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Immunogenetic Characterization of a Captive Colony of Sooty Mangabeys (*Cercocebus atys*) Used for SIV Research

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Abstract

Background—African nonhuman primates are SIV natural hosts and do not develop disease following infection. Understanding disease avoidance mechanisms in these species is important for HIV vaccine development. The largest captive population of sooty mangabeys, a SIV natural host species, resides at the Yerkes National Primate Research Center.

Methods—Thirteen primer sets that amplify polymorphic microsatellite loci within the MHC region were used to genotype 144 animals. Immunogenetic Management Software (IMS) was used to identify MHC haplotypes and organize data.

Results—Seventy-three haplotypes were identified. Limited haplotype diversity was observed in this population with 88.2% of included animals carrying one of 18 haplotypes. Differences in haplotype frequency were observed between SIV (+) and SIV (–) populations.

Conclusions—We have developed a novel tool for others to use in analysis of the role of the MHC in a natural host nonhuman primate model species used for SIV research.

Keywords

MHC; haplotype; microsatellite marker; HIV

Introduction

More than thirty species of African nonhuman primates have been identified as natural hosts for species-specific strains of simian immunodeficiency virus (SIV), the ancestral virus of human immunodeficiency virus (HIV) [25, 44, 56]. Unlike HIV infection in humans and SIV infection in Asian nonhuman primates, SIV infection in African nonhuman primates does not lead to acquired immunodeficiency syndrome (AIDS) [11, 25, 29, 45, 46, 48]. The difference in basic clinical outcome between SIV-infected natural host species and SIV-

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infected Asian nonhuman primate species and HIV-infected humans has been shown to be related to the difference in evolutionary history between SIV and its natural host species versus SIV and Asian nonhuman primate species and HIV and humans. African nonhuman primates evolved in the presence of SIV-infection for at least the last 32,000 years while HIV has infected humans for approximately the previous 100 years, and SIV infection does not exist in wild Asian nonhuman primate species populations [26, 42, 59]. Over time, evolutionary pressures selected for African nonhuman primate individuals that mount host responses to SIV infection that allow disease avoidance, which are not present in Asian nonhuman primate species or humans. Comparing host responses to SIV infection in natural host species to those in Asian nonhuman primates and responses to HIV infection in humans revealed some of the mechanisms important for the avoidance of disease following SIV infection in natural host species [11, 13, 19]. These mechanisms are likely necessary for the development of an effective HIV vaccine [50].

The majority of our understanding of host response to SIV infection in natural host species originates from the use of sooty mangabeys and African green monkeys as research models [25, 50]. Sooty mangabeys are of particular interest because the strain of SIV infecting them, SIV_{sm}, is the ancestral virus of both SIV_{mac}, which is used to produce simian AIDS in Asian nonhuman primates for HIV studies, and HIV-2, a strain of HIV endemic to West Africa [3, 4, 22, 28]. Sooty mangabeys and African green monkeys possess similar host responses to SIV infection that are thought to allow them to avoid the clinical development of AIDS despite equal or increased viral replication levels compared to HIV-infected humans and SIV-infected Asian nonhuman primates. These mechanisms include the maintenance of normal peripheral CD4⁺ T-cell numbers in the chronic phase of infection, the selective preservation of memory T-cells, the maintenance of T-cell regenerative capacity, the maintenance of mucosal integrity in the gastrointestinal system, the lack of chronic immune system activation, and the maintenance of normal lymph node architecture and function [45–48]. In addition, sooty mangabeys lack target cell availability early in development, which hinders mother-to-infant transmission of SIV [12].

Within human and Asian nonhuman primate populations, individuals exist that are capable of sustaining HIV or SIV infections without progressing to AIDS. This phenotype has been repeatedly correlated with specific major histocompatibility complex haplotypes [1, 6, 10, 23, 33, 34, 36, 51]. Proteins encoded within the major histocompatibility complex function as antigen presentation proteins that initiate either CD8⁺ T-cell cytotoxic activity to intracellular infections (MHC class I) or CD4⁺ T-cell mediated humoral responses to extracellular infections (MHC class II). Both MHC class I and MHC class II proteins are important for the host response to HIV or SIV infection. Individuals with MHC haplotypes that allow for the presentation of highly conserved regions of HIV or SIV proteins are better able to control infection and avoid progressing to AIDS [2, 14, 34]. In cynomolgus macaques, an Asian nonhuman primate species, cellular immune responses to SIV infection were found to be similar in individuals with the same MHC haplotype [9]. The role of MHC proteins is less well understood within natural host species, such as the sooty mangabey. It is likely that MHC haplotypes in sooty mangabeys also play a role in avoidance of clinical AIDS. However, MHC defined populations of sooty mangabeys are not available for research use. Currently, the only captive colony of sooty mangabeys resides at the Yerkes

National Primate Research Center Field Station. This study sought to create a database of individual animal's MHC haplotypes within this colony of sooty mangabeys. Combined with ongoing efforts to maintain detailed pedigree information, this resource will be valuable for future studies of SIV pathogenesis in a well-defined SIV natural host species, the sooty mangabey. We hypothesized that MHC haplotype diversity within this population of sooty mangabeys would be limited due to a founder effect and MHC frequencies would differ between the physically separated naturally infected SIV (+) and SIV (–) subpopulations of animals.

Large quantities of data are necessary to maintain a colony of animals that are MHC-defined. Here, we used software developed for the management of an MHC-defined colony of rhesus macaques used in transplant research, Immunogenetic Management Software (IMS). This system allows for pedigree visualization, MHC haplotype assignment, MHC RNA expression analysis, and calculation of MHC allele and expression sharing between individual members of the colony [24]. We aimed to expand the use of the IMS system to the colony of sooty mangabeys housed at the Yerkes National Primate Research Center.

Materials and Methods

Humane Care Guidelines

This study was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*. Animals included in this study were housed at the Yerkes National Primate Research Center Field Station (Lawrenceville, GA, USA) in accordance with all procedures approved by the Emory University Institutional Animal Care and Use Committee. The Yerkes National Primate Research Center Field Station is fully accredited by AAALAC, International.

Animals

144 naïve and naturally SIV-infected sooty mangabeys were included in this study. These animals were all captive-born and part of the Yerkes National Primate Research Center sooty mangabey colony that was established with 22 individuals in 1968 [43]. This colony has been maintained as a closed colony since its formation. Therefore, all animals included in this study are direct descendants of the original 22 founding animals. Animals were housed in large indoor/outdoor compounds and fed a standard monkey chow (Purina LabDiet 5037, St. Louis, MO, USA) supplemented with fresh fruit and vegetables for the Behavioral Management Unit enrichment program. Naturally infected SIV (+) and SIV (–) animals were housed in separate compounds.

Blood Sample Collection

Blood for this project was collected during three separate semiannual health monitoring surveys performed by the veterinary staff in the Division of Animal Resources and the Field Station Colony Management Unit from 2004 until 2006. Animals were fasted overnight and sedated with 10 mg/kg ketamine hydrochloride (Fort Dodge, Fort Dodge, IA, USA). A complete physical exam was performed on every animal prior to blood collection. After ensuring no abnormalities were noted on physical exam, blood was collected from the

femoral vein in Vacutainer Cell Preparation Tubes with Sodium Heparin (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). Blood was stored at room temperature until processing.

Blood Sample Processing

Within two hours of collection, blood was centrifuged at 1500 RCF for 20 minutes to separate white blood cells (WBCs). WBCs were frozen and stored at -20°C . DNA from WBCs was extracted using the Promega Maxwell System [20]. DNA was quantified using a Nanodrop spectrophotometer.

Existing primer sets shown to be polymorphic in the rhesus macaque MHC were gathered from both existing studies of rhesus macaque MHC genetics and the published rhesus macaque microsatellite map [38, 41]. The most centromeric and the most telomeric microsatellite markers used in this study were the same as those used in the Penedo, *et al.*, 2005 [38]. The approximate size of the MHC region is 5,000 kb, and it includes centromeric and telomeric flanking regions as well as the MHC I and MHC II regions. Due to the relatively close evolutionary relationship between sooty mangabeys and rhesus macaques, many of the primer sets amplified polymorphic regions in both species, however significant regions with no suitable loci existed within the sooty mangabey genome. To address this problem, we leveraged previously identified repeat units within the rhesus macaque genome and attempted to design primers using the techniques described within Raveendran, *et al.* [39]. Table 1 lists the forward and reverse primer sequences used for each of the thirteen microsatellite makers, and Figure 1 illustrates the relative genomic location of each microsatellite marker. These microsatellite markers were located within the MHC I region, within the MHC II region, and within the centromeric and telomeric flanking regions of the MHC on chromosome 6.

PCR amplification and genotyping of the entire sooty pedigree ($n = 144$ individuals) was performed with 25 ng of genomic DNA in a 6- μl reaction volume using Applied Biosystems (ABI) dual 384-well 9700 thermal cyclers under the following conditions: 12 min denaturation at 95°C followed by 10 cycles of 15 s at 94°C , 15 s at $48-55^{\circ}\text{C}$, 30 s at 72°C and 20 cycles of 15 s at 89°C , 15 s at $48-55^{\circ}\text{C}$, 30 s at 72°C , with a final 10-min extension at 72°C . PCR mixtures contained AmpliTaq Gold 10X PCR buffer, dNTPs, MgCl_2 , 0.5 μmol forward and reverse primers, and 0.5 units AmpliTaq Gold DNA polymerase (ABI). PCR products from a single panel were pooled, and the pooling ratio could be adjusted to achieve approximately even peak heights across all loci. The appropriate pooling volume was mixed with a cocktail containing genetic analysis grade Hi-Di formamide (ABI) and labeled size standard (GeneScan-500 Liz; ABI). The electrophoresis was performed in 3730 genetic analyzers (ABI) using data collection software v3.0. Genotyping analysis used Genemapper 3.7 software. Supplemental Table 1 lists the markers and their expected product lengths included in each of the three PCR panels used to fully haplotype each animal.

Haplotypes were identified using the web based Immunogenic Management Software (IMS) [24]. IMS is capable of utilizing both complex pedigree relationships and large genotypic data files in ways to quickly and visualize these complex data sets.

Polymorphism Information Content Calculation

The polymorphism information content (PIC) for each microsatellite marker was calculated according to Nagy, *et al.* [32]. Briefly, frequencies for all alleles at each microsatellite marker were entered into the online tool, Polymorphism Information Content Calculator, available at <http://w3.georgikon.hu/pic/english/kezi.aspx> (accessed 11/25/2014). This online tool calculates the PIC using the mathematical formula discussed in the above-mentioned reference. For this study, the PIC was calculated for each microsatellite marker in both the SIV (+) and the SIV (–) subpopulations.

Results

Microsatellite-based MHC Haplotypes

MHC haplotypes were determined for 144 animals (288 chromosomes) using PCR reactions with primer sets (Table 1) for 13 microsatellite markers (Figure 1).. Maternal and paternal haplotypes were determined by combining reproduction data identifying parentage with allelic data based on the size of the PCR products produced from each of the 288 chromosomes analyzed. Table 2 lists the 13 microsatellite markers used for haplotype determination, the alleles identified for each of these markers, the frequency of these alleles in both the SIV (+) and the SIV (–) subpopulations, the observed heterozygosity for these markers in both the SIV (+) and the SIV (–) subpopulations, and the PIC value calculated for each marker in both the SIV (+) and the SIV (–) subpopulations.. Seventy-three unique MHC haplotypes were identified. Eighteen of these haplotypes were carried by more than three animals. One hundred twenty-seven (88.2%) animals included in this study carried one of these 18 haplotypes. Figure 2 and Table 3 illustrate the prevalence of the 18 haplotypes present in three or more animals. *Ceat-004* was the most common MHC haplotype in this population, being present in 22.2% of the animals. Table 4 lists the allele profiles for each of these 18 haplotypes.

Differences between SIV (+) and SIV (–) Subpopulations

Within the 127 animals carrying at least one of the 18 haplotypes carried by more than three individuals, 70 animals were naturally infected with SIV and 57 animals were seronegative for SIV. Haplotype frequencies within these subpopulations varied. The greatest differences in haplotype frequencies were observed in *Ceat-028*, *Ceat-008*, and *Ceat-004*. As seen in Figure 2 and Table 3, *Ceat-028* and *Ceat-008* were present more frequently in SIV (+) animals while *Ceat-004* was present more frequently in SIV (–) animals. In addition, as noted in Table 2, nine marker alleles were present in the SIV(+) subpopulation and absent in the SIV (–) subpopulation while one marker allele was present in the SIV (–) subpopulation and absent in the SIV (+) subpopulation.

Discussion

Understanding immunologic responses to SIV infection in natural-host nonhuman primate species has been identified as a necessary component of HIV vaccine development [50]. Comparisons between sooty mangabeys and rhesus macaques have been used extensively to delineate these responses [12, 13, 19, 27–29, 31, 37, 40, 45–47, 52–54]. The colony of sooty

mangabeys at Yerkes National Primate Research Center is the largest source of animals naturally-infected with SIV and animals that are SIV-naïve, and as such, it represents a valuable resource for HIV research.

Immune system responses to infectious agents, including SIV and HIV, have been shown to vary with MHC haplotype. Specific MHC haplotypes in both Asian nonhuman primates and humans have been associated with aberrant, desirable disease progression patterns that prolong the chronic phase of immunodeficiency virus infection, which occurs prior to the onset of clinical AIDS. [1, 7, 10, 34, 49, 60]. Based on data collected in this study, it is clear that MHC microsatellite marker allele profiles differ among humans, Asian nonhuman primates, and sooty mangabeys. However, it is likely that the expressed protein profiles are similar in specific haplotypes identified across species. Additionally, due to the close evolutionary relationship between sooty mangabeys, Asian nonhuman primates, and humans, it is likely that specific sooty mangabey MHC haplotypes identified in this study will also be associated with desirable immune response phenotypes. Identification and characterization of the mechanisms resulting in these phenotypes will lead to improved HIV treatment or vaccination strategies.

In nonhuman primates, the MHC region is both highly polygenic and highly polymorphic [18, 38]. Consequently, variability in MHC genetic content is inherent in most populations of nonhuman primates used for research. This genetic variability results in levels of data variance in infectious disease research that could mask important but subtle differences in immune responses. As shown by studies completed in Mauritian cynomolgus macaques, a population of Asian nonhuman primates that display a limited MHC haplotype repertoire due a founder effect and their continued geographical isolation from other cynomolgus macaque populations, improved experimental design and data interpretation in SIV research projects are possible when the MHC haplotypes of individual animals included in the study are known and limited [5, 7–10, 30, 35, 36, 57, 58]. Here, we provide a tool for future researchers using sooty mangabeys from the Yerkes colony to include MHC haplotype information in their experimental design and data interpretation.

We used microsatellite-based haplotyping to characterize the MHC at the level of DNA. Alternative approaches for defining individual animal's MHC include using full-length cDNA sequencing of MHC transcripts or using high-resolution pyrosequencing of MHC transcripts. These alternative methods are complicated by the presence of duplicate regions of the MHC genome yielding multiple copies of individual loci in nonhuman primates that are absent in humans [57]. The consequence of this duplication is that multiple transcripts per gene may be present in each animal. Additionally, it has been shown that expression of MHC transcripts is dependent upon cell type [21]. By using genomic DNA as opposed to RNA transcripts, microsatellite-based MHC haplotyping presents the most straightforward, efficient, and cost-effective method of defining individual immunogenetics in nonhuman primates [15–17, 38].

Samples were collected from 144 animals for this study. A total of 73 unique microsatellite-based MHC haplotypes were identified within these animals. However, only 18 of these haplotypes were present in more than three individuals within the population. These 18

haplotypes had prevalence rates up to 22.2% of the population, and 127 out of the 144 (88.2%) animals tested carried one of these 18 haplotypes. This pattern of relatively few haplotypes being present in the majority of the animals in this population is similar to that observed by Wiseman, *et al.* when they used microsatellite-based MHC haplotyping to characterize the cynomolgus macaque population of the island of Mauritius [58]. Mauritian cynomolgus macaques are descended from a small founder population that was brought to the island approximately 500 years ago, and the population has been geographically isolated since that time [55]. Similarly, the sooty mangabey colony at Yerkes National Primate Research Center was founded by a small group of 22 animals 36 years prior to sample collection for this project [43]. Due to their protected status as a CITES appendix II animal and the subsequent limits placed on importation of this species, the colony of sooty mangabeys at Yerkes has remained genetically isolated. Therefore, the relative homogeneity of MHC haplotypes present in this population is expected, and it may prove to be useful in future studies that seek to use animals with identical MHC haplotypes.

The 22 founding animals of the Yerkes sooty mangabey colony could have carried at most 44 different MHC haplotypes. Considering that this colony has been closed since its inception, it is potentially paradoxical that our data suggest a minimum of 73 MHC haplotypes are present in the colony currently. A likely explanation for this potential paradox is the occurrence of recombination events. Most of these events would likely occur between our most centromeric microsatellite marker, D6S291, and our most telomeric microsatellite marker, D6S1691. Table 5 illustrates one such possible recombination event that may have occurred in an animal carrying both the *Ceat*-006 and the *Ceat*-019 haplotypes, which could have generated the *Ceat*-008 haplotype. Differences in MHC haplotype frequencies between naturally infected SIV (+) animals and SIV (–) animals were observed within the Yerkes sooty mangabey colony. Some MHC haplotypes were present more frequently in the SIV (+) subpopulation while some MHC haplotypes were present more frequently in the SIV (–) subpopulation. These differences are likely due to the management practices in place at the Yerkes National Primate Research Center Field Station, which physically separate naturally infected SIV (+) and SIV (–) animals.

In conclusion, the colony of sooty mangabeys at the Yerkes National Primate Research Center Field Station represents a valuable resource for HIV vaccine development research. The use of Immunogenetics Management Software to create a microsatellite-based database of MHC haplotypes for this colony has created a tool for future projects to use in order to give their experiments greater power. This increase in power could potentially unmask subtle differences in immune responses between experimental groups and lower the number of animals necessary to reliably identify effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Primer set locations. Relative positions of the 13 microsatellite marker primer sets, within and flanking the MHC region of the genome, used to genotype each animal. Primer sets were chosen to provide allelic data at loci from the MHC flanking regions, the MHC class I region, and the MHC class II region. The telomeric end of the chromosome is located to the left, and the centromere is located to the right.

Figure 2.

Haplotype frequencies. Histogram illustrating the frequency of the 18 MHC haplotypes identified in the Yerkes sooty mangabey colony that are carried by more than three individuals. *Ceat-004*, the most common MHC haplotype identified in the entire colony, is carried by 22.2% of the animals included in this study. Differences in frequencies of these 18 MHC haplotypes between SIV (+) and SIV (–) animals are also presented. The greatest differences in prevalence rates were seen in *Ceat-028* and *Ceat-008*, which were present more frequently in SIV (+) animals, and *Ceat-004*, which was present more frequently in SIV (–) animals.

Table 1

Primers. Forward and reverse primers for each microsatellite marker used to haplotype each animal.

Marker	Forward Primer Sequence	Reverse Primer Sequence
D6S1691	AGGACAGAATTTGCCTC	GCTGCTCCTGTATAAGTAATAAAC
D6S276	TTCCAGTGTATACATCAATCAAATCA	GGGTGCAACTTGTTCCTCCT
D6S1571	GGACCTACGCATCTGGTG	TGGCTCTAATGGTTACTTTTACA
D6S1621	AAAGATTTAGAGTAAATGCTGATGA	ACCACAGATGAGAATGCCTT
MML4SJ3	GGGAGACGGTTTCTTTCACA	TGGCAGATAATGTGTGGTTG
MML4SJ5	CTCCCAGCCTCACTCATTA	GAGGTTGTGGGTAGGGAGGT
MML4SJ16	TCAGATTTCTGTGAGGTG	TCCTTTCCTTTCACAGTAGCC
STRMICA	CCTTTTTTTCAGGGAAAGTGC	CCTTACCATCTCCAGAACTGC
DRACA	GATACTTTCCTAATTCTCCTCCTTC	ATGGAATCTCATCAAGGTCAG
D6S2876	GGTAAATTCCTGACTGGCC	GACAGCTCTTCTTAACCTGC
D6S2741	AGACTAGATGTAGGGCTAGC	CTGCACTTGGCTATCTCAAC
D6S1568	ACATGACCAGAACTTCCCAG	AGCTAGGCCAGGCCGT
D6S291	CTCAGAGGATGCCATGTCTAAAATA	GGGGATGACGAATTATCTACTAACT

Table 2

Microsatellite characterization. Allele sizes (bp) observed at each microsatellite locus genotyped, frequencies of each allele in both the SIV (+) and SIV (−) subpopulations, calculated polymorphism information content (PIC) for each microsatellite marker in both the SIV (+) and SIV (−) subpopulations, and observed heterozygosities of each microsatellite marker in both the SIV (+) and SIV (−) subpopulations.

Marker	Allele Sizes (bp)	Frequency SIV (+)	PIC SIV(+)	Heterozygosity SIV(+)	Frequency SIV(−)	PIC SIV(−)	Heterozygosity SIV(−)
D6S1691	258	0.12	0.77	0.86	0.10	0.78	0.86
	260	0.22			0.24		
	261	0.01			Not Present		
	265	0.20			0.13		
	268	0.29			0.25		
	270	0.08			0.10		
	272	0.08			0.18		
D6S276	210	0.59	0.57	0.62	0.50	0.65	0.64
	211	0.13			0.14		
	214	0.08			0.13		
	216	0.13			0.15		
	218	0.07			0.08		
D6S1571	114	0.11	0.73	0.80	0.11	0.75	0.80
	119	0.01			Not Present		
	124	0.07			0.06		
	126	0.42			0.37		
	127	0.16			0.14		
	131	0.01			0.01		
	137	0.09			0.17		
	139	0.13			0.14		
D6S1621	269	0.01	0.19	0.20	Not Present	0.18	0.23
	280	0.88			0.89		
	282	0.11			0.11		
MML4S13	204	0.27	0.68	0.74	0.32	0.71	0.76

Marker	Allele Sizes (bp)	Frequency SIV (+)	PIC SIV (+)	Heterozygosity SIV (+)	Frequency SIV (-)	PIC SIV (-)	Heterozygosity SIV (-)
	206	0.04			0.09		
	210	0.08			0.10		
	212	0.37			0.29		
	214	0.24			0.20		
MML4SJ5	143	0.05	0.80	0.84	0.02	0.81	0.82
	146	0.29			0.19		
	149	0.07			0.09		
	154	0.14			0.11		
	156	0.07			0.15		
	165	0.10			0.11		
	167	0.23			0.28		
	169	Not Present			0.03		
	182	0.01			Not Present		
	184	0.04			0.02		
MML4SJ16	161	0.16	0.75	0.79	0.13	0.77	0.76
	171	0.09			0.17		
	173	0.25			0.17		
	176	0.16			0.14		
	177	0.01			0.03		
	180	0.31			0.33		
	181	0.02			0.03		
STRMICA	207	0.20	0.29	0.35	0.28	0.32	0.41
	209	0.01			Not Present		
	224	0.79			0.72		
DRACA	248	0.41	0.73	0.76	0.46	0.72	0.80
	251	0.05			0.06		
	253	0.13			0.12		
	261	0.07			0.09		
	263	0.01			Not Present		

Marker	Allele Sizes (bp)	Frequency SIV (+)	PIC SIV (+)	Heterozygosity SIV (+)	Frequency SIV (-)	PIC SIV (-)	Heterozygosity SIV (-)
D6S2876	264	0.02			0.06		
	265	0.12			0.10		
	267	0.01			0.02		
	269	0.18			0.09		
D6S2741	224	0.13	0.69	0.71	0.06	0.63	0.66
	226	0.15			0.14		
	228	0.32			0.41		
	231	0.35			0.35		
	238	0.04			0.04		
	239	0.01			Not Present		
D6S1568	259	0.12	0.77	0.85	0.10	0.78	0.86
	261	0.22			0.24		
	262	0.01			Not Present		
	266	0.20			0.13		
	269	0.29			0.25		
	271	0.08			0.10		
	273	0.08			0.18		
D6S291	116	0.78	0.28	0.40	0.73	0.32	0.46
	128	0.22			0.27		
D6S291	202	0.06	0.46	0.62	0.05	0.44	0.45
	207	0.55			0.53		
	209	0.38			0.42		
	213	0.01			Not Present		

Table 3

Haplotype frequencies. Frequencies of the 18 MHC haplotypes carried by more than three individuals in the Yerkes sooty mangabey colony. Frequencies are also listed for these haplotypes in the SIV (+) subpopulation, and the SIV (–) subpopulation.

	Frequencies		
	Overall	SIV (+)	SIV (–)
<i>Ceat-001</i>	2.8%	2.4%	3.3%
<i>Ceat-004</i>	22.2%	16.7%	30.0%
<i>Ceat-005</i>	13.9%	10.7%	18.3%
<i>Ceat-006</i>	5.6%	2.4%	10.0%
<i>Ceat-007</i>	14.6%	11.9%	18.3%
<i>Ceat-008</i>	9.7%	13.1%	5.0%
<i>Ceat-009</i>	7.6%	6.0%	10.0%
<i>Ceat-015</i>	8.3%	10.7%	5.0%
<i>Ceat-016</i>	5.6%	4.8%	6.7%
<i>Ceat-018</i>	2.8%	2.4%	3.3%
<i>Ceat-019</i>	3.5%	1.2%	6.7%
<i>Ceat-020</i>	2.8%	3.6%	1.7%
<i>Ceat-021</i>	4.9%	4.8%	5.0%
<i>Ceat-022</i>	4.9%	6.0%	3.3%
<i>Ceat-026</i>	8.3%	7.1%	10.0%
<i>Ceat-028</i>	9.7%	15.5%	1.7%
<i>Ceat-033</i>	4.9%	4.8%	5.0%
<i>Ceat-034</i>	2.8%	3.6%	1.7%

Table 4

Allele profiles. Allele profiles for the 18 haplotypes carried by more than three individuals.

Allele Profile													
D6S1691	D6S276	D6S1571	D6S1621	MML4SJ3	MML4SJ5	MML4SJ6	STRMICA	DRACA	D6S2876	D6S2741	D6S1568	D6S291	
Ceat-001	258	210	114	282	212	146	180	207	265	228	259	116	207
Ceat-004	260	210	126	280	204	167	180	224	248	228	261	116	207
Ceat-005	270	211	139	280	210	149	180	224	261	226	271	116	207
Ceat-006	265	216	126	280	206	146	173	224	264	231	266	116	209
Ceat-007	272	214	137	280	212	156	171	207	248	228	273	128	209
Ceat-008	265	210	126	280	212	146	173	224	269	231	266	116	209
Ceat-009	260	216	139	280	204	154	161	224	248	231	261	116	202
Ceat-015	258	210	114	282	212	165	180	207	265	228	259	128	207
Ceat-016	268	211	127	280	214	167	176	224	253	231	269	116	207
Ceat-018	260	210	126	280	204	167	180	224	248	231	261	116	207
Ceat-019	265	210	126	280	212	146	173	224	269	224	266	128	209
Ceat-020	268	210	127	280	214	184	176	224	253	231	269	116	207
Ceat-021	268	210	124	280	214	143	161	224	248	226	269	116	207
Ceat-022	268	210	127	280	214	167	176	224	253	231	269	116	207
Ceat-026	268	210	114	280	204	154	161	224	251	231	269	116	209
Ceat-028	265	210	126	280	212	146	173	224	269	224	266	116	209
Ceat-033	268	216	126	280	214	146	173	224	253	231	269	116	209
Ceat-034	258	218	127	282	214	165	176	207	265	228	259	116	209

Table 5

Recombination event. Example of a possible recombination event between *Ceat-006* and *Ceat-019*, which created *Ceat-008*.

	Allele Profile												
	D6S1691	D6S276	D6S1571	D6S1621	MML4SJ3	MML4SJ5	MML4SJ16	STRMICA	DRACA	D6S2876	D6S2741	D6S1568	D6S291
<i>Ceat-019</i>	265	210	126	280	212	146	173	224	269	224	266	128	209
<i>Ceat-008</i>	265	210	126	280	212	146	173	224	269	231	266	116	209
<i>Ceat-006</i>	265	216	126	280	206	146	173	224	264	231	266	116	209