Title: Testing for post-copulatory selection for major histocompatibility complex genotype in a semi-free-ranging primate population

Authors: Joanna M Setchell[1], Kristin M Abbott[2], Jean-Paul Gonzalez[3,4,5], Leslie A Knapp[2]

Affiliations: [1] Evolutionary Anthropology Research Group, Department of Anthropology, Durham University

[2] Division of Biological Anthropology, University of Cambridge, Downing Street, Cambridge CB2 3QG, UK

[3] Health Ecology Unit, Centre International de Recherches Médicales de Franceville, BP 2105, Libreville, Gabon

[4] Institut de Recherche Pour le Développement, Chargé des Relations Bilatérales Santé et Biosécurité, France/USA

[5] METABIOTA Senior Advisor for Emerging Diseases & Biosecurity, USA

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Short title: Post-copulatory selection in mandrills

Address for correspondence: Joanna M Setchell, Department of Anthropology, Durham University, South Road, Durham DH1 3LE, UK. Tel: +44 (0)191 334 1633. Email: joanna.setchell@durham.ac.uk

ABSTRACT

A large body of evidence suggests that major histocompatability complex (MHC) genotype influences mate choice. However, few studies have investigated MHC-mediated post-copulatory mate choice under natural, or even semi-natural, conditions. We set out to explore this question in a large semi-free-ranging population of mandrills (*Mandrillus sphinx*) using MHC-DRB genotypes for 127 parent-offspring triads. First, we showed that offspring MHC heterozygosity correlates positively with parental MHC dissimilarity suggesting that mating among MHC dissimilar mates is efficient in increasing offspring MHC diversity. Second, we compared the haplotypes of the parental dyad with those of the offspring to test whether post-copulatory sexual selection favoured offspring with two different MHC haplotypes, more diverse gamete combinations, or greater within-haplotype diversity. Limited statistical power meant that we could only detect medium or large effect sizes. Nevertheless, we found no evidence for selection for heterozygous offspring when parents share a haplotype (large effect size), genetic dissimilarity between parental haplotypes (we could detect an odds ratio of $\geq$1.86), or within-haplotype diversity (medium-large effect). These findings suggest that comparing parental and offspring haplotypes may be a useful approach to test for post-copulatory selection when matings cannot be observed, as is the case in many study systems. However, it will be extremely difficult to determine conclusively whether post-copulatory selection mechanisms for MHC genotype exist, particularly if the effect sizes are small, due to the difficulty in obtaining a sufficiently large sample.

Key words: mate choice; post-copulatory selection; gamete choice; maternal-foetal interactions; sexual selection; cryptic female choice; selective fertilization.

INTRODUCTION

The major histocompatibility complex (MHC) is one of the most polymorphic regions of the vertebrate genome [Bernatchez and Landry 2003; Piertney and Oliver 2006]. This multigene family encodes cell-surface glycoproteins that play a critical role in the immune system by recognising foreign peptides, presenting them to specialist immune cells and initiating the appropriate immune response [Klein 1986]. MHC diversity is thought to be selectively maintained, at least in part, via pathogen-mediated selection and sexual selection [Apanius et al. 1997; Piertney and Oliver 2006; Sommer 2005]. Different MHC molecules recognise and bind different foreign peptides, meaning that MHC heterozygotes should be able to present more peptides to T cells than homozygotes and thus have improved resistance to pathogens (overdominance) [Doherty and Zinkernagel 1975]. Additionally, rare MHC alleles can provide pathogen resistance when the pathogen has adapted to the majority of MHC alleles in the population (negative frequency-dependency) [Piertney and Oliver 2006].

A large body of evidence suggests that MHC genotype influences mate choice [reviews in Jordan and Bruford 1998; Penn 2002; Penn and Potts 1999; Ziegler et al. 2005]. Mate choice may occur for MHC dissimilarity between partners (disassortative mating), offering two nonexclusive fitness benefits: production of MHC heterozygous offspring [Zeh and Zeh

1997] and/or prevention of inbreeding and increase in genome-wide genetic diversity [Brown and Eklund 1994]. Alternatively, mate choice may result in selection for an optimal number of MHC alleles in the offspring [Milinski 2006], or for specific MHC genotypes, including rare alleles [Penn 2002]. The potential for MHC-mediated mate choice exists before, during and after mating [Wedekind 1994]. Evidence is available for pre-copulatory mate choice based on the MHC in rodents [Yamazaki and Beauchamp 2007], fish [Agbali et al. 2010; Consuegra and Leaniz 2008; Eizaguirre et al. 2009; Forsberg et al. 2007; Reusch et al. 2001], reptiles [Olsson et al. 2003], birds [Ekblom et al. 2004; Freeman-Gallant et al. 2003; Richardson et al. 2005], and humans [Jacob et al. 2002; Wedekind et al. 1995]. However, pre-copulatory mate choice may not always result in inheritance of a particular advantageous MHC allele for offspring because males are often heterozygous at the locus of interest, and the haploid sperm of an individual diploid male differ in their genetic compatibility with the maternal genotype [Ober 1999]. Thus, females may need post-copulatory mechanisms to ensure transmission of the desired haplotype(s) and avoid the costs of investing in a sub-optimal embryo [Wedekind 1994]. Genetic compatibility may also be detected more easily after copulation than prior to copulation, via interactions between the sperm and the female reproductive tract and ovum [Zeh and Zeh 1997].

Post-copulatory selection cannot influence which maternal MHC haplotype is passed on to offspring, as the haplotype of the ovum is set prior to fertilisation [Tulsiani 2007]. Females may, however, be able to select which paternal MHC haplotype is passed on to the offspring. Some studies have suggested that MHC molecules are expressed on the surface of spermatozoids [review in Wedekind et al. 1996], at least under certain conditions [e.g.,

infectious status, Rulicke et al. 1998]. If sperm do express their MHC haplotype, this would present an opportunity for the female reproductive tract to choose those with compatible and/or dissimilar MHC alleles, or particular alleles. In support of this possibility, *in vitro* studies have shown that gamete fusion in mice is influenced by MHC genes [Wedekind et al. 1996]. However, other studies have concluded that MHC molecules are not expressed on mature spermatozoa [e.g., Desoye et al. 1991; Hutter and Dohr 1998], making this possible mechanism of sperm choice contentious. Intriguingly, MHC-linked olfactory receptor genes are transcribed in testicular tissue, and might indirectly, signal sperm MHC haplotype via linkage disequilibrium [Ziegler et al. 2002], via an Immuno-Olfactory Supercomplex [Ziegler 1997], providing a possible alternative mechanism for MHC-associated sperm choice.

While there is considerable evidence for post-copulatory biases in fertilisation success based on overall genetic similarity in insects [Bishop 1996; Bretman et al. 2004; Mack et al. 2002; Simmons et al. 2006; Stockley 1999; Wilson et al. 1997], reptiles [Jehle et al. 2007; Olsson et al. 1996], fish [Gasparini and Pilastro 2011], and birds [Marshall et al. 2003; Thuman and Griffith 2005], relatively few studies have examined the specific role of MHC genotype in post-copulatory mate choice [but see Skarstein et al. 2005; Yeates et al. 2009 for studies in fish]. This is particularly the case for animals living and reproducing under natural, or even semi-natural, conditions, as opposed to laboratory strains, and is readily understandable as such studies must disentangle the influence of sperm competition and female effects [Birkhead 1988]. For example, male mice are sensitive to clues indicating that females have already mated and respond by allocating more sperm in each ejaculate

[Ramm and Stockley 2007], and female jungle fowl respond to the MHC similarity of a female through allocating more sperm to the more MHC-dissimilar of two females [Gillingham et al. 2009].

Selection for (or against) particular MHC combinations may also occur post-fertilisation, via selective implantation or spontaneous abortion. The survival of the fetus in the maternal environment presents an immunological paradox, as the mother must accept the presences of the equivalent of a tissue transplant [Medawar 1953], although the fetus expresses foreign (i.e., paternal) genes. Studies of maternal-fetal interactions have concentrated on spontaneous abortion in humans [Beydoun and Saftlas 2005; Makrigiannakis et al. 2011; Ober 1999]. Some studies suggest that human conceptuses inheriting paternal MHC genotypes that differ from maternal genotypes (histoincompatible pregnancies) are more likely to survive than those inheriting paternal MHC genotypes that do not differ from maternal genotypes (histocompatible pregnancies) [reviews in Beydoun and Saftlas 2005; Ober 1999], possibly because proper implantation of the embryo requires an adequate immune response. However, there is, as yet, no consensus concerning the influence of MHC allele-sharing on the risk of spontaneous abortion in humans [Beydoun and Saftlas 2005; Makrigiannakis et al. 2011], and few studies of this topic in other species.

We attempted to address the question of whether post-copulation selection occurs for MHC genotype in a population of naturally reproducing, semi-free-ranging mandrills (*Mandrillus sphinx*). We have previously shown that reproduction in this population is biased in favour of MHC-dissimilar partners [Setchell et al. 2010]. Thus far, the underlying mechanism

142 remains unknown, but there are theoretical reasons to expect post-copulatory selection to
143 be common in mandrills, as in other primates [Birkhead and Kappeler 2004; Dixson 1998;
144 Setchell and Kappeler 2003]. First, female mandrills mate with multiple males during a
145 single fertile cycle [Setchell et al. 2005]. Second, mandrills possess very large testes relative
146 to their body mass, suggesting high levels of sperm competition [Dixson 1998]. Finally,
147 male coercion may limit a female's ability to express precopulatory choice; post-copulatory
148 selection mechanisms would allow the female to overcome these constraints and favour
149 particular males.
150
151 To test whether there is selection for or against sperm of different males, we would need to
152 determine exactly which sperm are present in a female's reproductive tract when
153 fertilization occurs. This requires knowledge of the exact timing of ovulation and the
154 identity, genotype, and order of mating for all males with whom she mated during her
155 fertile period. While the timing of ovulation can be determined using non-invasive faecal
156 endocrinology [Hodges and Heistermann 2003], it is impossible to know the identity of all
157 mates and the order of mating under field conditions. Moreover, the identity of the sire is
158 likely to be influenced by sperm competition, including factors such as timing of mating
159 relative to the optimal insemination period, ejaculate size, and position in the mating order
160 [Birkhead and Kappeler 2004], as well as cryptic male preference for genetically dissimilar
161 females [Gillingham et al. 2009]. We circumvented these issues by concentrating on post-
162 copulatory selection involving the sperm of just one male – the sire. If the sire of an
163 offspring is known, then we can be sure that his sperm were present in the mother's
164 reproductive tract at the right time. Meiosis results in each spermatozoid being haploid and

possessing only one of the sire's two copies of each chromosome, meaning that we can test whether selection occurs between the two gametes of the same male within the female reproductive tract, based on their different genetic characteristics. Restricting analyses to the sire alone allows us to remove most of the effects of sperm competition, although meiotic drive by selfish genetic elements may result in an over-representation of one haplotype in the sperm that we cannot control for. If we detect evidence for selection *within* males, then we can extrapolate to suggest that selection will also occur *between* males.

We concentrated on MHC-DRB genes, a highly variable group of MHC class II loci that encode proteins that are directly involved in the immune response and are under strong diversifying selection pressure in mandrills, with the peptide-binding region containing significantly more non-synonymous than synonymous changes [Abbott et al. 2006]. We began by testing whether parental dyads that are MHC-dissimilar produce offspring that are more MHC diverse than offspring of less MHC-dissimilar parents (Hypothesis 1). Next, taking advantage of the fact that MHC-DRB sequences are transmitted from parent to offspring as blocks of nucleotide sequence characterized by strong linkage disequilibrium, or haplotypes, we explored which of the two haplotypes the sire contributed to each offspring, to test whether gamete selection favours MHC heterozygosity in offspring. If this is the case, then when the parents share an MHC haplotype, MHC heterozygotes (those that inherit different haplotypes from their parents) should occur more often than predicted by random inheritance, while homozygous offspring (those that inherit the same haplotype from both parents) should occur less often (Hypothesis 2).

MHC-associated selection within the reproductive tract is more likely than selection on an early embryo or at the level of implantation, as it is less costly than the latter two possibilities, both of which would cost a female mandrill a minimum of one reproductive cycle (approx. 1 month). We tested whether gamete selection favours offspring with two haplotypes that are genetically dissimilar over those with two more similar haplotypes (Hypothesis 3). If this is the case, then the genetic dissimilarity between the paternal and maternal haplotypes inherited by the offspring should be greater than predicted from random inheritance. This differs from Hypothesis 2 because it concentrates on the genetic dissimilarity *between* different MHC haplotypes, rather than presence of the same vs. different haplotypes. Next, we tested whether gamete selection favours the inheritance of the most diverse MHC haplotype (the haplotype possessing more MHC sequences, or MHC sequences that are more functionally dissimilar) from the sire (Hypothesis 4). If this is the case, then the haplotype contributed should be more diverse than the alternative haplotype (i.e., it should possess more, or more functionally dissimilar, MHC sequences). This differs from Hypothesis 3 by examining diversity *within* the individual haplotypes, not dissimilarity *between* parental haplotypes. Finally, we examined the question of maternal-fetal compatibility [Ober 1999]. If histoincompatible foetuses are more likely to survive than those that are histocompatible (Hypothesis 5), then histoincompatible offspring should occur more often, while histocompatible offspring should occur less often, than predicted from random inheritance.

Despite a 20 year study, our conclusions concerning post-copulatory selection in mandrills are limited by an inability to detect small effect sizes in all analyses, and to detect even a large effect size reliably in some cases. Nevertheless, we present this study as the first exploration of gamete selection in a large primate, to propose the utility of within-sire comparisons, and as a cautionary tale in the logistical difficulties presented by such a study.

METHODS

Study population

We studied offspring born into in a large, semi-free-ranging population of mandrills at the Centre International de Recherches Médicales, Franceville (CIRMF), Gabon, over a 20 year period. The CIRMF mandrill colony was established in 1983/4, when 15 founder animals (seven males, eight females) originating from diverse locations in the wild, were released into a 6.5 ha naturally rain-forested enclosure. All further additions to the group have been due to reproduction of the founder animals and some animals have been removed. A second semi-free-ranging group was established in 1994 (3.5 ha) by transferring 17 mandrills (including four adult males and six adult females) from the first enclosure. The animals forage freely in the enclosure, and receive daily supplements of monkey chow and seasonal fruits. Water is available ad libitum. Group sizes ranged from 15 in 1983/4 to a maximum of 104 animals in 2002, similar to smaller groups observed in the wild [Rogers et al. 1996].

We assigned maternity using observations of maternal behaviour during daily observations of the colony, and subsequently confirmed these assignments using the published colony pedigree [Charpentier et al. 2005a]. The pedigree also provides an accurate paternity assignment for 193 (94 %) of the 205 offspring born [Charpentier et al. 2005a]. It was established using DNA extracted from blood samples obtained during annual captures of the colony and is based on microsatellite loci (mean loci typed per individual ± standard error 7.42 ± 0.07). Genotypes were available for all potential sires and paternity was assigned using CERVUS 2.0 [Marshall et al. 1998] and confirmed using PARENTE [Cercueil et al. 2002] [details in Charpentier et al. 2005a].

MHC genotyping

As reported previously [Setchell et al. 2010], we genotyped 155 members of the mandrill population for MHC-DRB, including 127 offspring and their parents. Insufficient DNA was available to genotype the remaining mandrills (N = 64). In particular, we were unable to genotype two stillborn individuals and 18 animals that died before they could be captured. While it is possible that these animals had sub-optimal MHC genotypes (e.g., were homozygotes) and thus bias our sample towards MHC-diverse animals, many of these deaths were accidental or occurred as a result of attack by other animals, events which are likely to be independent of their MHC genotype.

The molecular methods used for MHC-DRB genotyping this mandrill population have been described previously [Abbott et al. 2006]. Briefly, we used a combination of cloning and

sequencing and denaturing gradient gel electrophoresis (DGGE) and direct sequencing to initially characterise the MHC-DRB sequences of the mandrill population. We PCR-amplified MHC-DRB sequences using the primers 5'MDRB and 3'MDRB for both procedures and the reverse primer 3'MDRB+GC for DGGE [Knapp et al, 1997]. We obtained cloned sequences in triplicate and generated DGGE sequences by removing sections of DGGE bands for reamplification via PCR followed by direct sequencing. All cloned and DGGE bands were sequenced in both directions on an ABI 373 automated sequencer (Macrogen, Korea), allowing us to eliminate artefact heteroduplex (chimeric) sequences from our genotyping results. Using these methods, we identified a total of 35 different *Mandrillus sphinx Masp*-DRB sequences. We repeated all genotyping experiments to ensure that a sequence found in one individual was also detected, if present, in relatives and all other individuals in the population. We deposited MHC sequence data in GenBank (accession numbers DQ103715–DQ103732, DQ103734–DQ103746, EU693911–EU693914).

We used two methods to differentiate functional MHC-DRB genes from nonfunctional pseudogenes. First, we reviewed all sequences for stop codons [Abbott et al, 2006]. One sequence (*Masp*-DRB-6*0404) had a stop codon, so we removed this from the dataset for analysis. Next, we examined patterns of transcription using cDNA from a subset of seven mandrills representing all known *Masp*-DRB loci and lineages, and for whom mRNA samples were available [Setchell et al. 2010]. We found that 15 / 16 of the mandrill MHC-DRB sequences identified in these animals were transcribed and, therefore, possibly functional (although we did not investigate whether the sequences were translated). The one sequence that was undetected using cDNA (Masp-DRB 6*0402) had a 1 bp deletion,

which would disrupt the sequence reading frame and render it incapable of making a functional protein. Therefore, we also removed this sequence from our analyses. Human MHC-DRB6 sequences, traditionally characterised as pseudogenes due to mutations in exon 2, may only exhibit low levels of expression [Fernandez-Soria et al., 1998] so it is unsurprising that these two mandrill DRB6 sequences would be nonfunctional. Transcription of other *Masp*-DRB6 sequences, was uncertain, as we were unable to obtain mRNA for cDNA analyses, but these sequences had no stop codons or nonsense mutations to render them obviously nonfunctional. One of these, *Masp*-DRB6*0401, was found in a fairly large number of individuals (10 % of the population), and 10 of the offspring analysed (8 %). Two other *Masp*-DRB6 sequences were present in only eight (*Masp*-DRB6*0102) and one (*Masp*-DRB6*0101) individuals. Removing these individuals from the analysis did not alter our conclusions.

The MHC-DRB region in Old World primates frequently experiences expansion through gene duplication and contraction through deletion [Slierendregt et al. 1994]. Because of the extensive variation in DRB haplotype composition, individuals possess different numbers and types of DRB genes on each haplotype. We focused on these haplotypes, without making any assumptions about the number of loci involved. We deduced haplotypes (unique combinations of sequences inherited together from parent to offspring) via patterns of inheritance using known parent-offspring triads from the colony pedigree. For example, female 2's first offspring (mandrill 2A) was sired by male 7. The MHC genotypes of the triad are shown in Table 1. In this case, we can see that offspring 2A shares both *W7001 and *W7101 with female 2, but male 7 does not have these sequences, so we can

deduce that 2A must have inherited both from female 2. Thus 2A's maternal haplotype consists of sequences *W7001 and *W7101. Similarly, 2A shares only sequence 1*0404 with male 7. As female 2 does not possess this sequence, 2A must have inherited it from male 7. Thus 2A's paternal haplotype consists of only the sequence 1*0404. Further, sequences not passed to 2A by female 2, which must therefore be in her other, non-transmitted, haplotype, were 1*0302, 5*0302, and sequences not passed on to offspring 2A by male 7, the non-transmitted paternal haplotype, were 3*0402 and *W401.

We proceeded in a similar way for all offspring, identifying a total of 17 different MHC haplotypes in the 155 animals genotyped. Each haplotype consisted of 1-4 sequences (mean 2.4), and was present in 1-3 of the founder individuals (mean +/- SEM = 1.5 +/- 0.2) and 1-75 of all individuals (mean +/- SEM = 17.1 +/- 4.3). Each individual mandrill possessed 1–7 sequences, in two haplotypes; when we found only one haplotype in an individual we assumed the individual was homozygous for that haplotype. We detected no changes in MHC haplotype from parent to offspring in our dataset, suggesting that no major recombination occurred in our sample.

We examined MHC sequence diversity in terms of the number of sequences in each haplotype. As MHC sequences may differ in nucleotide composition, but still share the same amino acid sequences due to the presence of silent substitutions, we calculated the number of amino acid differences between each pair of MHC sequences as an estimate of genetic dissimilarity [Landry et al. 2001]. Additionally, since not all amino acids are involved in peptide binding, we also calculated the number of amino-acid differences in the predicted

peptide binding region (PBR, based on the PBR for human sequences) between each pair of MHC sequences as an estimate of genetic dissimilarity.

We calculated three measures of MHC-dissimilarity between the mother's haplotype and each of the sire's haplotypes:

MHC_{diff} The total number of different MHC sequences in the two haplotypes.

AA_{diff} The sum of all pairwise amino acid differences between the sequences of the two haplotypes.

PBR_{diff} The sum of all pairwise amino acid differences between the peptide binding sites of the two haplotypes.

We described the within-haplotype diversity of the two available MHC haplotypes for each parent as follows:

MHC_n The number of MHC sequences in the haplotype.

AA_n The sum of all pairwise amino acid differences between all sequences on the haplotype.

PBR_n The sum of all pairwise amino acid differences between the peptide binding sites of the sequences on the haplotype.

Statistical analyses

We used a mixed model (in SPSS) including dyad identity as a random effect to compare parental MHC-dissimilarity (measured as the total number of different MHC sequences

possessed by a mother x sire dyad) with the number of MHC sequences in the offspring (Hypothesis 1).

To test whether selection resulted in more heterozygous offspring than expected by chance (Hypothesis 2), we compared the inheritance patterns of paternal haplotypes, given the known maternal haplotype, with the 50:50 expected from chance using binomial tests.

To test whether selection occurred for more dissimilar combinations of haplotypes over similar combinations (Hypothesis 3) we compared MHC_{diff} and AA_{diff} for the maternal haplotype with each of the two alternative paternal haplotypes and tested for differences in similarity using Wilcoxon matched-paired tests. We also used Wilcoxon paired tests to test for selection for more MHC diverse paternal haplotypes, irrespective of the female haplotype (Hypothesis 4), by comparing MHC_n and AA_n in paternal haplotypes that were transmitted with those that were not.

Finally, to test whether an excess of histoincompatible offspring was born (Hypothesis 5), we split possible conceptuses into the following categories, following Ober (1999), and compared observed offspring with those predicted from random inheritance of haplotypes using a chi-squared test:

- Likely to be histoincompatible: paternally inherited allele different from both maternal alleles. (As there is very little information about acceptable or unacceptable MHC-DRB mismatches in Old World monkeys, it may be that not all non-identical MHC-DRB molecules are 'histoincomaptible').

- Homozygous histocompatible: paternally inherited allele is the same as maternally inherited allele.
- Heterozygous histocompatible: paternally inherited allele matches the maternal allele that was not inherited.

We used G*Power 3 [Faul et al. 2007] to determine the statistical power of our analyses. We used *sensitivity analyses* to compute the critical population effect size as a function of α (set as 0.05), $1 - \beta$ (set as 0.90) and N (the sample size) and *a priori* power analyses to determine the sample size necessary to detect Cohen's standardised *small*, *medium* and *large* effect size conventions [Cohen 1988] for each analysis. In the case of the MDC procedure we calculated power using a standard logistic regression.

We focussed our analyses at the level of the offspring. However, mothers (N = 31, range: 1-11, mean 4.1), sires (N = 15, range: 2-30, mean = 8.0), and mother-sire dyads (N = 75, range: 1-6, mean = 1.6) each contributed multiple offspring to the dataset, leading to the potential for pseudo-replication, if individuals or dyads show biased MHC transmission. With the exception of the initial mixed model, we were unable to control for this, as it was not possible to include parent or dyadic ID as a random factor in our analyses. Unfortunately, the relatively low numbers of offspring contributed by individual animals and the diversity of MHC haplotypes in the population meant that the occurrence of any particular MHC haplotype was too low to test for the transmission of particular MHC haplotypes with reasonable statistical power.

Ethics statement

This research complied with protocols approved by the Comité Régional d'Ethique Ile de France Paris Sud (registration number 02-010) and adhered to the legal requirements of the country in which the research was conducted (Gabon). The research adhered to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates. The CIRMF mandrills are housed in very large, naturally rain-forested enclosures, where they forage naturally and receive twice-daily provisioning. Animals remained in the enclosures during and after the study. The only invasive procedure involved was blood sampling for DNA, undertaken during routine annual veterinary controls of the mandrill colony, during which all efforts were made to ameliorate suffering.

RESULTS

MHC-dissimilar parents produce MHC-diverse offspring

Parents that were more MHC-dissimilar had offspring with a greater number of different MHC sequences than offspring from MHC-similar dyads (mixed model with dyad identity as a random effect: $F = 28.04$, d.f. = 1, 66, $P < 0.001$, Fig. 1).

Is there selection for heterozygous offspring when parents share a haplotype?

Of 18 cases where one of the two possible paternal haplotypes was the same as the

maternal haplotype, that paternal haplotype was passed on in 11 cases, which did not differ significantly from chance (one-tailed binomial exact test: $P = 0.240$). However, to detect even a large effect size (0.25) in this analysis would require a sample size of 33 offspring, so this may reflect Type 2 error (i.e., failure of the test to detect a real relationship). Thus, we cannot conclude that there is no selection for heterozygous offspring when parents share a haplotype.

Is there selection for more MHC dissimilar parental haplotypes?

We found no influence of genetic dissimilarity on whether a paternal haplotype was inherited (Table 2). With our sample size ($N = 127$), we would be able to detect an effect size of 0.267 (i.e., between a small (0.2) and medium (0.5) effect size). Detection of a small effect would require a sample size of 226. Thus we can conclude that there is no medium-large effect, but we are unable to rule out a small-medium effect of selection for more dissimilar haplotypes.

Is there selection for transmission of more diverse haplotypes?

Within-haplotype MHC diversity was not greater in the paternal haplotype that was passed on to the offspring than in the one that was not (Table 2). As above, we can conclude that there is no medium-large effect, but we are unable to rule out a small-medium effect of selection for more diverse haplotypes.

Is there selection for maternal-fetal MHC histoincompatibility?

The 96 offspring born to parents that shared no haplotypes (born to 51 dyads composed of 27 mothers and 14 sires) were likely to be histoincompatible. In the 31 cases where parents of an offspring shared an MHC haplotype (20 dyads composed of 14 mothers and 10 sires), they produced a heterozygous histocompatible offspring 6 times, a homozygous histocompatible offspring 11 times and a histoincompatible offspring 14 times. This distribution does not differ from chance (χ^2 = 1.903, df = 2, P = 0.386). However, to detect even a large effect (0.5) using this test would require a sample size of 51. Lumping heterozygous and homozygous histocompatible offspring did not improve this situation as the threshold to detect a large effect size in this comparison is 43.

DISCUSSION

We attempted to address an intriguing question in evolutionary biology - whether MHC-dependent post-copulatory mate choice occurs - in a large primate species. First, we showed that offspring MHC heterozygosity correlates positively with parental MHC dissimilarity in our study population. This is not surprising and shows that mating among MHC dissimilar parents, which is known to occur in mandrills [Setchell et al. 2010], is efficient in increasing offspring MHC diversity. Similar findings have been reported for white-toothed shrews, *Crocidura russula* [Duarte et al. 2003]; Seychelles warblers, *Acrocephalus sechellensis* [Richardson et al. 2004]; and house finches, *Carpodacus mexicanus* [Oh and Badyaev 2006].

Next, we attempted to test whether there is gamete selection for MHC heterozygosity, dissimilarity between parental haplotypes, or within-haplotype diversity. We genotyped 127 offspring born over 20 years, circumventing problems associated with differential sperm allocation by males by concentrating on within-sire haplotype selection. Problems with statistical power plague animal behaviour research [Smith et al. 2011], and it is often very difficult to increase the sample size [Taborsky 2010]. Our study is no exception, and our conclusions are limited by an inability to detect small effects in all analyses, and to detect even a large effect of maternal-fetal histocompatibility.

The lack of immigration into the CIRMF colony means that the potential for inbreeding has increased with subsequent generations. Previous studies of the colony have demonstrated the effects of inbreeding on fitness correlates: female body mass and size decrease with inbreeding, as does age at first birth, which may be an indirect consequence of the effect of inbreeding on body mass and size [Charpentier et al. 2006]. Moreover, there is a positive relationship between genetic diversity and reproductive success in both males and females [Charpentier et al. 2005b], and a positive relationship between MHC diversity and reproductive success in males [Setchell et al. 2010]. Theoretically, a risk of inbreeding should, if anything, lead to increased selection in favour of genetic diversity. For example, studies of inbred laboratory strains of rats [Michie and Anderson 1966; Palm 1969] and mice [Hamilton and Hellstrom 1978], where new-borns show a deficit of MHC homozygotes and increased heterozygosity. However, even in this closed colony, which results from a small number of founders, with no immigration, only a minority of offspring

(31/127) were born to parents that share MHC haplotypes, limiting our potential to examine questions of heterozygote excess and materno-fetal compatibility in particular. Nevertheless, we found no evidence for a large post-copulatory selection effect in favour of offspring with two different MHC haplotypes where the parents shared a haplotype, at least within males, although we did not have the power to detect medium or small effect sizes. We also found no evidence for a medium-large effect of selection for more dissimilar parental haplotypes, or for selection for more within-haplotype diversity, although we did not have the power to detect small effects.

Reproduction in the CIRMF mandrills is biased in favour of genetically dissimilar dyads and MHC-diverse males [Setchell et al. 2010]. The sexual dimorphism found in mandrills, and the polygynandrous nature of their mating system suggests that this may be due to post-copulatory selection, at least in part. However, we found no evidence of a medium-large effect of MHC-associated post-copulatory selection, although our sample size was too small to detect any small-medium effect. Possible MHC-associated post-copulatory selection has been found in mouse lemurs (*Microcebus murinus*), in which a study comparing sires and non-sires (i.e., *between* male comparisons) of 79 offspring found no evidence for pre-copulatory female choice based on male MHC genotype. However, sires differed significantly at the MHC from randomly assigned males, possessing fewer MHC sequences different to those of the female, but a higher number of MHC supertype differences different to those of the female, as well as fewer MHC sequences but more supertypes overall [Schwensow et al. 2008]. Intriguingly, studies of fish have provided contrasting results: MHC-heterozygous males had higher fertilisation success than MHC-homozygotes

in charr (*Salvelinus alpinus*) [Skarstein et al. 2005], whereas male Atlantic salmon (*Salmo salar*) obtained greater relative fertilization success when competing for eggs from MHC-similar females, a finding possibly related to the importance of avoiding outbreeding in this species [Yeates et al. 2009]. Together, these results suggest that post-copulatory selection for MHC can occur, at least *between* males, although the patterns observed differ between species. In a *within* male comparison in sedge warblers (*Acrocephalus schoenobaenus*), offspring had a higher overall genetic diversity (based on microsatellite genotype) than expected if fertilisation was random [Marshall et al. 2003], with a medium-large effect size (calculated as Z / sqrt (N) = 0.45), suggesting that selective fertilisation can occur within males, at least in birds. Our statistical power was sufficient to detect an effect of similar size in mandrills, but we did not find one.

Particular MHC haplotypes may be preferentially transmitted due to a selective advantage associated with the non-transmitted haplotype, for example in relation to specific parasites [review in Piertney and Oliver 2006]. Particular MHC haplotypes occurred at too low a rate in our study population to test for the transmission of particular MHC haplotypes with reasonable statistical power. However, Milinski (2006) has argued that females are not only unlikely to be able to detect the presence of individual MHC alleles, but they are probably also unlikely to know the precise relationship between specific parasites and MHC alleles, suggesting that mate selection for specific alleles is unlikely to occur. Alternatively, as noted for humans [Diamond 1987], certain MHC alleles may be linked to other genetic loci that have their own advantages or disadvantages and this linkage disequilibrium may result in biased transmission of the linked loci.

While we are unable to determine conclusively whether mandrills employ gamete selection for MHC diversity, a non-exclusive alternative mechanism underlying preferential reproduction with MHC-dissimilar mates [Setchell et al. 2010] relies on chemical communication. Both male and female mandrills possess a sternal gland, which produces a glandular secretion, which may play a role in the pre-copulatory assessment of MHC compatibility via 'fragrant genes' [Milinski 2006]. In support of this hypothesis, we have recently shown that odour similarity reflects similarity at the MHC in our study population, suggesting that odour provides information against which the receiver can compare its own genotype to assess genetic similarity [Setchell et al. 2011]. Without additional post-copulatory processes, pre-copulatory selection based on odour cannot select for particular haplotypes, as an individual transmits either of its two haplotypes. If MHC-associated mate choice does occur pre-copulation, then this may imply that the specific MHC Class II haplotype passed on by a chosen partner is less important than overall genetic dissimilarity, and that MHC-DRB diversity may be maintained as a consequence of selection for overall genetic dissimilarity [Brown and Eklund 1994], rather than selection for MHC diversity itself. However, our previous results suggest that the influence of MHC dissimilarity on reproduction was stronger than that of overall genetic dissimilarity [Setchell et al. 2010].

In conclusion, we set out to test for MHC-mediated post-copulatory selection in mandrills by genotyping as many of the 205 offspring born into the CIRMF mandrill colony over 20 years as possible (127). However, this sample size gave us the possibility of detecting

555 medium effect sizes at best, and in some cases we were unable to detect even a large effect
556 size. With these limitations, we found no evidence for large effect sizes of MHC-mediated
557 post-copulatory selection in this species. Our concentration on comparing the haplotypes
558 found in the sire and the mother with those of the resulting offspring may represent a way
559 forward in future, large-scale studies of the genetics of natural and semi-natural
560 populations, providing a window onto potential post-copulatory selection mechanisms.
561 Females of many primate species, including mandrills, mate with multiple males [Dixson
562 1998], and these are the species in which post-copulatory selection should be expected
563 [Setchell and Kappeler 2003]. However, field-workers are unlikely to be able to document
564 all mating events reliably, with the exception of species that experience a very short
565 receptive period [e.g., one night in mouse lemurs, Schwensow et al. 2008]. If gamete
566 selection occurs at the level of the individual sperm, then it should detect selection *between*
567 sperm of the same male. Any selection for particular characteristics of the sperm, resulting
568 from selection in the female reproductive tract, egg choice or selection following
569 conception in the oviduct would be detectable in such an analysis. While we cannot
570 pinpoint the exact timing of any such selection events, we suspect that any such selection
571 would occur relatively early, via sperm selection within the female reproductive tract or
572 egg choice for particular fertilising sperm. Later selection, for example selection on the
573 early embryo in oviduct, implantation, spontaneous abortion, and pre- and post-natal
574 investment [Ober 1999], would all incur relatively high costs for female primates, since
575 selection post-fertilisation would involve a delay in pregnancy of at least one menstrual
576 cycle, and possibly result in birth during a sub-optimal period in seasonal breeders.
577

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781

Table 1: MHC-DRB genotypes of one parent-offspring triad from the CIRMF mandrill population. M indicates a maternal haplotype, S a paternal haplotype. Sequences not present in these three individuals are not shown, for simplicity

	MHC-DRB sequences possessed						
	1*0302	1*0404	3*0402	5*0302	*W401	*W7001	*W7101
Mother (female 2)	M1			M1		M2	M2
Sire (male 7)		S1	S2		S2		
Offspring (2A)		S1				M2	M2

Table 2: Comparison of MHC diversity in paternal haplotypes inherited by offspring and those that were not (results of Wilcoxon paired tests, N = 127)

MHC variable [a]	Haplotype inherited (mean +/- SE)	Haplotype not inherited (mean +/- SE)	Z	P
MHC_{diff}	3.7 +/- 0.1	3.9 +/- 0.1	0.977	0.328
AA_{diff}	55.9 +/- 4.0	52.9 +/- 3.3	0.009	0.993
PBR_{diff}	33.4 +/- 1.8	32.7 +/- 1.6	0.215	0.830
MHC_n	1.9 +/- 0.1	2.0 +/- 0.1	0.447	0.655
AA_n	18.1 +/- 1.9	17.3 +/- 1.8	0.135	0.893
PBR_n	14.3 +/- 1.1	13.1 +/- 1.0	0.751	0.453

[a] MHC_{diff}: the number of different MHC sequences in the two haplotypes; AA_{diff}: the sum of all pairwise amino acid differences between the sequences of the two haplotypes; PBR_{diff}: the sum of all pairwise amino acid differences between the peptide binding sites of the two haplotypes; MHC_n: the number of MHC sequences in the haplotype; AA_n: the sum of all pairwise amino acid differences between all sequences on the haplotype; PBR_n: the sum of all pairwise amino acid differences between the peptide binding sites of the sequences on the haplotype.

Figure 1: Comparison of the number of MHC sequences in offspring with the number of different MHC sequences in the parents. Point size indicates number of overlapping data points.