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No postcopulatory selection against MHC-homozygous offspring: Evidence from a pedigreed captive rhesus macaque colony

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Abstract

The heterozygosity status of polymorphic elements of the immune system, such as the major histocompatibility complex (MHC), is known to increase the potential to cope with a wider variety of pathogens. Pre- and postcopulatory processes may regulate MHC heterozygosity. In a population where mating occurs among individuals that share identical MHC haplotypes, postcopulatory selection may disfavour homozygous offspring or ones with two MHC haplotypes identical to its mother. We tested these ideas by determining the incidence of MHC-heterozygous and MHC-homozygous individuals in a pedigreed, partially consanguineous captive rhesus monkey colony. Bayesian statistics showed that when parents share MHC haplotypes, the distribution of MHC-heterozygous and MHC-homozygous individuals significantly fitted the expected Mendelian distribution, both for the complete MHC haplotypes, and for MHC class I or II genes separately. Altogether, we found in this captive colony no evidence for postcopulatory selection against MHC-homozygous individuals. However, the distribution of paternally and maternally inherited MHC haplotypes tended to differ significantly from expected. Individuals with two MHC haplotypes identical to their mother were underrepresented and offspring with MHC haplotypes identical to their father tended to be overrepresented. This suggests that postcopulatory processes affect MHC haplotype combination in offspring, but do not prevent low MHC heterozygosity.

KEYWORDS

major histocompatibility complex class I or II genes, major histocompatibility complex heterozygosity, major histocompatibility complex homozygosity, maternal–foetal incompatibility, postcopulatory selection, rhesus macaque

1 | INTRODUCTION

Heterozygosity of the major histocompatibility complex (MHC) genes is important for an individual's pathogen resistance and MHC-homozygous individuals are thus supposed to be at a disadvantage (Carrington et al., 1999; Doherty & Zinkernagel, 1975; Huchard, Knapp, Wang, Raymond, & Cowlishaw, 2010) and may be selected against. The MHC represents a gene region which consists of multicopy gene families that play a key role in initiating adaptive immune responses. The MHC encompasses two main classes of genes that

encode for cell surface proteins with different functionalities, MHC I and II, both involved in the binding and presentation of peptides to specialized types of T cells belonging to the immune system (Falk, Rötzschke, Stevanović, Jung, & Rammensee, 2006; Klein, 1986). MHC I gene products are expressed on virtually all nucleated cells and are involved in generating immune responses to pathogens that cause intracellular infections, such as viruses and mycobacteria. In contrast, MHC II gene products are only expressed on professional antigen-presenting cells such as macrophages and dendritic cells and present peptides, mostly from extracellular origin. The MHC class I and II molecules may control susceptibility/resistance to many autoimmune and infectious diseases (McLaren & Carrington, 2015; Tiwari & Terasaki, 1981). The characteristics of the genes encoding for MHC I and II are their high degree of polymorphism (allelic variation) and their copy number variation (diversity) (Doxiadis, Otting, de Groot, Noort, & Bontrop, 2000; Otting et al., 2005; Robinson, Waller, Fail, & Marsh, 2006). This holds true not only for humans but also for nonhuman primates such as macaques (de Groot et al., 2012). MHC class I and II genes, which are inherited together on one chromosome, are called MHC haplotypes. MHC molecules are codominantly expressed and different MHC allotypes can bind and display a unique gradient of foreign peptides. As a consequence, MHC-heterozygous individuals can present more peptides selected from pathogens to T cells than MHC-homozygous ones and therefore have an improved fitness profile (heterozygous advantage hypothesis: Doherty & Zinkernagel, 1975; Carrington et al., 1999). MHC polymorphism itself appears to be maintained via pathogen-mediated selection and/or sexual selection expressed in mating preferences resulting in offspring heterozygous for their MHC haplotypes (Kamiya, O'Dwyer, Westerdahl, Senior, & Nakagawa, 2014; Penn, Damjanovich, & Potts, 2002; Piertney & Oliver, 2006; Winternitz et al., 2013). So, mechanisms that result in the production of MHC-heterozygous offspring will enhance their fitness.

The influence of MHC-dependent sexual selection on the maintenance of MHC diversity in natural populations has been described (Jordan & Bruford, 1998; Kamiya et al., 2014; Penn & Potts, 1999). Several processes may explain how the MHC influences mating behaviour and reproductive success such as precopulatory disassortative mating based on the MHC genotype (Piertney & Oliver, 2006) and postcopulatory MHC-related selective fertilization, implantation and/or selective abortion (Ziegler, Kentenich, & Uchanska-Ziegier, 2005). Precopulatory partner choice and postcopulatory processes may both prevent inbreeding and promote the best combination of polymorphic genes for the offspring. Inbreeding can lead to reduced viability in offspring. This may result from homozygosity of deleterious genes (Charpentier, Widdig, & Alberts, 2007; Leberg & Firmin, 2008; Pusey & Wolf, 1996) and from similarity of genes of the immune system, that is, the major histocompatibility complex (MHC). Partner choice for unrelated partners is a precopulatory process that will maintain heterozygosity of the relevant MHC genes, as nonrelated individuals are likely to have different MHC haplotypes. Multiple precopulatory mechanisms make mating outbreeding more likely, including differential dispersal patterns or distance of the sexes, extra-pair copulations and partner choice for unrelated individuals (Pusey & Wolf, 1996). Such partner choice may be guided by the genetic characteristics of the partner concerning its MHC haplotypes. Typically, partners with different MHC haplotypes (mandril, *Mandrillus sphinx*: Setchell, Charpentier, Abbott, Wickings, & Knapp, 2010), a specific MHC or a more heterozygous MHC (fat-tailed dwarf lemur, *Cheirogaleus medius*: Schwensow, Fietz, Dausmann, & Sommer, 2008) are preferred (meta-analyses: Kamiya et al., 2014; Winternitz, Abbate, Huchard, Havlícek, & Garamszegi, 2017). However, when precopulatory mechanisms do not prevent copulating and breeding with related individuals, postcopulatory processes may reduce the proportion of inbred offspring (Tregenza & Wedell, 2000) and MHC-homozygous offspring.

Postcopulatory selection is typically investigated in a polyandrous setting, where a female mates with both related and unrelated males. In these polyandrous matings, females select against inbreeding, as unrelated males father relatively more offspring than related males (decorated field cricket, *Gryllodes supplicans*: Stockley, 1999; Gouldian finch, *Erythrura gouldiae*: Pryke, Rollins, & Griffith, 2010). In addition, polyandrous mating may also enhance embryo viability (Simmons, 2005). However, these studies do not address whether postcopulatory mechanisms also operate to reduce the proportion of MHC-homozygous offspring. Such postcopulatory mechanisms may concern selection of MHC-specific sperm by the ovum. There is some evidence for sperm selection in inbred laboratory mouse strains. Although blastocysts are on average more often MHC-homozygous than heterozygous, the proportion of heterozygous blastocysts seems to increase with external circumstances, such as infections (Olsson, Madsen, Ujvari, & Wapstra, 2004; Scofield, Schlumpberger, West, & Weissman, 1982; Wedekind, Chapuisat, Macas, & Rulicke, 1996).

Another possible postcopulatory selection process concerns the MHC disparity or similarity between maternal and foetal MHC that may result in selective implantation or selective abortion of embryos. Data on the human MHC (human leucocyte antigen: HLA) matching and elevated foetal loss are reported of the Hutterites, U.S.A. (Ober, Hyslop, & Hauck, 1999), an inbred population that is characterized by a limited number of HLA haplotypes and a high natural fertility rate. In Hutterites, foetal loss is more likely to occur in couples sharing an entire HLA haplotype than not. In other human studies, HLA similarity of parents has also been linked to foetal loss (Ober & van der Ven, 1996). Similarly, pig-tailed macaque mothers that share MHC haplotypes with their mating partner have a lower fertility than females mating males with a different MHC haplotype (pig-tailed macaques, *Macaca nemestrina*: Knapp, Ha, & Sackett, 1996). Such reduced fecundity may (partly) be caused by a lower survival or implantation of MHC-homozygous embryos (review: Simmons, 2005). Surprisingly, in the Hutterite population, the number of surviving HLA homozygous individuals did not differ from expectations. However, the observed number of heterozygous-compatible individuals, namely individuals that are HLA identical to the mother, was lower than expected (Ober, Hyslop, Elias, Weitkamp, & Hauck, 1998; Ober et al., 1999). Therefore, maternal–foetal incompatibility from

the foetal perspective may be important in human pregnancy due to immune-related processes, which may need initiation/activation by HLA (MHC) incompatibility (humans: Ober et al., 1999; mice: Moldenhauer et al., 2009; Robertson et al., 2009).

The role of the MHC in pre- and postcopulatory selection may be due to the complete MHC haplotype or particular gene classes within the MHC haplotype. This research project investigates whether postcopulatory selection processes may affect the presence of particular combinations of MHC haplotypes in offspring from parents that share founder MHC haplotypes, that is, MHC haplotypes present in the founding rhesus macaques of the studied colony, relative to a Mendelian distribution of these haplotypes. This was investigated retrospectively in a pedigreed rhesus macaque (*M. mulatta*) colony. We measured both the representation of MHC haplotypes and the constituting MHC class I (Mamu-A and Mamu-B) and MHC class II (Mamu-DRB) alleles (Doxiadis et al., 2013).

We analysed whether there is selection against offspring with particular MHC haplotypes. The representation for two types of offspring may be relatively low: (i) when the offspring is MHC homozygous for its complete MHC haplotype (Simmons, 2005) or for MHC class I and MHC class II genes or (ii) for heterozygous-compatible offspring, that is, when mother and offspring match for their MHC haplotypes (cf. Ober et al., 1998).

2 | METHODS

2.1 | Study animals and cell lines

The data set consists of rhesus monkeys that were born at the BPRC facilities. The colony was founded in the 1970s by about 140 animals. Twenty-nine males and 69 females were sufficiently successful in breeding and are thus the founders of the analysed cohort. In the past, the colony has been pedigreed for more than seven generations based on segregation of polymorphic MHC markers (Bontrop, Otting, de Groot, & Doxiadis, 1999; Bontrop, Otting, Slierendregt, & Lanchbury, 1995; de Groot, Doxiadis, Otting, de Vos-Rouweler, & Bontrop, 2014). Today, BPRC houses a self-sustaining breeding colony of about 650 rhesus macaques of Indian origin consisting of around 28 simultaneous breeding groups. The macaques are group-housed, mimicking their social organization in the wild (Thierry, 2007). Groups consist of multiple nonrelated mothers and their offspring and one unrelated non-natal male. Females and their female offspring remain in the group, male offspring remains in the group until at approximately 4 years of age and can stay to an older age when aggression levels allow this. Breeding is seasonal and typically occurs from November to February. In this colony, males and females can breed from an age of 3 years. When multiple males older than 3 years reside in the group, precopulatory mate preferences can be exerted (Massen et al., 2012). In addition, inbreeding can potentially occur between females and natal males (i.e., mother–son back-crossing and sibling-crossing) or young females and long-resident non-natal alpha males (father–daughter back-crossing). Parentage of all animals included in this study had been defined by parentage analysis based on 24 microsatellites localized on 11 different chromosomes (Andrade et al., 2004; Massen et al., 2012). This high number of microsatellites was intentionally chosen to be able to distinguish between related males with regard to having fathered the offspring.

The individuals get a health check on a yearly basis. Immatures receive their first health check between 1 and 2 years of age, when also their MHC haplotypes are determined. Immature mortality almost only occurs in the first month of life; almost all individuals that survived to 1 month also survived to 1 year (A. Louwerse, personal communication, April 7, 2017: $N = 1749$ births; 8.3% is born dead; 3.9% dies in first week; 1.5% between 1 week and 1 month; 1.6% between 1 month and 1 year; and 1.2% between 1 and 2 years).

2.2 | Genomic DNA (gDNA), RNA and complementary DNA (cDNA) synthesis

gDNA was extracted from EDTA blood samples or from immortalized B lymphocytes (B-cell lines) by a standard salting-out method. RNA was isolated from B-cell lines (RNeasy kit, Qiagen, USA), and first-strand cDNA synthesis was performed on the RNA samples, using the Revertaid kit, as recommended by the supplier (Thermo Scientific, Leusden, the Netherlands).

Lymphoblastoid B-cell lines and genomic DNA (gDNA) have been available from most of the animals in the colony for the last 20 years so that animals could also be typed retrospectively.

2.3 | Haplotype definition

A haplotype is defined as the combination of alleles of different genes which are located next to each other on the chromosome and are usually inherited together. MHC haplotypes of Indian rhesus macaques were initially defined by cosegregation of serologically defined MHC class I Mamu-A and Mamu-B and MCH class II DR antigens (Bontrop et al., 1995). Due to sequencing techniques, a high number of alleles and loci have been defined in this colony at the haplotype level (Doxiadis et al., 2013). Currently, a haplotype is defined by cosegregation of the same microsatellite (STR) pattern in at least two animals of the same family with STR D6S2878 for DRB, and STRs D6S2854 and D6S2859 for Mamu-A. Furthermore, Mamu-A and Mamu-B as well as Mamu-DRB of a given founder haplotype have been ascertained by full-length complementary DNA (cDNA) or by exon 2 genomic DNA (gDNA) sequencing of selected animals, respectively, as described earlier (Doxiadis et al., 2013), and Mamu-A, Mamu-B and Mamu-DRB types, defined by the various methods, have been renamed by a nomenclature committee (de Groot et al., 2012). In the BPRC rhesus macaque colony, 2377 extended Mamu haplotypes (Mhc-A, Mhc-B, Mhc-DRB) of 1383 rhesus macaques have been determined of which 176 distinct and different founder MHC haplotypes were defined based on 137 founder animals (Doxiadis et al., 2013).

The 176 MHC haplotypes known for this population concern combinations of 17 different Mamu-A, 18 Mamu-B and 22 Mamu-DRB types. Individuals that differ for their MHC haplotype may share the same Mamu-A and Mamu-B or Mamu-DRB.

2.4 | Categorization of relatedness

The MHC haplotypes of both parents and the four grandparents were known for 1149 MHC-typed individuals. These individuals represent an unbiased sample of the breeding population. Between 1996 and 2012, 1196 individuals were born and survived to their first health check. For 1158 individuals, the four grandparents were known (Table 1), the remaining 38 individuals could not be included in the current analysis as not all grandparents were known.

The MHC haplotypes of individuals, their parents and grandparents were compared. The relatedness of their parents was determined (Table 1, Parental category). Several parental categories were distinguished: (i) back-crossings, that is, offspring of father and daughter or of mother and son; (ii) sibling-crossings, that is, offspring of full-siblings or of maternal or paternal half-siblings; and (iii) distantly related parents, that is, offspring of parents that shared at least one founder MHC haplotype and were distantly related (no shared grandparents).

If parents are both heterozygous and share only one haplotype (AB and AC), the expected Mendelian inheritance incidence heterozygous *versus* homozygous offspring is 3:1. If one parent is homozygous and shares this haplotype with the other heterozygous parent (AA and AB) or if parents are both heterozygous and share two times two parental MHC haplotypes (AB and AB), the expected Mendelian inheritance incidence of heterozygous *versus* homozygous offspring is 1:1 (Table 2, MHC haplotypes shared).

2.5 | Statistics

We employed a relatively new method to determine whether the data fit a Mendelian distribution: the Bayesian binomial test to accept or reject the null hypothesis (Rouder, Speckman, Sun, Morey, & Iverson, 2009). This test compares a found distribution with an expected, null distribution. In contrast to most statistical tests, this test can not only be used to determine whether an observed distribution differs significantly from an expected distribution but also to determine how well an observed distribution significantly fits the expected, null distribution. In other words, how strong the evidence is for the null hypothesis relative to the alternative hypothesis. The so-called Bayes factor, that measures the odds of the null hypothesis over the alternative hypothesis, is interpreted as follows: Bayes factor > 3 is considered "some evidence"; Bayes factor > 10 is considered "strong evidence"; and Bayes factor > n 30 is considered "very strong evidence" for the null hypothesis over the alternative one (Rouder et al., 2009). Note that finding a fit to the null hypothesis requires a substantial amount of data.

Other statistical analyses were calculated with the statistical programs SPSS version 20. All tests are two-tailed and considered significant at $p < .05$ and a trend at $p < .10$.

3 | RESULTS

3.1 | Incidence of same founder MHC haplotype sharing in parents

Of a total of 1158 individuals of the BPRC rhesus macaque breeding colony, the parents and grandparents were known (Table 1). The MHC haplotypes of the individual, its two parents and its four grandparents were known for 1149 individuals (995 + 154 individuals) and

TABLE 1 Parental category of individuals in the breeding colony with known major histocompatibility complex of the individual, both parents and all four grandparents

		Haplotypes parents			
Parental category	Type of crossing	Different	Shared	Unclear[a]	N summed
Back-crossing					
	Father–daughter	–	37	–	37
	Mother–son	–	5	–	5
	Subtotal back-crossing	–	42	–	42
Sibling-crossing					
	Full-sibling	–	5	–	5
	Paternal half-siblings	29	57	3	89
	Maternal half-siblings	3	1	1	5
	Subtotal sibling-crossing	32	63	4	99
Not (or distantly) related[b]		963	49	5	1017
Total		995	154	9	1158

[a]For the nine "unclear" (Table 1) individuals, the four grandparents were known, but only one MHC haplotype of the individual was known ($N = 3$) or not all MHC haplotypes from its parents were known ($N = 6$).
[b]four different grandparents; when a haplotype is shared, the relatedness is further back in the family tree.

TABLE 2 Distribution of heterozygous or homozygous haplotypes within the offspring of parents with major histocompatibility complex haplotype sharing

Parental category	Type of crossing	Expected distribution hetero-homozygosity	Heterozygous	Homozygous	N summed
Parents share one haplotype[a]		3:1			
Back-crossing	Father–daughter		29	6	35
	Mother–son		3	2	5
	Backcrossing subtotal		32	8	40
Sibling-crossing	Full-siblings		4	1	5
	Paternal half-siblings		35	17	52
	Maternal half-siblings		1	0	1
	Sibling-crossing subtotal		40	18	58
Distantly related[c]			35	11	46
Subtotal parents share one haplotype[b]			107	37	144
Parents share more than one haplotype		1:1			
Back-crossing	Father–daughter		1	1	2
Sibling-crossing	Paternal half-sibs		3	2	5
Distantly related[c]			1	2	3
Subtotal parents share more than one haplotype			5	5	10
Total			112	42	154

[a]Parents share one founder MHC haplotype (AB and AC).
[b]Parents share more than one founder MHC haplotype: (i) homozygous parent shares one haplotype in other parent (AA and AB), or (ii) parents share two different haplotypes (AB and AB).
[c]Distantly related: four different grandparents; when parents have shared founder MHC haplotype(s), the relatedness is further back in the family tree.

for nine individuals not all are known. Table 1 reveals that 141 individuals were closely related, concerning 42 offspring from back-crossings and 99 offspring from sibling-crossings. Most individuals (N = 1017) were not related or were distantly related.

Altogether, of 141 closely related individuals, all 42 offspring from back-crossings and 63 of 99 offspring from sibling-crossings had parents that shared founder MHC haplotypes. In addition, 49 individuals with distantly related parents shared founder MHC haplotypes. These 154 individuals may be homozygous for their MHC or share both haplotypes with their mother.

3.2 | Representation of MHC-homozygous offspring

3.2.1 | Distribution of complete MHC haplotypes

We investigated the representation of MHC-homozygous offspring. The analyses were separated for the individuals where the expected distribution is 3:1 (N = 144) and where it is 1:1 (N = 10). In total, 144 individuals had parents that shared one MHC haplotype (Table 2). Based on Mendelian genetics, a 3:1 distribution of heterozygous versus homozygous individuals is expected. The observed distribution, 107 heterozygous and 37 homozygous individuals (Table 2), shows strong evidence that it fits the expected distribution (Bayesian binomial test: successes = 107; trials = 144; null point = 0.75; prior (a,b) = (1, 1); odds of null model over alternative model: Bayes factor = 10.804).

Ten individuals had parents that shared more than one MHC haplotype (Table 2) and are expected to produce a 1:1 distribution of heterozygous versus homozygous offspring. The observed distribution, five heterozygous and five homozygous individuals, did not deviate strongly from 1:1, but there was also no evidence that it confirmed the expected distribution (Bayesian binomial test: successes = 5; trials = 10; null point = 0.5; prior (a,b) = (1, 1); Bayes factor = 2.707).

Further, we explored whether the type of crossing of parents sharing one haplotype (i.e., back-crossing, sibling-crossing, distantly related) showed a similar pattern as the main finding. The distribution of heterozygous and homozygous individuals resulting from back-crossings showed some evidence that did not differ from expected (Table 2; Bayesian binomial test: successes = 32; trials = 40; null point = 0.75; prior (a,b) = (1, 1); Bayes factor = 4.833), and neither did the distribution of heterozygous and homozygous individuals from sibling-crossing (Table 2; Bayesian binomial test: successes = 40; trials = 58; null point = 0.75; prior (a,b) = (1, 1); Bayes factor = 3.885). Also the distribution of heterozygous and homozygous individuals with distantly related parents showed some evidence that it did not deviate from the expected distribution (Table 2; Bayesian binomial test: successes = 35; trials = 46; null point = 0.75; prior (a,b) = (1, 1); Bayes factor = 6.335). Note that we did not correct for multiple comparisons.

Altogether, we found strong evidence that there was no selection against MHC-homozygous individuals.

3.2.2 | Distribution of Mamu-A, Mamu-B and Mamu-DRB alleles

We also tested whether there were fewer individuals homozygous for either MHC class I Mamu-A, or Mamu-B, or MHC class II Mamu-DRB alleles than expected (Appendix S1–S4). There was very strong evidence that the distribution of individuals heterozygous and homozygous for Mamu-A, Mamu-B or Mamu-DRB fitted an expected 3:1 Mendelian distribution and some evidence that it fitted an expected 1:1 Mendelian distribution (Table 3). Therefore, we found no indication for selection against Mamu-A, Mamu-B and Mamu-DRB homozygous individuals.

3.3 | MHC-matching of mother and embryo

We investigated whether the offspring showed over- or underrepresentation of particular MHC haplotype combinations if their parents share MHC haplotypes (e.g., AB-AC: shared haplotype A from father = A_f; shared haplotype A from mother A_m). It was expected that all four haplotype combinations were equally present. The distribution of MHC haplotypes of individuals with parents that shared one MHC haplotype was determined ($N = 144$) (Figure 1). The observed distribution of the MHC haplotypes tended to differ significantly from the expected distribution (Chi2 = 7.667; $p = .053$). Post hoc tests revealed that offspring sharing its haplotype with the father (A_mB) tended to be more often present than expected (individuals with haplotype Am-B; (o-e)/sqrt(e) = 2.167, $p = .03$; critical p after Bonferroni correction: .0125). Individuals with the same haplotype as their mother (individuals with haplotype A_f-C) were relatively underrepresented, but this was not significant ((o-e)/sqrt(e) = −1.500, $p = .134$). Homozygous individuals were not underrepresented.

4 | DISCUSSION

We determined in a pedigreed rhesus macaque colony whether there was postcopulatory selection for or against particular MHC haplotype combinations when parents shared at least one founder MHC haplotype. Bayesian statistics showed that the distribution of MHC-heterozygous and MHC-homozygous individuals significantly fitted the expected Mendelian inheritance incidence, and therefore, MHC-homozygous individuals were not selected against. However, the overall distribution of the four possible inherited MHC haplotypes tended to differ significantly from expected, indicating selection on the combination of MHC haplotypes. As predicted, relatively few individuals share both MHC haplotypes with their mother, yet this was not significant. Individuals with a set of MHC haplotypes that were identical to their paternal MHC haplotypes tended to be overrepresented.

Postcopulatory processes may promote MHC-heterozygous offspring, resulting in a relatively low number of MHC-homozygous individuals among offspring from parents that share founder MHC haplotypes. However, Bayesian statistics that can confirm the null hypothesis (Rouder et al., 2009) show that the MHC haplotypes from 144 offspring of parents that share one inherited founder MHC haplotype significantly fit the expected 3:1 Mendelian distribution. Also the 10 offspring of parents that share more than one MHC haplotype have a distribution similar to the expected 1:1, but due to the low number of individuals, this cannot significantly fit the expected distribution (Rouder et al., 2009). When inbreeding per se, and not selection on MHC haplotype combinations, determined the effects, we would expect that they differed between on the one hand back-crossings and sibling-crosses, and on the other hand paring among distantly related individuals. We determined whether the type of paring affected the incidence of MHC-heterozygous offspring. However, we found some evidence that the proportion of MHC-homozygous offspring fitted the 3:1 distribution for all three types of parings, indicating that the relatedness among parents did not affect the proportion of MHC-homozygous offspring. These outcomes encompass several processes, including sperm selection during fertilization, implantation, embryo survival and early infant survival. We cannot differentiate between these diverse processes, while each may affect the distribution of MHC haplotypes. Altogether, we found no evidence for postcopulatory selection against MHC-homozygous offspring.

TABLE 3 Representation of individuals that are homozygous or heterozygous for MHC class I Mamu-A, Mamu-B or MHC class II Mamu-DRB, respectively, and fit to the expected distribution based on Bayesian statistics

Haplotype	Expected distribution hetero-homozygosity	Heterozygous	Homozygous	N summed	Bayes factor
Mamu-A					
	3:1	249	74	323	11.65
	1:1	69	72	141	9.23
Mamu-B					
	3:1	235	72	307	13.55
	1:1	47	45	92	7.55
Mamu-DRB					
	3:1	184	65	249	13.26
	1:1	46	35	81	3.46

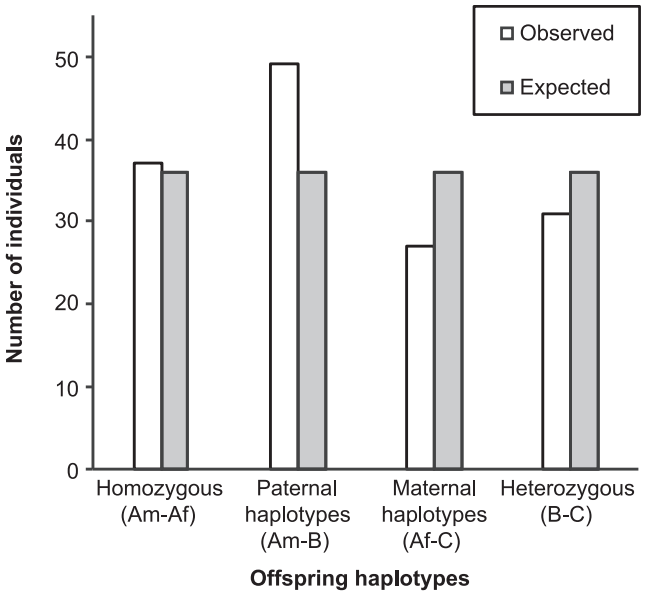

FIGURE 1 Observed and expected distribution of major histocompatibility complex (MHC) haplotypes of offspring whose parents share one founder haplotype A (father: A_fB; mother: A_mC; A_f and A_m concern the same founder haplotype derived from father or mother, respectively). The overall differences tended to be significant ($p = .053$). Note that the offspring MHC haplotypes are labelled according to the parent from whom it originated: an offspring that has the same two MHC haplotypes as its father, AfB, obtained the B from its father and the Af from its mother

As the MHC class I molecules, Mamu-A and Mamu-B, and the MHC class II molecules, DR, have distinct and different functions, it seems possible that one or two of these loci show a differential distribution of heterozygosity vs homozygosity. Moreover, many field studies use only one of these classes, mainly DRB, to determine whether individuals have the same or different MHC antigens (Grob, Knapp, Martin, & Anzenberger, 1998; Knapp et al., 1996; Pechouskova et al., 2015; Setchell & Huchard, 2010; Setchell, Abbott, Gonzalez, & Knapp, 2013; Setchell et al., 2010). Therefore, we calculated heterozygosity *versus* homozygosity frequencies for each locus independently. However, the distributions measured all showed very strong (3:1) or some (1:1) evidence that they fitted the expected 3:1 or 1:1 distributions, indicating that selection against homozygous MHC I A or B or MHC II DR genes was absent. These results suggest that none of the different functions of these genes promote MHC-heterozygous offspring through postcopulatory processes. In addition, the lack of postcopulatory selection based on specific MHC class I and/or MHC class II genes, as mostly defined in field studies, suggests that similarity in part of the MHC haplotype also does not incite postcopulatory selection against MHC-homozygous offspring. As these results were based on larger subsets (Appendix S1–S4) than those available for individuals that are homozygous for their complete MHC haplotype and a broader application, we are convinced that these data have general biological significance for studies on MHC-matching between parents.

As the results indicate postcopulatory selection on the combination of MHC haplotypes of offspring and parents, post hoc analyses investigated what caused this effect. Their representation may depend on the viability of the individual offspring or its match with its mother's MHC haplotype. The overall distribution tended to differ significantly from expected and post hoc analyses did not identify one MHC haplotype combination that significantly differed from expected. Still, three points deserve attention. First, consistent with the Hutterite study, we found a relatively low number of individuals that shared its MHC haplotypes with its mother. The lower number of these offspring may be due to the lower amount of microchimerism resulting from maternal–foetal cell exchange during pregnancies. Such microchimerism appears to be of importance to avoid miscarriages (Gammill, Stephenson, Aydelotte, & Nelson, 2014). We cannot confirm this pattern, but as we did find an effect in the same direction, further research is called for. Second, the actual number of MHC-homozygous individuals was close to the expected number. This fits the other comparisons in this study and the outcome of the Hutterite study. Third, we found that offspring that had the same MHC haplotypes as their father tended to be more often present than expected. Unfortunately, the study on the Hutterites does not distinguish this category, so no comparison is possible. Both the paternal haplotypes (Am-B) and the heterozygous (B-C) haplotypes recognize the maternal tissues as nonself (Ober et al., 1998) and a similar overrepresentation was expected. However, heterozygous (B-C) haplotypes seem present as expected, while the relative overrepresentation of the paternal haplotypes (Am-B) was unexpected. Alternatively, this overrepresentation may result from MHC-matching with the father's seminal fluid. In humans, seminal fluids activate immune tolerance in females (Sharkey, Tremellen, Jasper, Gemzell-Danielsson, & Robertson, 2012) and thereby enhance the survival of sperm and the implantation and survival of embryos (Robertson et al., 2009). As this tolerance is MHC-specific in mice (Moldenhauer et al., 2009; Robertson et al., 2009) and humans (Sharkey et al., 2012), seminal effects may be enhanced and increase implantation and survival of embryos when the seminal fluid matches not only one, but for two MHC haplotypes with the embryo. However, whether maternal–foetus MHC-matching reduces or paternal–foetus MHC-matching enhances their survival or that both processes are relevant remains to be conclusively established.

Sexual selection may favour the production of MHC-heterozygous offspring through pre- and postcopulatory processes (e.g., Setchell & Huchard, 2010; Tregenza & Wedell, 2000). The postcopulatory processes may concern two different phenomena: differentiation between sperm from kin and nonkin males in polyandrous matings, and within sire selection of specific MHC haplotypes resulting in MHC-heterozygous offspring. Our findings indicate that within sire selection based on offspring MHC haplotypes may take place among the heterozygous offspring, yet does not reduce the incidence of MHC-homozygous offspring. This suggests that when sexual selection processes increase the presence of MHC-heterozygous offspring, this may result from preferential fertilization by unrelated males, either through postcopulatory

processes favouring fertilization by unrelated males in polyandrous matings; or through pre-copulatory processes that favour mating with unrelated males. Whether this is also present in rhesus macaques could not be determined in our study, as we did not systematically document the number of mating partners of females. However, female rhesus macaques in our colony typically express mate choice and mate with multiple males when they have this option (Massen et al., 2012; Overduin-De Vries, Massen, Spruijt, & Sterck, 2012). Moreover, this is consistent with other studies, which showed that unrelated males are favoured over related males (Pusey & Wolf, 1996). Therefore, precopulatory processes favouring mating with unrelated males and postcopulatory processes in polyandrous matings favouring sperm from unrelated males may be the major reason for the observation that MHC-heterozygous offspring are favoured, and not postcopulatory selection against offspring with MHC-homozygous haplotypes.

Altogether, postcopulatory selection based on rhesus macaque offspring's MHC haplotypes when parents share MHC haplotypes in a pedigreed colony takes place between fertilization and infant birth. This postcopulatory selection does not disfavour MHC-homozygous offspring, yet may favour particular MHC-heterozygous offspring. However, the processes involved in this postcopulatory MHC selection remain to be established. Altogether, this study suggests that a high incidence of MHC heterozygosity in offspring will not result from postcopulatory selection against offspring with homozygous MHC haplotypes, but from the prevalence of paternity by unrelated males favoured by both pre- and postcopulatory processes.

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DATA ACCESSIBILITY

The data sets used for the analyses are provided in the supplementary data.

AUTHOR CONTRIBUTION

E.H.M.S. designed research, N.G. and A.J.M.V-R. performed genetic research, E.H.M.S. and G.G.M.D. analysed data, E.H.M.S., R.E.B. and G.G.M.D. wrote the paper.

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