy number variation

between species, populations, and individuals (Lighten et al. 2014; Llaurens, McMullan, and Van Oosterhout 2012; Vincze, Loiseau, and Giraudeau 2021). The resulting multi-allelic templates are prone to higher rates of sequencing error, especially the formation of chimeras, which are sequence artifacts containing motifs from two true alleles (Lenz and Becker 2008). Furthermore, older sequencing technologies such as Sanger sequencing require alleles to be separated prior to sequencing individually, for example, by cloning them into separate plasmids. However, the cloning process is labor intensive for large sample sizes. Using next-generation sequencing (NGS) technologies simplifies the process by allowing all alleles to be sequenced at once, but NGS requires much more complex bioinformatic analyses to process its high-throughput sequencing data and to distinguish true alleles from artifacts (Lighten, van Oosterhout, and Bentzen 2014).

Second, collecting high-quality genetic samples, such as blood or tissue, from wildlife is often difficult, and may pose ethical concerns if the target species of one of conservation concern. The use of non-invasive samples such as feces presents a possible alternative, but DNA from such samples tends to be highly degraded (Taberlet, Waits, and Luikart 1999), further complicating the sequencing process. One advantage of sampling feces is that researchers are sometimes able to collect more samples from the wild than would be available using more invasive methods such as blood or tissue collection (Kohn and Wayne 1997). However, cloning and sequencing may not be feasible with large sample sizes. In such cases it may be preferable to use NGS, but applying these technologies to sequence the complex MHC gene family using degraded fecal DNA has only rarely been attempted (Hans et al. 2015). If non-invasive samples can serve as a viable source of DNA for NGS sequencing of multi-allelic MHC loci, this would be a promising avenue to study adaptive genetic diversity in vulnerable species like mandrills, and to better understand the broader roles that the MHC can play in individual fitness and population health.

For instance, MHC variation in a population can lead to differing levels of survivorship, when some individuals are more or less vulnerable to pathogens (Osborne et al. 2015; Penn, Damjanovich, and

Potts 2002). This results not only from differing MHC genotypes, but also individual traits that may affect pathogen prevalence. Male-biased parasitism, for example, is common in a variety of species (Lynsdale et al. 2017; Müller-Graf et al. 1997; Poulin 1996; Wilson et al. 2004). In some cases, increased parasite pressure on males has led to sex-specific selection on MHC genotypes (Dorak et al. 2002; Roved et al. 2018; Schaschl et al. 2012). Males with disadvantageous MHC alleles may be removed from the population through early parasite-associated mortality, since juveniles tend to be at increased risk of parasitism (Brzeski et al. 2015; Clutton-Brock and Pemberton 2004; Lynsdale et al. 2017). Alternatively, postcopulatory selection can remove these males prior to birth via sperm competition or cryptic female choice favoring sperm with optimal MHC alleles (Dixson 2018; Mytilinaiou et al. 2019), or by spontaneous abortion (Knapp, Ha, and Sackett 1996). Any of these mechanisms can result in males being more MHC-diverse than females or possessing alleles at different frequencies (Dorak et al. 2002; Schaschl et al. 2012).

Mandrills are an interesting candidate species in which to study sex-specific selection on the MHC. Certain pathogens, such as the malaria-causing parasite *Plasmodium gonderi*, have been shown to vary by sex in mandrills, with infection prevalence generally higher in males (Charpentier et al. 2019). Male-biased parasitism is common in sexually dimorphic species like mandrills (Moore and Wilson 2002). Reproductive males can attain more than three times the body mass of females (Setchell et al. 2001), with canine teeth that measure 4.5cm on average (Leigh et al. 2008) and dramatic red facial coloration, among other adornments (Hill 1970). The sexes also differ in their life history and behavior patterns, which can contribute to differences in parasite pressure (Bundy 1988; Müller-Graf et al. 1997; Nunn and Altizer 2004). Males have a shorter lifespan, yet mature more slowly than females (Setchell, Charpentier, and Wickings 2005), and females are thought to be philopatric while males tend to become solitary upon reaching maturity (Abernethy, White, and Wickings 2002). These patterns of strong sexual dimorphism, behavioral differences, and evidence for higher rates of *Plasmodium* infection in males, all

indicate that male-biased parasitism, and thus sex-specific selection on MHC, is likely to operate in mandrills.

Despite a range of studies on the MHC, sexual selection, and parasite pressure in captive and free-ranging mandrills (Charpentier et al. 2019; Dibakou et al. 2020; Fouchet et al. 2012; Setchell et al. 2007, 2010, 2016; Setchell, Charpentier, and Wickings 2005), sex-based differences in the mandrill MHC have never been evaluated. It is a challenge to quantify the role of parasitism in the dynamics and evolution of wild populations, but studying sex-specific differences in MHC markers may provide insight into pathogen-induced selective pressures endured by male and female mandrills.

The topics outlined above, including demographic history, MHC diversity, and sex-specific selection on the MHC, all have important implications for the conservation status and evolutionary history of mandrills. However, these issues have thus far been addressed only in semi-captive populations, or have not been addressed at all. In this dissertation, markers of neutral and adaptive genetic diversity are used to explore the gaps in our knowledge of wild mandrill population dynamics and evolution.

First, I use coalescent theory of microsatellite evolution to test for population bottlenecks in mandrills and three other sympatric forest mammals (western lowland gorillas [*Gorilla gorilla*], Peters's duikers [*Cephalophus callipygus*], and blue duikers [*Philantomba monticola*]) since the last glacial maximum 18,000 years ago. The forest landscape has undergone major contractions and expansions over the millennia, with the most recent period of significant forest loss occurred around 2,500 years ago during a period of aridity and increased seasonality (Maley 1996; Maley et al. 2018). Understanding how these changes affected wildlife may aid in predicting the effects of future environmental change. Because mandrills and both species of duiker are known to utilize forest fragments, while gorillas are

more restricted to continuous forests (Tutin, White, and Mackanga-Missandzou 1997), we expect gorillas to have been more affected by Holocene-era loss of forest cover than other species.

Second, I compare the replicability of MHC sequencing of DNA extracted from feces with that extracted from higher quality tissue samples. Non-invasive samples are a valuable source of genetic material for wildlife, and this test will show whether degraded DNA can be used with NGS to produce reliable MHC allele assignments. I then present a new method for MHC allele assignment from error-prone non-invasive samples and employ this method to characterize the genetic diversity found in the class II MHC in wild mandrills. This chapter provides new information on immunogenetic diversity and evolution in wild mandrills, and the method presented here will aid in future wildlife studies that rely on non-invasive samples.

Lastly, I test for differences in selection on MHC genes between male and female mandrills. Due to male-biased parasitism, males with disadvantageous alleles or low heterozygosity may experience higher mortality than females, and post-copulatory selection may cause nonrandom inheritance of MHC alleles (Dorak et al. 2002). If such selective pressure on the MHC is stronger in males than females, then I would expect males to have generally higher diversity in their MHC alleles, or for the frequencies of specific alleles to vary by sex. This study will contribute to our understanding of disease risk and immunogenetic capacity in male and female mandrills.

Literature Cited

Abbott, Kristin M., E. Jean Wickings, and Leslie A. Knapp. 2006. "High Levels of Diversity Characterize Mandrill (Mandrillus Sphinx) Mhc-DRB Sequences." *Immunogenetics* 58(8): 628–40.

Abernethy, Katharine et al. 2013. "Extent and Ecological Consequences of Hunting in Central African Rainforests in the Twenty-First Century." *Philosophical Transactions of the Royal Society B: Biological Sciences* 368(1625).

Abernethy, Katharine, and F Maisels. 2019. "Mandrillus Sphinx." *The IUCN Red List of Threatened Species*. https://www.iucnredlist.org/species/12754/17952325.

Abernethy, Katharine, Fiona Maisels, and Lee J.T. White. 2016. "Environmental Issues in Central Africa." *Annual Review of Environment and Resources* 41: 1–33.

Abernethy, Katharine, Lee J.T. White, and E J Wickings. 2002. "Hordes of Mandrills (Mandrillus Sphinx): Extreme Group Size and Seasonal Male Presence." *Journal of Zoology, Proceedings of the Zoological Society of London* 258: 131–37.

Anthony, Nicola M. et al. 2007. "The Role of Pleistocene Refugia and Rivers in Shaping Gorilla Genetic Diversity in Central Africa." *Proceedings of the National Academy of Sciences of the United States of America* 104(51): 20432–36.

Azevedo, Luisa, Catarina Serrano, Antonio Amorim, and David N. Cooper. 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(1): 4–9. http://dx.doi.org/10.1186/s40246-015-0043-1.

Born, Ceine et al. 2011. "Insights into the Biogeographical History of the Lower Guinea Forest Domain: Evidence for the Role of Refugia in the Intraspecific Differentiation of Aucoumea Klaineana." *Molecular Ecology* 20: 131–42.

Bracamonte, Seraina E. et al. 2022. "Divergent and Non-Parallel Evolution of MHC IIB in the Neotropical Midas Cichlid Species Complex." *BMC Ecology and Evolution* 22(1): 1–17. https://doi.org/10.1186/s12862-022-01997-9.

Brockmeyer, Timo et al. 2015. "Social Organization and Space Use of a Wild Mandrill (Mandrillus Sphinx) Group." *American Journal of Primatology* 77(10): 1036–48.

Brzeski, Kristin E. et al. 2015. "Infectious Disease and Red Wolf Conservation: Assessment of Disease Occurrence and Associated Risks." *Journal of Mammalogy* 96(4): 751–61.

Bundy, A P. 1988. "Gender-Dependent Patterns of Infection and Disease." *Parasitology Today* 4(7): 186–89.

Bush, Emma R. et al. 2020. "Long-Term Collapse in Fruit Availability Threatens Central African Forest Megafauna." *Science* 370(6521): 1219–22.

Charpentier, Marie et al. 2006. "Life History Correlates of Inbreeding Depression in Mandrills (Mandrillus Sphinx)." *Molecular Ecology* 15: 21–28.

———. 2019. "A Longitudinal Molecular Study of the Ecology of Malaria Infections in Free-Ranging Mandrills." *International Journal for Parasitology: Parasites and Wildlife* 10(August): 241–51.

https://doi.org/10.1016/j.ijppaw.2019.09.009.

Clutton-Brock, Tim, and Josephine M. Pemberton. 2004. Cambridge University Press *Dynamics and Selection in an Island Population*.

Dibakou, Serge Ely et al. 2020. "Ecological, Parasitological and Individual Determinants of Plasma Neopterin Levels in a Natural Mandrill Population." *International Journal for Parasitology: Parasites and Wildlife* 11(February): 198–206. https://doi.org/10.1016/j.ijppaw.2020.02.009.

Disotell, Todd R, Rodney L Honeycutt, and Maryellen Ruvolo. 1992. "Mitochondrial DNA Phylogeny of the Old-World Monkey Tribe Papionini." *Molecular Biology and Evolution* 9(1): 1–13.

Dixson, Alan F. 2018. "Copulatory and Postcopulatory Sexual Selection in Primates." *Folia Primatologica* 89(3–4): 258–86.

Dorak, M. T. et al. 2002. "Increased Heterozygosity for MHC Class II Lineages in Newborn Males." *Genes and Immunity* 3(5): 263–69.

Doxiadis, Gaby G M et al. 2006. "Extensive Sharing of MHC Class II Alleles between Rhesus and Cynomolgus Macaques." *Immunogenetics* 58(4): 259–68.

Fouchet, David et al. 2012. "Natural Simian Immunodeficiency Virus Transmission in Mandrills: A Family Affair?" *Proceedings of the Royal Society B: Biological Sciences* 279(1742): 3426–35.

Frankham, Richard. 1995. "Conservation Genetics." *Annual Review of Genetics* 29: 305–27.

Funk, W. Chris, John K. McKay, Paul A. Hohenlohe, and Fred W. Allendorf. 2012. "Harnessing Genomics for Delineating Conservation Units." *Trends in Ecology and Evolution* 27(9): 489–96. http://dx.doi.org/10.1016/j.tree.2012.05.012.

Guibinga Mickala, Amour et al. 2022. "Estimation of the Census (Nc) and Effective (Ne) Population Size of a Wild Mandrill (Mandrillus Sphinx) Horde in the Lope National Park, Gabon Using a Non-Invasive Genetic Approach." *Conservation Genetics*.

Hacking, Jessica D., Devi Stuart-Fox, Stephanie S. Godfrey, and Michael G. Gardner. 2018. "Specific MHC Class I Supertype Associated with Parasite Infection and Color Morph in a Wild Lizard Population." *Ecology and Evolution* 8(19): 9920–33.

Hans, Jörg B. et al. 2015. "Characterization of MHC Class II B Polymorphism in Multiple Populations of Wild Gorillas Using Non-Invasive Samples and next-Generation Sequencing." *American Journal of Primatology* 77(11): 1193–1206.

von der Heyden, Sophie. 2017. "Making Evolutionary History Count: Biodiversity Planning for Coral Reef Fishes and the Conservation of Evolutionary Processes." *Coral Reefs* 36(1): 183–94.

Hill, WCO. 1970. *Primates, Comparative Anatomy and Taxonomy, Volume 8, Cynopithecinae, Papio, Mandrillus, Theropithecus. Edinburgh:* Edinburgh: Edinburgh University Press.

Holderegger, Rolf, Urs Kamm, and Felix Gugerli. 2006. "Adaptive vs. Neutral Genetic Diversity: Implications for Landscape Genetics." *Landscape Ecology* 21(6): 797–807.

Huchard, Elise et al. 2010. "MHC, Mate Choice and Heterozygote Advantage in a Wild Social Primate." *Molecular Ecology* 19(12): 2545–61.

Huchard, Elise, Alice Baniel, Susanne Schliehe-Diecks, and Peter M. Kappeler. 2013. "MHC-Disassortative

Mate Choice and Inbreeding Avoidance in a Solitary Primate." *Molecular Ecology* 22(15): 4071–86.

James, Rachel, and Richard Washington. 2013. "Changes in African Temperature and Precipitation Associated with Degrees of Global Warming." *Climatic Change* 117(4): 859–72.

Junker, Jessica et al. 2012. "Recent Decline in Suitable Environmental Conditions for African Great Apes." *Diversity and Distributions* 18(11): 1077–91.

Kelley, James, Lutz Walter, and John Trowsdale. 2005. "Comparative Genomics of Major Histocompatibility Complexes." *Immunogenetics* 56(10): 683–95.

Knapp, Leslie A., James C Ha, and Gene P Sackett. 1996. "Parental MHC Anitgen Sharing and Pregnancy Wastage in Captive Pigtailed Macaques." *Journal of Reproductive Immunology* 32: 73–88.

Kohn, Michael H., and Robert K. Wayne. 1997. "Facts from Feces Revisited." *Trends in Ecology and Evolution* 12(6): 223–27.

Kulski, Jerzy K et al. 2002. "Comparative Genomic Analysis of the MHC: The Evolution of Class I." *Immunological Reviews* 190(1): 95–122.

Küper, Wolfgang et al. 2014. "Africa's Hotspots of Biodiversity Redefined." *Annals of the Missouri Botanical Garden* 91(4): 525–35.

Lahm, Sally A. 1986. "Diet and Habitat Preference of Mandrillus Sphinx in Gabon: Implications of Foraging Strategy." *American Journal of Primatology* 26: 9–26.

Leigh, Steven R. et al. 2008. "Canine Tooth Size and Fitness in Male Mandrills (Mandrillus Sphinx)." *Journal of Human Evolution* 55(1): 75–85.

Lenz, Tobias L., and Sven Becker. 2008. "Simple Approach to Reduce PCR Artefact Formation Leads to Reliable Genotyping of MHC and Other Highly Polymorphic Loci - Implications for Evolutionary Analysis." *Gene* 427(1–2): 117–23. http://dx.doi.org/10.1016/j.gene.2008.09.013.

Lighten, Jackie et al. 2014. "Ultra-Deep Illumina Sequencing Accurately Identifies MHC Class IIb Alleles and Provides Evidence for Copy Number Variation in the Guppy (Poecilia Reticulata)." *Molecular Ecology Resources* 14(4): 753–67.

Lighten, Jackie, Cock van Oosterhout, and Paul Bentzen. 2014. "Critical Review of NGS Analyses for de Novo Genotyping Multigene Families." *Molecular Ecology* 23(16): 3957–72.

Llaurens, V., M. McMullan, and C. Van Oosterhout. 2012. "Cryptic MHC Polymorphism Revealed but Not Explained by Selection on the Class IIB Peptide-Binding Region." *Molecular Biology and Evolution* 29(6): 1631–44.

Lynsdale, Carly L. et al. 2017. "Parasite-Associated Mortality in a Long-Lived Mammal: Variation with Host Age, Sex, and Reproduction." *Ecology and Evolution* 7(24): 10904–15.

MacKinnon, John et al. 2017. "Regional Analysis-Central Africa." In *Larger than Elephants: Inputs for an EU Strategic Approach to Wildlife Conservation in Africa*, Publications Office of the Eurpean Union, 204–77.

Maley, Jean. 1996. 104 Proceedings of the Royal Society of Edinburgh Section B: Biological Sciences *The African Rain Forest - Main Characteristics of Changes in Vegetation and Climate from the Upper Cretaceous to the Quaternary*.

———. 2018. “Late Holocene Forest Contraction and Fragmentation in Central Africa.” *Quaternary Research (United States)* 89(1): 43–59.

Maley, Jean, and Patrice Brenac. 1998. “Vegetation Dynamics, Palaeoenvironments and Climatic Changes in the Forests of Western Cameroon during the Last 28,000 Years B.P.” *Review of Palaeobotany and Palynology* 99: 157–87.

“Mandrillus Project.” http://www.projetmandrillus.com/ (January 9, 2023).

Milinski, Manfred. 2006. “The Major Histocompatibility Complex, Sexual Selection, and Mate Choice.” *Annual Review of Ecology, Evolution, and Systematics* 37: 159–86.

Moore, Sarah L., and Kenneth Wilson. 2002. “Parasites as a Viability Cost of Sexual Selection in Natural Populations of Mammals.” *Science* 297: 2015–18.

Müller-Graf, C.D.M., D.A. Collins, C. Packer, and M.E.J. Woolhouse. 1997. “Schistosoma Mansoni Infection in a Natural Population of Olive Baboons (Papio Cynocephalus Anubis) in Gombe Stream National Park, Tanzania.” *Parasitology* 115: 621–27.

Mytilinaiou, Irene et al. 2019. “Immune Molecules in the Female Reproductive Path and Sperm: Relevance to Mate Choice.” *Reproduction* 158(2): 159–67.

Nicolas, V et al. 2011. “The Roles of Rivers and Pleistocene Refugia in Shaping Genetic Diversity in Praomys Misonnei in Tropical Africa.” *Journal of Biogeography* 38: 191–207.

Nunn, Charles L., and Sonia M. Altizer. 2004. “Sexual Selection, Behaviour and Sexually Transmitted Diseases.” In *Sexual Selection in Primates: New and Comparative Perspectives*, eds. Peter M. Kappeler and Carel P. van Schaik. Cambridge University Press, 117–30.

Osborne, Amy J. et al. 2015. “Heterozygote Advantage at MHC DRB May Influence Response to Infectious Disease Epizootics.” *Molecular Ecology* 24(7).

Peignot, Patricia et al. 2008. “Learning from the First Release Project of Captive-Bred Mandrills Mandrillus Sphinx in Gabon.” *Oryx* 42(1): 122–31.

Penn, D. J., K. Damjanovich, and W. K. Potts. 2002. “MHC Heterozygosity Confers a Selective Advantage against Multiple-Strain Infections.” *Proceedings of the National Academy of Sciences* 99(17): 11260–64.

Piertney, S B, and M K Oliver. 2006. “The Evolutionary Ecology of the Major Histocompatibility Complex.” *Heredity* 96(1): 7–21. http://www.ncbi.nlm.nih.gov/pubmed/16094301.

Poulin, Robert. 1996. “Helminth Growth in Vertebrate Hosts: Does Host Sex Matter?” *International Journal for Parasitology* 26(11): 1311–15.

Reed, David H, and Richard Frankham. 2003. “Correlation between Fitness and Genetic Diversity.” *Conservation Biology* 17(1): 230–37. https://www-jstor-org.ezproxy.its.uu.se/stable/pdf/3095289.pdf?refreqid=excelsior%3A5ae5d015a5621dbf078f7cc90dd3716f.

Roved, Jacob et al. 2018. “Evidence for Sexual Conflict over Major Histocompatibility Complex Diversity in a Wild Songbird.” *Proceedings of the Royal Society B: Biological Sciences* 285(1884).

Sauermann, U. et al. 2001. “Increased Reproductive Success of MHC Class II Heterozygous Males among

Free-Ranging Rhesus Macaques." *Human Genetics* 108(3): 249–54.

Savage, Anna E., Kevin P. Mulder, Taina Torres, and Stuart Wells. 2018. "Lost but Not Forgotten: MHC Genotypes Predict Overwinter Survival despite Depauperate MHC Diversity in a Declining Frog." *Conservation Genetics* 19(2): 309–22.

Schaschl, Helmut et al. 2012. "Sex-Specific Selection for MHC Variability in Alpine Chamois." *BMC Evolutionary Biology* 12(1).

Schwensow, Nina, Manfred Eberle, and Simone Sommer. 2008. "Compatibility Counts: MHC-Associated Mate Choice in a Wild Promiscuous Primate." *Proceedings of the Royal Society B: Biological Sciences* 275(1634): 555–64.

Selmoni, Oliver et al. 2020. "Seascape Genomics as a New Tool to Empower Coral Reef Conservation Strategies: An Example on North-Western Pacific Acropora Digitifera." *Evolutionary Applications* 13(8): 1923–38.

Setchell, J.M. et al. 2007. "Parasite Prevalence, Abundance, and Diversity in a Semi-Free-Ranging Colony of Mandrillus Sphinx." *International Journal of Primatology* 28(6): 1345–62.

———. 2009. "Is Brightest Best? Testing the Hamilton-Zuk Hypothesis in Mandrills." *International Journal of Primatology* 30(6): 825–44.

———. 2010. "Opposites Attract: MHC-Associated Mate Choice in a Polygynous Primate." *Journal of Evolutionary Biology* 23(1): 136–48.

Setchell, J.M., Marie Charpentier, and E. Jean Wickings. 2005. "Sexual Selection and Reproductive Careers in Mandrills (Mandrillus Sphinx)." *Behavioral Ecology and Sociobiology* 58(5): 474–85.

Setchell, J.M., Phyllis C. Lee, E. Jean Wickings, and Alan F. Dixson. 2001. "Growth and Ontogeny of Sexual Size Dimorphism in the Mandrill (Mandrillus Sphinx)." *American Journal of Physical Anthropology* 115(4): 349–60.

Setchell, J.M., Shane A. Richards, Kristin M. Abbott, and Leslie A. Knapp. 2016. "Mate-Guarding by Male Mandrills (Mandrillus Sphinx) Is Associated with Female MHC Genotype." *Behavioral Ecology* 27: arw106.

Taberlet, Pierre, Lisette P Waits, and Gordon Luikart. 1999. "Noninvasive Genetic Sampling: Look before You Leap." *Trends in Ecology and Evolution* 14(8): 323–27.

Teixeira, João C., and Christian D. Huber. 2021. "The Inflated Significance of Neutral Genetic Diversity in Conservation Genetics." *Proceedings of the National Academy of Sciences of the United States of America* 118(10): 1–10.

Telfer, Paul T et al. 2003. "Molecular Evidence for Deep Phylogenetic Divergence in Mandrillus Sphinx." *Molecular Ecology* 12(7): 2019–24.

Ting, Nelson et al. 2012. "Genetic Signatures of a Demographic Collapse in a Large-Bodied Forest Dwelling Primate (Mandrillus Leucophaeus)." *Ecology and Evolution* 2(3): 550–61.

Trujillo, Alexa L., Eric A. Hoffman, C. Guilherme Becker, and Anna E. Savage. 2021. "Spatiotemporal Adaptive Evolution of an MHC Immune Gene in a Frog-Fungus Disease System." *Heredity* 126(4): 640–55. http://dx.doi.org/10.1038/s41437-020-00402-9.

Tutin, Caroline E.G., Lee J.T. White, and A. Mackanga-Missandzou. 1997. “The Use by Rain Forest Mammals of Natural Forest Fragments in an Equatorial African Savanna.” *Conservation Biology* 11(5): 1190–1203.

Vincze, Orsolya, Claire Loiseau, and Mathieu Giraudeau. 2021. “Avian Blood Parasite Richness Decreases with Major Histocompatibility Complex Class i Loci Number.” *Biology Letters* 17(8).

Walsh, Peter D. et al. 2003. “Catastrophic Ape Decline in Western Equatorial Africa.” *Nature* 422(6932): 611–14.

Wilson, K et al. 2004. “Parasites and Their Impact.” In *Dynamics and Selection in an Island Population*, eds. Tim Clutton-Brock and Josephine M. Pemberton. Cambridge University Press, 113–65.

Worley, Kirsty et al. 2010. “MHC Heterozygosity and Survival in Red Junglefowl.” *Molecular Ecology* 19(15): 3064–75.

Wroblewski, Emily E. et al. 2015. “Signature Patterns of MHC Diversity in Three Gombe Communities of Wild Chimpanzees Reflect Fitness in Reproduction and Immune Defense against SIVcpz.” *PLoS Biology* 13(5): 1–36.

Zhu, Ying et al. 2013. “Patterns of Genetic Differentiation at MHC Class i Genes and Microsatellites Identify Conservation Units in the Giant Panda.” *BMC Evolutionary Biology* 13(1).

Chapter 1

Demographic history of central African forest mammals since the last glacial maximum

Abstract

The forests of central Africa have expanded and contracted repeatedly over the millennia due to changing environmental conditions. Considerable research has examined the influences of these changes on species diversification, but most studies have focused on Pleistocene-era effects. Less information is available about the consequences of more recent shifts, including the abrupt decline of forest cover that occurred in the region between 2,500 and 2,000 years ago, in an event known as the "rainforest crisis." Central African tropical forests are home to a diversity of wildlife, and the sudden loss of forest habitat could have led to severe demographic declines. Here, we model the demographic history of four forest-associated species that may have been affected by the rainforest crisis. We test for evidence of population decline in western lowland gorillas (*Gorilla gorilla*), blue duikers (*Philantomba monticola*), Peters's duikers (*Cephalophus callipygus*), and mandrills (*Mandrillus sphinx*) using microsatellite data collected at several sites in Gabon. Our results suggest that all four species maintained stable populations throughout the Holocene, including during the rainforest crisis. This finding indicate that these species have historically been able to tolerate some amount of forest fragmentation, possibly by moving between forest patches to pursue resources. Central African forests are expected to continue to fluctuate in the future due to climate change and anthropogenic effects. Our findings indicate that these species may be able to withstand some changes to their habitat, although we cannot necessarily predict future population stability in light of hunting and contemporary climate change.

Introduction

Throughout the mid- to late Pleistocene and Holocene, the climate and landscape of central Africa has undergone major shifts in forest cover (Maley 1991). During glacial cycles, glacial maxima were accompanied by periods of aridity and low temperatures in the region, whereas during interglacial periods, forest cover expanded with warming temperatures. The most recent arid period occurred between 24,000 and 12,000 years ago, corresponding with the last glacial maximum (LGM) 18,000 years ago (Maley 1996). During the LGM, the forest was at is minimum and formed patches that are thought to have served as refugia for the region's flora and fauna (Anthony et al. 2007; Born et al. 2011; Colyn, Gautier-Hion, and Verheyen 1991; Haffer 1969; Muloko-Ntoutoume et al. 2000; Telfer et al. 2003). It is well-established that these refugia helped to shape current species distributions, for instance, leading to population structure in an endemic tree (Born et al. 2011; Muloko-Ntoutoume et al. 2000), in rodents (Nicolas et al. 2011), and in primates (Telfer et al. 2003; Anthony et al. 2007). Speciation within the insect genus *Leiodontocercus* has also been attributed to the isolation of populations within refugia (Massa 2020). Lastly, Pleistocene-era forest expansion during warming periods is thought to have led to range expansion in forest duikers (Ntie et al. 2017).

Despite the well-developed body of knowledge about the effects of Pleisotcene-era climatic change on species diversification and distributions, less is known about the impacts of environmental and anthropogenic change in central Africa since the LGM 18,000 years ago. Forest cover has continued to fluctuate, with one particularly large shift occurring between 2,500 and 2,000 years ago in an event known as the "rainforest crisis" (RFC) (Maley 2002). Palynological evidence suggests that during this time, the central African forest fragmented rapidly, with concurrent savannah expansion (Maley 2002; Maley et al. 2018; Ngomanda, Neumann, et al. 2009). Since that time, the forest has been recovering, although it never reached the same extent as earlier in the Holocene (Maley 1996). The RFC was associated with a period of increased aridity and seasonality across tropical Africa, which may have

contributed to forest loss (Maley 2002; Ngomanda, Chepstow-Lusty, et al. 2009). At the same time, Bantu populations were expanding through central Africa, bringing with them agriculture and iron-smelting (Neumann et al. 2012; Oslisly 2001). Slash-and-burn farming and extracting of wood resources for iron-working furnaces may also have played a role in declining forest cover (Willis, Gillson, and Brncic 2004). The question of whether climate change or increased land use was the primary driver of the RFC remains unresolved (Bayon et al. 2012; Clist et al. 2018; Garcin, Deschamps, Ménot, de Saulieu, Schefub, et al. 2018; Garcin, Deschamps, Ménot, de Saulieu, Schefuß, et al. 2018; Maley 2002).

Despite lingering debates over the cause of the RFC, the decline in forest cover could have impacted wildlife populations. Mid-Holocene declines have been reported in multiple species in east Africa, such as African buffalo (Heller et al. 2008) and elephants (Okello et al. 2008). These population bottlenecks roughly correspond in time with the widespread climatic changes associated with central Africa's RFC. Despite these findings, less is known about the impacts of the RFC or other mid-Holocene environmental changes on central African wildlife. Understanding the effects of events such as these is crucial, since they can play an important role in shaping contemporary population genetic variation and can have important implications for species evolution and conservation. While Pleistocene-era fluctuations in forest cover occurred cyclically over many millennia, mid-Holocene loss of forest cover during the RFC happened relatively rapidly, over the course of just a few hundred years (Maley 2002). The pace of forest fragmentation during the RFC may be analogous to modern habitat loss and fragmentation as a result of anthropogenic pressure, which is known to have detrimental effects on many species (Cushman 2006; Keinath et al. 2017; Laurance et al. 1998; Püttker et al. 2020). Understanding past demographic changes can therefore be very informative in predicting how species will respond to future challenges.

In this study, we compare the demographic histories of four central African forest mammals in the middle Ogooué valley in Gabon during the time of the RFC, when environmental conditions were

arid and forest cover was in rapid decline (Maley 2002). We focus specifically on two primate species, western lowland gorillas (*Gorilla gorilla gorilla*) and mandrills (*Mandrillus sphinx*), and two duiker species, Peters's duikers (*Cephalophus callipygus*) and blue duikers (*Philantomba monticola*). All four focal species can be found in Lopé National Park (LNP) in central Gabon, which is characterized by a forest-savannah mosaic in its northern region that transitions further south into contiguous lowland tropical rainforest (L. J. T. White 2001). The transition zone between the mosaic and contiguous forest is characterized by Marantaceae forest, which is thought to have grown between 1,400 and 800 years BP, when humans were absent from LNP and seasonal burning did not occur (L. J. T. White 2001). The biomass of wildlife in the mosaic's gallery forests and island "bosquets" has been shown to be more than twice that of the continuous forest, and the four focal species utilize these forest patches to varying degrees (Tutin, White, and Mackanga-Missandzou 1997). Gorillas occupy both primary and secondary forest, but in LNP, their biomass appears highest in the Marantaceae forest (Tutin et al. 1995; Tutin and Fernandez 1984). Mandrills and duikers, on the other hand, appear to use the gallery forests and bosquets of the forest-savannah mosaic more intensively than gorillas (Tutin, White, and Mackanga-Missandzou 1997). Mandrill hordes can make use of all forest types, although they avoid the savanna except to cross between forest patches (E. C. White et al. 2010). Peters's duikers are difficult to differentiate visually from other *Cephalophus* species (Hedwig et al. 2018), so less is known about their ecology. They are thought to have large home ranges compared to blue duikers (Feer 1988), but both species are known to utilize forest fragments and the continuous forest, with less time spent in the Marantaceae zone (Tutin, White, and Mackanga-Missandzou 1997).

Of the four species, gorillas appear to be the most specialized in their habitat needs and are more dependent on continuous forests (Tutin et al. 1995; Tutin and Fernandez 1984; Tutin, White, and Mackanga-Missandzou 1997). Their visits to the forest fragments appear to be seasonal and driven primarily by a need for fruit resources (Williamson, Tutin, and Fernandez 1988). For this reason, they

may have been more vulnerable to historical loss of forest cover than the other three species. A previous study tested for bottlenecks in mountain gorillas (*G. beringei*) and western gorillas, including the western lowland and Cross River subspecies, and detected bottlenecks in all except the western lowland subspecies (Bergl et al. 2008). However, the analysis of western lowland gorillas included only a single population residing at a Mondika research station, near the border of the Central African Republic and the Republic of the Congo. The population in the middle Ogooué valley, where LNP is located, has never been assessed for historical demographic declines, although they have been shown to have undergone population fragmentation and expansion during Pleistocene-era glacial cycles of forest contraction and regrowth (Anthony et al. 2007).

Like gorillas, neither mandrills nor duikers have been tested for population bottlenecks occurring since the LGM, although both blue and Peters's duikers are hypothesized to have undergone post-glacial population expansions during the Pleistocene (Ntie et al. 2017). The closest relative of mandrills, the drills, did undergo a population bottleneck 3-5 thousand years ago (Ting et al. 2012), but because drills avoid crossing marginal habitats (Astaras 2009), we hypothesize that mandrills may have been better able to tolerate habitat fragmentation than their congener. Likewise, the lack of contemporary population structure in duikers (Ntie et al. 2017) and their apparent ability to cross savannas with relative ease suggests that they may have been resilient to Holocene-era fragmentation.

In the present study, we set out to test the hypothesis that one or more of these four species experienced mid-Holocene population bottlenecks associated with the RFC. The demographic history of each species was modeled using full likelihood Bayesian analyses of microsatellite data implemented in the program MSVAR (Beaumont 1999; Storz and Beaumont 2002). By exploring past demographic shifts in forest-associated species, we will gain a better understanding of how environmental change has shaped the contemporary vertebrate communities of central Africa. This study also sheds light on the

ability of each of these species to tolerate rapid loss of forest cover, which may be analogous to future environmental changes.

Methods

Sampling and Microsatellite Genotyping

Western lowland gorillas

Microsatellite data for western lowland gorillas was collected from a previous study conducted by Anthony et al. (2007). Details can be found in the original study, but in brief, shed gorilla hairs were collected from nests at six sites spread across Nigeria, Gabon, the Central African Republic, the Congo, the Democratic Republic of Congo, and Uganda. Because this study centered on the Ogooué River valley and environmental effects on wildlife likely varied spatially, only genotypes from the site in Gabon at Lopé National Park were used here. Each sample was genotyped for seven polymorphic microsatellite loci.

Duikers

Data for both blue and Peters's duikers were originally generated by Ntie et al. (2017). Although the previous authors sampled from 46 sites in nine central African countries, here we use only the samples collected from sites in Gabon where more than five individuals were sampled. For blue duiker, ten such sites were available: the Gamba complex of protected areas, Lopé National Park, Monts de Cristal National Park, Birougou National Park, Ivindo National Park, and five additional sites on both sides of the Ogooué and Ivindo rivers. Three of these sites were in the province of Ogooué Ivindo, and the provinces of Ogooué Lolo, Moyen Ogooué, and Haute Ogooué each included one site. A total of nine sites were used for the Peters's duiker: Waka National Park, Lopé National Park, Birougou National Park, and Minkebe National Park, plus other sites in Haute Ogooué, Ogooué Lolo, and Ogooué Ivindo. For

both species, DNA was extracted primarily from opportunistically-collected fecal samples, and each sample was genotyped for twelve microsatellite loci (Ntie et al. 2010).

Mandrills

Microsatellite data for mandrills was also generated using a non-invasive approach. During 2016, 2017, and 2018, fecal samples were collected from a single mandrill horde, generally residing in the forest-savannah mosaic of northeastern Lopé National Park. This horde is known as the "SEGC" horde due to its long history of study by the Station d'Etudes des Gorilles et Chimpanzes (SEGC) field research station (Rogers, Abernethy, and Fontaine 1996; E. C. White et al. 2010). In 2019, two additional hordes were sampled, the "Mikongo" and "ECOFAC" hordes. These hordes are found further south of LNP, and the extent of migration and gene flow between the hordes is currently unknown. Sampling was always conducted during July or August, when adult males and females are both present in the horde (Abernethy, White, and Wickings 2002). Radio telemetry was used to locate the SEGC horde daily, and researchers then followed the mandrills and collected fresh fecal samples (<6 hours old), storing them in 50mL Falcon tubes with ~25mL silica gel since this has been shown to be an effective method for preserving nuclear DNA (Soto-Calderón et al. 2009).

After storage at ambient temperature in the field for one to four weeks, samples were transferred to a -20°C freezer. DNA was extracted using QIAmp DNA Stool Mini Kits (Qiagen, California). Each sample of extracted DNA was then subjected to polymerase chain reaction to amplify a panel of 16 microsatellite markers (Benoit et al. 2014), as well as two sex diagnostic loci on the X and Y chromosomes (Di Fiore 2005). Microsatellite loci were arranged into multiplex PCRs of four or five loci each (Table 1), and each PCR included a negative control to monitor cross-contamination. Each multiplex PCR contained 200 nmol of each forward and reverse primer, 0.5 µL of 20mg/mL bovine serum albumin, 5 µL of 2X Multiplex PCR Kit (Qiagen, CA), 1.7 mL of molecular grade water, and 2µL of DNA extract. All

four multiplexes were amplified using a touchdown PCR procedure to improve specificity of primer binding early in the cycling process. As per the instructions for the Multiplex PCR Kit, all PCRs began with an initial Taq polymerase activation step at 95°C for 15 minutes. For multiplexes 1 and 3, ten cycles were then performed, consisting of 95°C for 30 seconds, 60°C for 90 seconds with a 1°C decrease per cycle, and 60 seconds of extension at 72°C. After these ten cycles, an additional 30 were performed with the annealing temperature held constant at 50°C. Cycling conditions were identical for multiplexes 2 and 4, except that the annealing temperature began at 63°C and was reduced by 1°C per cycle until reaching 53°C. Following the total set of 40 cycles, the temperature was held at 60°C for 30 minutes as a final extension step.

Table 1 Mandrill microsatellite markers and primer sequences

Locus	Multiplex	Forward Primer	Reverse Primer
MaCh868	1	TCATCTGTCATTATCTGTCTGACTGT	GGCGGAATGAATAGATAGAGAC
MaCh726	1	TTCCATCTGTCCATCCTTTCTT	GATCCCAGTGACCTAGCCTG
MaCh303	1	CCCTGCATCTATCCGTCATT	TGTATCCCTGGAGTGCCTTT
MaCh834	1	TGTCTGCGACCCATGAGTAT	AGCCCAACTGAGACTGCCTA
MaCh866	2	GATGCTGAGTTTTCTGGAAGC	CAGTTGTCTTTGGATTGCCC
MaCh070	2	CTATCGTGGAACCTTGCGAT	CTATTTTTCACCCTGCCCAA
MaCh184	2	ATGGCAAGGATGTGACCTTT	AGGGTTACCCGTAGAACTGAG
MaCh372	2	TCACAAAGGCACAAAGAACG	AAACTCTTTGCCAAGACCGA
MaCh419	3	ATGAAGCTGCCATTTCAACC	CTATGTCCCATCCATCCACC
MaCh129	3	AGTGCAATGTGGGTAGGCTC	CCAGGCGGTTTTGAGAATTA
MaCh409	3	AGCTCTTGCCCTCTCCTTTC	CAAGCTGGATGCTGTGAAGA
MaCh141	3	CTGAGGGCCTAACAGGAACA	GCCTGGCCTACAAAGGTACA
Amelogenin	3	ACCACCAGCTTCCCAGTTTA	GCTGGGWTAGAACCAAGCTG
MaCh581	4	CACTCACTTCCTTTTTCGTG	AGATCTAGTGTGGCAGAAAG
MaCh007	4	TGGAATTTAGTCAGGGGTTCC	TGCCAGCTTCCATAATCACA
MaCh312	4	GCATGCACCTCTGTCTCAAA	TGTGCATGTAAAGGTTAGTACATCA
MaCh262	4	AGGACCCCTCTTGCAAGTTT	CCTGGCTAGCAGTCAGCTCT
SRY	4	AGTGAAGCGACCCATGAACG	TGTGCCTCCTGGAAGAATGG

Because data collected from noninvasive samples are known to be error-prone (Gagneux, Boesch, and Woodruff 1997; Taberlet et al. 1996), we performed a pilot study to quantify genotyping error rates and determine the number of PCR replicates required for a reliable genotype. A total of 19 mandrill fecal samples were genotyped by six replicates of PCR, with fragment analysis performed on an

ABI3130xl sequencer. Consensus genotypes from these six replicates were generated using the program Gimlet (Valière 2002). The rate of allelic dropout (R_{AD}), or random non-amplification of an allele, was quantified by comparing each of the six replicate genotypes with their consensus and calculating the percentage of replicates in which dropout occurred. To minimize the risk of allelic dropout occurring in a consensus genotype, we estimated the minimum number of PCR replicates required to reduce the probability of dropout occurring in all replicates to <5%. This was achieved by multiplying the estimated allelic dropout rate across replicates ($R_{AD}^{\#replicates}$) for each locus, until the cumulative R_{AD} fell to <0.05. From the results of this pilot study, we determined that three PCR replicates were sufficient to reduce genotyping error to <5% if the consensus genotype is generated using the following criteria: for a sample to be genotyped as homozygous, a single allele must appear alone in all three replicates, and for a sample to be genotyped as heterozygous, two distinct alleles must appear at least twice across the three replicates. Genotypes that did not adhere to either of these criteria were discarded. After an initial PCR amplification of all samples, those successfully amplifying at nine or more loci underwent two additional PCR replicates to generate consensus genotypes. Samples that amplified at fewer than nine loci in the first replicate were excluded from further analysis. The raw allele sizes were binned into allele classes using the program Tandem (Matschiner and Salzburger 2009), which allows for easy visual identification of any imperfect loci, i.e. loci with alleles deviating from the expected microsatellite repeat pattern (Ellegren 2004). Imperfect loci would be problematic for our downstream analyses and must be excluded, as they violate the strict single-step mutation assumption of the coalescent model (Beaumont 1999; Ellegren 2000).

From the consensus genotypes, we also calculated the per-locus probability of identity, defined as the probability that two individuals share a genotype by chance, using a conservative estimator, PID_{sibs}, that assumes that a dataset may contain full siblings (Waits, Luikart, and Taberlet 2001). By multiplying each locus's PID_{sibs} together in descending order, we determined that a minimum of six loci

are required to differentiate individuals (probability < 0.01 of two full siblings sharing a genotype at six loci), so any genotype with fewer than six loci in its multilocus consensus was discarded. Finally, a custom Python script was used to perform pairwise comparisons of all multilocus genotypes in order to detect any sets of samples originating from the same animal. The script searched for duplicates within and between hordes and sampling years. If a pair of genotypes was identical at six or more loci, with no more than two mismatching alleles, they were considered to represent the same animal, and the genotype with fewer amplified loci was discarded. Likewise, if missing data made it impossible to confirm whether a pair of genotypes represented two unique individuals, the less informative genotype of the pair was discarded. After discarding all genotypes in the mandrill dataset with insufficient data, and those that were duplicates of already-sampled individuals, a total of 348 genotypes were available, including 212 genotypes from the SEGC horde, 80 from the ECOFAC horde, and 56 from the Mikongo horde.

Population genetic analyses

In all four species, we tested for the presence of null alleles using the program Micro-Checker (van Oosterhout et al. 2004) and for deviations from Hardy-Weinberg equilibrium and linkage equilibrium using Arlequin v3.5 (Excoffier and Lischer 2010). Holm-Bonferroni corrections were applied to all statistical tests performed across multiple loci (Gaetano 2018; Holm 1979). Loci with evidence for null alleles, or those with excessive missing data, were excluded from the final dataset. In the program FSTAT (Goudet 2003), we calculated F_{is} for each population in all species and tested for significance by randomly permuting alleles at each locus among individuals. P values were determined based on the proportion of randomized datasets that had F_{is} values higher or lower than the observed data. The significance level was Bonferroni-corrected to evaluate across loci and populations. Allelic richness (A_R) was also estimated using the program HP-RARE v1.0 (Kalinowski 2005). The number of alleles detected at a locus is strongly influenced by sample size, so HP-RARE uses rarefaction to estimate the number of

alleles present in a sample of a specified number of chromosomes. In the present study, sample size varied widely across species and study site. To allow comparisons between species and sites, we estimated the allelic richness assuming the minimum number of chromosomes sampled at any locus for any species or site. For Peters duikers sampled from Parc National Minkébé, only three individuals amplified successfully at microsatellite locus BM121, yielding six chromosomes as the minimum number sampled.

Demographic modeling in MSVAR

Demographic history was assessed using a full likelihood approach as implemented in MSVAR v1.3 (Beaumont 1999; Storz and Beaumont 2002), under a model of exponential population decline. MSVAR uses a coalescent-based approach to model four parameters using a strict single-step mutation model: current effective population size (N_0), historical effective population size (N_1), microsatellite mutation rate (μ), and time since change in population size (xa, where x = number of generations since the change and a = generation time in years). This set of parameters is modeled independently for each locus using a Markov Chain Monte Carlo (MCMC), with parameters in each MCMC step drawn from log-normal prior distributions. Means across loci of these prior distributions are normally distributed and defined by hyperpriors α (mean) and σ (standard deviation), and standard deviations are likewise normally distributed and defined by β and τ (mean and standard deviation respectively).

The hyperpriors used for mutation rate (μ) and time since decline (xa) were the same for all four species. Mutation rate hyperpriors (α=-3.3, σ=1, β=0, τ=1) allowed support for a wide range of values averaging $5x10^{-4}$, since this value is considered a typical value for microsatellite mutation rates in mammals (Ellegren 2004). Hyperpriors for xa (α=3.5, σ=0.8, β=0, τ=0.5) allowed the model to explore decline scenarios temporally centered around the RFC, yet provided enough flexibility to detect bottlenecks that were much older (>20,000 years) or much more recent (within the last 500 years). Hyperpriors for N_0 and N_1 varied by species but generally allowed the past population sizes to be as

much as one thousand times larger than the current size. The parameters were also broad enough to allow support scenarios of population expansion ($N_0 \geq N_1$). Furthermore, to ensure that the hyperpriors did not bias the model, an uninformative model was also assessed for each species in which hyperpriors for current and ancestral population sizes were equal, both set to the mean between N_0 and N_1's α values from the informative models. The distributions used for N_0 and N_1 are shown in Table 2 and explained in species-specific sections below.

Table 2 Prior distributions used in MSVAR

	N_0 α, σ, β, τ	Approx. N_0 range	N_1 α, σ, β, τ	Approx. N_1 range
Gorilla	2, 1, 0, 0.5	1-10,000	3, 1, 0, 0.5	10-100,000
uninformative	2.5, 1, 0, 0.5	3-31,622	2.5, 1, 0, 0.5	3-31,622
Peters duiker	3.3, 1, 0, 0.5	20-199,526	4.3, 1, 0, 0.5	200-1,995,262
uninformative	3.8, 1, 0, 0.5	63-630,957	3.8, 1, 0, 0.5	63-630,957
Blue duiker	2.7, 1, 0, 0.5	5-50,119	3.7, 1, 0, 0.5	50-501,187
uninformative	3.2, 1, 0, 0.5	2-158,489	3.2, 1, 0, 0.5	2-158,489
Mandrill	2.6, 0.7, 0, 0.5	16-10,000	3.6, 1, 0, 0.5	40-398,107
uninformative	3.1, 1, 0, 0.5	13-125,893	3.1, 1, 0, 0.5	13-125,893

α,σ = hyperpriors describing mean and standard deviations of mean current effective size (N_0) or historical effective size (N_1) across loci; β, τ = mean and standard deviation of standard deviations of effective sizes across loci. Approx. ranges describe the range of population sizes (in diploid number of individuals) most commonly tested by the model (within 2 standard deviations of the mean)

For each species, five MCMC chains were run for $8x10^9$ steps per chain, with draws from the posterior distribution every hundred thousand steps. The first half of each chain was discarded as burn-in. Model convergence was measured from the remainder of the chains using the Gelman-Rubin statistic (Brooks and Gelman 1998) as implemented in the R package *boa* (Smith 2007; R Core Team 2020). Parameter means, medians and 95% highest posterior density (HPD) were calculated from the posterior distribution, and model support was assessed using Bayes Factors (BF) (Kass and Raftery 1995), with a BF>10 taken as evidence for strong support of the model. For each of the four demographic parameters, MSVAR also produces an estimate of variance across loci. If all loci behave similarly with respect to the

demographic model, these variances should converge near zero. Therefore, variances from combined chains were plotted in histograms to ensure that there were no aberrant loci present that may bias the demographic parameter estimates.

Western lowland gorillas

Values for the prior distribution of gorilla N_0 (Table 2) were determined using the linkage disequilibrium method (Waples 2006), as implemented in NeEstimator v2.01 (Do et al. 2014). A generation time of 22 years was assumed, based on estimates reported by the IUCN Red List of Threatened Species (Maisels, Bergl, and Williamson 2018).

All 34 gorilla genotypes available from Lopé National Park were used in the demographic model. Past studies have shown the importance of sampling from multiple populations for coalescent models of population decline, since population structure combined with low levels of gene flow can cause false bottleneck signals when a single deme is examined (Chikhi et al. 2010). However, the LNP gorilla population is relatively isolated, and insufficient microsatellite data was available from populations near enough to LNP to feasibly exchange migrants. Furthermore, the other populations for which microsatellite data were available were distant enough that they may have not have experienced the same historical pressure due to environmental change.

Duikers

Like for gorillas, duiker N_0 hyperpriors (Table 2) were also determined by estimating effective population size using NeEstimator's linkage disequilibrium method (Do et al. 2014; Waples 2006). Generation time was assumed to be 4.5 and 5.2 years respectively for blue and Peters's duiker (IUCN SSC Antelope Specialist Group 2016a, 2016b). To avoid the risk of false bottleneck detection, four genotypes were used for each species from each site. The four genotypes were selected based on their

completeness, and they were combined into datasets of 36 genotypes for Peters's duikers and 40 genotypes for blue duikers.

Mandrills

For mandrills, hyperpriors for N_0 (Table 2) were determined based on observational data in the field and from Guibinga Mickala et al., (2022), who estimated that the SEGC horde alone is made up of close to one thousand animals, with an effective population size between 135 and 292. Here we assume that the other two hordes are of similar size, but the N_0 hyperprior distributions were kept sufficiently broad to allow for considerable error in these estimates, such as unobserved breeding adults or migration between mandrill hordes.

The generation time of mandrills in the wild is not well resolved, but studies in captive populations show a marked difference in the age of first reproduction between males and females, with females typically having their first offspring at around four years old and males siring for the first time at around 10-11 years of age (Setchell, Charpentier, and Wickings 2005). Therefore, an approximate value of five years was selected as the mandrill generation time for the model, acknowledging that if a bottleneck was detected, the time since decline may be underestimated if the true generation time is longer than five years. Finally, the twenty genotypes with the least missing data from each of the three hordes were pooled into a single dataset of 60 individuals for the model.

Results

Microsatellite genotyping

After removing microsatellite loci showing evidence for null alleles, imperfect alleles, or poor amplification success in the subset of genotypes used for MSVAR, four loci were available for gorillas. Seven and six loci remained for Peters's and blue duiker respectively, and thirteen were used for mandrills. None of these remaining loci showed any evidence of significant linkage disequilibrium or

deviation from Hardy-Weinberg equilibrium. Summarized population statistics for each species are shown in Table 3.

The average number of observed alleles across loci ranged from 5.5 in gorillas to 13.8 in blue duikers, and cumulative probabilities of identity were less than 0.005 for all species. The mean A_R across sites was similar for all species when assuming a sample size of six chromosomes (3 diploid individuals), although this value is slightly higher in blue duiker. Comparing all sites for all species, A_R ranged from 2.84 in Peters's duiker in the Ogooué Lolo province to 5.08 in blue duiker in Ivindo National Park. Average heterozygosity varied from 0.61 (Peters's duiker) to 0.77 (mandrills). Average F_{is} values across loci and study sites were near zero for all species, although for duikers, some sites with very small sample sizes had higher F_{is} values that were nevertheless still insignificant.

Demographic history modeling in MSVAR

There was no strong evidence in any of the four species for a population bottleneck having occurred, whether using informative or uninformative priors, with Bayes Factors < 10 in all cases. All chains in all models for gorillas and duikers were shown to have converged according to the Gelman-Rubin statistic (Brooks and Gelman 1998). In the mandrill model, one of the five chains in the model using informative priors failed due to computational problems. However, convergence was reached for the remaining four chains and for the model with uninformative priors. Plots of the distribution of interlocus variation trended towards zero, indicating that all loci behaved similarly during coalescent simulations. Because the evidence does not support a model of $N_0 < N_1$, the models using uninformative hyperpriors ($N_0=N_1$) likely provide a more accurate representation of the demographic parameters of these species. Parameter estimates from these uninformative models are shown in Table 4, although these values have limited usefulness considering the breadth of the 95% HPDs.

Table 3 Summary statistics from each species across sampled populations

Species	Loci	Pop.	Samples	Mean A_R	A_R Range	Mean H_e	H_e Range	Mean H_o	H_o Range	Mean F_{is}	F_{is} Range
Gorilla	4	1	34	3.24	NA	0.71	NA	0.67	NA	0.056	NA
Peters's Duiker	7	9	6-143	3.53	2.84-4.46	0.61	0.52-0.75	0.58	0.48-0.71	0.043	-0.205
Blue Duiker	6	10	6-40	4.29	3.5-5.08	0.76	0.69-0.81	0.69	0.56-0.78	0.099	0.029-0.231
Mandrill	13	3	56-212	3.67	3.64-3.69	0.77	0.77-0.78	0.82	0.81-0.83	-0.07	-0.036

Pop.=number of populations

Table 4 Posterior distributions of parameters from MSVAR analyses

Species	BF_{uninf}	BF_{inf}	$\log(N_0/N_1)$	Parameter	Lower 95% HPD	Mean	Median	Upper 95% HPD
Gorilla	0.54	1.19	0.3	N_0	30	797	757	23,067
				N_1	8	397	422	17,750
				xa	69	3,463	3,471	154,064
Peters's duiker	3.89	8.75	-0.58	N_0	125	3,223	2,932	97,195
				N_1	618	12,479	12,653	248,788
				xa	55	1,822	1,667	81,503
Blue duiker	3.13	5.71	-0.45	N_0	103	2,065	2,000	41,495
				N_1	195	5,876	6,250	164,286
				xa	41	1,696	1,425	112,309
Mandrill	1.65	3.14	-0.17	N_0	92	1,962	1,891	42,914
				N_1	86	2,893	3,099	85,039
				xa	39	2,257	1,900	184,596

Bayes Factors from models using uninformative priors (BF_{uninf}) and informative priors (BF_{inf}), as well as $\log(N_0/N_1)$, which is a measure of decline severity based on mean values of N_0 and N_1. Negative values indicate a decline, positive values indicate an expansion, and a value of 0 suggests population stability. Mean, median, and 95% HPD for each parameter as estimated by the uninformative models are also shown, with N_0 and N_1 expressed in number of diploid individuals, and xa expressed in years.

Prior and posterior distributions for N_0 and N_1 for the uninformative models are shown in Figure 1. A small shift in the distribution between N_0 and N_1 is evident for all species, however, as described above, these shifts are not large enough to provide strong evidence for a population decline. In gorillas, mean N_1 is slightly smaller than N_0, but there is nearly complete overlap between the two distributions.

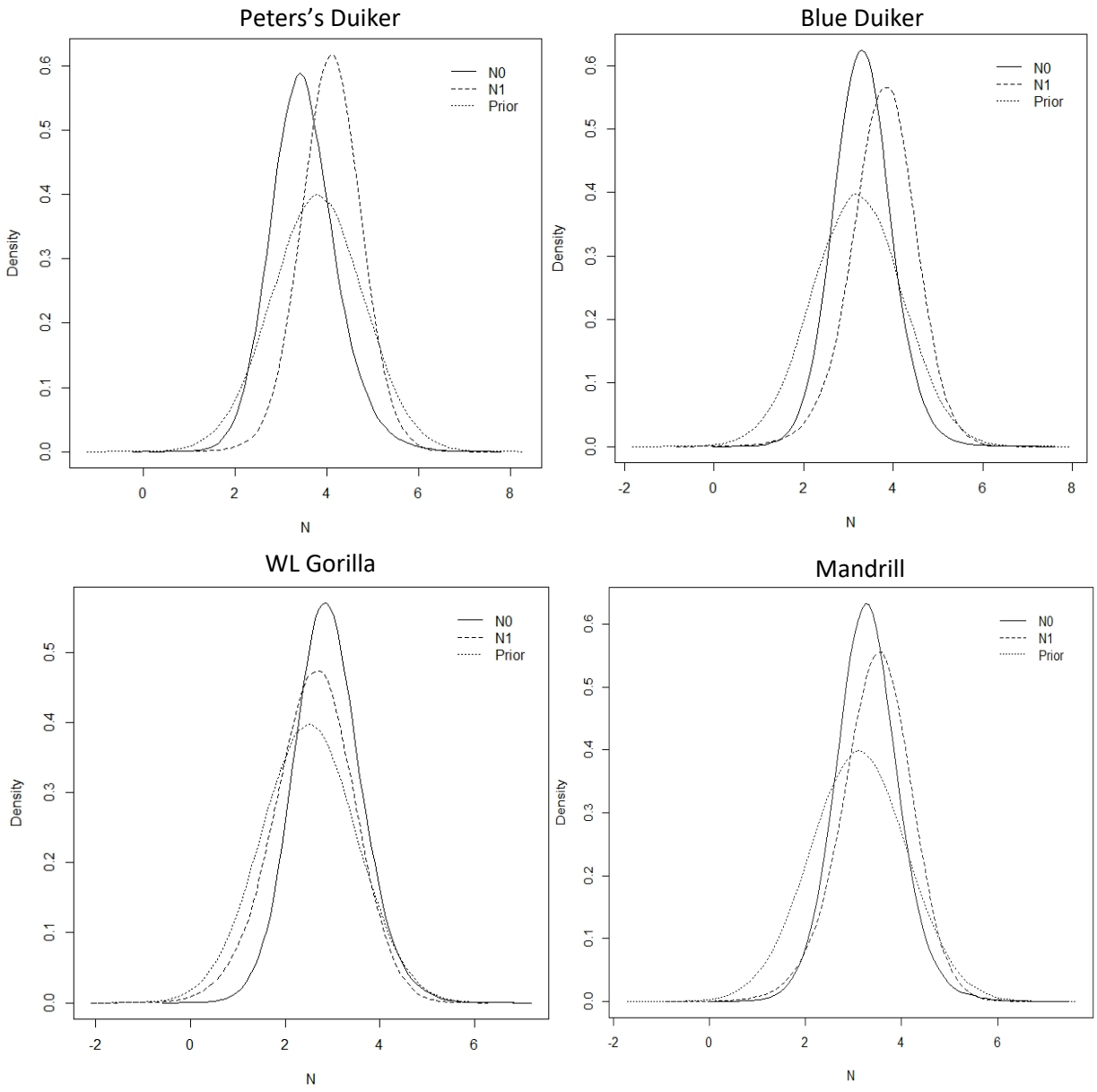

Figure 1 Prior and posterior distributions for current (N0) and historical (N1) effective population size from the uninformative models

Discussion

In this study, we present our evaluation of the demographic history of four central African forest mammals. After testing for evidence of population declines, we found that historical populations of all four species appear to have remained stable despite the increased aridity, seasonality, and forest cover loss that occurred during the RFC around 2-3,000 years ago (Maley 2002; Ngomanda, Chepstow-Lusty,

et al. 2009). These findings align with the previously proposed hypothesis that populations of blue and Peters's duikers expanded to their current ranges during the period of forest growth following the LGM (Ntie et al. 2017), although we did not directly test for population expansion. The stability of the mandrill population also suggests that the species' ability to traverse savannas may have increased their resilience to Holocene-era habitat fragmentation. This hypothesis is supported by the finding that the nearest relative of mandrills, the drills, underwent a climate-attributed decline during this period (Ting et al. 2012). Unlike their congener, drills do not appear to readily cross farmland or marginal habitats, potentially reducing their ability to tolerate forest fragmentation (Astaras 2009).

Our results were somewhat more surprising for gorillas, since in our study site, they use forest fragments minimally (Tutin 1999; Tutin, White, and Mackanga-Missandzou 1997) and were therefore expected to be more vulnerable to RFC-associated loss of forest cover. Western lowland gorillas in Lopé National Park instead appear to have been resilient to historical Holocene-era changes to their habitat. This finding is also supported by a similar study that found no evidence for bottlenecks in a population of western lowland gorillas at the Mondika Research Center (Bergl et al. 2008). Western gorillas maintain a large home range on the scale of 22.9 km^2 and can travel several kilometers per day in search of food and mates (Cipolletta 2004; Remis 1997, 1999). It might then be expected that their ranging in search of fruit resources would be limited by the loss of continuous forest. However, if their population density was relatively low during the RFC, then they may not have reached the ecosystem's carrying capacity despite the reduction of forest extent. Furthermore, around 1,800 years ago, when humid conditions returned after the RFC, savannas were colonized by the tree species that today form the Marantaceae forest (L. J. T. White 2001), the preferred habitat of western lowland gorillas (Tutin, White, and Mackanga-Missandzou 1997). The expansion of this particular type of forest may have been particularly beneficial to gorillas as it could have provided additional fruit resources and nesting sites (Tutin et al. 1995).

Our findings generally have positive implications for viability of the focal species in the face of fragmentation, aridity, and increased seasonality. In central Africa, the coming decades are expected to bring increased temperatures and changes to precipitation patterns resulting from climate change (James and Washington 2013), as well as increasingly fragmented forests as a consequence of agriculture, logging, and urban growth (Taubert et al. 2018). Habitat patches with environmental conditions suitable for wildlife, particularly great apes, are expected to decline (Junker et al. 2012). Although the four focal species have withstood substantial environmental change in the past, the threats today may be compounded by other factors. For instance, a negative relationship may exist between fragmentation and hunting pressure, since forest fragments are generally more accessible to hunters than contiguous forest (Péres 2001). All four species studied here face pressure from hunting and are thought to be in decline according to the IUCN. In fact, the ape population of Gabon is known to have declined by more than half between 1983 and 2000, largely due to commercial hunting as well as outbreaks of the Ebola virus (Bermejo et al. 2006; Walsh et al. 2003). This decline was not detected in the present study, but this is not surprising as coalescent methods are most robust on longer time scales (Girod et al. 2011).

Additionally, recent research has documented a marked decrease in fruit availability in Lopé National Park, thought to be a result of increasing temperatures (Bush et al. 2020). It is unknown whether the loss of this resource affected wildlife during the RFC, but the current trend has been associated with declining body condition in elephants and may pose a threat to other frugivorous species, including mandrills and gorillas.

In short, our finding of historical stability does not necessarily predict future viability, in light of novel climatic and anthropogenic threats. Further research is needed to better understand potential impacts of future environmental change on central African forest wildlife.

Literature Cited

Abernethy, Katharine, Lee J.T. White, and E J Wickings. 2002. "Hordes of Mandrills (Mandrillus Sphinx): Extreme Group Size and Seasonal Male Presence." *Journal of Zoology, Proceedings of the Zoological Society of London* 258: 131–37.

Anthony, Nicola M. et al. 2007. "The Role of Pleistocene Refugia and Rivers in Shaping Gorilla Genetic Diversity in Central Africa." *Proceedings of the National Academy of Sciences of the United States of America* 104(51): 20432–36.

Astaras, Christos. 2009. PhD diss. "Ecology and Status of the Drill (Mandrillus Leucophaeus) in Korup National Park, Southwest Cameroon: Implications for Conservation." University of Gottingen.

Bayon, Germain et al. 2012. "Intensifying Weathering and Land Use in Iron Age Central Africa." *Science* 335(6073): 1219–22.

Beaumont, Mark A. 1999. "Detecting Population Expansion and Decline Using Microsatellites." *Genetics* 153(4): 2013–29.

Benoit, Laure et al. 2014. "Using Next-Generation Sequencing Methods to Isolate and Characterize 24 Simple Sequence Repeat Loci in Mandrills (Mandrillus Sphinx)." *Conservation Genetics Resources* 6(4): 903–5.

Bergl, Richard A., Brenda J. Bradley, Anthony Nsubuga, and Linda Vigilant. 2008. "Effects of Habitat Fragmentation, Population Size and Demographic History on Genetic Diversity: The Cross River Gorilla in a Comparative Context." *American Journal of Primatology* 70(9): 848–59.

Bermejo, Magdalena et al. 2006. "Ebola Outbreak Killed 5000 Gorillas." *Science* 314(5805): 1564.

Born, Ceine et al. 2011. "Insights into the Biogeographical History of the Lower Guinea Forest Domain: Evidence for the Role of Refugia in the Intraspecific Differentiation of Aucoumea Klaineana." *Molecular Ecology* 20: 131–42.

Brooks, Stephen P., and Andrew Gelman. 1998. "General Methods for Monitoring Convergence of Iterative Simulations)?" *Journal of Computational and Graphical Statistics* 7(4): 434–55.

Bush, Emma R. et al. 2020. "Long-Term Collapse in Fruit Availability Threatens Central African Forest Megafauna." *Science* 370(6521): 1219–22.

Chikhi, Lounès et al. 2010. "The Confounding Effects of Population Structure, Genetic Diversity and the Sampling Scheme on the Detection and Quantification of Population Size Changes." *Genetics* 186(3): 983–95.

Cipolletta, Chloé. 2004. "Effects of Group Dynamics and Diet on the Ranging Patterns of a Western Gorilla Group (Gorilla Gorilla Gorilla) at Bai Hokou, Central African Republic." *American Journal of Primatology* 64(2): 193–205.

Clist, Bernard et al. 2018. "Did Human Activity Really Trigger the Late Holocene Rainforest Crisis in Central Africa?" *Proceedings of the National Academy of Sciences of the United States of America* 115(21): E4733–34.

Colyn, Marc, Annie Gautier-Hion, and Walter Verheyen. 1991. "A Re-Appraisal of Palaeoenvironmental History in Central Africa: Evidence for a Major Fluvial Refuge in the Zaire Basin." *Journal of*

Biogeography 18(4): 403.

Cushman, Samuel A. 2006. “Effects of Habitat Loss and Fragmentation on Amphibians: A Review and Prospectus.” *Biological Conservation* 128(2): 231–40.

Do, C. et al. 2014. “NeEstimator v2: Re-Implementation of Software for the Estimation of Contemporary Effective Population Size (Ne) from Genetic Data.” *Molecular Ecology Resources* 14(1): 209–14.

Ellegren, Hans. 2000. “Microsatellite Mutations in the Germline: Implications for Evolutionary Inference.” *Trends in Genetics* 16(12): 551–58.

———. 2004. “Microsatellites: Simple Sequences with Complex Evolution.” *Nature Reviews Genetics* 5(6): 435–45.

Excoffier, Laurent, and Heidi E L Lischer. 2010. “Arlequin Suite Ver 3.5: A New Series of Programs to Perform Population Genetics Analyses under Linux and Windows.” *Molecular Ecology Resources* 10(3): 564–67.

Feer, F. 1988. “Stratégies Écologiques de Deux Espèces de Bovides Sym- Patriques de La Forêt Sempervirente Africaine (Cephalphus Callipygus et C. Dorsalis): Influence Du Rhythme d’activité.” Université de Pierre et Marie Curie, Paris.

Di Fiore, Anthony. 2005. “A Rapid Genetic Method for Sex Assignment in Non-Human Primates.” *Conservation Genetics* 6: 1053–58.

Gaetano, Justin. 2018. “Holm-Bonferroni Sequential Correction: An Excel Calculator (1.3).” https://www.researchgate.net/publication/322569220_Holm-Bonferroni_sequential_correction_An_Excel_calculator_13.

Gagneux, P., C. Boesch, and D. S. Woodruff. 1997. “Microsatellite Scoring Errors Associated with Noninvasive Genotyping Based on Nuclear DNA Amplified from Shed Hair.” *Molecular Ecology* 6: 861–68.

Garcin, Yannick, Pierre Deschamps, Guillemette Ménot, Geoffroy de Saulieu, Enno Schefub, et al. 2018. “Human Activity Is the Most Probable Trigger of the Late Holocene Rainforest Crisis in Western Central Africa.” *Proceedings of the National Academy of Sciences of the United States of America* 115(21): E4735–36.

Garcin, Yannick, Pierre Deschamps, Guillemette Ménot, Geoffroy de Saulieu, Enno Schefuß, et al. 2018. “No Evidence for Climate Variability during the Late Holocene Rainforest Crisis in Western Central Africa.” *Proceedings of the National Academy of Sciences of the United States of America* 115(29): E6674–75.

Girod, Christophe, Renaud Vitalis, Raphaël Leblois, and Hélène Fréville. 2011. “Inferring Population Decline and Expansion from Microsatellite Data: A Simulation-Based Evaluation of the Msvar Method.” *Genetics* 188(1): 165–79.

Goudet, Jérôme. 2003. “Fstat (Ver. 2.9.4), a Program to Estimate and Test Population Genetics Parameters.” https://www2.unil.ch/popgen/softwares/fstat.htm.

Guibinga Mickala, Amour et al. 2022. “Estimation of the Census (Nc) and Effective (Ne) Population Size of a Wild Mandrill (Mandrillus Sphinx) Horde in the Lope National Park, Gabon Using a Non-Invasive Genetic Approach.” *Conservation Genetics*.

Haffer, Jurgen. 1969. “Speciation in Amazonian Forest Birds.” *Science* 165(3889): 131–37.

Hedwig, Daniela et al. 2018. “A Camera Trap Assessment of the Forest Mammal Community within the Transitional Savannah-Forest Mosaic of the Batéké Plateau National Park, Gabon.” *African Journal of Ecology* 56(4): 777–90.

Heller, R. et al. 2008. “Mid-Holocene Decline in African Buffalos Inferred from Bayesian Coalescent-Based Analyses of Microsatellites and Mitochondrial DNA.” *Molecular Ecology* 17(22): 4845–58.

Holm, Sture. 1979. “A Simple Sequentially Rejective Multiple Test Procedure.” *Scandinavian Journal of Statistics* 6(2): 65–70.

IUCN SSC Antelope Specialist Group. 2016a. “Cephalophus Callipygus.” *The IUCN Red List of Threatened Species*.

———. 2016b. “Philantomba Monticola.” *The IUCN Red List of Threatened Species*. http://dx.doi.org/10.2305/IUCN.UK.2016- 1.RLTS.T4143A50183103.en.

James, Rachel, and Richard Washington. 2013. “Changes in African Temperature and Precipitation Associated with Degrees of Global Warming.” *Climatic Change* 117(4): 859–72.

Junker, Jessica et al. 2012. “Recent Decline in Suitable Environmental Conditions for African Great Apes.” *Diversity and Distributions* 18(11): 1077–91.

Kalinowski, Steven T. 2005. “HP-RARE 1.0: A Computer Program for Performing Rarefaction on Measures of Allelic Richness.” *Molecular Ecology Notes* 5(1): 187–89.

Kass, Robert E., and Adrian E. Raftery. 1995. “Bayes Factors.” *Journal of the American Statistical Association* 90(430): 773–95.

Keinath, Douglas A. et al. 2017. “A Global Analysis of Traits Predicting Species Sensitivity to Habitat Fragmentation.” *Global Ecology and Biogeography* 26(1): 115–27.

Laurance, William F., Leandro V. Ferreira, Judy M Rankin-de Merona, and Susan G. Laurance. 1998. “Rain Forest Fragmentation and the Dynamics of Amazonian Tree Communities.” *EcologyEcology* 79(6): 2032–40.

Maisels, Fiona, R. A. Bergl, and E. A. Williamson. 2018. “Gorilla Gorilla (Amended Version of 2016 Assessment).” *The IUCN Red List of Threatened Species*. https://www.iucnredlist.org/species/9404/136250858.

Maley, Jean. 1991. “The African Rain Forest Vegetation and Palaeoenvironments during Late Quaternary.” *Climate Change* 19(1–2): 79–98.

———. 1996. 104 Proceedings of the Royal Society of Edinburgh Section B: Biological Sciences *The African Rain Forest - Main Characteristics of Changes in Vegetation and Climate from the Upper Cretaceous to the Quaternary*.

———. 2002. “A Catastrophic Destruction of African Forests about 2,500 Years Ago Still Exerts a Major Influence on Present Vegetation Formations.” *IDS Bulletin* 33(1): 13–30.

———. 2018. “Late Holocene Forest Contraction and Fragmentation in Central Africa.” *Quaternary Research (United States)* 89(1): 43–59.

Massa, Bruno. 2020. “Revision of the Afrotropical Genus Leiodontocercus (Orthoptera, Tettigoniidae ,

Phaneropterinae) with a Description of Four New Species." *ZooKeys* 951: 47–65.

Matschiner, Michael, and Walter Salzburger. 2009. "TANDEM: Integrating Automated Allele Binning into Genetics and Genomics Workflows." *Bioinformatics* 25(15): 1982–83.

Muloko-Ntoutoume, N, R J Petit, Lee J.T. White, and Katharine Abernethy. 2000. "Chloroplast DNA Variation in a Rainforest Tree (Aucoumea Klaineana, Burseraceae) in Gabon." *Molecular Ecology* 9(3): 359–63.

Neumann, Katharina et al. 2012. "First Farmers in the Central African Rainforest: A View from Southern Cameroon." *Quaternary International* 249: 53–62.

Ngomanda, Alfred, A. Chepstow-Lusty, et al. 2009. "Western Equatorial African Forest-Savanna Mosaics: A Legacy of Late Holocene Climatic Change?" *Climate of the Past* 5: 647–59.

Ngomanda, Alfred, Katharina Neumann, Astrid Schweizer, and Jean Maley. 2009. "Seasonality Change and the Third Millennium BP Rainforest Crisis in Southern Cameroon (Central Africa)." *Quaternary Research* 71(3): 307–18. http://dx.doi.org/10.1016/j.yqres.2008.12.002.

Nicolas, V et al. 2011. "The Roles of Rivers and Pleistocene Refugia in Shaping Genetic Diversity in Praomys Misonnei in Tropical Africa." *Journal of Biogeography* 38: 191–207.

Ntie, Stephan et al. 2017. "Evaluating the Role of Pleistocene Refugia, Rivers and Environmental Variation in the Diversification of Central African Duikers (Genera Cephalophus and Philantomba)." *BMC Evolutionary Biology* 17(1): 1–17.

Ntie, Stephan, Iván D. Soto-Calderón, Mitchell J. Eaton, and Nicola M. Anthony. 2010. "Cross-Species Amplification of Bovid Microsatellites in Central African Duikers (Genus Cephalophus) and Other Sympatric Artiodactyls." *Molecular Ecology Resources* 10(6): 1059–65.

Okello, J. B.A. et al. 2008. "Effective Population Size Dynamics Reveal Impacts of Historic Climatic Events and Recent Anthropogenic Pressure in African Elephants." *Molecular Ecology* 17(17): 3788–99.

van Oosterhout, Cock, William F. Hutchinson, Derek P M Wills, and Peter Shipley. 2004. "MICRO-CHECKER: Software for Identifying and Correcting Genotyping Errors in Microsatellite Data." *Molecular Ecology Notes* 4(3): 535–38.

Oslisly, Richard. 2001. "The History of Human Settlement in the Middle Ogooue Valley (Gabon)." In *African Rain Forest Ecology and Conservation: An Interdisciplinary Perspective*, , 101–18.

Péres, Carlos A. 2001. "Synergistic Effects of Subsistence Hunting and Habitat Fragmentation on Amazonian Forest Vertebrates." *Conservation Biology* 15(6): 1490–1505.

Püttker, Thomas et al. 2020. "Indirect Effects of Habitat Loss via Habitat Fragmentation: A Cross-Taxa Analysis of Forest-Dependent Species." *Biological Conservation* 241: 108368. https://doi.org/10.1016/j.biocon.2019.108368.

Remis, Melissa J. 1997. "Western Lowland Gorillas (Gorilla Gorilla Gorilla) as Seasonal Frugivores: Use of Variable Resources." *American Journal of Primatology* 43(2): 87–109.

———. 1999. "Ranging and Grouping Patterns of a Western Lowland Gorilla Group at Bai Hokou, Central African Republic." *American Journal of Primatology* 43(2): 111–33.

Rogers, M, Katharine Abernethy, and B Fontaine. 1996. "Ten Days in the Life of a Mandrill Horde in the

Lope Reserve Gabon." *American Journal of Primatology* 40(4): 297–313.

Setchell, J.M., Marie Charpentier, and E. Jean Wickings. 2005. "Sexual Selection and Reproductive Careers in Mandrills (Mandrillus Sphinx)." *Behavioral Ecology and Sociobiology* 58(5): 474–85.

Smith, Brian J. 2007. "Boa: An R Package for MCMC Output Convergence." *Journal of Statistical Software* 21(11).

Soto-Calderón, Iván D. et al. 2009. "Effects of Storage Type and Time on DNA Amplification Success in Tropical Ungulate Faeces." *Molecular Ecology Resources* 9(2): 471–79.

Storz, Jay, and Mark A. Beaumont. 2002. "Testing for Genetic Evidence of Population Expansion and Contraction: An Emprical Analysis of Microsatellite DNA Variation Using a Hierarchical Bayesian Model." *Evolution* 56(1): 156–66.

Taberlet, Pierre et al. 1996. "Reliable Genotyping of Samples with Very Low DNA Quantities Using PCR." *Nucleic Acids Research* 24(16): 3189–94.

Taubert, Franziska et al. 2018. "Global Patterns of Tropical Forest Fragmentation." *Nature* 554(7693): 519–22. http://dx.doi.org/10.1038/nature25508.

Team, R Core. 2020. "R: A Language and Environment for Statistical Computing." https://www.r-project.org/.

Telfer, Paul T et al. 2003. "Molecular Evidence for Deep Phylogenetic Divergence in Mandrillus Sphinx." *Molecular Ecology* 12(7): 2019–24.

Thalmann, O. H. et al. 2007. "The Complex Evolutionary History of Gorillas: Insights from Genomic Data." *Molecular Biology and Evolution* 24(1): 146–58.

Ting, Nelson et al. 2012. "Genetic Signatures of a Demographic Collapse in a Large-Bodied Forest Dwelling Primate (Mandrillus Leucophaeus)." *Ecology and Evolution* 2(3): 550–61.

Tutin, Caroline E.G. 1999. "Fragmented Living: Behavioural Ecology of Primates in a Forest Fragment in the Lopé Reserve, Gabon." *Primates* 40(1): 249–65.

Tutin, Caroline E.G., and M. Fernandez. 1984. "Nationwide Census of Gorilla (Gorilla g. Gorilla) and Chimpanzee (Pan t. Troglodytes) Populations in Gabon." *American Journal of Primatology* 6(4): 313–36.

Tutin, Caroline E.G., Richard J. Parnell, Lee J.T. White, and Michel Fernandez. 1995. "Nest Building by Lowland Gorillas in the Lopé Reserve, Gabon: Environmental Influences and Implications for Censusing." *International Journal of Primatology* 16(1): 53–76.

Tutin, Caroline E.G., Lee J.T. White, and A. Mackanga-Missandzou. 1997. "The Use by Rain Forest Mammals of Natural Forest Fragments in an Equatorial African Savanna." *Conservation Biology* 11(5): 1190–1203.

Valière, Nathaniel. 2002. "GIMLET: A Computer Program for Analysing Genetic Individual Identification Data." *Molecular Ecology Notes* 2(3): 377–79.

Waits, Lisette P, Gordon Luikart, and Pierre Taberlet. 2001. "Estimating the Probability of Identity among Genotypes in Natural Populations : Cautions and Guidelines." *Molecular Ecology* 10(1): 249–56.

Walsh, Peter D. et al. 2003. "Catastrophic Ape Decline in Western Equatorial Africa." *Nature* 422(6932):

611–14.

Waples, Robin S. 2006. “A Bias Correction for Estimates of Effective Population Size Based on Linkage Disequilibrium at Unlinked Gene Loci.” *Conservation Genetics* 7: 167–84.

White, Elizabeth C. et al. 2010. “Home-Range Use by a Large Horde of Wild Mandrillus Sphinx.” *International Journal of Primatology* 31(4): 627–45.

White, Lee J.T. 2001. “Forest-Savannah Dynamics and the Origins of Marantaceae Forest in Central Gabon.” In *African Rain Forest Ecology and Conservation: An Interdisciplinary Perspective*, , 165–82.

Williamson, E A, Caroline E.G. Tutin, and M Fernandez. 1988. “Western Lowland Gorillas Feeding in Streams and on Savannas.” *Primate Report* 19: 29–34.

Willis, K. J., L. Gillson, and T. M. Brncic. 2004. “How ‘Virgin’ Is Virgin Rainforest?” *Science* 304(5669): 402–3.

Chapter 2

Targeted amplicon sequencing of complex loci from noninvasive samples: Identifying pitfalls and solutions

(under review at Molecular Ecology Resources)

Abstract

Fecal samples are a valuable source of genetic material for wildlife and are often used in population genetic and genomic studies. However, DNA from such samples tends to be highly degraded, and its reliability for targeted amplicon sequencing of complex gene families has not been thoroughly assessed. Here, we quantify replicability of Illumina-generated allele assignments for a 157-base fragment of the hypervariable DRB gene of the major histocompatibility complex (MHC) amplified from wild mandrills (*Mandrillus sphinx*). We show that R_A, the average proportion of variants that replicate between sequencing runs, is significantly lower in fecal samples (R_A=0.32) than in tissue samples (R_A=0.76). We also found that high-depth sequence variants in fecal samples are not necessarily replicable across runs, contrary to the common assumption that depth indicates "true" alleles. Furthermore, excluding low-depth amplicons did not improve replicability. To circumvent these issues, we present a method of allele assignment that uses variant replicability rather than read depth, and we apply this method to generate an in-depth characterization of the wild mandrill MHC-DRB. We observe trans-species polymorphism and shared alleles between mandrills and olive baboons, crab-eating macaques, and western lowland gorillas. We also note the presence of the putative DRB9 pseudogene lineage, which has rarely been documented in non-human primates. Although the lack of known MHC allele assignments in the study population precludes rigorous verification of our method, it shows promise for studies utilizing high throughput sequencing of degraded DNA, where contamination, allelic dropout, and PCR bias can hinder reliable allele identification.

Introduction

Adaptive genetic variation has important effects on species viability (Manlik et al., 2019; Muñoz et al., 2015; Sgrò et al., 2011; Sommer, 2005), especially in a rapidly-changing world where organisms may have to adapt over short time frames (Hoffmann & Sgró, 2011). Organisms lacking sufficient standing variation may be less adaptable to environmental changes and more vulnerable to novel pathogens (Meyers & Bull, 2002). Therefore, functional genetic variation is an important indicator of adaptive capacity (Holderegger et al., 2006) that can be used to guide conservation planning (Morgan et al., 2020).

Loci within the class II major histocompatibility complex (MHC) have drawn considerable attention from conservationists over past decades due to their role in vertebrate immunity (Manlik et al., 2019; Sommer, 2005). Class II MHC genes encode glycoproteins on the surface of antigen presenting cells. These glycoproteins bind pathogenic peptides and present them to T cells to initiate an immune response (Kaufman et al., 1984; Unanue, 1984). The amino acids present in the glycoprotein's antigen binding groove determine its binding properties, so variability in these amino acids enables immune responses against multiple pathogens (Matsumura et al., 1992; Ou et al., 1998). The class II MHC genes are thought to be under strong balancing selection and include some of the most polymorphic loci in the vertebrate genome (Hughes & Nei, 1989; Klein et al., 1993). For instance, the MHC DRB gene complex within humans (or Human Leukocyte Antigen, HLA) is currently known to include 4,018 alleles spread across nine lineages denoted DRB1-9 (Robinson et al., 2020). Of the nine lineages, DRB1, 3, 4, and 5 are functional and highly polymorphic, each containing hundreds or thousands of alleles (Klein et al., 2007). In an example of trans-species polymorphism (TSP), these MHC lineages predate primate speciation and appear to be conserved across species (Geluk et al., 1993; Kelley et al., 2005; Slierendregt et al., 1992). With its extreme functional diversity, the MHC has proven to be an important study system for research in conservation and evolution. It has been used to study host-parasite co-evolution (Biedrzycka et al.,

2018; Hedrick, 2002), delineate conservation units (Vásquez-Carrillo et al., 2014; Zhu et al., 2013), and infer migratory connectivity (Rodríguez et al., 2011). In many species, the MHC also plays a role in mate choice and reproductive success, since offspring fitness may be affected by MHC genotype (Dandine-Roulland et al., 2019; Gasparini et al., 2015; Huchard et al., 2013; Rekdal et al., 2019).

Despite the value of diversity in the class II MHC, its complexity presents a challenge for sequencing and genotyping (Lighten, van Oosterhout, & Bentzen, 2014; Lighten, van Oosterhout, Paterson, et al., 2014). Many studies seeking to characterize the MHC have found success in next-generation sequencing (NGS) (Bracamonte et al., 2022; Grogan et al., 2016; Lighten, van Oosterhout, Paterson, et al., 2014; Million & Lively, 2022). Since massively parallel sequencing produces large amounts of data, numerous methods have been developed to synthesize thousands of reads into concise MHC allele assignments or genotypes (Grogan et al., 2016; Lighten, van Oosterhout, Paterson, et al., 2014; Radwan et al., 2012; Sebastian et al., 2016; Sommer et al., 2013). A primary goal of these methods is to differentiate true alleles from sequence artifacts. The exact approach differs among methods, but some common assumptions are that true alleles should have higher read depth than sequence artifacts (Babik, 2010; Cummings et al., 2010) and should appear in more than one individual given a large enough sample size (Sommer et al., 2013). Artifacts are often detected as low-depth sequences that can be highly similar to a true allele if single-base substitution or indel errors occur. These artifacts may also appear as a combination of two parental alleles, in the case of a chimeric sequence (Cummings et al., 2010). The previously developed allele calling methods have been shown to have good repeatability across sequencing runs. For example, Biedrzycka et al. (2017) showed that when sequence depth is greater than 5,000 reads per sample, repeatability between sequencing runs ranges from 90% to 98% depending on the genotyping method chosen. Similarly, Million and Lively (2022) found an 87% replicability for their samples with average read depth of 4,205, and Lighten et al. (2014) found 83.6%, with 2,484 reads per sample on average.

Although NGS is a powerful tool to quantify MHC variability, past studies using NGS have almost always relied upon high quality genetic material, such as blood or tissue samples (Biedrzycka et al., 2020; Gagnon et al., 2020; Kessler et al., 2021; Million & Lively, 2022). Such samples are generally difficult to obtain from wildlife. As an alternative, researchers may turn to other sources of DNA that require minimal to no contact with the focal species, such as feces, feathers, shed hair, or eggshells (Carroll et al., 2018). Such non-invasive samples generally contain highly degraded DNA and are prone to allelic dropout and cross-contamination (Morin et al., 2001; Taberlet et al., 1999). Nevertheless, fecal DNA has been used to generate genetic information from a wide range of species, such as duikers (Ntie et al., 2017), gorillas (Clifford et al., 2004), tigers (Natesh et al., 2019), and bonobos (Gerloff et al., 1999).

Fecal samples have been used with success in studies of the MHC (Arguello-Sánchez et al., 2018; de Winter et al., 2019; Zhang et al., 2018). These studies have rarely utilized NGS, however, with most relying on older sequencing methods such as Sanger sequencing (Arguello-Sánchez et al., 2018; Yu et al., 2018; Zhang et al., 2018), single-stranded conformation polymorphism (Maruya et al., 1996), or denatured gradient gel electrophoresis (Huchard et al., 2006; Knapp et al., 1997; Myers et al., 1985; Setchell et al., 2009). These methods are labor-intensive and may not be feasible for large sample sizes. Hans et al. (2015) pioneered the use of targeted amplicon sequencing using NGS with fecal DNA in a study characterizing MHC in two species of gorilla (*Gorilla gorilla* and *G. beringei*). In this case, non-invasive sampling combined with NGS allowed the most comprehensive description of gorilla MHC that was available at the time. Despite the demonstrated potential of these methods, few, if any, other species have had their MHC characterized in this manner. In addition, although Hans et al. (2015) employed rigorous quality controls in their study, the reliability and replicability of MHC alleles identified from non-invasive samples using NGS has not been thoroughly evaluated.

Here, we aim to use NGS of non-invasive samples to characterize the class II MHC-DRB in another African primate, the mandrill (*Mandrillus sphinx*). Mandrills are endemic to Gabon, Cameroon,

Equatorial Guinea, and the Republic of Congo and are a species of conservation concern due to habitat loss and hunting (Abernethy & Maisels, 2019). They are categorized as Vulnerable by the International Union for the Conservation of Nature (IUCN) and listed under Appendix I of the Convention on International Trade in Endangered Species (CITES). The mandrill MHC has been well studied in a single captive population (Abbott et al., 2006; Charpentier et al., 2006; Setchell et al., 2009), and its variation appears to play a role in interactions among conspecifics. Females have been shown to preferentially mate with MHC-dissimilar males (Setchell et al., 2010), and specific MHC alleles are associated with the male's dramatic red facial coloration (Setchell et al., 2009). However, despite the apparently important role of MHC diversity in mandrill reproduction and signaling, it has never been studied in a wild population. Furthermore, the captive population is highly inbred, having originated from fifteen founder individuals in 1983 (Charpentier et al., 2005; Wickings, 1995), so it cannot be assumed that studies of this colony adequately represent natural variation in wild mandrill populations. Although wild mandrills can be difficult to study in their dense forest habitat, non-invasive sampling is feasible in certain landscapes. The ancient forest-savannah mosaic in northern Lopé National Park, Gabon, provides an opportunity to sample mandrill feces, as it contains a wild mandrill horde of close to a thousand individuals (Abernethy et al., 2002; Guibinga Mickala et al., 2022; White et al., 2010). Males are only present seasonally, and the horde is thought to use a fission-fusion system to take advantage of patchy resources (Abernethy et al., 2002; White et al., 2010). Because of the mosaic habitat, this horde, known as the SEGC horde, is relatively easy to sample non-invasively.

In this study, we test whether NGS-derived MHC allele assignments from fecal samples of the SEGC horde are equally replicable as those from tissue samples. We quantify assignment repeatability between NGS runs for fecal and tissue samples and between duplicate samples of feces. We also test whether the common assumptions that true alleles should be at a higher depth and appear in more than one individual hold true for non-invasive samples. After showing that previously-published assignment

methods are problematic in fecal samples, we apply a novel method of MHC allele assignment to assess functional genetic diversity in the SEGC horde to better understand the species' adaptive potential. Finally, we compare MHC alleles present in the wild horde to those found in the captive colony, and to that of other primates to examine trends of TSP.

Methods

Sample Collection

Fresh samples of feces (typically <6 hours) were collected daily from the SEGC horde during July or August of 2016 and 2017, when both sexually mature males and females are present (Abernethy et al., 2002; Hongo et al., 2016). Researchers located the horde using radio telemetry, then followed on foot, collecting ~1 cm^3 of dung per sample. Feces were stored immediately in Falcon tubes with 25mL of silica (Soto-Calderón et al. 2009). Various samples were also collected between 2016 and 2018 from a total of 22 radio-collared mandrills: fourteen samples of whole blood, nine samples of plucked hair with attached bulb tissue, and nine fecal samples. DNA from fecal samples was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, CA), while the DNeasy Blood & Tissue Kit (Qiagen, CA) was used for blood and hair samples. Negative controls for DNA extraction were also prepared regularly by performing all extraction steps with a blank containing no extracted DNA.

PCR Amplification and Sequencing

A 157-nucleotide fragment of the MHC DRB gene was amplified from extracted DNA using the following PCR primers: forward primer sequence 5'-TTCTTCAAYGGGACGGAGC-3', reverse primer sequence 5'-GTGTCTGCAGTAGGTGTCC-3'. The standard Illumina linker sequences were also added to the 5' end of each primer to facilitate library preparation and Illumina sequencing. To improve amplification success of the fecal DNA extractions, the primers were designed interior to the binding sites used in previous studies of mandrill and macaque MHC (Knapp et al. 1997; Abbott, Wickings, and Knapp 2006). The redesign of these primers resulted in a relatively short sequence, but the amplicon

encompasses much of the peptide binding region (PBR), which contains amino acid residues that are important targets of selection (Brown et al., 1993). All PCRs were performed in a total volume of 25µl, with 12.5 µL 2X GoTaq HotStart Polymerase Master Mix (Promega), 0.5 mM $MgCl_2$, 400 nM of each primer, 8.25 µL of water, and 2 µL of template DNA. Cycling conditions were as follows: 5 minutes initial denaturation at 98°C, followed by 38 cycles of 95°C for 30 seconds, 65°C for 30 seconds, and 72°C for 60 seconds, and finally a ten-minute final extension at 72°C. Successful amplification of each sample was confirmed by agarose gel electrophoresis.

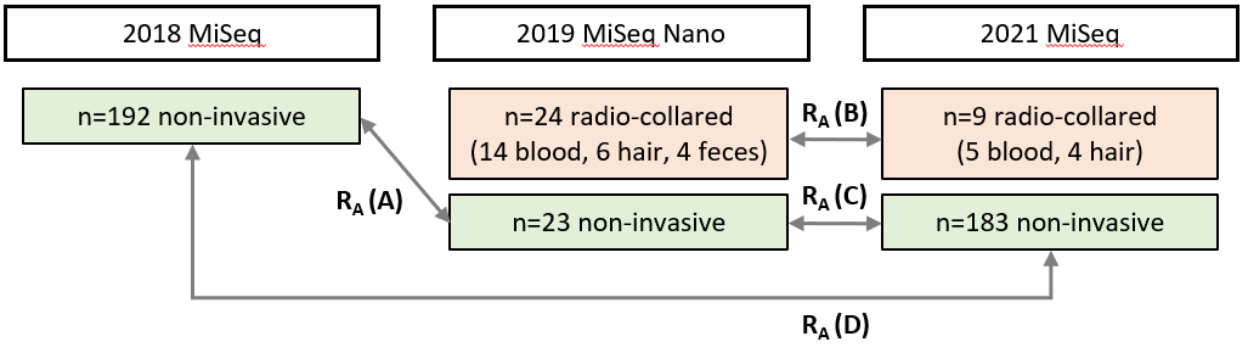

Figure 1 Replicability scores calculated between different sample types among three independent Illumina runs. Gray arrows show the pairs of Illumina runs used to calculate R_A scores, and letters A, B, C, or D denote comparisons between runs. Mann-Whitney U tests were used to test for differences in the average R_A values from comparisons (i) B and C, (ii) A and C, and (iii) C and D. Because comparisons B and D have very different sample sizes, their average R_A scores were compared by randomly selecting 1000 sets of nine values from D to generate a distribution of R_A scores. Statistical significance was assessed based on whether the average value of B was greater than the 95th percentile of the permuted distribution of scores from non-invasive samples.

To quantify replicability and establish robust allele assignments for each sample, three independent paired-end Illumina runs were performed, all using 150 sequencing cycles (Figure 1). The first, performed in 2018, was a standard Illumina MiSeq run of 192 pooled PCR products generated exclusively from non-invasive fecal samples. In 2019, a MiSeq Nano run was conducted to sequence the 24 successfully-amplified samples of blood, hair, and feces from the radio-collared mandrills, along with

replicates of 23 of the non-invasive samples originally sequenced in the 2018 MiSeq run. A PCR-amplified DNA sample from the first author was also included in this run to monitor contamination from human DNA. Finally, 192 replicate PCR products (comprising nine blood and hair samples and 183 previously sequenced noninvasive samples) were pooled into another standard MiSeq run in 2021. For repeatability calculations and development of consensus allele assignments, these three runs were considered in pairs as shown in Figure 1. A schematic of the overall workflow is shown in Figure 2.

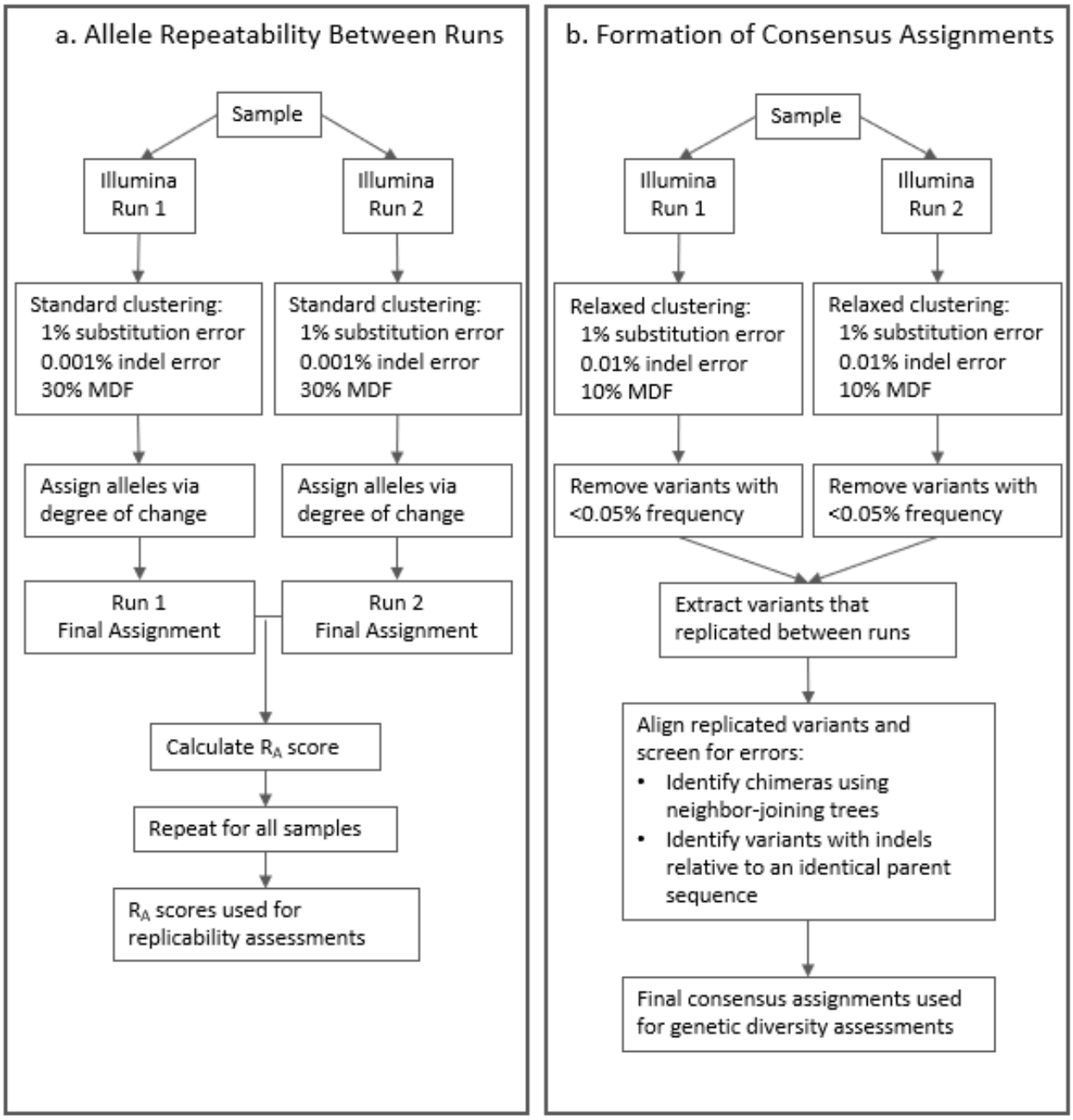

Figure 2 The above figure shows the processes undertaken for each sample to determine its repeatability score between runs (*a*) and its consensus allele assignment (*b*). Pairs of sequencing runs used for each sample are shown in Figure 1. For within-run repeatability of duplicate fecal samples, the same repeatability quantification process as in *a* was applied to the pair of samples within the run. R_A scores were also calculated between the consensus assignments for duplicate sample pairs. MDF = minimum dominant frequency threshold

Repeatability of MHC allele assignments

To quantify allele replicability between sequencing runs, data from each Illumina run was processed independently (Figure 2a). Illumina adapter sequences were trimmed using Trimmomatic (Bolger et al., 2014), and primer sequences were removed using the Python program cutPrimers (Kechin et al., 2017). Paired end reads were merged and cleaned using tools in the ampliSAT pipeline, ampliMERGE and ampliCLEAN (Sebastian et al., 2016). Merged reads with an average Phred quality score below 30 were discarded, as were reads falling outside the range of 152-160 nucleotides in length. Using the ampliSAS tool, artificial sequences were identified and clustered with their putative parent sequences, with each artifact's read depth added to that of its parent (Sebastian et al., 2016). Clustering parameters allowed a 1% substitution error threshold, a 0.001% indel error threshold, and a 30% minimum dominant frequency threshold (MDF), which allows sequences that are similar to a higher-depth variant to become the dominant sequence of a new cluster if they have sufficiently high read depth. We used the degree of change (DOC) method (Lighten, van Oosterhout, Paterson, et al., 2014) as implemented in ampliSAS to differentiate true alleles from artefacts in amplicons containing at least one hundred reads after filtering. In the DOC method, the depths of each variant cluster in an amplicon are added cumulatively in descending order, and the first and second derivative of the resulting cumulative depth curve is used to identify a breakpoint in read depth that separates true alleles from artifacts.

After applying the DOC method to all three Illumina datasets, replicability scores (R_A), defined as the proportion of variants that replicated between sequencing runs, were calculated for each sample as shown in Figure 1. Statistical details are explained in Figure 1, but in brief, R_A scores of blood and hair samples were compared to R_A scores of non-invasive samples using Mann-Whitney U tests and permutation tests. The proportion of samples of each type with "perfect" R_A scores (R_A=1) were compared using Fisher's Exact Test.

In addition to R_A scores, we also tested for associations between a variant's replicability and its relative frequency within the amplicon (depth of a variant divided by total amplicon depth), as well as and its status as a singleton (whether the allele only appears in one individual or not). We focused on non-invasive samples for these assessments and therefore used only the 2018 and 2021 MiSeq datasets, which contain the bulk of the fecal samples. In each of these two datasets, we used a Mann-Whitney U test to compare the relative frequencies of replicated and unreplicated variants. We also ranked each amplicon's variants in descending order of relative frequency and compared rankings of replicated and unreplicated variants using a Mann-Whitney U test. Lastly, we counted the number of replicated and unreplicated singleton alleles in each run and used a chi-square test to test for a difference in replicability between singletons and non-singletons.

In addition to comparing MHC allele assignments from each sample between runs, we also examined repeatability between duplicate fecal samples collected from the same mandrill. Duplicates were detected using a panel of 16 highly polymorphic microsatellite markers selected from Benoit et al. (2014). For full details on methods for microsatellite genotyping and duplicate detection, see Guibinga Mickala et al. (2022a) (data from Guibinga Mickala et al., 2022b). Briefly, multilocus genotypes matching for at least six loci, with two or fewer mismatches, were considered duplicates. R_A scores were calculated between MHC allele assignments of duplicate samples within each sequencing run, and between consensus assignments for each member of the pair (see below for consensus assignment procedure).

Individual Allele Assignment

Due to the potential error in each Illumina run, we devised a method to generate consensus allele assignments from paired runs (Figure 2b). Our strategy relies on several assumptions. First, we assumed that given sufficiently high sequencing depth, all alleles should be detected even if at very low read depth. Past studies show that some alleles amplify less efficiently than others, resulting in

consistently lower read depths (Sommer et al., 2013). True alleles may therefore appear at depths even lower than putative sequence artifacts (Sommer et al., 2013). This problem may be exacerbated in fecal samples because they tend to be highly degraded and subject to substantial allelic dropout (Morin et al., 2001). However, given high read depths for each amplicon, we assume detection of all alleles, even if depth for a particular allele is very low (<1% of amplicon depth). Second, artificial variants resulting from random single-base substitution or indels are unlikely to replicate at an appreciable depth between runs. Some bases are more error-prone than others (Gilles et al., 2011), which would result in a higher probability of replicable errors at these positions. However, because artifacts are clustered with their parent sequences in the ampliSAT pipeline (Sebastian et al., 2016), such repeatable errors are still unlikely to be mistaken for true alleles in two independent runs. Third, chimeras and indels in homopolymeric regions may be more likely to replicate between runs, but they are also easily identifiable by alignment to parent sequence(s). Fourth, alleles resulting from cross-contamination between samples during PCR are unlikely to be replicated, as PCR products for each Illumina run were prepared independently.

Considering these four assumptions, our strategy to assign individual MHC alleles using pairs of sequencing runs was as follows (Figure 2b): each Illumina dataset was reanalyzed using the ampliSAS tool (Sebastian et al., 2016), this time using less stringent clustering and filtering parameters. Relaxed parameters retain many low-depth variants from each run, while still clustering most artificial variants resulting from substitution or indel errors with their parent alleles. Substitution, indel, and minimum dominant frequency thresholds were therefore set to 1%, 0.01%, and 10% respectively. Minimal filtering parameters were also applied, retaining all variants with a relative frequency greater than 0.05%. For each sample, the ampliSAS-generated fasta files of variants from two Illumina runs were then entered into a custom Python program that extracted all replicated variants. For each non-invasive sample, replicated variants were extracted from the 2018 and the 2021 Illumina runs. For the blood and hair

samples, the 2019 and 2021 runs were used. Resulting variants were then aligned in MEGA-X (Kumar et al., 2018), where replicated artifacts resulting from indels in homopolymer regions were easily identified by their introduction of alignment gaps relative to otherwise-identical parent sequences. Replicated chimeras were also detected visually and by generating neighbor-joining trees for each sample, since chimeras result from recombination of parent sequences and contain no unique mutations. After removing replicated sequence artifacts, all remaining variants were considered putative true alleles.

Our method is similar to that of Sommer et al., (2013) in that we rely on paired sequencing runs. A key difference is that the previous approach assumes that the most deeply-sequenced variant in an amplicon is a true allele, and all other alleles are identified based on their replicability, relative frequency, and their similarity to other variants. Our method makes no assumptions about relative read depth indicating allele veracity.

Using all alleles identified in the SEGC horde, as well as all previously-published mandrill MHC-DRB sequences downloaded from the Immuno-Polymorphism Database (IPD) (Maccari et al., 2020), a neighbor-joining tree was generated in MEGA-X (Kumar et al., 2018). When possible, lineages of novel variants were identified based on monophyly with previously described DRB loci (Abbott et al., 2006; Setchell et al., 2009). Because our sequences do not include the full exon, novel alleles were named arbitrarily by number within their putative lineages instead of attempting to follow the formal MHC nomenclature described by de Groot et al. (2019). Here, putative lineage for novel alleles is given a prefix “p,” so, for instance, alleles that form a monophyletic group with the DRB3 lineage would be named pDRB3-1, pDRB3-2, pDRB3-3, and so forth. Alleles that do not form a monophyletic group with a previously known lineage are simply designated pDRB, and sequences that are identical to a previously described allele are given the same name, with the addition of the “p” prefix.

Assessing functional diversity

Once consensus MHC allele assignments were generated, alleles were classified into supertypes following Doytchinova and Flower (2005). Each supertype represents a group of alleles with similar physio-chemical properties in the amino acids comprising the antigen binding sites (ABS). To identify the ABS, per-codon signatures of positive selection were evaluated by comparing rates of synonymous (dS) and nonsynonymous (dN) mutations. Because ABS diversity is functionally important, amino acid sites involved in peptide binding are likely to be under positive selection. The comparison of dN and dS was performed using the tool MEME (Murrell et al., 2012) within the DataMonkey server (Weaver et al., 2018). MEME compares the dN and dS rates at each amino acid site, accounting for the possibility that positive selection may vary across phylogenetic branches. The position of codons comprising the ABS is likely to be relatively conserved across populations, so to increase statistical power, this analysis was applied to a dataset including both novel and previously-published alleles.

Amino acid sites under positive selection (i.e., putative antigen binding sites) were then extracted from the sequences. Five physiochemical measurements (z-scores) were used to quantify each allele's binding properties based on the putative ABS codons: z1 (hydrophobicity), z2 (steric bulk), z3 (polarity) and z4 and z5 (electronic effects) (Sandberg et al., 1998). Z-scores were entered into a matrix and transformed by principal components analysis. Alleles were then classified into supertypes using a Discriminant Analysis of Principal Components (DAPC) (Jombart et al., 2010) using the R package adegenet (Jombart & Ahmed, 2011). This classification method maximizes the between-group z-score variance while minimizing within-group variance, generating groups of alleles with functionally distinct antigen-binding sites.

Results

Replicability assessment

After filtering poor quality and improper length reads, both 2018 and 2021 Illumina MiSeq runs had comparable read depths, although nearly 60% of the reads from the 2021 run were lost during the filtering process (Table 1). As an expected consequence of the sequencing technology used, the 2019 MiSeq Nano run had much lower read depth. According to a Fisher Exact test comparing allele count distributions in each sequencing run, the 2021 run detected fewer alleles per sample than the 2018 run (p=0.005), although there was no significant difference in the number of alleles detected between the 2018 MiSeq and the 2019 MiSeq Nano runs (p=0.86) or the 2019 and 2021 runs (p=0.07). Data from each Illumina run is available from Weber et al. (2022).

Table 1 Summary information from each of the three sequencing runs

	Raw reads	Filtered reads	Mean depth per amplicon	Mean allele count
2018 Miseq	7,429,011	4,215,792	22,072	3.91
2019 Miseq Nano	289,775	120,608	2,566	3.48
2021 Miseq	13,952,325	5,633,320	29,649	3.83

Replicate sequence data was successfully obtained from 181 of the 183 non-invasive samples sequenced in the 2018 and 2021 MiSeq runs. Twenty-two non-invasive samples and nine blood/hair samples sequenced successfully in both the 2019 and 2021 runs. R_A scores from all compared datasets are shown in Table 2, along with the percentage of "perfect" scores ($R_A = 1$) and the percentage of samples for which no variants replicated ($R_A = 0$). The average R_A score from the nine blood/hair samples (R_A=0.76, dataset B in Figure 1 and Table 2) is higher than the entire distribution of average scores calculated from 1000 randomly-drawn sets of nine non-invasive samples (dataset D), indicating a significant difference in replicability. The blood/hair samples also had a significantly higher percentage of perfect R_A scores (dataset B, 55.6%) than the replicated non-invasive samples (dataset C, 13.6%)

(Fisher Exact Test, p=0.016). The 22 non-invasive samples sequenced in both the 2019 and 2021 runs also had significantly lower R_A scores (dataset C, R_A=0.31) than the blood/hair samples (dataset B, R_A=0.76) (p=0.003). There was no significant difference in R_A scores between any of the sets of non-invasive samples (datasets A and C, and C and D, in Figure 1 and Table 2). Past studies have shown that for the degree of change method, allele repeatability improves when read depth is above 5000 (Biedrzycka et al., 2017), so we recalculated R_A scores from the non-invasive samples, excluding those with fewer than 5000 reads in either replicate. Using only high-depth amplicons (n=154 from dataset D) did not change replicability levels (mean R_A = 0.32).

Table 2 Replicability statistics for all Illumina runs

Runs Compared	R_A Score Dataset	Sample Type	Mean R_A	SD R_A	%R_A=1	%R_A=0
2018 Miseq & 2019 Miseq Nano	A	Non-invasive (n=22)	0.44	0.30	13.6	9.1
2019 Miseq Nano & 2021 Miseq	B	Blood/Hair (n=9)	0.76	0.29	55.6	0
	C	Non-invasive (n=22)	0.31	0.33	13.6	27.3
2018 Miseq & 2021 Miseq	D	Non-invasive (n=181)	0.32	0.30	9.9	21.5
	D	1000 random draws of n=9	0.05-0.69	0.07-0.48	0-44.4	0-77.8

R_A= proportion of variants that replicated between runs. Letters A, B, C, and D correspond to sequencing run comparisons shown in Figure 1. Significant R_A score comparisons: B&C (p=0.003), D&B (R_A score from B > the 95th percentile of score distribution from D).

For the non-invasive samples sequenced in the 2018 and 2021 runs, relative frequencies of replicated variants were higher than unreplicated variants, both in terms of calculated frequency (2018: p<0.001, 2021: p<0.001) and rank within the amplicon (2018: p<0.001, 2021: p<0.001) (Table 3). However, the distributions of replicated and unreplicated variant frequencies and ranks overlap almost completely (Figure 3), indicating that either can occur at high or low frequencies. Similarly, the top-ranking variant within an amplicon is not necessarily repeatable across runs, although some genotyping

methods assume that variants in this position are true alleles (Lighten, van Oosterhout, Paterson, et al., 2014; Sommer et al., 2013).

Table 3 Mean relative frequencies, and median frequency rank of replicated and unreplicated variants in non-invasive samples in each Illumina Miseq run

	Replicated variants		Unreplicated variants	
	Relative frequency	Rank	Relative frequency	Rank
2018 Miseq	28.44	2	12.63	3
2021 Miseq	19.46	2	10.57	4

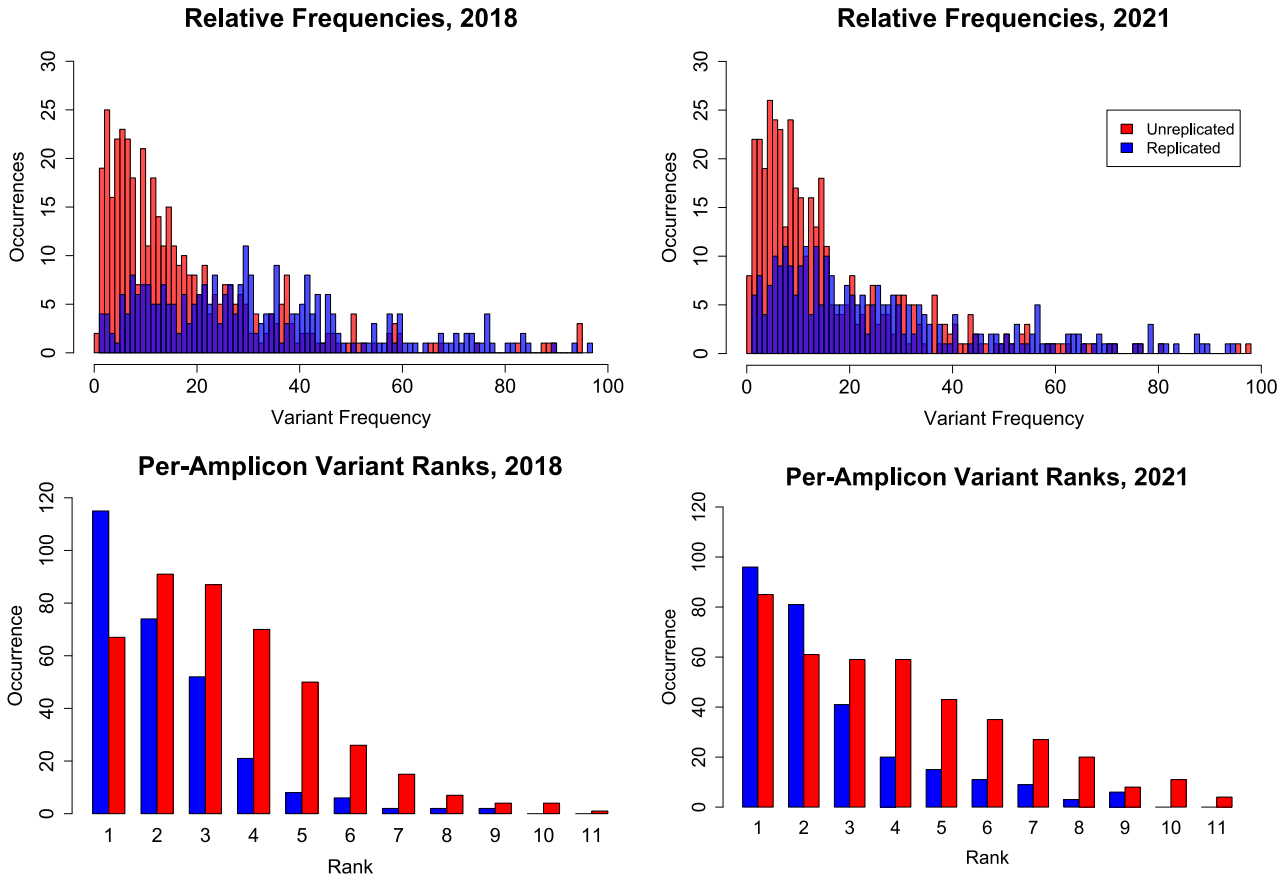

Figure 3 Graphs showing relative frequencies (a-b) and per amplicon ranks (c-d) of variants that replicated (blue) and those that did not (red), in both the 2018 and 2021 Illumina Miseq runs.

Variants that were singletons within each run were also significantly less likely to replicate between runs (2018: $p<0.001$, 2021: $p<0.001$). Across the two sequencing runs, a total of 233 singleton variants were identified (125 singletons in the 2018 run and 108 in the 2021 run). Only six of these (2.6% of the 227 unique variants) appeared in both runs. This suggests that within a single Illumina run, the majority of singleton variants are errors.

For the eight pairs of duplicate fecal samples, the within-run average R_A scores were significantly lower in the 2021 run (mean $R_A = 0.12$ [±0.14]) than in the 2018 run (mean $R_A = 0.72$ [±0.34]) (Mann-Whitney U test, W=58, $p = 0.006$). Consensus allele assignments were generated for all sixteen samples, resulting in two distinct assignments for each of the eight mandrills. The mean of the R_A scores calculated between these pairs of consensus assignments was 0.58 (±0.34).

MHC characterization from consensus genotypes

Of the 181 non-invasive samples that successfully replicated in the 2018 and 2021 runs, consensus allele assignments could be generated for 170 samples (data available from Weber et al., 2022). For the remaining 11, no variants replicated between runs, despite the relaxed clustering and filtering parameters used for this step. All nine replicated blood and hair samples had replicated variants and could therefore be assigned alleles.

A total of 62 alleles were detected in the focal horde, 17 of which match those previously reported in mandrills (Abbott et al., 2006; Setchell et al., 2009). We found a maximum of 11 alleles per sample, with an average of 3.62 (± 1.88), suggesting the presence of up to six MHC-DRB loci. The most common allele in our dataset appeared in 111 out of 190 samples (58.4%). Eighteen alleles were only identified in a single animal, but in accordance with our assignment procedure, they were accepted as true alleles because they replicated across runs. One singleton matched the previously published

mandrill allele Masp-DRB1*04:02 (Abbott et al., 2006), lending support to the validity of these singletons.

When the alleles found in this study were combined in a neighbor-joining tree with an additional 23 previously published alleles (Abbott et al., 2006; Setchell et al., 2009), two monophyletic groups are apparent (Figure 4). The first ("clade 1") contains 40 alleles, including representatives of DRB lineages 1, 3, 5, and 6, each producing a unique amino acid sequence. All previously described mandrill alleles belong to this clade. The second monophyletic group ("clade 2") includes 22 alleles, with 20 unique amino acid sequences, that are highly divergent from the other described alleles. The most frequently-occurring allele in the dataset belongs to clade 2, and when subjected to a BLAST search of the Immuno-Polymorphism Database (Maccari et al., 2020), it was found to share 97% identity with the DRB9 allele identified in crab-eating macaques (*Macaca fascicularis)* (GenBank accession number MW679616.1). Clade 2 is characterized by mutations that may reduce functionality of transcribed amino acids, further supporting the classification of clade 2 as the nonfunctional gene fragment DRB9 (Gongora et al. 1997). Two alleles, Masp-pDRB9-12 and Masp-pDRB9-21, contain a premature stop codon caused by a G to T substitution at amino acid sites 35 and 53 respectively, as well an additional 2-base deletion. Seven clade 2 sequences have deletions of 1-3 bases with no stop codons. All clade 2 alleles contain a single base deletion between bases 82 and 83 and an insertion at position 170. The same compensatory indels have also been identified in human DRB9 alleles (Haas et al., 1987). By contrast, clade 1 contains no deletions, and the only stop codon present is in a sequence belonging to the DRB6 lineage, another known pseudogene (Bontrop et al., 1999).

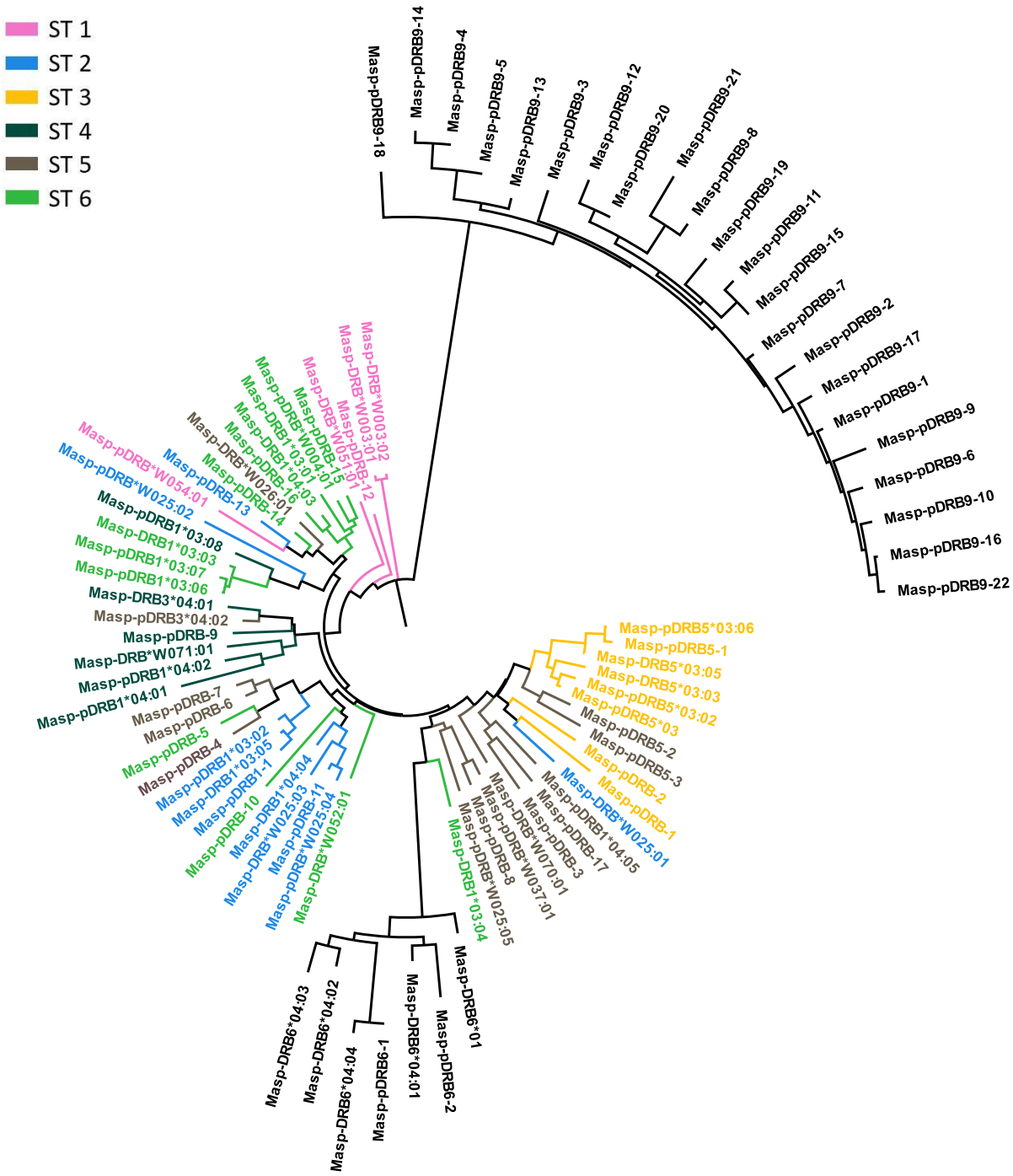

Figure 4 Neighbor-joining tree of all known mandrill MHC-DRB alleles. Alleles are colored by supertype (ST), and nonfunctional loci (DRB6 and putative DRB9) are represented in black.

Functional diversity

Clade 1 and clade 2 alleles may be subjected to different selective pressures and so were analyzed separately in MEME. The pseudogenic DRB6 sequences were also excluded. There was no evidence for selection on the clade 2 alleles, which is characteristic of loss of functionality. In the novel and previously published clade I alleles, nine codons had significantly higher rates of nonsynonymous than synonymous mutations ($p<0.1$), indicating the presence of positive selection. The nine sites (amino acids 5, 9, 15, 33, 34, 41, 47, 48, and 51) correspond to DRB exon sites 28, 32, 38, 56, 57, 64, 70, 71, and 74 (Brown et al., 1993). Seven of these match the antigen-binding sites that have been identified in humans using x-ray crystallography (Figure 5) (Brown et al., 1993). Therefore, these sites can be presumed to represent the ABS of this portion of the mandrill DRB molecule, and all nine were used for supertype analysis.

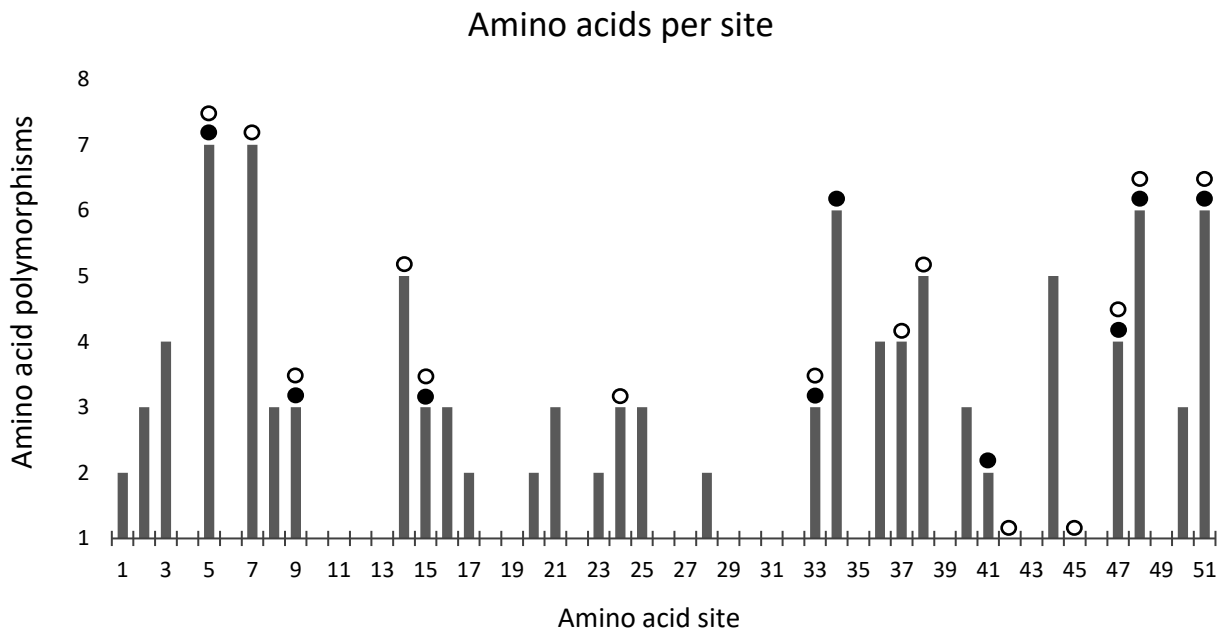

Figure 5 A plot of amino acid polymorphisms present at each codon. Black circles indicate sites from the current study with significant evidence for positive selection, and open circles indicate sites identified in Brown et al. (1993) as part of the HLA peptide binding region.

According to the DAPC performed on these nine amino acids, functional diversity in these alleles can be represented by six supertypes, each containing between 5 and 14 alleles. Mandrills possess an average of 1.9 (±1.03) unique supertypes each, with a maximum of five. One supertype, termed "ST1" and including alleles DRBW*003:01, DRBW*003:02, DRBW*051:01, pDRBW*054:01, and pDRB-12, is strongly separated from the others by the first discriminant function, suggesting that ST1 could be somewhat functionally divergent.

Alleles shared with other species

Of the 62 sequences in this dataset (available from Weber et al. (2022)), four are identical to alleles in other primate species. A total of 30 mandrill samples possessed the allele Masp-pDRB-17, which matches Paan-DRB*W001:02 (IPD accession NHP04483), previously identified in olive baboons (*Papio anubis*). Two individuals carry a DRB6 allele, here designated as pDRB6-2, from the same species (Paan-DRB6*01:05, IPD accession NHP04517). An allele (Masp-pDRB5-3) matching crab-eating macaque's Mafa-DRB5*03:02 (*Macaca fascicularis,* IPD accession NHP00322) (Blancher et al., 2006; Leuchte et al., 2004), appears in two mandrill samples. Finally, one sample includes Masp-pDRB5-2, which matches Patr-DRB5*03:11 (IPD accession NHP00891), previously found in chimpanzees (*Pan troglodytes*) (Fan et al., 1989; Kenter et al., 1992).

One possible explanation is that these identical sequences are a result of using a shorter amplicon than is typically used, and the length was insufficient to capture polymorphisms distinguishing alleles from different species. To test this, all MHC-DRB alleles were downloaded for 15 non-human primate species, including nine species of Old World Monkey, three New World Monkey species, and three ape species (Table S1). The alleles were aligned with those from the present study and trimmed to equal length. By counting the number of unique sequences per species before and after trimming, we determined that an average of 13.46% (±12.18) of each species' allelic diversity was lost by using the shorter amplicon. When the shortened sequences (n=856 alleles from all species combined) were

compared across species, some alleles were identical, leaving 684 unique alleles. However, alleles identical across the shortened length were almost always between species in the same genus. The only exception was between gorillas (*Gorilla gorilla*) and chimpanzees, for which alleles Gogo-DRB6*01:03N and Patr-DRB6*01:05N were identical after trimming. While some allelic diversity within mandrills was likely lost due to our use of a short amplicon, the shared sequences between mandrills and baboons, macaques, and chimpanzees are unusual and may be indicative of biological phenomena.

Discussion

Replicability of MHC sequencing from non-invasive samples

We found that between-run repeatability of MHC allele assignment is poor in fecal samples compared to samples of blood and plucked hair. Past studies of non-invasive genotyping methods have shown that replication is critical to producing reliable data from degraded samples (Morin et al., 2001; Taberlet et al., 1996), and it is unsurprising that the same would apply to next-generation sequencing of the MHC. Although Illumina sequencing yields a great deal of information, more research is needed to determine a suitable number of replicates for reliable allele assignments from non-invasive samples, and an appropriate method to differentiate alleles from artifacts.

One caveat to this study is that the quality of the 2021 MiSeq run was lower than the 2018 or 2019 runs. Additionally, the 2019 sequencing run was performed using MiSeq Nano, which produces lower read depth than a standard MiSeq run. Repeatability decreases at lower read depths, especially for the degree of change method (Biedrzycka et al., 2017). The poor quality of the 2021 sequencing run, and the low read depth of the 2019 run, could have negatively biased replicability scores. However, this issue would have affected all sample types, so the score comparison between types is still valid. R_A scores of non-invasive samples also remained low regardless of which pairs of runs were compared. This suggests that low read depth in the 2019 MiSeq Nano run and the poor quality of the 2021 sequencing run had only minor effects on R_A scores.

In addition to quantifying replicability between runs, we also found that replicated variants generally had higher relative frequency and appear in multiple individuals. However, low frequency sequences (<3%, for example) are still sometimes repeatable, and high frequency or high-ranking sequences do not necessarily replicate. If repeatability reflects a variant's status as an allele or an artifact, then neither frequency nor rank appear to be particularly useful in classifying sequences from non-invasive samples. However, all MHC genotyping methods thus far have, to some extent, relied on these factors to identify alleles (Babik et al., 2009; Grogan et al., 2016; Lighten, van Oosterhout, Paterson, et al., 2014; Sommer et al., 2013; Stutz & Bolnick, 2014). This includes the method of Sommer et al. (2013), which utilizes replication to assign alleles, similar to the present study. However, this previous method assumes that the most deeply sequenced variant in an amplicon is a true allele. While this assumption may be valid for high-quality tissue samples, it appears unsubstantiated for non-invasive samples. DNA degradation is common in fecal samples, likely exacerbating the stochasticity and amplification biases inherent in PCR, in turn affecting relative variant frequency. Our consensus allele-calling method circumvents this issue, since it considers all replicated variants regardless of depth as potential alleles prior to error screening.

We also found that non-invasive samples of the same animal do not necessarily yield the same alleles within a single Illumina run. Although only eight pairs of duplicate fecal samples were available in this study, agreement between samples was highly variable ($0 \leq R_A \leq 1$). The reduced between-sample repeatability observed in the 2021 run may be due to the lower quality of that run. It is also possible that sample quality degraded over time, since several years passed between the first and final sequencing runs. However, DNA is known to be very stable (Madisen et al., 1987), and repeatability scores remained fairly consistent between different pairs of sequencing runs.

Agreement between allele assignments for pairs of duplicate fecal samples remained poor when considering consensus assignments generated from sequence replicates. This may reflect cross-

contamination prior to PCR, since the low concentration of host DNA makes fecal samples especially prone to contamination (Taberlet et al., 1999). Since our assignment procedure only considered replicated variants, any cross-contamination in one member of a pair must have occurred prior to PCR. Contamination may occur due to human error or due to the animal's behavior, such as ingestion of hairs from another individual during mutual grooming (Poirotte et al., 2017). These types of errors are difficult to predict in wild populations, and the discrepancies between sample pairs merit further investigation.

The conflicting alleles between sample pairs could also be explained by false allele discovery due to the high read depth achieved in sequencing. Biedrzycka et al. (2017) showed that consensus assignment methods relying on sequence replication (Sommer et al., 2013) are prone to a high false discovery rate when read depths are high, presumably because higher depth increases the probability of recovering replicated sequencing errors. We did observe such artifacts in our data, but all replicated variants were screened to remove errors from the final consensus. Furthermore, if our assignment method frequently accepted replicated sequence artifacts as true alleles, we would expect the consensus assignments to include more alleles per sample than the assignments generated by either replicate alone. Instead, the mean number of alleles per sample contained in the consensus (3.62) was slightly less than the means found in the MiSeq runs alone when processed using the DOC method (3.91 and 3.83 for the 2018 and 2021 runs, respectively). Biedrzyckia et al. (2017) also recommended adding a minimum relative frequency to the Sommer et al. method as a final threshold, whereby variants below the minimum frequency would be discarded as errors. However, because we expect that true alleles may amplify at very low depths in fecal samples, using such a threshold may not be appropriate for our study system.

MHC diversity in wild mandrills

Although individual allele assignments in this study must be interpreted with caution, we have confidence in the alleles identified within the SEGC horde, and our results point to some interesting conclusions.

In our wild focal population, we identified nearly twice as many alleles as have been detected in a captive horde (Setchell et al., 2009). This is not surprising considering that the previously-studied captive colony originated from only fifteen individuals (Charpentier et al., 2005) and is known to be inbred, likely causing an underestimate of MHC allelic diversity. Our study also identified a maximum of eleven alleles per animal, four more than in the captive horde (Setchell et al., 2009). This finding is partially explained by our detection of "clade 2 alleles," which account for a maximum of three alleles per animal. These alleles likely represent a homolog of the human DRB9 pseudogene. The DRB9 is thought to be the most ancient of the MHC lineages and has previously been amplified in gorillas, orangutans, chimpanzees, and crab-eating macaques (Gongora, Figueroa, and Klein 1996), although publicly available sequence data for this locus in non-human primates remains limited. Here, the lineage may have been revealed by our use of novel primer sequences. All studies of mandrill MHC to date have used the same primer sequences that were thought to amplify all MHC DRB sequences across primates (Abbott et al., 2006; Setchell et al., 2009). However, in the DRB9 sequence from the crab-eating macaque, the binding site for the reverse primer used by Setchell et al. (2009) contains mutations that would likely preclude primer binding at the 3' end. By contrast, the primer pair used in the present study are complementary to alleles in clade 1 (DRB1-6) and clade 2 (DRB9) in macaques and mandrills. This suggests that using more than one pair of primers to sequence the MHC DRB may be necessary to uncover the full allelic diversity present in a species (see also Llaurens et al., 2012).

In addition to the apparent preservation of the DRB9 locus in mandrills, we have also noted four alleles that appear to be shared between mandrills and olive baboons (Masp-pDRB-17 and Masp-pDRB6-

2), crab-eating macaques (Masp-pDRB5-3), and chimpanzees (Masp-pDRB5-2). Extensive trans-species polymorphism is known to occur in the primate MHC (Geluk et al., 1993; Suárez et al., 2006; Yasukochi & Satta, 2014), and shared alleles have been reported among macaque species in the MHC DRB, DPB, DQA, and DQB loci (Doxiadis et al., 2006; Otting et al., 2002). Mutations may have occurred outside the length of the short fragment sequenced here, but according to our post-hoc tests, the sequences are still more highly conserved than is typically found across genera.

As mentioned above, these sequence similarities could be explained by trans-species polymorphism, in which alleles may be preserved by natural selection over time, since before the divergence of a taxonomic group. For instance, MHC alleles preserved between macaque species have been attributed to strong selection (Doxiadis et al., 2006). If this is the case, then the shared sequences identified here have been maintained since the divergence of the tribe Papionini over six million years ago (Liedigk et al., 2014), or 24-27 million years ago for the allele shared with chimpanzees (Springer et al., 2012). Selective pressure would have had to be very intense to maintain sequences over this time, suggesting that these alleles are particularly important for response to pathogens.

Contrary to this hypothesis, one of the shared sequences, Masp-pDRB6-2, is expected to be a pseudogene, having lost its function a very long time ago (Figueroa et al., 1991). It is unclear why this allele would have been preserved through positive selection, but pseudogenes may instead be maintained through balancing selection on the peri-MHC region. The MHC accumulates recessive deleterious mutations as a “sheltered load” (van Oosterhout, 2009). Due to the MHC’s high diversity, these mutations rarely become expressed in homozygous condition and can accumulate without efficient purifying selection. After selection on the functional MHC loci has been relaxed, for example when the locus has evolved into a pseudogene, purifying selection may continue to act on the peri-MHC region. This could retain the same haplotypes over long evolutionary time and explain trans-species polymorphism of non-functional pseudogenes (van Oosterhout, 2009).

Finally, another possible explanation for the sequences shared with olive baboons (pDRB-17 and pDRB6-2) is historical introgression. Introgression of olive and common baboons (*Papio hamadryas*) has been previously documented in geladas (*Theropithecus gelada*) (Dunbar & Dunbar, 1974) and kipunji (*Rungwecebus kipunji*) (Roberts et al. 2010; Zinner, Arnold, and Roos 2009). As central African forest cover has shifted over the millennia (Maley, 1996; Maley et al., 2018), mandrill and baboon habitats may have overlapped. The two species are both group-living and primarily terrestrial (Abernethy et al., 2002; Higham et al., 2009), and they may be infected by similar pathogens. Since MHC alleles are thought to be under pathogen-mediated selection (Spurgin & Richardson, 2010), if a beneficial allele in baboons was introduced to the mandrill population, positive selection may result in an increased frequency of that allele. Although this is a highly speculative hypothesis, it could explain the high incidence of the pDRB-17 allele in mandrills.

Future directions

The present study highlights both the advantages and challenges of conducting non-invasive genetic research on elusive primates. Our use of fecal samples enabled MHC characterization from many wild individuals, but technical obstacles still exist that inhibit effective use of targeted NGS with poor quality samples. Further optimization of sampling, laboratory, and sequencing procedures may be required. For instance, the importance of sequence replication in generating accurate consensus allele assignments merits further investigation. It remains to be seen whether two replicates are sufficient for consensus assignment in fecal samples. To validate our method, it would be necessary to perform the procedure on an individual with known MHC alleles, and such an individual was unavailable in this study.

In addition to performing sequence replicates, alternative sequencing approaches may be explored. Genomic approaches such as genotyping-in-thousands by sequencing (GT-seq) (Campbell et al., 2015) and targeted capture enrichment (Fontsere et al., 2021) show promise for non-invasive samples (Hayward et al., 2022; Natesh et al., 2019; Schmidt et al., 2020), but these may be less useful

for *de novo* sequencing specific MHC loci. Other studies have employed MHC-linked microsatellite loci to quantify MHC diversity from fecal samples (Müller et al. 2014; de Groot et al. 2017), but this approach is feasible only for species in which the entire MHC region is already well-characterized. Portable sequencers such as the Oxford Nanopore's MinION offer the potential to sequence samples in the field immediately after collection, which may improve data quality since fecal DNA degrades with time until DNA extraction (Soto-Calderón et al. 2009). With this technology, the target sequence can be enriched through adaptive sampling (Payne et al., 2021; Wanner et al., 2021), eliminating the need for PCR and thus PCR-generated sequence errors such as chimeras. However, the MinION sequencer is known to have a high sequence error rate (Loit et al., 2019), and further experimentation would be required to optimize methods for fecal samples. Regardless, technological advances for field sequencing show promise for genetic research in wildlife.

Despite the problems with replicability documented here, a strong signal of trans-species polymorphism could still be detected. The alleles identified in the SEGC horde, including the DRB9 locus, support the widely-reported hypothesis that trans-species polymorphism is extensive in the primate MHC (Geluk et al., 1993; Slierendregt et al., 1992; Suárez et al., 2006). The degree to which the purportedly nonfunctional locus DRB9 is preserved among primates warrants further investigation, as it is likely widespread despite its having been reported in only a few species. Allele sharing between mandrills and other primates should also be confirmed through sequencing of the entire DRB region. These shared alleles merit further exploration, as this could have important implications on the evolution of disease resistance among free-ranging primates and captive breeding programs.

Our study focuses on mandrills south of the Ogooué River in Gabon, but collecting MHC data in the northern part of their range may reveal insights into mandrill evolution and historical introgression by olive baboons. Northern and southern mandrills are known to be genetically distinct (Telfer et al., 2003), but gene flow between the two lineages has never been assessed. Due to their proximity to the

baboon range, northern mandrills may have been more likely to be involved in introgression events. If introgression has occurred, baboon genetic material may also be detectable in northern mandrill populations.

Overall, our data suggest that diverse immune genes have arisen in mandrills due to balancing selection. Although the exact role of MHC diversity in species viability is not well understood (Radwan et al., 2010), these findings bode well for the species' ability to adapt to shifting pathogen pressures over time. Our results also reveal interesting evolutionary relationships between mandrills and other primates, and studies of other populations will be integral to uncovering the roles of trans-species polymorphism and potential introgression in the evolution of primate MHC.

Literature Cited

Abbott, K. M., Wickings, E. J., & Knapp, L. A. (2006). High levels of diversity characterize mandrill (Mandrillus sphinx) Mhc-DRB sequences. *Immunogenetics*, *58*(8), 628–640. https://doi.org/10.1007/s00251-006-0132-3

Abernethy, K., & Maisels, F. (2019). *Mandrillus sphinx*. The IUCN Red List of Threatened Species. https://www.iucnredlist.org/species/12754/17952325

Abernethy, K., White, L. J. T., & Wickings, E. J. (2002). Hordes of mandrills (Mandrillus sphinx): Extreme group size and seasonal male presence. *Journal of Zoology, Proceedings of the Zoological Society of London*, *258*, 131–137.

Arguello-Sánchez, L. E., Arguello, J. R., García-Feria, L. M., García-Sepúlveda, C. A., Santiago-Alarcon, D., & Espinosa de los Monteros, A. (2018). MHC class II DRB variability in wild black howler monkeys (Alouatta pigra), an endangered new world primate. *Animal Biodiversity and Conservation*, *41*(2), 389–404. https://doi.org/10.32800/abc.2018.41.0389

Babik, W. (2010). Methods for MHC genotyping in non-model vertebrates. *Molecular Ecology Resources*, *10*(2), 237–251. https://doi.org/10.1111/j.1755-0998.2009.02788.x

Babik, W., Taberlet, P., Ejsmond, M. J., & Radwan, J. (2009). New generation sequencers as a tool for genotyping of highly polymorphic multilocus MHC system. *Molecular Ecology Resources*, *9*(3), 713–719. https://doi.org/10.1111/j.1755-0998.2009.02622.x

Benoit, L., Mboumba, S., Willaume, E., Kappeler, P. M., & Charpentier, M. J. E. (2014). Using next-generation sequencing methods to isolate and characterize 24 simple sequence repeat loci in mandrills (Mandrillus sphinx). *Conservation Genetics Resources*, *6*(4), 903–905. https://doi.org/10.1007/s12686-014-0237-1

Biedrzycka, A., Bielański, W., Ćmiel, A., Solarz, W., Zając, T., Migalska, M., Sebastian, A., Westerdahl, H., & Radwan, J. (2018). Blood parasites shape extreme major histocompatibility complex diversity in a migratory passerine. *Molecular Ecology*, *27*(11). https://doi.org/10.1111/mec.14592

Biedrzycka, A., Konopiński, M., Hoffman, E., Trujillo, A., & Zalewski, A. (2020). Comparing raccoon major histocompatibility complex diversity in native and introduced ranges: Evidence for the importance of functional immune diversity for adaptation and survival in novel environments. *Evolutionary Applications*, *13*(4), 752–767. https://doi.org/10.1111/eva.12898

Biedrzycka, A., Sebastian, A., Migalska, M., Westerdahl, H., & Radwan, J. (2017). Testing genotyping strategies for ultra-deep sequencing of a co-amplifying gene family: MHC class I in a passerine bird. *Molecular Ecology Resources*, *17*(4), 642–655. https://doi.org/10.1111/1755-0998.12612

Blancher, A., Tisseyre, P., Dutaur, M., Apoil, P. A., Maurer, C., Quesniaux, V., Raulf, F., Bigaud, M., & Abbal, M. (2006). Study of Cynomolgus monkey (Macaca fascicularis) MhcDRB (Mafa-DRB) polymorphism in two populations. *Immunogenetics*, *58*(4), 269–282. https://doi.org/10.1007/s00251-006-0102-9

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bontrop, R. E., Otting, N., De Groot, N. G., & Doxiadis, G. G. M. (1999). Major Histocompatibility Complex Class II polymorphisms in primates. *Immunological Reviews*, *167*, 339–350.

https://doi.org/10.1016/B978-0-12-374994-9.10001-4

Bracamonte, S. E., Hofmann, M. J., Lozano-Martín, C., Eizaguirre, C., & Barluenga, M. (2022). Divergent and non-parallel evolution of MHC IIB in the Neotropical Midas cichlid species complex. *BMC Ecology and Evolution*, *22*(1), 1–17. https://doi.org/10.1186/s12862-022-01997-9

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

Campbell, N. R., Harmon, S. A., & Narum, S. R. (2015). Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. *Molecular Ecology Resources*, *15*(4), 855–867. https://doi.org/10.1111/1755-0998.12357

Carroll, E. L., Bruford, M. W., DeWoody, J. A., Leroy, G., Strand, A., Waits, L., & Wang, J. (2018). Genetic and genomic monitoring with minimally invasive sampling methods. *Evolutionary Applications*, *11*(7), 1094–1119. https://doi.org/10.1111/eva.12600

Charpentier, M. J. E., Setchell, J. M., Prugnolle, F., Knapp, L. A., Wickings, E. J., Peignot, P., & Hossaert-McKey, M. (2005). Genetic diversity and reproductive success in mandrills (Mandrillus sphinx). *Proceedings of the National Academy of Sciences of the United States of America*, *102*(46), 16723–16728. https://doi.org/10.1073/pnas.0507205102

Charpentier, M., Setchell, J. M., Prugnolle, F., Wickings, E. J., Peignot, P., Balloux, F., & Hossaert-McKey, M. (2006). Life history correlates of inbreeding depression in mandrills (Mandrillus sphinx). *Molecular Ecology*, *15*, 21–28. https://doi.org/10.1111/j.1365-294X.2005.02760.x

Clifford, S. L., Anthony, N. M., Bawe-Johnson, M., Abernethy, K. A., Tutin, C. E. G., White, L. J. T., Bermejo, M., Goldsmith, M. L., Mcfarland, K., Jeffery, K. J., Bruford, M. W., & Wickings, E. J. (2004). Mitochondrial DNA phylogeography of western lowland gorillas (Gorilla gorilla gorilla). *Molecular Ecology*, *13*, 1551–1565. https://doi.org/10.1111/j.1365-294X.2004.02140.x

Cummings, S. M., McMullan, M., Joyce, D. A., & van Oosterhout, C. (2010). Solutions for PCR, cloning and sequencing errors in population genetic analysis. *Conservation Genetics*, *11*(3), 1095–1097. https://doi.org/10.1007/s10592-009-9864-6

Dandine-Roulland, C., Laurent, R., Dall'Ara, I., Toupance, B., & Chaix, R. (2019). Genomic evidence for MHC disassortative mating in humans. *Proceedings. Biological Sciences*, *286*(1899), 20182664. https://doi.org/10.1098/rspb.2018.2664

de Groot, N. G., Otting, N., Maccari, G., Robinson, J., Hammond, J. A., Blancher, A., Lafont, B. A. P., Guethlein, L. A., Wroblewski, E. E., Marsh, S. G. E., Shiina, T., Walter, L., Vigilant, L., Parham, P., O'Connor, D. H., & Bontrop, R. E. (2019). Nomenclature report 2019: major histocompatibility complex genes and alleles of Great and Small Ape and Old and New World monkey species. *Immunogenetics*. https://doi.org/10.1007/s00251-019-01132-x

de Groot, N., Stanbury, K., de Vos-Rouweler, A. J. M., de Groot, N. G., Poirier, N., Blancho, G., de Luna, C., Doxiadis, G. G. M., & Bontrop, R. E. (2017). A quick and robust MHC typing method for free-ranging and captive primate species. *Immunogenetics*, *69*(4), 231–240. https://doi.org/10.1007/s00251-016-0968-0

de Winter, I. I., van Hooft, P., Heitkönig, I. M. A., Prins, H. H. T., Qurkhuli, T., de Groot, N., de Vos-Rouweler, A. J. M., Bontrop, R. E., & Doxiadis, G. G. M. (2019). Determining Mhc-DRB profiles in

wild populations of three congeneric true lemur species by noninvasive methods. *Immunogenetics*, *71*(2), 97–107. https://doi.org/10.1007/s00251-018-1085-z

Doxiadis, G. G. M., Rouweler, A. J. M., De Groot, N. G., Louwerse, A., Otting, N., Verschoor, E. J., & Bontrop, R. E. (2006). Extensive sharing of MHC class II alleles between rhesus and cynomolgus macaques. *Immunogenetics*, *58*(4), 259–268. https://doi.org/10.1007/s00251-006-0083-8

Doytchinova, I. A., & Flower, D. R. (2005). In Silico Identification of Supertypes for Class II MHCs. *The Journal of Immunology*, *174*(11), 7085–7095. https://doi.org/10.4049/jimmunol.174.11.7085

Dunbar, R. I. M., & Dunbar, P. (1974). On hybridization between Theropithecus gelada and Papio anubis in the wild. *Journal of Human Evolution*, *3*(3), 187–192. https://doi.org/10.1016/0047-2484(74)90176-6

Fan, W., Kasahara, M., Gutknecht, J., Klein, D., Mayer, W. E., Jonker, M., & Klein, J. (1989). Shared class II MHC polymorphisms between humans and chimpanzees. *Human Immunology*, *26*(2), 107–121. https://doi.org/10.1016/0198-8859(89)90096-7

Figueroa, F., Colm, O., Inoki, H., & Klein, J. (1991). Primate DRB6 pseudogenes: clue to evolutionary origin of the HLA-DR2 haplotype. *Immunogenetics*, *34*, 324–337.

Fontsere, C., Frandsen, P., Hernandez-Rodriguez, J., Niemann, J., Scharff-Olsen, C. H., Vallet, D., Le Gouar, P., Ménard, N., Navarro, A., Siegismund, H. R., Hvilsom, C., Gilbert, M. T. P., Kuhlwilm, M., Hughes, D., & Marques-Bonet, T. (2021). The genetic impact of an Ebola outbreak on a wild gorilla population. *BMC Genomics*, *22*(1), 1–12. https://doi.org/10.1186/s12864-021-08025-y

Gagnon, M., Yannic, G., Boyer, F., & Côté, S. D. (2020). Adult survival in migratory caribou is negatively associated with MHC functional diversity. *Heredity*, *125*(5), 290–303. https://doi.org/10.1038/s41437-020-0347-3

Gasparini, C., Congiu, L., & Pilastro, A. (2015). Major histocompatibility complex similarity and sexual selection: Different does not always mean attractive. *Molecular Ecology*, *24*(16). https://doi.org/10.1111/mec.13222

Geluk, A., Elferink, D. G., Slierendregt, B. L., Van Meijgaarden, K. E., Vries, R. R. P., Ottenhoff, T. H. M., & Bontrop, R. E. (1993). Evolutionary conservation of major histocompatibility complex-DR/peptide/T cell interactions in primates. *Journal of Experimental Medicine*, *177*(4), 979–987. https://doi.org/10.1084/jem.177.4.979

Gerloff, U., Hartung, B., Fruth, B., Hohmann, G., & Tautz, D. (1999). Intracommunity relationships, dispersal pattern and paternity success in a wild living community of Bonobos (Pan paniscus) determined from DNA analysis of faecal samples. *Proceedings of the Royal Society B: Biological Sciences*, *266*(1424), 1189–1195. https://doi.org/10.1098/rspb.1999.0762

Gilles, A., Meglécz, E., Pech, N., Ferreira, S., Malausa, T., & Martin, J. F. (2011). Accuracy and quality assessment of 454 GS-FLX Titanium pyrosequencing. *BMC Genomics*, *12*. https://doi.org/10.1186/1471-2164-12-245

Gongora, R., Figueroa, F., & Klein, J. (1996). The HLA-DRB9 gene and the origin of HLA-DR haplotypes. *Human Immunology*, *51*(1), 23–31. https://doi.org/10.1016/S0198-8859(96)00189-9

Gongora, R., Figueroa, F., O'hUigin, C., & Klein, J. (1997). HLA-DRB9 - Possible remnant of an ancient functional DRB subregion. *Scandinavian Journal of Immunology*, *45*(5), 504–510.

https://doi.org/10.1046/j.1365-3083.1997.d01-428.x

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*(1), 1–16. https://doi.org/10.1186/s12864-016-2503-y

Guibinga Mickala, A., Weber, A., Ntie, S., Gahlot, P., Lehmann, D., Mickala, P., Abernethy, K., & Anthony, N. (2022). Estimation of the census (Nc) and effective (Ne) population size of a wild mandrill (Mandrillus sphinx) horde in the Lope National Park, Gabon using a non-invasive genetic approach. *Conservation Genetics*.

[dataset] Guibinga Mickala, A., Weber, A., Ntie, S., Gahlot, P., Lehmann, D., Mickala, P., Abernethy, K., & Anthony, N. (2022). Microsatellite data from a horde of mandrills (Mandrillus sphinx) in Lope National Park, Gabon. DataDryad.org; https://doi.org/10.5061/dryad.s4mw6m99m

Haas, D. A., Boss, J. M., Strominger, J. L., & Spies, T. (1987). A highly diverged B1 Exon in the DR region of the human MHC: Sequence and evolutionary implications. *Immunogenetics*, *25*, 15–20.

Hans, J. B., Haubner, A., Arandjelovic, M., Bergl, R. A., Fünfstück, T., Gray, M., Morgan, D. B., Robbins, M. M., Sanz, C., & Vigilant, L. (2015). Characterization of MHC class II B polymorphism in multiple populations of wild gorillas using non-invasive samples and next-generation sequencing. *American Journal of Primatology*, *77*(11), 1193–1206. https://doi.org/10.1002/ajp.22458

Hayward, K. M., Clemente-Carvalho, R. B. G., Jensen, E. L., de Groot, P. V. C., Branigan, M., Dyck, M., Tschritter, C., Sun, Z., & Lougheed, S. C. (2022). Genotyping-in-thousands by sequencing (GT-seq) of noninvasive faecal and degraded samples: A new panel to enable ongoing monitoring of Canadian polar bear populations. *Molecular Ecology Resources*, *June 2021*, 1–13. https://doi.org/10.1111/1755-0998.13583

Hedrick, P. W. (2002). Pathogen resistance and genetic variation at MHC loci. *Evolution*, *56*(10), 1902–1908. https://doi.org/10.1111/j.0014-3820.2002.tb00116.x

Higham, J. P., Warren, Y., Adanu, J., Umaru, B. N., Maclarnon, A. M., Sommer, V., & Ross, C. (2009). Living on the edge: Life-history of olive baboons at Gashaka-Gumti National Park, Nigeria. *American Journal of Primatology*, *71*(4), 293–304. https://doi.org/10.1002/ajp.20651

Hoffmann, A. A., & Sgró, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*(7335), 479–485. https://doi.org/10.1038/nature09670

Holderegger, R., Kamm, U., & Gugerli, F. (2006). Adaptive vs. neutral genetic diversity: Implications for landscape genetics. *Landscape Ecology*, *21*(6), 797–807. https://doi.org/10.1007/s10980-005-5245-9

Hongo, S., Nakashima, Y., Akomo-Okoue, E. F., & Mindonga-Nguelet, F. L. (2016). Female Reproductive Seasonality and Male Influxes in Wild Mandrills (Mandrillus sphinx). *International Journal of Primatology*, *37*(3), 416–437. https://doi.org/10.1007/s10764-016-9909-x

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*(15), 4071–4086. https://doi.org/10.1111/mec.12349

Huchard, E., Cowlishaw, G., Raymond, M., Weill, M., & Knapp, L. A. (2006). Molecular study of Mhc-DRB in wild chacma baboons reveals high variability and evidence for trans-species inheritance.

Immunogenetics, *58*(10), 805–816. https://doi.org/10.1007/s00251-006-0156-8

Hughes, A. L., & Nei, M. (1989). Nucleotide substitution at major histocompatibility complex class II loci: evidence for overdominant selection. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(3), 958–962. https://doi.org/10.1073/pnas.86.3.958

Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics*, *27*(21), 3070–3071. https://doi.org/10.1093/bioinformatics/btr521

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). https://doi.org/10.1371/journal.pcbi.1000455

Kaufman, J. F., Auffray, C., Korman, A. J., Shackelford, D. A., & Strominger, J. (1984). The class II molecules of the human and murine major histocompatibility complex. *Cell*, *36*(1), 1–13. https://doi.org/10.1016/0092-8674(84)90068-0

Kechin, A., Boyarskikh, U., Kel, A., & Filipenko, M. (2017). cutPrimers: A New Tool for Accurate Cutting of Primers from Reads of Targeted Next Generation Sequencing. *Journal of Computational Biology*, *24*(11), 1138–1143. https://doi.org/10.1089/cmb.2017.0096

Kelley, J., Walter, L., & Trowsdale, J. (2005). Comparative genomics of major histocompatibility complexes. *Immunogenetics*, *56*(10), 683–695. https://doi.org/10.1007/s00251-004-0717-7

Kenter, M., Otting, N., Anholts, J., Jonker, M., Schipper, R., & Bontrop, R. E. (1992). Mhc-DRB diversity of the chimpanzee (Pan troglodytes). *Immunogenetics*, *37*(1), 1–11. https://doi.org/10.1007/BF00223539

Kessler, C., Brambilla, A., Waldvogel, D., Camenisch, G., Biebach, I., Leigh, D. M., Grossen, C., & Croll, D. (2021). A robust sequencing assay of a thousand amplicons for the high-throughput population monitoring of Alpine ibex immunogenetics. *Molecular Ecology Resources*, *22*(1), 66–85. https://doi.org/10.1111/1755-0998.13452

Klein, J., Sato, A., & Nikolaidis, N. (2007). MHC, TSP, and the Origin of Species: From Immunogenetics to Evolutionary Genetics. *Annual Review of Genetics*, *41*(1), 281–304. https://doi.org/10.1146/annurev.genet.41.110306.130137

Klein, J., Satta, Y., & O'hUigin, C. (1993). The molecular descent of the major histocompatibility complex. *Annual Review of Immunology*, *11*, 269–295.

Knapp, L. A., Cadavid, L. F., Eberle, M. E., Knechtle, S. J., Bontrop, R. E., & Watkins, D. I. (1997). Identification of new Mamu-DRB alleles using DGGE and direct sequencing. *Immunogenetics*, *45*, 171–179.

Knapp, L. A., Lehmann, E., Hennes, L., Eberle, M. E., & Watkins, D. I. (1997). High-resolution HLA-DRB typing using denaturing gradient gel electrophoresis and direct sequencing. *Tissue Antigens*, *50*(2), 170–177. https://doi.org/10.1111/j.1399-0039.1997.tb02856.x

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

Leuchte, N., Berry, N., Köhler, B., Almond, N., LeGrand, R., Thorstensson, R., Titti, F., & Sauermann, U.

(2004). MhcDRB-sequences from cynomolgus macaques (Macaca fascicularis) of different origin. *Tissue Antigens*, *63*(6), 529–537. https://doi.org/10.1111/j.0001-2815.2004.0222.x

Liedigk, R., Roos, C., Brameier, M., & Zinner, D. (2014). Mitogenomics of the Old World monkey tribe Papionini. *BMC Evolutionary Biology*, *14*(1), 1–12. https://doi.org/10.1186/s12862-014-0176-1

Lighten, J., van Oosterhout, C., & Bentzen, P. (2014). Critical review of NGS analyses for de novo genotyping multigene families. *Molecular Ecology*, *23*(16), 3957–3972. https://doi.org/10.1111/mec.12843

Lighten, J., van Oosterhout, C., Paterson, I. G., Mcmullan, M., & Bentzen, P. (2014). Ultra-deep Illumina sequencing accurately identifies MHC class IIb alleles and provides evidence for copy number variation in the guppy (Poecilia reticulata). *Molecular Ecology Resources*, *14*(4), 753–767. https://doi.org/10.1111/1755-0998.12225

Llaurens, V., McMullan, M., & Van Oosterhout, C. (2012). Cryptic MHC polymorphism revealed but not explained by selection on the class IIB peptide-binding region. *Molecular Biology and Evolution*, *29*(6), 1631–1644. https://doi.org/10.1093/molbev/mss012

Loit, K., Adamson, K., Bahram, M., Puusepp, R., Anslan, S., Kiiker, R., Drenkhan, R., & Tedersood, L. (2019). Relative performance of MinION (Oxford Nanopore Technologies) versus Sequel (Pacific Biosciences) thirdgeneration sequencing instruments in identification of agricultural and forest fungal pathogens. *Applied and Environmental Microbiology*, *85*(21). https://doi.org/10.1128/AEM.01368-19

Maccari, G., Robinson, J., Hammond, J. A., & Marsh, S. G. E. (2020). The IPD Project: a centralised resource for the study of polymorphism in genes of the immune system. *Immunogenetics*, *72*(1–2), 49–55. https://doi.org/10.1007/s00251-019-01133-w

Madisen, L., Hoar, D. I., Holroyd, C. D., Crisp, M., & Hodes, M. E. (1987). DNA banking: The effects of storage of blood and isolated DNA on the integrity of DNA. *American Journal of Medical Genetics*, *27*(2), 379–390. https://doi.org/10.1002/ajmg.1320270216

Maley, J. (1996). The African rain forest - Main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. In *Proceedings of the Royal Society of Edinburgh Section B: Biological Sciences* (Vol. 104, Issue 1996). https://doi.org/10.1017/S0269727000006114

Maley, J., Doumenge, C., Giresse, P., Mahé, G., Philippon, N., Hubau, W., Lokonda, M. O., Tshibamba, J. M., & Chepstow-Lusty, A. (2018). Late Holocene forest contraction and fragmentation in central Africa. *Quaternary Research (United States)*, *89*(1), 43–59. https://doi.org/10.1017/qua.2017.97

Manlik, O., Krützen, M., Kopps, A. M., Mann, J., Bejder, L., Allen, S. J., Frère, C., Connor, R. C., & Sherwin, W. B. (2019). Is MHC diversity a better marker for conservation than neutral genetic diversity? A case study of two contrasting dolphin populations. *Ecology and Evolution*, *9*(12), 6986–6998. https://doi.org/10.1002/ece3.5265

Maruya, E., Saji, H., & Yokoyama, S. (1996). PCR-LIS-SSCP (low ionic strength single-stranded conformation polymorphism) - A simple method for high-resolution allele typing of HLA-DRB1, -DQB1, and -DPB1. *Genome Research*, *6*(1), 51–57. https://doi.org/10.1101/gr.6.1.51

Matsumura, M., Fremont, D. H., Peterson, P. A., & Wilson, I. A. (1992). Emerging principles for the recognition of peptide antigens by MHC class I molecules. *Science*, *257*(5072), 927–934. https://doi.org/10.1126/science.1323878

Meyers, L. A., & Bull, J. J. (2002). Fighting change with change: Adaptive variation in an uncertain world. *Trends in Ecology and Evolution*, *17*(12), 551–557. https://doi.org/10.1016/S0169-5347(02)02633-2

Million, K. M., & Lively, C. M. (2022). Trans-specific polymorphism and the convergent evolution of supertypes in major histocompatibility complex class II genes in darters (Etheostoma). *Ecology and Evolution*, *12*(1), 1–10. https://doi.org/10.1002/ece3.8485

Morgan, K., Mboumba, J. F., Ntie, S., Mickala, P., Miller, C. A., Zhen, Y., Harrigan, R. J., Le Underwood, V., Ruegg, K., Fokam, E. B., Tasse Taboue, G. C., Sesink Clee, P. R., Fuller, T., Smith, T. B., & Anthony, N. M. (2020). Precipitation and vegetation shape patterns of genomic and craniometric variation in the central African rodent Praomys misonnei: Genomic diversity in an African rodent. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1930). https://doi.org/10.1098/rspb.2020.0449rspb20200449

Morin, P. A., Chambers, K. E., Boesch, C., & Vigilant, L. (2001). Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (Pan troglodytesverus). *Molecular Ecology*, *10*(7), 1835–1844.

Müller, N., Ostner, J., Schülke, O., & Walter, L. (2014). Towards the non-invasive assessment of MHC genotype in wild primates: Analysis of wild assamese macaque MHC-DRB from fecal samples. *American Journal of Primatology*, *76*(3), 230–238. https://doi.org/10.1002/ajp.22225

Muñoz, N. J., Farrell, A. P., Heath, J. W., & Neff, B. D. (2015). Adaptive potential of a Pacific salmon challenged by climate change. *Nature Climate Change*, *5*(2), 163–166. https://doi.org/10.1038/nclimate2473

Murrell, B., Wertheim, J. O., Moola, S., Weighill, T., Scheffler, K., & Kosakovsky Pond, S. L. (2012). Detecting individual sites subject to episodic diversifying selection. *PLoS Genetics*, *8*(7). https://doi.org/10.1371/journal.pgen.1002764

Myers, R. M., Fischer, S. G., Lerman, L. S., & Maniatis, T. (1985). Nearly all single base substitutions in DNA fragments joined to a GC-clamp can be detected by denaturing gradient gel electrophoresis. *Nucleic Acids Research*, *13*(9), 3131–3145.

Natesh, M., Taylor, R. W., Truelove, N. K., Hadly, E. A., Palumbi, S. R., Petrov, D. A., & Ramakrishnan, U. (2019). Empowering conservation practice with efficient and economical genotyping from poor quality samples. *Methods in Ecology and Evolution*, *10*(6), 853–859. https://doi.org/10.1111/2041-210X.13173

Ntie, S., Davis, A. R., Hils, K., Mickala, P., Thomassen, H. A., Morgan, K., Vanthomme, H., Gonder, M. K., & Anthony, N. M. (2017). Evaluating the role of Pleistocene refugia, rivers and environmental variation in the diversification of central African duikers (genera Cephalophus and Philantomba). *BMC Evolutionary Biology*, *17*(1), 1–17. https://doi.org/10.1186/s12862-017-1054-4

Otting, N., De Groot, N. G., Doxiadis, G. G. M., & Bontrop, R. E. (2002). Extensive Mhc-DQB variation in humans and non-human primate species. *Immunogenetics*, *54*(4), 230–239. https://doi.org/10.1007/s00251-002-0461-9

Ou, D., Mitchell, L. A., & Tingle, A. J. (1998). A new categorization of HLA DR alleles on a functional basis. *Human Immunology*, *59*(10), 665–676. https://doi.org/10.1016/S0198-8859(98)00067-6

Payne, A., Holmes, N., Clarke, T., Munro, R., Debebe, B. J., & Loose, M. (2021). Readfish enables targeted nanopore sequencing of gigabase-sized genomes. *Nature Biotechnology*, *39*(4), 442–450.

https://doi.org/10.1038/s41587-020-00746-x

Poirotte, C., Charpentier, M. J. E., Massol, F., Herbert, A., Willaume, E., Bomo, P. M., & Kappeler, P. M. (2017). Mandrills use olfaction to socially avoid parasitized conspecifics. *Science Advances*, *3*(4). https://doi.org/10.1126/sciadv.1601721

Radwan, J., Biedrzycka, A., & Babik, W. (2010). Does reduced MHC diversity decrease viability of vertebrate populations? *Biological Conservation*, *143*(3), 537–544. https://doi.org/10.1016/j.biocon.2009.07.026

Radwan, J., Zagalska-Neubauer, M., Cichoń, M., Sendecka, J., Kulma, K., Gustafsson, L., & Babik, W. (2012). MHC diversity, malaria and lifetime reproductive success in collared flycatchers. *Molecular Ecology*, *21*(10), 2469–2479. https://doi.org/10.1111/j.1365-294X.2012.05547.x

Rekdal, S. L., Anmarkrud, J. A., Lifjeld, J. T., & Johnsen, A. (2019). Extra-pair mating in a passerine bird with highly duplicated major histocompatibility complex class II: Preference for the golden mean. *Molecular Ecology*, *28*(23), 5133–5144. https://doi.org/10.1111/mec.15273

Roberts, T. E., Davenport, T. R. B., Hildebrandt, K. B. P., Jones, T., Stanley, W. T., Sargis, E. J., & Olson, L. E. (2010). The biogeography of introgression in the critically endangered African monkey Rungwecebus kipunji. *Biology Letters*, *6*(2), 233–237. https://doi.org/10.1098/rsbl.2009.0741

Robinson, J., Barker, D., Georgiou, X., Cooper, M., Flicek, P., & Marsh, S. (2020). The IPD-IMGT/HLA Database. *Nucleic Acids Research*, *43*, D948–D955.

Rodríguez, A., Alcaide, M., Negro, J. J., & Pilard, P. (2011). Using major histocompatibility complex markers to assign the geographic origin of migratory birds: Examples from the threatened lesser kestrel. *Animal Conservation*, *14*(3), 306–313. https://doi.org/10.1111/j.1469-1795.2010.00431.x

Sandberg, M., Eriksson, L., Jonsson, J., Sjöström, M., & Wold, S. (1998). New chemical descriptors relevant for the design of biologically active peptides. A multivariate characterization of 87 amino acids. *Journal of Medicinal Chemistry*, *41*(14), 2481–2491. https://doi.org/10.1021/jm9700575

Schmidt, D. A., Campbell, N. R., Govindarajulu, P., Larsen, K. W., & Russello, M. A. (2020). Genotyping-in-Thousands by sequencing (GT-seq) panel development and application to minimally invasive DNA samples to support studies in molecular ecology. *Molecular Ecology Resources*, *20*(1), 114–124. https://doi.org/10.1111/1755-0998.13090

Sebastian, A., Herdegen, M., Migalska, M., & Radwan, J. (2016). Amplisas: A web server for multilocus genotyping using next-generation amplicon sequencing data. *Molecular Ecology Resources*, *16*(2), 498–510. https://doi.org/10.1111/1755-0998.12453

Setchell, J. M., Charpentier, M. J. E., Abbott, K. M., Wickings, E. J., & Knapp, L. A. (2009). Is brightest best? Testing the Hamilton-Zuk hypothesis in Mandrills. *International Journal of Primatology*, *30*(6), 825–844. https://doi.org/10.1007/s10764-009-9371-0

Setchell, J. M., Charpentier, M. J. E., Abbott, K. M., Wickings, E. J., & Knapp, L. A. (2010). Opposites attract: MHC-associated mate choice in a polygynous primate. *Journal of Evolutionary Biology*, *23*(1), 136–148. https://doi.org/10.1111/j.1420-9101.2009.01880.x

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

Slierendregt, B. L., van Noort, J. T., Bakas, R. M., Otting, N., Jonker, M., & Bontrop, R. E. (1992). Evolutionary stability of transspecies major histocompatibility complex class II DRB lineages in humans and rhesus monkeys. *Human Immunology*, *35*(1), 29–39. https://doi.org/10.1016/0198-8859(92)90092-2

Sommer, S. (2005). The importance of immune gene variability (MHC) in evolutionary ecology and conservation. *Frontiers in Zoology*, *2*, 1–18. https://doi.org/10.1186/1742-9994-2-16

Sommer, S., Courtiol, A., & Mazzoni, C. J. (2013). MHC genotyping of non-model organisms using next-generation sequencing: A new methodology to deal with artefacts and allelic dropout. *BMC Genomics*, *14*(1). https://doi.org/10.1186/1471-2164-14-542

Soto-Calderón, I. D., Ntie, S., Mickala, P., Maisels, F., Wickings, E. J., & Anthony, N. M. (2009). Effects of storage type and time on DNA amplification success in tropical ungulate faeces. *Molecular Ecology Resources*, *9*(2), 471–479. https://doi.org/10.1111/j.1755-0998.2008.02462.x

Springer, M. S., Meredith, R. W., Gatesy, J., Emerling, C. A., Park, J., Rabosky, D. L., Stadler, T., Steiner, C., Ryder, O. A., Janečka, J. E., Fisher, C. A., & Murphy, W. J. (2012). Macroevolutionary Dynamics and Historical Biogeography of Primate Diversification Inferred from a Species Supermatrix. *PLoS ONE*, *7*(11). https://doi.org/10.1371/journal.pone.0049521

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

Stutz, W. E., & Bolnick, D. I. (2014). Stepwise threshold clustering: A new method for genotyping MHC loci using next-generation sequencing technology. *PLoS ONE*, *9*(7), 25–27. https://doi.org/10.1371/journal.pone.0100587

Suárez, C. F., Patarroyo, M. E., Trujillo, E., Estupiñán, M., Baquero, J. E., Parra, C., & Rodriguez, R. (2006). Owl monkey MHC-DRB exon 2 reveals high similarity with several HLA-DRB lineages. *Immunogenetics*, *58*(7), 542–558. https://doi.org/10.1007/s00251-006-0127-0

Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L. P., & Bouvet, J. (1996). Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, *24*(16), 3189–3194.

Taberlet, P., Waits, L. P., & Luikart, G. (1999). Noninvasive genetic sampling: look before you leap. *Trends in Ecology and Evolution*, *14*(8), 323–327.

Telfer, P. T., Souquière, S., Clifford, S. L., Abernethy, K., Bruford, M. W., Disotell, T. R., Sterner, K. N., Roques, P., Marx, P. A., & Wickings, E. J. (2003). Molecular evidence for deep phylogenetic divergence in Mandrillus sphinx. *Molecular Ecology*, *12*(7), 2019–2024. https://doi.org/10.1046/j.1365-294X.2003.01877.x

Unanue, E. R. (1984). Antigen-presenting function of the macrophage. *Annual Review of Immunology*, *2*, 395–428.

van Oosterhout, C. (2009). A new theory of MHC evolution: Beyond selection on the immune genes. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1657), 657–665. https://doi.org/10.1098/rspb.2008.1299

Vásquez-Carrillo, C., Friesen, V., Hall, L., & Peery, M. Z. (2014). Variation in MHC class II B genes in

marbled murrelets: Implications for delineating conservation units. *Animal Conservation*, *17*(3), 244–255. https://doi.org/10.1111/acv.12089

Wanner, N., Larsen, P. A., McLain, A., & Faulk, C. (2021). The mitochondrial genome and Epigenome of the Golden lion Tamarin from fecal DNA using Nanopore adaptive sequencing. *BMC Genomics*, *22*(1), 1–11. https://doi.org/10.1186/s12864-021-08046-7

Weaver, S., Shank, S. D., Spielman, S. J., Li, M., Muse, S. V., & Kosakovsky Pond, S. L. (2018). Datamonkey 2.0: A modern web application for characterizing selective and other evolutionary processes. *Molecular Biology and Evolution*, *35*(3), 773–777. https://doi.org/10.1093/molbev/msx335

[dataset] Weber, A., Lighten, J., van Oosterhout, C., Guibinga Mickala, A., Ntie, S., Mickala, P., Lehmann, D., Abernethy, K., Anthony, N. (2022). Targeted sequencing of the major histocompatibility complex from mandrill fecal samples: replicated sequencing and consensus allele assignments. DataDryad.org; https://doi.org/10.5061/dryad.w9ghx3fst

White, E. C., Dikangadissi, J. T., Dimoto, E., Karesh, W. B., Kock, M. D., Abiaga, N. O., Starkey, R., Ukizintambara, T., White, L. J. T., & Abernethy, K. A. (2010). Home-range use by a large horde of wild Mandrillus sphinx. *International Journal of Primatology*, *31*(4), 627–645. https://doi.org/10.1007/s10764-010-9417-3

Wickings, E. J. (1995). Genetic self-management in a captive colony of mandrills (Mandrillus sphinx) as revealed by DNA minisatellite fingerprints. *Electrophoresis*, *16*, 1678–1683.

Yasukochi, Y., & Satta, Y. (2014). A human-specific allelic group of the MHC DRB1 gene in primates. *Journal of Physiological Anthropology*, *33*(1), 1–9. https://doi.org/10.1186/1880-6805-33-14

Yu, L., Nie, Y., Yan, L., Hu, Y., & Wei, F. (2018). No evidence for MHC-based mate choice in wild giant pandas. *Ecology and Evolution*, *8*(17), 8642–8651. https://doi.org/10.1002/ece3.4419

Zhang, P., Huang, K., Zhang, B., Dunn, D. W., Chen, D., Li, F., Qi, X., Guo, S., & Li, B. (2018). High polymorphism in MHC-DRB genes in golden snub-nosed monkeys reveals balancing selection in small, isolated populations. *BMC Evolutionary Biology*, *18*(1), 1–13. https://doi.org/10.1186/s12862-018-1148-7

Zhu, Y., Wan, Q. H., Yu, B., Ge, Y. F., & Fang, S. G. (2013). Patterns of genetic differentiation at MHC class i genes and microsatellites identify conservation units in the giant panda. *BMC Evolutionary Biology*, *13*(1). https://doi.org/10.1186/1471-2148-13-227

Zinner, D., Arnold, M. L., & Roos, C. (2009). Is the new primate genus Rungwecebus a baboon? *PLoS ONE*, *4*(3). https://doi.org/10.1371/journal.pone.0004859

Chapter 3

No evidence for sex-specific selection on immunity genes in a sexually dimorphic primate

Abstract

Parasite-mediated selection is thought to drive high polymorphism in the genes of the major histocompatibility complex (MHC), a gene family that plays an important role in vertebrate adaptive immunity. In many mammals, parasite prevalence differs between males and females, potentially causing sex-specific variation in the strength of selection on the MHC. In this study, we investigate whether MHC DRB allelic richness or allele frequencies vary between the sexes in mandrills (*Mandrillus sphinx*), a highly sexually dimorphic primate with documented evidence for male-biased parasitism. We also test for differences between the sexes in functional diversity, quantified by the number of supertypes and level of amino acid diversity included in each individual's allele assignments. If males with high MHC homozygosity are at a greater risk of mortality due to parasitism than females, then we would expect sampled males to have higher heterozygosity and functional diversity than females. Similarly, if the sexes are affected by different pathogens, we might expect specific alleles to occur at different frequencies in males and females. Contrary to our expectations, we found no evidence of sex-specific selection on MHC DRB loci. Sampled males did not possess more alleles or greater functional diversity than females on average, and common alleles occurred at equal frequencies in both sexes. This finding suggests that MHC DRB alleles provide equal protection for males and females from parasite-mediated selection. Other mechanisms, such as behavioral avoidance of infection, may also protect males from fitness consequences from increased parasite load or greater parasite diversity.

Introduction

The major histocompatibility complex (MHC) is a crucial component of immunity in vertebrates. Located on the surface of antigen presenting cells, MHC glycoproteins bind to pathogenic peptides and present them to T-cells to initiate an immune response (Rock et al., 2016). MHC molecules are classified as either class I or class II. Class I MHC proteins are found on the surface of all cells and present endogenously produced peptides, such as those derived from viral infections or cancerous cells. On the other hand, class II molecules bind extracellular proteins and are limited to the surfaces of immune cells such as macrophages, monocytes, dendritic cells, and B cells. Both MHC class I and II loci have varying levels of polymorphism and copy number variation (Lighten et al., 2014), but generally speaking, the class II MHC is considered one of the most complex and diverse regions of the vertebrate genome (Hedrick, 1994).

One set of class II loci in particular, the MHC DRB, is known for its extremely high levels of polymorphism, with over 4,000 alleles documented in humans alone (Maccari et al., 2020). With their important role in immunity, the diversity of MHC class II molecules is maintained by one or more forms of pathogen-mediated selection: heterozygote advantage (Doherty & Zinkernagel, 1975), frequency-dependent selection (Slade & McCallum, 1992), and temporally fluctuating selection (A. V. S. Hill, 1991). There is considerable evidence showing that the MHC plays an important role in individual fitness and pathogen resistance in many taxa including: humans (A. Hill et al., 1991; Kuniholm et al., 2013), non-human primates (O'Connor et al., 2010; Schad et al., 2005), fish (Evans & Neff, 2009; Phillips et al., 2018; Smallbone et al., 2021), rodents (Doherty & Zinkernagel, 1975; Kloch et al., 2010; Lenz et al., 2009; Penn et al., 2002), birds (Biedrzycka et al., 2018; Radwan et al., 2012; Westerdahl et al., 2005; Worley et al., 2010), and other vertebrates (Biedrzycka et al., 2020; Brambilla et al., 2018; Hacking et al., 2018; Osborne et al., 2015; Schwensow et al., 2017).

In addition to MHC variation, other factors such as age, sex, and nutrition also lead to variability in immune function (Clutton-Brock & Pemberton, 2004; Siva-Jothy & Thompson, 2002). In particular, males of multiple species have been shown to be more vulnerable to parasites than females (Lynsdale et al., 2017; Müller-Graf et al., 1997; Poulin, 1996; Wilson et al., 2004). This trend is generally attributed to ecological or physiological differences between males and females. For species in which males are physically larger, the male's likelihood of parasitic infection may be elevated based on the rationale that larger animals provide more habitat for parasites, as suggested by the principles of island biogeography (Kuris et al., 1980). Larger animals may also be more detectable and potentially more exposed due to their greater consumption of resources (Bell & Burt, 1991; Kennedy et al., 1986; C. Nunn & Altizer, 2010). The sexes may also occupy different habitats, consume different prey, or engage in different social behaviors with conspecifics, potentially exposing each sex to different sets of pathogens (Bundy, 1988; Müller-Graf et al., 1997; C. L. Nunn & Altizer, 2004). From a physiological perspective, male sex hormones such as testosterone have been shown to have immunosuppressive effects (Alexander & Stimson, 1988; Zuk & McKean, 1996), sometimes leading to tradeoffs between immunity and showy secondary sexual characteristics in males (Folstad & Karter, 1992).

As might be expected if sex biases in parasite prevalence are caused by physiological and ecological differences, past research has shown that male-biased parasitism is commonly associated with species that have strong sexual dimorphism (Moore & Wilson, 2002). The present study focuses on one such species, the mandrill (*Mandrillus sphinx*), for which males and females exhibit dramatic physical and behavioral differences as a result of sexual selection (Setchell, 2016). Dominant males are up to three times the size of females, and social rank is determined by intense physical combat in males and by inheritance of rank in females (Setchell et al., 2006, 2008). High-ranking males also possess dramatic secondary sexual characteristics that are associated with high testosterone, such as brightly colored faces and genitals and extremely long canine teeth (Wickings & Dixson, 1992). Mandrills form

large groups, or “hordes,” of up to one thousand individuals (Guibinga Mickala et al., 2022), with adult males often remaining solitary and re-joining the horde during the summer months when females are receptive to breeding (Abernethy et al., 2002). From a limited body of evidence, the pattern of male-biased parasitism in sexually dimorphic species appears to hold true in mandrills, at least for certain parasites. For example, Charpentier et al., (2019) showed that males are more susceptible to infection by the malaria-causing parasite *Plasmodium gonderii*, and some evidence suggests that SIVmnd-2, a mandrill-specific strain of simian immunodeficiency virus (SIV), may be more prevalent and spread more quickly among males (Dibakou et al., 2020; Fouchet et al., 2012).

If male mandrills are more vulnerable to parasites than females, then the MHC genotype may be more important for male fitness than for females, leading to differences in pathogen-mediated selection between the sexes. In a natural population, this selection could manifest in two ways. First, due to their higher parasite load, males with MHC genotypes offering insufficient immune capacity may be less likely to survive to adulthood than females with the same alleles. For example, a 2012 study in Alpine chamois (*Rupicapra rupicapra*) concluded that males with lower MHC heterozygosity had reduced longevity compared to more diverse males, but the same pattern did not apply to females (Schaschl et al., 2012). Thus, adult male animals had higher MHC diversity than females. Second, sex-specific selection may occur through prenatal or postcopulatory selection against embryos or sperm cells with disadvantageous MHC alleles or genotypes. It has been theorized that preferred gametes can be selected by cryptic female choice, sperm competition, and selective abortion (Jennions & Petrie, 2000; J. Kamiya et al., 2020; Knapp et al., 1996; Wedekind et al., 1996). Evidence for postcopulatory selection for MHC heterozygosity has been observed in studies of mice (Hamilton & Hellström, 1978) and gray mouse lemurs (Schwensow et al., 2008), and the effect was found to be specific to male offspring in newborn humans (Dorak et al., 2002) and rats (Palm, 1969).

These two mechanisms of sex-specific selection are difficult to distinguish in wild populations, but either could lead to the sexes having different patterns of MHC diversity. Depending on the context of host-parasite interactions, selection could favor different levels of heterozygosity in males and females, or specific beneficial alleles, if the sexes are exposed to different sets of pathogens due to their extensive behavioral differences (Abernethy et al., 2002; Setchell et al., 2006, 2008).

Due to their sexual dimorphism and male-biased parasitism, mandrills are an ideal system in which to study sex-specific selection on the MHC. The MHC is known to play an important role in social signaling, mate choice, and fitness in this species. A male's MHC genotype is related to the diversity of his odor profile (Setchell et al., 2011), and particular MHC alleles are associated with bright red facial coloration (Setchell et al., 2009). Females also preferentially mate with MHC dissimilar males to produce more heterozygous offspring (Setchell et al., 2010). A 2013 study found no evidence of post-copulatory selection for MHC heterozygosity, but the study was limited by an insufficient number of samples in the face of small effect sizes (Setchell et al., 2013).

In the present study, we sequence the MHC DRB loci from non-invasive samples collected from a wild population of mandrills in Lopé National Park, Gabon. After assigning sex to the samples using a genetic sex diagnostic, we compare MHC diversity and allele frequencies in males and females to test the hypothesis that pathogen-mediated selection operates differently in the two sexes. If males with low MHC variation are under stronger selection than females, experiencing higher mortality or stronger post-copulatory selection, then sampled males should have higher MHC diversity than females. Similarly, if males and females experience mortality due to different sets of pathogens, then we might expect specific MHC alleles to occur at different frequencies between sampled males and females. Like many studies in natural populations, this research is limited by the lack of data definitively connecting parasitism to fitness or mortality. However, this study presents the first examination of sex-specific selection and potential connections between MHC and survivorship in a wild mandrill population.

Methods

Sampling and DNA extraction

The present study focuses on a population of mandrills located in Lopé National Park, Gabon, specifically in the region north of the Station d'Etudes des Gorille et Chimpanzés (SEGC). While the southern section of Lopé National Park is densely forested with lowland rainforest, the area north of the SEGC research station and south of the Ogooué River is characterized by an ancient forest-savannah mosaic (Oslisly & White, 2000). The forest fragments of this region support a variety of wildlife (Tutin et al., 1997), including a large horde of mandrills generally referred to as the SEGC horde. The SEGC horde has been estimated to include close to one thousand individuals during the summer months, when both reproductive males and females are present (Abernethy et al., 2002; Guibinga Mickala et al., 2022). The mosaic habitat's relatively open landscape helps to ameliorate the challenges of studying mandrills in dense forest, where they normally reside. Daily location of the SEGC horde during fieldwork was aided by radio telemetry, as several individuals have been fitted with radio collars for population monitoring.

Samples of feces were collected daily from the focal horde during July or August of 2016 through 2018, although only samples from 2016 and 2017 were used in this study. Approximately 1 cm^3 of fecal material was collected per sample and stored in a 50 mL Falcon tube containing 25 mL of silica gel, as this method has been shown to be effective for preserving DNA from fecal samples (Soto-Calderón et al., 2009). DNA was then extracted from the samples using the QIAamp DNA Stool Mini Kit (Qiagen, CA). In addition to the non-invasive samples of feces, samples of blood, plucked hair, and feces were also collected from individuals anesthetized for radio collaring for an unrelated project. DNA was extracted from these blood and hair samples using the DNeasy Blood & Tissue Kit (Qiagen, CA).

MHC sequencing and allele assignment

The extracted DNA samples were amplified for a 157-base fragment of the MHC DRB gene using a forward primer with sequence 5'-TTCTTCAAYGGGACGGAGC-3' and a reverse primer sequence of 5'-GTGTCTGCAGTAGGTGTCC-3'. To aid in library preparation for Illumina sequencing, the forward and reverse Illumina linker sequences were added to the 5' end of the target-specific primers. Polymerase chain reactions of 25µL total volume were performed using 12.5 µL 2X GoTaq HotStart Polymerase Master Mix (Promega), 0.5 mM $MgCl_2$, 400 nM of each primer, 8.25 µL of water, and 2 µL of template DNA. Cycling conditions were as follows: 5 minutes initial denaturation at 98°C, followed by 38 cycles of 95°C for 30 seconds, 65°C for 30 seconds, and 72°C for 60 seconds, and finally a ten-minute final extension at 72°C. Each PCR product was checked on an agarose gel to verify successful amplification prior to sequencing.

Our methods for MHC sequencing and allele assignment have been previously described elsewhere (see Chapter 2). To summarize, each sample was sequenced in two separate Illumina paired-end sequencing runs of 150 cycles, and the data from each run was processed separately. Trimmomatic and cutPrimers were used respectively to remove adapter and primer sequences (Bolger et al., 2014; Kechin et al., 2017). The ampliSAT pipeline was then used to process the resulting data (Sebastian et al., 2016). Specifically, ampliMERGE was used to merge paired-end reads, and ampliCLEAN was used to discard reads with Phred quality score below 30 or with sequence length outside the range of 152-160 nucleotides.

After pre-processing, the ampliSAS tool was used to cluster artificial sequences with their parent variants and filter clusters that may represent false alleles. We used relaxed clustering and filtering parameters that retained low-depth reads because past studies show that read depth may not be a reliable indicator of true alleles in fecal samples (Chapter 2). The substitution error rate was set to 1%, and the indel error rate was set to 0.01%. In ampliSAS's clustering algorithm, a "minimum dominant

frequency threshold" of read depth relative to that of a cluster's dominant sequence can be implemented. This allows a variant within a cluster to become the dominant sequence of a new cluster if the threshold is exceeded. Here, a minimum dominant frequency threshold of 10% was used. In the filtering step, only clusters with read depth comprising less than 0.05% of the total amplicon were discarded to avoid discarding low-depth true alleles.

Once each sequencing run had been processed in this manner, a custom Python script was used to extract the variants that replicated between runs for each sample. Variants that did not replicate were discarded. The replicated variants from each amplicon were then aligned in MEGA-X (Kumar et al., 2018), and sequence artifacts were visually detected and removed. All remaining sequences were then considered to be true alleles.

Microsatellite genotyping and sex diagnostic

To ensure that each sample represented a unique mandrill, samples were also genotyped for a set of sixteen highly polymorphic microsatellite markers (Benoit et al., 2014). Details on genotyping methods can be found in Guibinga Mickala et al. (2022), but in brief, the sixteen markers were assembled into four multiplex PCRs of four microsatellite loci each. The markers were amplified using fluorolabeled primers and sized on an ABI3130xl sequencer. Three replicates of each PCR were performed, and each sample was considered heterozygous if two alleles appeared in at least two replicates, and homozygous if a single allele appeared alone in all three replicates. Any genotypes not satisfying either condition were considered unknown and treated as missing data. Genotypes from all samples were compared pairwise, and a pair was considered to have originated from the same mandrill if the genotypes matched at a minimum of six loci, with no more than two mismatches allowed.

To determine the sex of sampled individuals, markers on the X and Y chromosomes were also amplified with fluorolabeled primers and included in the multiplex PCRs. The X marker was a 193-base

fragment of the amelogenin gene, and the Y chromosome marker was a 163-base section of the sex-determining region (SRY) (Di Fiore, 2005). A pilot study using samples from the radio collared individuals, for which the sex is known, showed that both markers amplify reliably in fecal samples, and dropout of the Y marker in males is uncommon. On the other hand, the Y marker occasionally amplified at low levels in known females, likely due to minor cross-contamination. Therefore, samples were designated male if at least two of the three PCR replicates contained the Y marker, and females were permitted to have low-level Y amplification in no more than one replicate. Any other genotypes were considered to be of unknown sex, as were genotypes in which the X marker failed, since non-amplification of the X marker was likely to indicate a poor quality sample.

Statistical analysis

To test whether sampled males possess greater allelic richness than females, the total number of alleles were counted for each individual, and the distribution of counts in each sex were compared using a Fisher's Exact test. Past studies have suggested the presence of at least two non-functional loci, known as DRB6 and DRB9 (Chapter 2; Abbott et al., 2006). Alleles belonging to such loci are not expected to contribute to individual immunity, so these statistical tests were run with and without these alleles to ensure that they did not affect the results.

Because not all mutations lead to an amino acid change, and not all amino acid changes will affect the binding properties of the MHC protein, allelic richness may not necessarily indicate functional diversity. Two additional tests were therefore assessed to evaluate whether functional diversity differs between males and females. The putatively functional alleles present in this study have previously been allocated into six supertypes designated ST1 through ST6 (Chapter 2), which represent groups of alleles with similar physiochemical properties in their predicted peptide binding sites (Doytchinova & Flower, 2005). Similar to the test performed for allelic richness, the number of supertypes possessed by each

individual were counted, and the distribution of those counts in each sex were also compared using Fisher's exact test.

The second test of sex-specific differences in functional diversity compared individual MHC protein variability between sexes. In each sample, all putatively functional alleles were converted to amino acid sequences, and a measure of within-individual amino acid diversity, AA_{div}, was calculated. To calculate amino acid diversity, each individual's set of amino acid sequences were compared pairwise, and the number of amino acid differences between each pair was summed and then divided by the total number of pairwise combinations. After checking for data normality using a Shapiro-Wilks test, a two-sample t-test was used to compare the average AA_{div} between males and females. Homoscedasticity was confirmed using an F-test. To eliminate noise from amino acids not directly involved in peptide binding, the same test was also performed using only the nine amino acid sites that have been previously identified as putative antigen binding sites (Chapter 2). Alleles ascribed to nonfunctional lineages DRB6 and DRB9 were excluded from this test, as they are highly divergent and cause a positive bias in AA_{div}. Samples in which one or zero functional alleles were detected were also excluded since it was not possible to calculate pairwise amino acid differences in allele assignments for these samples.

Finally, we used a series of two-by-two contingency tables to test whether frequencies of any allele or supertype varied by sex. These tests were applied to all six supertypes, and to all alleles occurring in ten or more mandrills. Fisher's exact tests were again used to test for significant differences in frequency, and p-values were corrected for multiple hypothesis testing using the Holm-Bonferroni correction (Gaetano, 2018; Holm, 1979).

Results

MHC allele assignments were successfully produced for 170 fecal samples and nine samples of blood or hair. Sex was assigned to 96 of the 170 fecal samples and sex was known for all nine samples of

blood and hair from radio collared individuals. After removal of duplicate fecal samples from the same individual (one pair in females and two pairs in males), we obtained a total of 50 unique multi-locus microsatellite genotypes for females (four blood samples and 46 fecal samples) and 52 for males (four of plucked hair, one of blood, and 47 of feces). A total of 51 MHC alleles were present in this group of 102 mandrills. Representative alleles were identified from the DRB1 (9 alleles), DRB3 (1 allele), DRB5 (4 alleles), DRB6 (2 alleles), and DRB9 (16 alleles) lineages. The remaining 19 alleles could not be assigned by monophyly to any particular lineage, although they all cluster phylogenetically with the functional lineages (DRB1, DRB3, and DRB5) (see Chapter 2, Fig. 4).

The 50 females possessed 184 total alleles of the sequenced MHC fragment, 110 of which belonged to putatively functional loci and therefore could be assigned a supertype. The set of allele assignments for males included a total of 214 alleles, with 134 ascribed to functional loci. Females possessed between one and eleven alleles, with a mean of 3.68 (SD=1.94) per individual, while males had a mean of 4.12 alleles (SD=1.67) with a maximum of ten (Figure 1a). When alleles belonging to DRB6 and DRB9 lineages are excluded, the average number of alleles falls to 2.58 (SD=1.45) in males and 2.20 (SD=1.56) in females (Figure 1b). Although the average allele count is higher in males than females, the difference is not significant regardless of whether nonfunctional alleles are included or not ($p=0.80$ when all alleles are included and $p=0.40$ with functional alleles only).

Males and females both possessed a maximum of four unique supertypes per individual (Figure 2). In four females and three males, no functional alleles were detected, so no supertypes could be assigned. In the remaining 49 males and 46 females, males had an average of 2.20 (SD=0.88) unique supertypes, while females had 1.96 (SD=0.83). The difference in the number of supertypes per individual was not significantly different between the sexes ($p=0.61$).

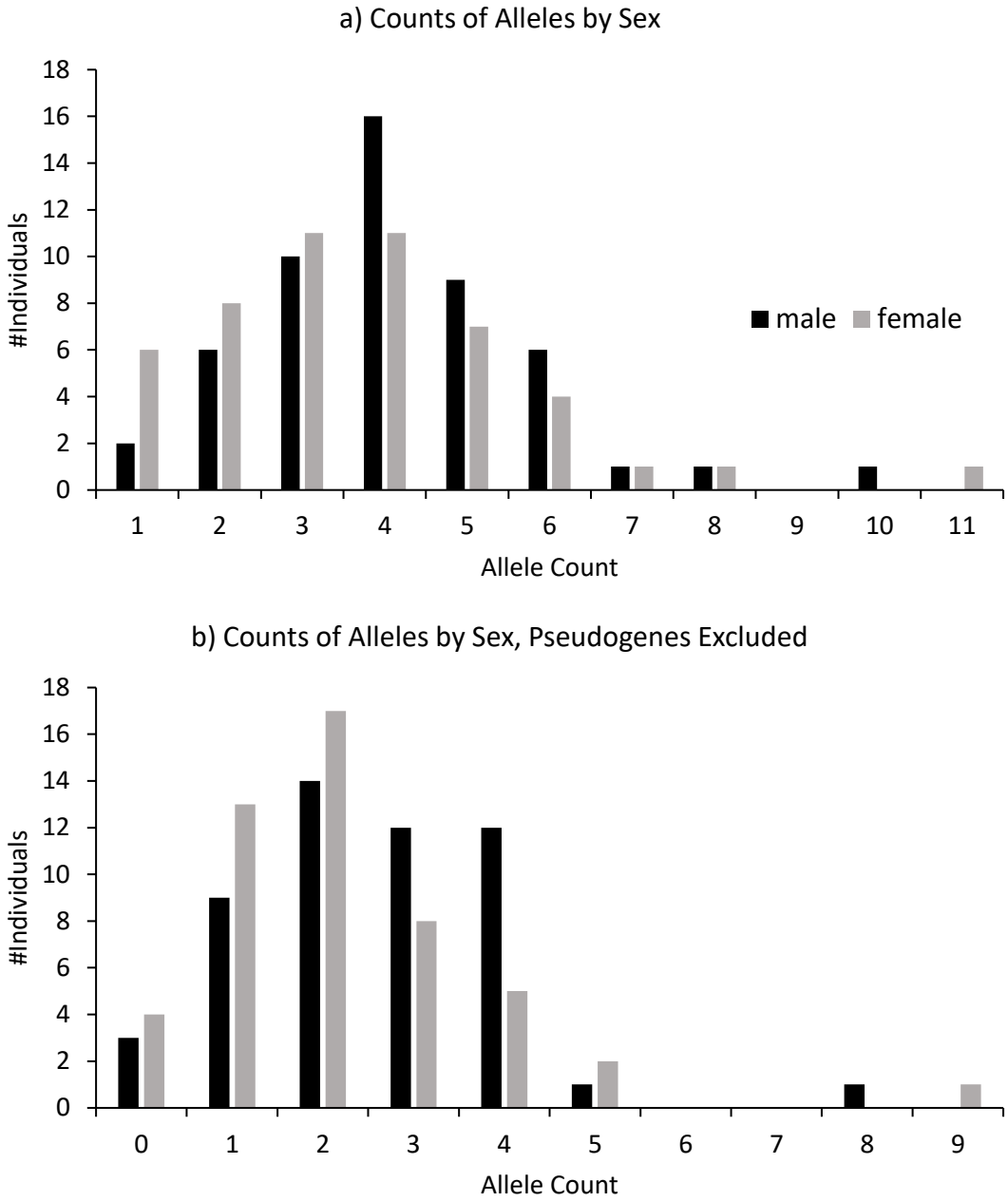

Figure 1 Distribution of allele counts per individual in males (black) and females (gray). In a) all alleles are included, and b) shows allele counts with pseudogenes excluded.

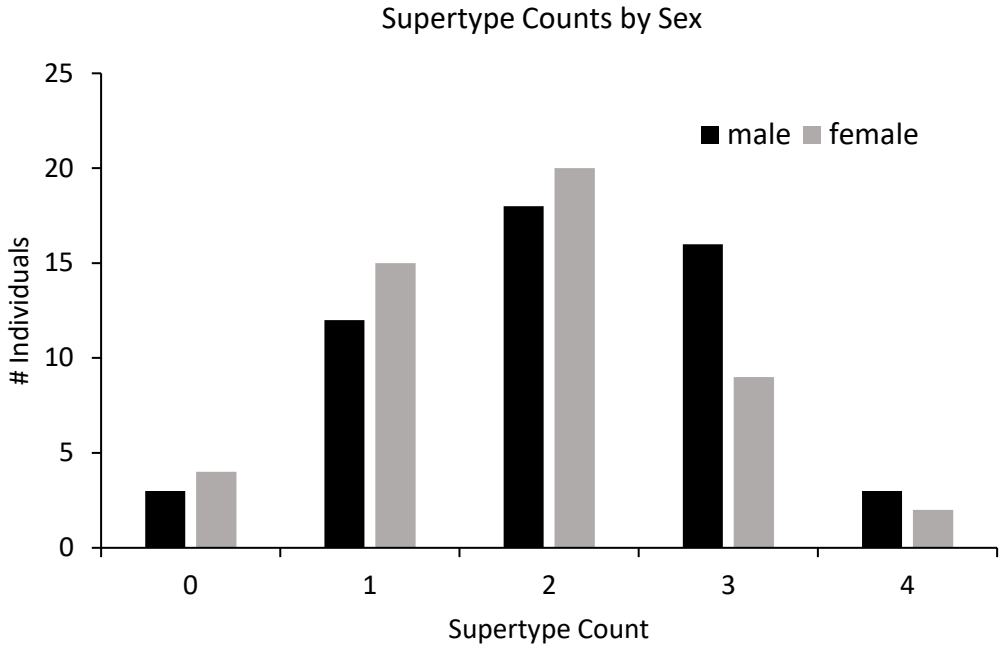

Figure 2 The number of supertypes per individual in males (black) and females (gray).

For AA_{div} calculations, a total of fifteen females and eleven males were excluded because they included one or zero functional alleles, leaving a total of 35 females and 41 males available for comparison. Using the full amino acid sequence, the average female AA_{div} was 11.13 (SD=2.29), compared to 11.05 (SD=2.28) in males (Figure 3). According to a two-sample t-test, this difference was not significant (two-tailed p = 0.883). When only the amino acids expected to be involved in antigen binding were considered, a slight right skew was evident in the data, so the AA_{div} values were log-transformed to conform to a normal distribution. The average AA_{div} in the peptide binding region of females was 5.63 (SD=1.42) (after transformation, mean AA_{div}=0.74, SD=0.11), with an average of 5.41 (SD=1.24) in males (after transformation, mean AA_{div}=0.72, SD=0.10). This difference was also not significant (two-tailed p=0.55).

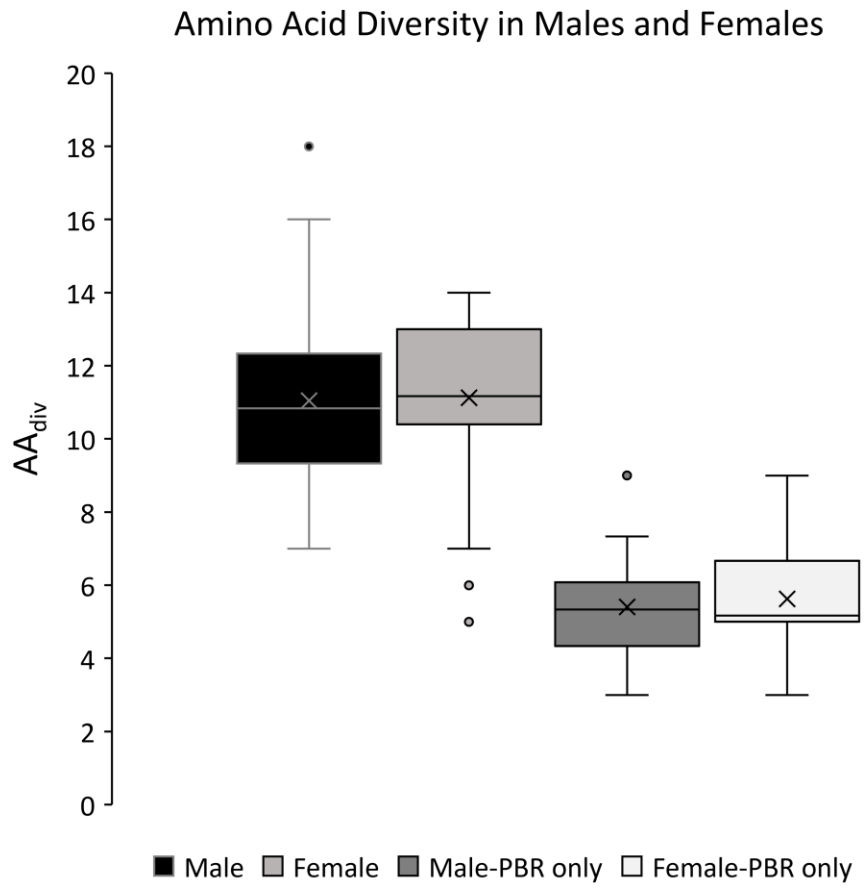

Figure 3 AA_{div} values calculated for males (darker gray) and females (lighter gray), for the full sequence (left two boxes) and for the peptide binding sites only (right two boxes).

Of the 51 alleles detected in this group of mandrills, fifteen appeared only in females and five appeared only in males. However, each of these alleles was rare and occurred in only one or two individuals total, so the sample size was not sufficient to test whether the sex-specificity was due to chance. There were thirteen alleles that appeared in at least ten individuals, with the most common (Masp-pDRB9-1) appearing in 68 mandrills (Table 1). Frequency did not vary significantly between males and females for any of these alleles. There was also no significant difference in frequency between sexes for any of the six identified supertypes. The least common supertype, ST1, occurred in only four individuals, while ST6, the most common, occurred in 62 individuals (Table 2).

Table 1 Frequencies of the most common MHC alleles in males and females

Allele	Male	Female	*p*	$p_{H\text{-}B}$
Masp-pDRB9-1	15.89 (34)	18.48 (34)	0.5071	>0.99
Masp-pDRB1*03:06	9.81 (21)	13.04 (24)	0.3429	>0.99
Masp-pDRB5*03	9.35 (20)	7.07 (13)	0.4685	>0.99
Masp-pDRB9-2	6.54 (14)	5.98 (11)	0.8394	>0.99
Masp-pDRB-17	6.07 (13)	2.72 (5)	0.1465	>0.99
Masp-pDRB9-3	5.61 (12)	2.17 (4)	0.1228	>0.99
Masp-pDRB1*0405	5.61 (12)	1.63 (3)	0.06132	0.80
Masp-pDRB5*03:02	4.21 (9)	2.17 (4)	0.3973	>0.99
Masp-pDRB*W401	2.8 (6)	2.17 (4)	0.7579	>0.99
Masp-pDRB1*0302	3.27 (7)	1.63 (3)	0.3517	>0.99
Masp-pDRB-14	1.87 (4)	3.26 (6)	0.5236	>0.99
Masp-pDRB1-3	2.34 (5)	2.72 (5)	1	>0.99
Masp-pDRB-2	2.34 (5)	2.72 (5)	1	>0.99

*Values in parentheses are the number of individuals possessing the allele. P values are shown before and after Holm-Bonferroni correction (*p *and* $p_{H\text{-}B}$*, respectively)*

Table 2 Supertype Frequency in Males and Females

Supertype	#Alleles	Occurrence	Frequency		*p*	$p_{H\text{-}B}$
			Male	Female		
ST1	2	4	0.75	2.73	0.330	0.990
ST2	4	23	13.43	6.36	0.090	0.537
ST3	4	50	25.37	20.91	0.450	0.990
ST4	4	8	1.49	5.45	0.145	0.726
ST5	11	51	29.85	26.36	0.571	0.990
ST6	8	62	29.1	38.18	0.172	0.726

*#Alleles represents the number of alleles included in the supertype. Occurrence is the number of individuals possessing at least one copy of the supertype. P values are shown before and after Holm-Bonferroni correction (*p *and* $p_{H\text{-}B}$*, respectively).*

Discussion

In this study, we test for differences in MHC DRB diversity and allele frequencies in male and female mandrills to evaluate the hypothesis that selection may favor different levels of heterozygosity or different alleles between the sexes. This hypothesis is based on the premise that males are likely to be more heavily parasitized than females (Charpentier et al., 2019; Lynsdale et al., 2017). Therefore, we expected that males with lower MHC heterozygosity or specific disadvantageous alleles may be less likely to be sampled due to higher mortality or prenatal/postcopulatory selection. However, our findings do not support this hypothesis, as sampled males and females appeared to have similar levels of diversity in MHC alleles, supertypes, and amino acids, as well as equal frequencies of common alleles and supertypes. We did observe a slight trend in that males have higher allelic and supertype MHC diversity than females, but this difference did not approach statistical significance.

Based on these results, there is no evidence that MHC genotypes at the DRB locus impact male success specifically, for instance through parasite-associated early mortality or prenatal/postcopulatory selection based on MHC DRB genotype. This apparent lack of sex-specific selection could indicate that males and females experience similar levels of parasitism. Multiple studies across various taxa have shown a general trend of increased parasitism in males (Clutton-Brock & Pemberton, 2004; Lynsdale et al., 2017; Zuk & McKean, 1996), and the same appears to be true in mandrills (Charpentier et al., 2019; Dibakou et al., 2020; Fouchet et al., 2012). However, varied results in past studies suggest that this pattern is not universal across parasitic taxa. One study showed equal levels of gastrointestinal parasites such as amoebas, ciliates, and nematodes in semi-captive males and females, but since the focal horde in that study is confined, provisioned, and provided with veterinary care, the relevance of those findings to wild populations is unclear (Setchell et al., 2007). Free-ranging male mandrills in Lékédi Park in Gabon were shown to have a higher prevalence of *Plasmodium gonderi* than females, and prevalence of *P. mandrilli* was also increased although statistical significance was marginal (Charpentier et al., 2019).

Prevalence of certain strains of SIV are higher in males in both the CIRMF and Lékédi mandrill populations (Dibakou et al., 2020; Fouchet et al., 2012).

Overall, these past studies point to a tendency for males to be infected more often with certain types of parasites than females. However, even if males have higher prevalence of certain parasites, this may not necessarily lead to higher mortality and consequent selection on MHC genes. If a male's higher parasite load is primarily comprised of non-lethal or non-pathogenic parasites, the sex-specific selective pressure would be weak, and we would thus be unlikely to detect resulting sex-based differences in the MHC. According to the Global Mammal Parasite Database (Stephens et al., 2017), mandrills are infected by a variety of viruses, bacteria, protozoans, and helminths, and many more likely remain undetected in this understudied species. In most cases, the effects of these parasites on fitness or health are unknown, especially for wild populations. It is interesting to note, however, that male mandrills have a markedly shorter lifespan than females (~13 years compared to >22) (Setchell et al., 2005). With the exception of the mortality that occurs due to male-male conflict, especially in captivity (Setchell et al., 2006), the proximate cause of their earlier death remains unclear. High parasite loads could be a contributing factor, as parasitic infections have been shown to be associated with reduced survivorship in multiple species, such as spotted hyenas (*Crocuta Crocuta*) (Ferreira et al., 2019), cliff swallows (*Hirundo pyrrhonota*) (Brown et al., 1995), and great tits (*Parus major*) (Pigeault et al., 2018).

As an alternative to the hypothesis that parasites exert strong selective pressure on the male mandrill MHC, it is possible that parasite-mediated mortality is minimized through behavioral mechanisms. Free-ranging mandrills utilize several behaviors that may reduce parasite transmission and therefore relax pathogen-mediated selective pressure. Adult males tend to spend less time than females in the horde and remain solitary for part of the year (Abernethy et al., 2002), potentially reducing their likelihood of contracting directly transmitted parasites. Both males and females avoid grooming and coming into contact with feces of conspecifics with active parasitic infections (Poirotte, Charpentier, et

al., 2017), and they return more slowly to nest sites that may be contaminated by gastrointestinal parasites (Poirotte, Benhamou, et al., 2017). Poirotte et al. (2017) suggested that these behaviors may provide some level of so-called "behavioral immunity." If mortality is lessened by avoiding infections, selective pressure may be reduced for immunity-related genes. It is difficult to directly assess the impacts of such behaviors on parasite transmission in the population, but the role of host behavior in host-parasite coevolutionary dynamics certainly merits further consideration.

One caveat of this study centers on our assumption that individuals with insufficient immune protection are often removed from the population before sampling, either through early mortality or prenatal/post-copulatory selection, which could remove individuals with disadvantageous MHC genotypes prior to birth. Because we are unlikely to sample very young mandrills due to their small feces, the majority of our samples should come from animals that had adequate immune protection to survive the vulnerable juvenile stage. Multiple studies have shown that young animals tend to have high parasite loads and are heavily affected by pathogen-mediated mortality (Brzeski et al., 2015; Clutton-Brock & Pemberton, 2004; Lynsdale et al., 2017). This trend is generally attributed to immune systems that are not yet fully developed (Simon et al., 2015), or to energetic tradeoffs between rapid growth and immunity (Tschirren & Richner, 2006). If our samples are indeed primarily from adults, we should observe sex-specific differences in the MHC of survivors if pathogen-mediated juvenile mortality varies by sex. However, if mortality occurs later in life, individuals that are particularly vulnerable may still have been sampled, which would obscure the signal of sex-specific selection. Charpentier et al. (2019) found that the probability of *P. gonderi* infection is highest in male mandrills of around 13 years, and nematode prevalence appears to increase with age in females (Setchell et al., 2007). However, to our knowledge, no information is currently available on age-specific pathogen-mediated mortality in mandrills, even though parasitism plays a role in juvenile mortality in multiple other species (Lynsdale et al., 2017; Souchay et al., 2013; Tanaka et al., 2013). It would be preferable to examine differences in

MHC genotypes across age classes as has been performed in other studies (Schaschl et al., 2012), but because most samples in this study are not collected from known individuals, there is no data available regarding the age of sampled mandrills.

Further caveats to this study are related to sample size and our choice of the MHC DRB marker. Effect sizes in MHC studies are generally small in wild populations (Gaigher et al., 2019; Hoover & Nevitt, 2016; T. Kamiya et al., 2014), and if there was only a weak signal of sex-based differences in MHC diversity, our sample size may have been insufficient to detect a significant difference. Furthermore, this study focuses exclusively on a single gene of the class II MHC, which binds to antigens from exogenous pathogens (Rock et al., 2016). To understand the full scope of sex-specific pathogen-mediated selection, it may be necessary to examine markers from other immunogenetic loci, such as the class I MHC, which provides protection against exogenous pathogens like viruses.

The caveats and questions outlined above highlight the overall lack of information available about parasites and their role in the ecology of wild mandrills and the evolution of their immune systems. Only a few parasitic taxa, namely *Plasmodium* and certain viruses such as SIV, have been studied in depth in mandrills, and the prevalence of most other groups remains unknown, especially in wild populations. The effect of infections on individual health, mortality, and fitness has also not been thoroughly explored. To better understand the evolution of MHC genes in mandrills and other primates, and to enable management of disease for conservation purposes, more information is needed to describe which parasites affect wild populations, their degree of pathogenicity, and which individuals are most vulnerable. Future studies should focus on characterization of important parasitic taxa through direct sampling of parasites, and quantifying their role in fitness and mortality. Other immune-related genes also should be examined, such as the class I MHC, since it provides protection against a different set of pathogens and may be subject to different levels of parasite-mediated selection.

Literature Cited

Abbott, K. M., Wickings, E. J., & Knapp, L. A. (2006). High levels of diversity characterize mandrill (Mandrillus sphinx) Mhc-DRB sequences. *Immunogenetics*, *58*(8), 628–640. https://doi.org/10.1007/s00251-006-0132-3

Abernethy, K., White, L. J. T., & Wickings, E. J. (2002). Hordes of mandrills (Mandrillus sphinx): Extreme group size and seasonal male presence. *Journal of Zoology, Proceedings of the Zoological Society of London*, *258*, 131–137.

Alexander, J., & Stimson, W. H. (1988). Sex hormones and the course of parasitic infection. *Parasitology Today*, *4*(7), 189–193. https://doi.org/10.1016/0169-4758(88)90077-4

Bell, G., & Burt, A. (1991). The comparative biology of parasite species diversity: Internal heminths of freshwater fish. *Journal of Animal Ecology*, *60*, 1047–1064.

Benoit, L., Mboumba, S., Willaume, E., Kappeler, P. M., & Charpentier, M. (2014). Using next-generation sequencing methods to isolate and characterize 24 simple sequence repeat loci in mandrills (Mandrillus sphinx). *Conservation Genetics Resources*, *6*(4), 903–905. https://doi.org/10.1007/s12686-014-0237-1

Biedrzycka, A., Bielański, W., Ćmiel, A., Solarz, W., Zając, T., Migalska, M., Sebastian, A., Westerdahl, H., & Radwan, J. (2018). Blood parasites shape extreme major histocompatibility complex diversity in a migratory passerine. *Molecular Ecology*, *27*(11), 2594–2603. https://doi.org/10.1111/mec.14592

Biedrzycka, A., Konopiński, M., Hoffman, E., Trujillo, A., & Zalewski, A. (2020). Comparing raccoon major histocompatibility complex diversity in native and introduced ranges: Evidence for the importance of functional immune diversity for adaptation and survival in novel environments. *Evolutionary Applications*, *13*(4), 752–767. https://doi.org/10.1111/eva.12898

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Brambilla, A., Keller, L., Bassano, B., & Grossen, C. (2018). Heterozygosity–fitness correlation at the major histocompatibility complex despite low variation in Alpine ibex (Capra ibex). *Evolutionary Applications*, *11*(5), 631–644. https://doi.org/10.1111/eva.12575

Brown, C. R., Brown, M. B., & Rannala, B. (1995). Ectoparasites reduce long-term survival of their avian host. *Proceedings of the Royal Society B: Biological Sciences*, *262*(1365), 313–319. https://doi.org/10.1098/rspb.1995.0211

Brzeski, K. E., Harrison, R. B., Waddell, W. T., Wolf, K. N., Rabon, D. R., & Taylor, S. S. (2015). Infectious disease and red Wolf conservation: Assessment of disease occurrence and associated risks. *Journal of Mammalogy*, *96*(4), 751–761. https://doi.org/10.1093/jmammal/gyv080

Bundy, A. P. (1988). Gender-dependent Patterns of Infection and Disease. *Parasitology Today*, *4*(7), 186–189.

Charpentier, M., Boundenga, L., Beaulieu, M., Dibakou, S. E., Arnathau, C., Sidobre, C., Willaume, E., Mercier-Delarue, S., Simon, F., Rougeron, V., & Prugnolle, F. (2019). A longitudinal molecular study of the ecology of malaria infections in free-ranging mandrills. *International Journal for Parasitology: Parasites and Wildlife*, *10*(August), 241–251. https://doi.org/10.1016/j.ijppaw.2019.09.009

Clutton-Brock, T., & Pemberton, J. M. (2004). Dynamics and Selection in an Island Population. In *Cambridge University Press*. https://doi.org/10.1017/cbo9780511550669

Di Fiore, A. (2005). A rapid genetic method for sex assignment in non-human primates. *Conservation Genetics*, *6*, 1053–1058. https://doi.org/10.1007/s10592-005-9086-5

Dibakou, S. E., Souza, A., Boundenga, L., Givalois, L., Mercier-Delarue, S., Simon, F., Prugnolle, F., Huchard, E., & Charpentier, M. (2020). Ecological, parasitological and individual determinants of plasma neopterin levels in a natural mandrill population. *International Journal for Parasitology: Parasites and Wildlife*, *11*(February), 198–206. https://doi.org/10.1016/j.ijppaw.2020.02.009

Doherty, P. C., & Zinkernagel, R. M. (1975). Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature*, *256*, 50–52.

Dorak, M. T., Lawson, T., Machulla, H. K. G., Mills, K. I., & Burnett, A. K. (2002). Increased heterozygosity for MHC class II lineages in newborn males. *Genes and Immunity*, *3*(5), 263–269. https://doi.org/10.1038/sj.gene.6363862

Doytchinova, I. A., & Flower, D. R. (2005). In Silico Identification of Supertypes for Class II MHCs. *The Journal of Immunology*, *174*(11), 7085–7095. https://doi.org/10.4049/jimmunol.174.11.7085

Evans, M. L., & Neff, B. D. (2009). Major histocompatibility complex heterozygote advantage and widespread bacterial infections in populations of Chinook salmon (Oncorhynchus tshawytscha). *Molecular Ecology*, *18*(22), 4716–4729. https://doi.org/10.1111/j.1365-294X.2009.04374.x

Ferreira, S. C. M., Hofer, H., Madeira de Carvalho, L., & East, M. L. (2019). Parasite infections in a social carnivore: Evidence of their fitness consequences and factors modulating infection load. *Ecology and Evolution*, *9*(15), 8783–8799. https://doi.org/10.1002/ece3.5431

Folstad, I., & Karter, A. J. (1992). Parasites, bright males, and the immunocompetence handicap. *American Naturalist*, *139*(3), 603–622. https://doi.org/10.1086/285346

Fouchet, D., Verrier, D., Ngoubangoye, B., Souquière, S., Makuwa, M., Kazanji, M., Gonzalez, J. P., & Pontier, D. (2012). Natural simian immunodeficiency virus transmission in mandrills: A family affair? *Proceedings of the Royal Society B: Biological Sciences*, *279*(1742), 3426–3435. https://doi.org/10.1098/rspb.2012.0963

Gaetano, J. (2018). *Holm-Bonferroni sequential correction: An Excel calculator (1.3)*. https://www.researchgate.net/publication/322569220_Holm-Bonferroni_sequential_correction_An_Excel_calculator_13

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. In *Molecular Ecology* (Vol. 28, Issue 23). https://doi.org/10.1111/mec.15276

Guibinga Mickala, A., Weber, A., Ntie, S., Gahlot, P., Lehmann, D., Mickala, P., Abernethy, K., & Anthony, N. (2022). Estimation of the census (Nc) and effective (Ne) population size of a wild mandrill (Mandrillus sphinx) horde in the Lope National Park, Gabon using a non-invasive genetic approach. *Conservation Genetics*.

Hacking, J. D., Stuart-Fox, D., Godfrey, S. S., & Gardner, M. G. (2018). Specific MHC class I supertype associated with parasite infection and color morph in a wild lizard population. *Ecology and Evolution*, *8*(19), 9920–9933. https://doi.org/10.1002/ece3.4479

Hamilton, M. S., & Hellström, I. (1978). Selection for histoincompatible progency in mice. *Biology of Reproduction*, *19*, 267–270.

Hedrick, P. W. (1994). Evolutionary Genetics of the Major Histocompatibility Complex. *The American Naturalist*, *143*(6), 945–964.

Hill, A., Allsop, C., Kwiatkowski, D., Anstey, N., Twumasi, P., Rowe, P., Bennett, S., Brewster, D., McMichael, A., & Greenwood, B. (1991). Common west African HLA antigens are associated with protection from severe malaria. *Nature*, *352*, 595–600.

Hill, A. V. S. (1991). HLA Associations with Malaria in Africa: Some Implications for MHC Evolution. In *Molecular Evolution of the Major Histocompatibility Complex* (pp. 403–420). https://doi.org/10.1007/978-3-642-84622-9_33

Holm, S. (1979). A Simple Sequentially Rejective Multiple Test Procedure. *Scandinavian Journal of Statistics*, *6*(2), 65–70.

Hoover, B., & Nevitt, G. (2016). Modeling the Importance of Sample Size in Relation to Error in MHC-Based Mate-Choice Studies on Natural Populations. *Integrative and Comparative Biology*, *56*(5), 925–933. https://doi.org/10.1093/icb/icw105

Jennions, M. D., & Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. *Biological Reviews*, *75*(1), 21–64. https://doi.org/10.1111/j.1469-185X.1999.tb00040.x

Kamiya, J., Kang, W., Yoshida, K., Takagi, R., Kanai, S., Hanai, M., Nakamura, A., Yamada, M., Miyamoto, Y., Miyado, M., Kuroki, Y., Hayashi, Y., Umezawa, A., Kawano, N., & Miyado, K. (2020). Suppression of non-random fertilization by MHC class i antigens. *International Journal of Molecular Sciences*, *21*(22), 1–13. https://doi.org/10.3390/ijms21228731

Kamiya, T., O'Dwyer, K., Westerdahl, H., Senior, A., & Nakagawa, S. (2014). A quantitative review of MHC-based mating preference: The role of diversity and dissimilarity. *Molecular Ecology*, *23*(21), 5151–5163. https://doi.org/10.1111/mec.12934

Kechin, A., Boyarskikh, U., Kel, A., & Filipenko, M. (2017). cutPrimers: A New Tool for Accurate Cutting of Primers from Reads of Targeted Next Generation Sequencing. *Journal of Computational Biology*, *24*(11), 1138–1143. https://doi.org/10.1089/cmb.2017.0096

Kennedy, C. R., Bush, A. O., & Aho, J. M. (1986). Patterns in helminth communities: why are birds and fish different? *Parasitology*, *93*, 205–215.

Kloch, A., Babik, W., Bajer, A., Siński, E., & Radwan, J. (2010). Effects of an MHC-DRB genotype and allele number on the load of gut parasites in the bank vole Myodes glareolus. *Molecular Ecology*, *19*(SUPPL. 1), 255–265. https://doi.org/10.1111/j.1365-294X.2009.04476.x

Knapp, L. A., Ha, J. C., & Sackett, G. P. (1996). Parental MHC anitgen sharing and pregnancy wastage in captive pigtailed macaques. *Journal of Reproductive Immunology*, *32*, 73–88.

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

Kuniholm, M. H., Anastos, K., Kovacs, A., Gao, X., Marti, D., Sette, A., Greenblatt, R. M., Peters, M., Cohen, M. H., Minkoff, H., Gange, S. J., Thio, C. L., Young, M. A., Xue, X., Carrington, M., & Strickler,

H. D. (2013). Relation of HLA class I and II supertypes with spontaneous clearance of hepatitis C virus. *Genes and Immunity*, *14*(5), 330–335. https://doi.org/10.1038/gene.2013.25

Kuris, A. M., Blaustein, A. R., & Alio, J. J. (1980). Hosts as Islands. *The American Naturalist*, *116*(4), 570–586. https://doi.org/10.1086/283647

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*(1), 1–13. https://doi.org/10.1186/1471-2148-9-269

Lighten, J., van Oosterhout, C., Paterson, I. G., Mcmullan, M., & Bentzen, P. (2014). Ultra-deep Illumina sequencing accurately identifies MHC class IIb alleles and provides evidence for copy number variation in the guppy (Poecilia reticulata). *Molecular Ecology Resources*, *14*(4), 753–767. https://doi.org/10.1111/1755-0998.12225

Lynsdale, C. L., Mumby, H. S., Hayward, A. D., Mar, K. U., & Lummaa, V. (2017). Parasite-associated mortality in a long-lived mammal: Variation with host age, sex, and reproduction. *Ecology and Evolution*, *7*(24), 10904–10915. https://doi.org/10.1002/ece3.3559

Maccari, G., Robinson, J., Hammond, J. A., & Marsh, S. G. E. (2020). The IPD Project: a centralised resource for the study of polymorphism in genes of the immune system. *Immunogenetics*, *72*(1–2), 49–55. https://doi.org/10.1007/s00251-019-01133-w

Moore, S. L., & Wilson, K. (2002). Parasites as a viability cost of sexual selection in natural populations of mammals. *Science*, *297*, 2015–2018. https://doi.org/10.1126/science.1079746

Müller-Graf, C. D. M., Collins, D. A., Packer, C., & Woolhouse, M. E. J. (1997). Schistosoma mansoni infection in a natural population of olive baboons (Papio cynocephalus anubis) in Gombe Stream National Park, Tanzania. *Parasitology*, *115*, 621–627.

Nunn, C., & Altizer, S. (2010). *Infectious Diseases in Primates: Behavior, Ecology and Evolution* (P. H. Harvey & R. M. May (eds.)). Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198565857.001.0001

Nunn, C. L., & Altizer, S. M. (2004). Sexual selection, behaviour and sexually transmitted diseases. In P. M. Kappeler & C. P. van Schaik (Eds.), *Sexual Selection in Primates: New and Comparative Perspectives* (pp. 117–130). Cambridge University Press. https://doi.org/10.1017/cbo9780511542459.009

O'Connor, S. L., Lhost, J. J., Becker, E. A., Detmer, A. M., Johnson, R. C., MacNair, C. E., Wiseman, R. W., Karl, J. A., Greene, J. M., Burwitz, B. J., Bimber, B. N., Lank, S. M., Tuscher, J. J., Mee, E. T., Rose, N. J., Desrosiers, R. C., Hughes, A. L., Friedrich, T. C., Carrington, M., & O'Connor, D. H. (2010). MHC heterozygote advantage in simian immunodeficiency virus-infected Mauritian cynomolgus macaques. *Science Translational Medicine*, *2*(22), 1–10. https://doi.org/10.1126/scitranslmed.3000524

Osborne, A. J., Pearson, J., Negro, S. S., Louise Chilvers, B., Kennedy, M. A., & Gemmell, N. J. (2015). Heterozygote advantage at MHC DRB may influence response to infectious disease epizootics. *Molecular Ecology*, *24*(7). https://doi.org/10.1111/mec.13128

Oslisly, R., & White, L. J. T. (2000). La relation homme/milieu dans la Reserve de la Lope (Gabon) au cours de l'Holocene; les implications sur l'environnement. In *Dynamique à long terme des écosystèmes forestiers intertropicaux, CNRS/ORSTROM* (Issue July 2020). File Attachments

Palm, J. (1969). Association of maternal genotype and excess heterozygosity for Ag-B histocompatibility antigens among male rats. *Transplantation Proceedings*, *1*(1), 82–84.

Penn, D. J., Damjanovich, K., & Potts, W. K. (2002). MHC heterozygosity confers a selective advantage against multiple-strain infections. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(17), 11260–11264. https://doi.org/10.1073/pnas.162006499

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*. *115*(7), 1–6. https://doi.org/10.5061/dryad.72262

Pigeault, R., Cozzarolo, C. S., Choquet, R., Strehler, M., Jenkins, T., Delhaye, J., Bovet, L., Wassef, J., Glaizot, O., & Christe, P. (2018). Haemosporidian infection and co-infection affect host survival and reproduction in wild populations of great tits. *International Journal for Parasitology*, *48*(14), 1079–1087. https://doi.org/10.1016/j.ijpara.2018.06.007

Poirotte, C., Benhamou, S., Mandjembe, A., Willaume, E., Kappeler, P. M., & Charpentier, M. (2017). Gastrointestinal parasitism and recursive movements in free-ranging mandrills. *Animal Behaviour*, *134*, 87–98. https://doi.org/10.1016/j.anbehav.2017.10.013

Poirotte, C., Charpentier, M., Massol, F., Herbert, A., Willaume, E., Bomo, P. M., & Kappeler, P. M. (2017). Mandrills use olfaction to socially avoid parasitized conspecifics. *Science Advances*, *3*(4). https://doi.org/10.1126/sciadv.1601721

Poulin, R. (1996). Helminth growth in vertebrate hosts: Does host sex matter? *International Journal for Parasitology*, *26*(11), 1311–1315. https://doi.org/10.1016/S0020-7519(96)00108-7

Radwan, J., Zagalska-Neubauer, M., Cichoń, M., Sendecka, J., Kulma, K., Gustafsson, L., & Babik, W. (2012). MHC diversity, malaria and lifetime reproductive success in collared flycatchers. *Molecular Ecology*, *21*(10), 2469–2479. https://doi.org/10.1111/j.1365-294X.2012.05547.x

Rock, K. L., Reits, E., & Neffjes, J. (2016). Present Yourself! By MHC Class I and MHC Class II Molecules. *Trends in Immunology*, *37*(11), 724–737. https://doi.org/10.1016/j.it.2016.08.010.Present

Schad, J., Ganzhorn, J. U., & Sommer, S. (2005). Parasite burden and constitution of major histocompatibility complex in the Malagasy mouse lemur, Microcebus murinus. *Evolution*, *59*(2), 439–450. https://doi.org/10.1111/j.0014-3820.2005.tb01002.x

Schaschl, H., Suchentrunk, F., Morris, D. L., Slimen, H. Ben, Smith, S., & Arnold, W. (2012). Sex-specific selection for MHC variability in Alpine chamois. *BMC Evolutionary Biology*, *12*(1). https://doi.org/10.1186/1471-2148-12-20

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

Schwensow, N., Mazzoni, C. J., Marmesat, E., Fickel, J., Peacock, D., Kovaliski, J., Sinclair, R., Cassey, P., Cooke, B., & Sommer, S. (2017). High adaptive variability and virus-driven selection on major histocompatibility complex (MHC) genes in invasive wild rabbits in Australia. *Biological Invasions*, *19*(4), 1255–1271. https://doi.org/10.1007/s10530-016-1329-5

Sebastian, A., Herdegen, M., Migalska, M., & Radwan, J. (2016). Amplisas: A web server for multilocus genotyping using next-generation amplicon sequencing data. *Molecular Ecology Resources*, *16*(2),

498–510. https://doi.org/10.1111/1755-0998.12453

Setchell, J. M. (2016). Sexual Selection and the Differences Between the Sexes in Mandrills (Mandrillus sphinx). *Yearbook of Physical Anthropology*, *159*, S105–S129. https://doi.org/10.1002/ajpa.22904

Setchell, J. M., Abbott, K. M., Gonzalez, J. P., & Knapp, L. A. (2013). Testing for post-copulatory selection for major histocompatibility complex genotype in a semi-free-ranging primate population. *American Journal of Primatology*, *75*(10), 1021–1031. https://doi.org/10.1002/ajp.22166

Setchell, J. M., Bedjabaga, I. Ben, Goossens, B., Reed, P., Wickings, E. J., & Knapp, L. A. (2007). Parasite prevalence, abundance, and diversity in a semi-free-ranging colony of Mandrillus sphinx. *International Journal of Primatology*, *28*(6), 1345–1362. https://doi.org/10.1007/s10764-007-9225-6

Setchell, J. M., Charpentier, M., Abbott, K. M., Wickings, E. J., & Knapp, L. A. (2009). Is brightest best? Testing the Hamilton-Zuk hypothesis in Mandrills. *International Journal of Primatology*, *30*(6), 825–844. https://doi.org/10.1007/s10764-009-9371-0

Setchell, J. M., Charpentier, M., Abbott, K. M., Wickings, E. J., & Knapp, L. A. (2010). Opposites attract: MHC-associated mate choice in a polygynous primate. *Journal of Evolutionary Biology*, *23*(1), 136–148. https://doi.org/10.1111/j.1420-9101.2009.01880.x

Setchell, J. M., Charpentier, M., & Wickings, E. J. (2005). Sexual selection and reproductive careers in mandrills (Mandrillus sphinx). *Behavioral Ecology and Sociobiology*, *58*(5), 474–485. https://doi.org/10.1007/s00265-005-0946-2

Setchell, J. M., Smith, T., Wickings, E. J., & Knapp, L. A. (2008). Factors Affecting Fecal Glucocorticoid Levels in Semi-Free-Ranging Female Mandrills (Mandrillus sphinx). *American Journal of Primatology*, *70*, 1023–1032. https://doi.org/10.1002/ajp.20594

Setchell, J. M., Vaglio, S., Abbott, K. M., Moggi-Cecchi, J., Boscaro, F., Pieraccini, G., & Knapp, L. A. (2011). Odour signals major histocompatibility complex genotype in an Old World monkey. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1703), 274–280. https://doi.org/10.1098/rspb.2010.0571

Setchell, J. M., Wickings, E. J., & Knapp, L. A. (2006). Life history in male mandrills (Mandrillus sphinx): Physical development, dominance rank, and group association. *American Journal of Physical Anthropology*, *131*, 498–510. https://doi.org/10.1002/ajpa

Simon, A. K., Hollander, G. A., & McMichael, A. (2015). Evolution of the immune system in humans from infancy to old age. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1821). https://doi.org/10.1098/rspb.2014.3085

Siva-Jothy, M. T., & Thompson, J. J. W. (2002). Short-term nutrient deprivation affects immune function. *Physiological Entomology*, *27*, 206–212.

Slade, R. W., & McCallum, H. I. (1992). Overdominant vs. frequency-dependent selection at MHC loci. *Genetics*, *132*, 861–862. https://doi.org/10.1597/13-053

Smallbone, W., Ellison, A., Poulton, S., van Oosterhout, C., & Cable, J. (2021). Depletion of MHC supertype during domestication can compromise immunocompetence. *Molecular Ecology*, *30*(3), 736–746. https://doi.org/10.1111/mec.15763

Soto-Calderón, I. D., Ntie, S., Mickala, P., Maisels, F., Wickings, E. J., & Anthony, N. M. (2009). Effects of storage type and time on DNA amplification success in tropical ungulate faeces. *Molecular Ecology Resources*, *9*(2), 471–479. https://doi.org/10.1111/j.1755-0998.2008.02462.x

Souchay, G., Gauthier, G., & Pradel, R. (2013). Temporal variation of juvenile survival in a long-lived species: The role of parasites and body condition. *Oecologia*, *173*(1), 151–160. https://doi.org/10.1007/s00442-013-2613-z

Stephens, P. R., Pappalardo, P., Huang, S., Byers, J. E., Farrell, M. J., Gehman, A., Ghai, R. R., Haas, S. E., Han, B., Park, A. W., Schmidt, J. P., Altizer, S., Ezenwa, V. O., & Nunn, C. L. (2017). Global Mammal Parasite Database version 2.0. *Ecology*, *98*(5). https://doi.org/10.1111/ijlh.12426

Tanaka, T., Lerche, N. W., Farver, T. B., Ardeshir, A., & Kass, P. H. (2013). Specific-pathogen-free status is associated with lower infant mortality rate in rhesus macaque (Macaca mulatta) colonies at the California National Primate Research Center. *Journal of Medical Primatology*, *42*(4), 186–191. https://doi.org/10.1111/jmp.12049

Tschirren, B., & Richner, H. (2006). Parasites shape the optimal investment in immunity. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1595), 1773–1777. https://doi.org/10.1098/rspb.2006.3524

Tutin, C. E. G., White, L. J. T., & Mackanga-Missandzou, A. (1997). The use by rain forest mammals of natural forest fragments in an equatorial African savanna. *Conservation Biology*, *11*(5), 1190–1203. https://doi.org/10.1046/j.1523-1739.1997.96211.x

Wedekind, C., Chapuisat, M., Macas, E., & Rülicke, T. (1996). Non-random fertilization in mice correlates with the MHC and something else. *Heredity*, *77*(4), 400–409. https://doi.org/10.1038/hdy.1996.160

Westerdahl, H., Waldenstrom, J., Hansson, B., Hasselquist, D., Von Schantz, T., & Bensch, S. (2005). Associations between malaria and MHC genes in a migratory songbird. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1571), 1511–1518. https://doi.org/10.1098/rspb.2005.3113

Wickings, E. J., & Dixson, A. F. (1992). Testicular function, secondary sexual development, and social status in male mandrills (Mandrillus sphinx). *Physiology & Behavior*, *52*, 909–916.

Wilson, K., Grenfell, B., Pilkington, J., Boyd, H., & Gulland, F. (2004). Parasites and their impact. In T. Clutton-Brock & J. M. Pemberton (Eds.), *Dynamics and Selection in an Island Population* (pp. 113–165). Cambridge University Press.

Worley, K., Collet, J., Spurgin, L. G., Cornwallis, C., Pizzari, T., & Richardson, D. S. (2010). MHC heterozygosity and survival in red junglefowl. *Molecular Ecology*, *19*(15), 3064–3075. https://doi.org/10.1111/j.1365-294X.2010.04724.x

Zuk, M., & McKean, K. A. (1996). Sex differences in parasite infections: Patterns and processes. *International Journal for Parasitology*, *26*(10), 1009–1024. https://doi.org/10.1016/S0020-7519(96)00086-0

Conclusion

Biodiversity data is lacking for many tropical species (Collen et al., 2008), but in the face of increasing threats from climate change and anthropogenic pressure, it is crucial to develop a baseline understanding of threatened species' evolutionary history, genetic diversity, tolerance to habitat change, and vulnerability to pathogens. In the preceding studies, we provide some insight on these topics for a vulnerable species of primate known to be in decline due to pressure bushmeat hunting and habitat loss.

The central African landscape is subject to ongoing climatic and anthropogenic stressors that may lead to major ecosystem shifts (Réjou-Méchain et al., 2021). Forest area in western and central Africa, spanning from Senegal to the Democratic Republic of the Congo, has declined in recent decades due to deforestation, decreasing from 346,581 K ha in 1990 to 313,000 K ha in 2015 (Keenan et al., 2015), and temperature and precipitation variables are expected to change dramatically by the end of the 21st century (Williams et al., 2007). Long-term environmental datasets in LNP show a significant trend of climate warming and drying since 1984 (Bush, Jeffery, et al., 2020), and researchers have also noted changes in flowering and fruiting patterns in the park which have lead to an extreme decline in fruit availability and body condition in megafauna (Bush, Whytock, et al., 2020).

Central African forests and savannas also have a history of significant environmental fluctuations, particularly changes in forest cover (Maley, 1993, 1996; Maley & Brenac, 1998). Regardless of these changes, the data presented here suggest that multiple species of forest mammals weathered extreme forest loss in the past without undergoing major population declines. This conclusion provides a hopeful outlook on species viability in the face of projected forest cover loss, although past climate change is not necessarily a useful analog for contemporary climate change (Crowley, 1990). While gorillas, mandrills, blue duikers, and Peters's duikers may be robust to forest fragmentation, additional

threats exist today that cannot be disregarded, such as increased bushmeat hunting, urbanization, and disease (Abernethy et al., 2016; Bermejo et al., 2006; Ripple et al., 2016), all of which continue to threaten animal populations across the region.

Because MSVAR analyses show that these four species have not undergone historical population bottlenecks, they may retain sufficient genetic diversity to enable adaptation as their habitats change. However, neutral diversity does not necessarily predict adaptive capacity (Teixeira & Huber, 2021), so in threatened and endangered species, it is important to study functional genetic markers like the MHC to understand the ability of a species to adapt to new environmental conditions and, more specifically, to mount an effective immune response to introduced pathogens (Holderegger et al., 2006). The MHC's extremely high diversity makes it a particularly useful marker of adaptive genetic variation, and it can provide a great deal of information about patterns of parasite-mediated and sexual selection in populations (Sommer, 2005). However, the extreme polymorphism of the MHC presents a challenge for sequencing (Lighten et al., 2014), especially in studies of wildlife for which high quality samples may not be available.

To facilitate the study of the MHC based on a non-invasive sampling approach, we present a new method to assign MHC alleles to individual samples that have been deeply sequenced using next-generation sequencing. Our method requires further verification and should be tested using fecal samples from individuals with known MHC allele assignments. Nevertheless, it provides a promising avenue to sequencing complex loci using degraded DNA that is typically obtained from non-invasive samples. When applied to the focal population of mandrills at LNP, higher levels of genetic diversity were observed that had not yet been documented in this species. In addition to detecting an MHC pseudogene previously identified only rarely in non-human primates (Gongora et al., 1996), we also identified strong patterns of trans-species polymorphism, with identical alleles shared between mandrills and olive baboons, gorillas, and crab-eating macaques. This finding reaffirms those from

previous studies which have suggested that MHC lineages are maintained across various primate species by pathogen-mediated selective pressure (Azevedo et al., 2015).

As demonstrated by evidence of trans-species polymorphism (Klein et al., 2007), pathogens and parasites have undoubtedly shaped the evolutionary trajectory of many species (Nunn et al., 2004). Pathogens and MHC genes are known to co-evolve (Woolhouse et al., 2002), so studying the MHC in wild populations can provide insight to patterns of pathogen-mediated selection (Garamszegi & Nunn, 2011; Hedrick, 2002; Hill, 1991). MHC diversity can also be an indicator of species' ability to adapt to novel pathogens (Sommer, 2005), which is especially relevant for primate conservation in the face of increasing human encroachment (Ryan & Walsh, 2011; Walsh et al., 2003).

Despite apparent male-biased parasitism in mandrills (Charpentier et al., 2019), we found no evidence of sex-specific selection on the MHC DRB gene. Sampled males and females were equally MHC-diverse, both in terms of allelic richness and amino acid variability, and there was no significant sex-specific difference in the frequencies of any of the common alleles. This finding suggests that MHC DRB variation protects males and females equally against pathogen-associated mortality, despite males having apparently higher parasite prevalence. Possible explanations for this observation are that the species of parasites that are more prevalent in males do not cause significant mortality, or that infection is avoided through behavioral mechanisms. However, much more research is needed to fully explore this topic, as the role that pathogens play in mandrill evolution, ecology, and conservation remains poorly understood.

Overall, the findings of this dissertation paint a picture of a species that has maintained high levels of neutral and functional genetic diversity over time. Mandrills at LNP appear to have tolerated historical forest fragmentation without undergoing major population declines, and their levels of immunogenetic diversity will likely aid them in adapting to new and changing pathogen pressures. More

information is still needed to understand how mandrills will cope with increasing threats, as bushmeat hunting, habitat loss, and climate change continue to affect central African ecosystems (Bush, Jeffery, et al., 2020; Bush, Whytock, et al., 2020; Réjou-Méchain et al., 2021; Ripple et al., 2016; Williams et al., 2007). However, the results generated here provide valuable new insights into the evolution and ecology of this iconic species.

Literature Cited

Abernethy, K., Maisels, F., & White, L. J. T. (2016). Environmental Issues in Central Africa. *Annual Review of Environment and Resources*, *41*, 1–33. https://doi.org/10.1146/annurev-environ-110615-085415

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*(1), 4–9. https://doi.org/10.1186/s40246-015-0043-1

Bermejo, M., Rodríguez-Teijeiro, J. D., Illera, G., Barroso, A., Vilà, C., & Walsh, P. D. (2006). Ebola outbreak killed 5000 gorillas. *Science*, *314*(5805), 1564. https://doi.org/10.1126/science.1133105

Bush, E. R., Jeffery, K., Bunnefeld, N., Tutin, C. E. G., Musgrave, R., Moussavou, G., Mihindou, V., Malhi, Y., Lehmann, D., Ndong, J. E., Makaga, L., & Abernethy, K. (2020). Rare ground data confirm significant warming and drying in western equatorial Africa. *PeerJ*, *2020*(4), 1–29. https://doi.org/10.7717/peerj.8732

Bush, E. R., Whytock, R. C., Bahaa-El-din, L., Bourgeois, S., Bunnefeld, N., Cardoso, A. W., Dikangadissi, J. T., Dimbonda, P., Dimoto, E., Ndong, J. E., Jeffery, K. J., Lehmann, D., Makaga, L., Momboua, B., Momont, L. R. W., Tutin, C. E. G., White, L. J. T., Whittaker, A., & Abernethy, K. (2020). Long-term collapse in fruit availability threatens Central African forest megafauna. *Science*, *370*(6521), 1219–1222. https://doi.org/10.1126/science.abc7791

Charpentier, M., Boundenga, L., Beaulieu, M., Dibakou, S. E., Arnathau, C., Sidobre, C., Willaume, E., Mercier-Delarue, S., Simon, F., Rougeron, V., & Prugnolle, F. (2019). A longitudinal molecular study of the ecology of malaria infections in free-ranging mandrills. *International Journal for Parasitology: Parasites and Wildlife*, *10*(August), 241–251. https://doi.org/10.1016/j.ijppaw.2019.09.009

Collen, B., Ram, M., Zamin, T., & McRae, L. (2008). The Tropical Biodiversity Data Gap: Addressing Disparity in Global Monitoring. *Tropical Conservation Science*, *1*(2), 75–88. https://doi.org/10.1177/194008290800100202

Crowley, T. J. (1990). Are There Any Satisfactory Geologic Analogs for a Future Greenhouse Warming ? *Journal of Climate*, *3*(11), 1282–1292.

Garamszegi, L. Z., & Nunn, C. L. (2011). Parasite-mediated evolution of the functional part of the MHC in primates. *Journal of Evolutionary Biology*, *24*(1), 184–195. https://doi.org/10.1111/j.1420-9101.2010.02156.x

Gongora, R., Figueroa, F., & Klein, J. (1996). The HLA-DRB9 gene and the origin of HLA-DR haplotypes. *Human Immunology*, *51*(1), 23–31. https://doi.org/10.1016/S0198-8859(96)00189-9

Hedrick, P. W. (2002). Pathogen resistance and genetic variation at MHC loci. *Evolution*, *56*(10), 1902–1908. https://doi.org/10.1111/j.0014-3820.2002.tb00116.x

Hill, A. V. S. (1991). HLA Associations with Malaria in Africa: Some Implications for MHC Evolution. In *Molecular Evolution of the Major Histocompatibility Complex* (pp. 403–420). https://doi.org/10.1007/978-3-642-84622-9_33

Holderegger, R., Kamm, U., & Gugerli, F. (2006). Adaptive vs. neutral genetic diversity: Implications for landscape genetics. *Landscape Ecology*, *21*(6), 797–807. https://doi.org/10.1007/s10980-005-5245-9

Keenan, R. J., Reams, G. A., Achard, F., de Freitas, J. V., Grainger, A., & Lindquist, E. (2015). Dynamics of global forest area: Results from the FAO Global Forest Resources Assessment 2015. *Forest Ecology and Management*, *352*, 9–20. https://doi.org/10.1016/j.foreco.2015.06.014

Klein, J., Sato, A., & Nikolaidis, N. (2007). MHC, TSP, and the Origin of Species: From Immunogenetics to Evolutionary Genetics. *Annual Review of Genetics*, *41*(1), 281–304. https://doi.org/10.1146/annurev.genet.41.110306.130137

Lighten, J., van Oosterhout, C., & Bentzen, P. (2014). Critical review of NGS analyses for de novo genotyping multigene families. *Molecular Ecology*, *23*(16), 3957–3972. https://doi.org/10.1111/mec.12843

Maley, J. (1993). The climatic and vegetational history of the equatorial regions of Africa during the upper Quaternary. In *The Archaeology of Africa* (pp. 43–52). https://doi.org/10.4324/9780203754245-12

Maley, J. (1996). The African rain forest - Main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. In *Proceedings of the Royal Society of Edinburgh Section B: Biological Sciences* (Vol. 104, Issue 1996). https://doi.org/10.1017/S0269727000006114

Maley, J., & Brenac, P. (1998). Vegetation dynamics, palaeoenvironments and climatic changes in the forests of western Cameroon during the last 28,000 years B.P. *Review of Palaeobotany and Palynology*, *99*, 157–187. https://doi.org/10.1109/ICCE.2015.7066569

Nunn, C. L., Altizer, S., Sechrest, W., Jones, K. E., Barton, R. A., & Gittleman, J. L. (2004). Parasites and the evolutionary diversification of primate clades. *American Naturalist*, *164*(5 SUPPL.). https://doi.org/10.1086/424608

Réjou-Méchain, M., Mortier, F., Bastin, J. F., Cornu, G., Barbier, N., Bayol, N., Bénédet, F., Bry, X., Dauby, G., Deblauwe, V., Doucet, J. L., Doumenge, C., Fayolle, A., Garcia, C., Kibambe Lubamba, J. P., Loumeto, J. J., Ngomanda, A., Ploton, P., Sonké, B., … Gourlet-Fleury, S. (2021). Unveiling African rainforest composition and vulnerability to global change. *Nature*, *593*(7857), 90–94. https://doi.org/10.1038/s41586-021-03483-6

Ripple, W. J., Abernethy, K., Betts, M. G., Chapron, G., Dirzo, R., Galetti, M., Levi, T., Lindsey, P. A., Macdonald, D. W., Machovina, B., Newsome, T. M., Peres, C. A., Wallach, A. D., & Wolf, C. (2016). Bushmeat hunting and extinction risk to the world's mammals. *Royal Society Open Science*, *3*(10). https://doi.org/10.1098/rsos.160498

Ryan, S. J., & Walsh, P. D. (2011). Consequences of non-intervention for infectious disease in African great apes. *PLoS ONE*, *6*(12). https://doi.org/10.1371/journal.pone.0029030

Sommer, S. (2005). The importance of immune gene variability (MHC) in evolutionary ecology and conservation. *Frontiers in Zoology*, *2*, 1–18. https://doi.org/10.1186/1742-9994-2-16

Teixeira, J. C., & Huber, C. D. (2021). The inflated significance of neutral genetic diversity in conservation genetics. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(10), 1–10. https://doi.org/10.1073/pnas.2015096118

Walsh, P. D., Abernethy, K., Bermejo, M., Beyers, R., De Wachter, P., Akou, M. E., Huljbregts, B., Mambounga, D. I., Toham, A. K., Kilbourn, A. M., Lahm, S. A., Latour, S., Maisels, F., Mbina, C., Mihindou, Y., Ndong Obiang, S., Effa, E. N., Starkey, M. P., Teifer, P., … Wilkie, D. S. (2003). Catastrophic ape decline in western equatorial Africa. *Nature*, *422*(6932), 611–614.

https://doi.org/10.1038/nature01566

Williams, J. W., Jackson, S. T., & Kutzbach, J. E. (2007). Projected distributions of novel and disappearing climates by 2100 AD. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(14), 5738–5742. https://doi.org/10.1073/pnas.0606292104

Woolhouse, M. E. J., Webster, J. P., Domingo, E., Charlesworth, B., & Levin, B. R. (2002). Biological and biomedical implications of the co-evolution of pathogens and their hosts. *Nature Genetics*, *32*(4), 569–577. https://doi.org/10.1038/ng1202-569

Appendix

Chapter 2: Table S1

Group	Scientific name	Species	Available seqs	Unique w. 157 bases	% loss
NWM	*Aotus nancymaae*	Aona	110	78	29.09
NWM	*Aotus nigriceps*	Aoni	32	28	12.50
NWM	*Callicebus moloch*	Camo	14	13	7.14
OWM	*Chlorocebus aethiops*	Chae	12	11	8.33
OWM	*Chlorocebus sabaeus*	Chsa	12	12	0.00
Ape	*Gorilla gorilla*	Gogo	33	30	9.09
OWM	*Macaca fascicularis*	Mafa	330	223	32.42
OWM	*Mandrillus leucophaeus*	Male	2	2	0.00
OWM	*Macaca leonina*	Malo	21	19	9.52
OWM	*Macaca mulatta*	Mamu	246	193	21.54
OWM	*Macaca nemestrina*	Mane	151	90	40.40
OWM	*Mandrillus sphinx*	Masp	42	40	4.76
OWM	*Papio anubis*	Paan	49	46	6.12
Ape	*Pan paniscus*	Papa	5	5	0.00
Ape	*Pan troglodytes*	Patr	81	64	20.99
				average	13.46
				sd	12.18
			pooled sequences unique within species	854	
			sequences unique across species	684	19.91

OWM=Old World Monkey, NWM=New World Monkey
Data not including LNP mandrills

Vita

The author was born in Indiana and grew up in Michigan, receiving her Bachelors of Science in Biology from Alma College in December 2014. She began her graduate career as Masters' student at UNO in August 2016, transitioning to the PhD program in Spring 2019. Her graduate research was completed under the supervision of Dr. Nicola Anthony.