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Opposites attract: MHC-associated mate choice in a polygynous primate

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heterozygosity;
major histocompatability complex;
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Abstract

We investigated reproduction in a semi-free-ranging population of a polygynous primate, the mandrill, in relation to genetic relatedness and male genetic characteristics, using neutral microsatellite and major histocompatibility complex (MHC) genotyping. We compared genetic dissimilarity to the mother and genetic characteristics of the sire with all other potential sires present at the conception of each offspring (193 offspring for microsatellite genetics, 180 for MHC). The probability that a given male sired increased as pedigree relatedness with the mother decreased, and overall genetic dissimilarity and MHC dissimilarity with the mother increased. Reproductive success also increased with male microsatellite heterozygosity and MHC diversity. These effects were apparent despite the strong influence of dominance rank on male reproductive success. The closed nature of our study population is comparable to human populations for which MHC-associated mate choice has been reported, suggesting that such mate choice may be especially important in relatively isolated populations with little migration to introduce genetic variation.

Introduction

Mate choice, particularly female choice, has been the focus of extensive research over the past two decades (Andersson & Simmons, 2006). Where there is little or no direct benefit of mate choice to an individual or its offspring, females may choose for genetic benefits that will be inherited by their offspring (choice for 'good genes'). These indirect benefits may include increased offspring attractiveness (Fisher, 1958) or other heritable qualities (Zahavi, 1975), such as immunocompetence and parasite resistance (Hamilton & Zuk, 1982; Folstad & Karter, 1992). Adaptive complementarity may also be an important factor in mate selection (Trivers, 1972; Zeh & Zeh, 1996) as offspring born to closely related parents often show reduced fitness (inbreeding depression) (Keller & Waller, 2002). Estimators of genetic diversity are correlated with a range of fitness components, including survival, disease susceptibility and reproductive success (review in Hansson & Westerberg, 2002). Females should therefore benefit by mating preferentially with genetically different males, thereby increasing the heterozygosity of their progeny. However, choice for genetically dissimilar mates may trade-off against the loss of locally adaptive gene complexes, leading to choice for some optimal level of dissimilarity (Bateson, 1983).

The major histocompatibility complex (MHC) is among the best candidates for the genetic basis of mate choice in vertebrates (Jordan & Bruford, 1998; Penn & Potts, 1999). The MHC is a multigene family encoding cell-surface glycoproteins (MHC molecules) that play a critical role in the immune system by recognizing foreign peptides, presenting them to specialist immune cells and initiating the appropriate immune response (Klein, 1986). Expressed loci are highly polymorphic and this diversity is selectively maintained, at least in part, via two mechanisms of pathogen-mediated selection:

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heterozygote advantage and frequency-dependent selection (Apanius *et al.*, 1997; Sommer, 2005). In the former mechanism, heterozygote individuals are able to resist a wider range of pathogens, rendering them fitter than less diverse individuals (Doherty & Zinkernagel, 1975). In the latter, a particular allele is beneficial when rare, but disadvantageous when common, because natural selection favours parasites that can evade the MHC-dependent immunity of the most common host genotypes, decreasing the fitness of individuals possessing common alleles. Rare alleles are thus favoured, because they escape recognition by the MHC-dependent immune system, until they increase in frequency and parasites evolve to evade them, in a co-evolutionary arms race (Penn & Potts, 1999).

Major histocompatibility complex-based mate choice may favour individuals that possess particular MHC alleles, those with diverse MHC genotypes, or those with MHC genotypes that are dissimilar to the chooser (review in Penn & Potts, 1999; Penn, 2002). Choice for particular beneficial alleles may provide offspring with resistance to particular parasites (Penn & Potts, 1999). Choice for an MHC-diverse mate may be advantageous because heterozygotes possess more rare alleles than homozygotes, which can be inherited by offspring, and because an MHC-diverse mate is less likely to share alleles with the chooser, leading to MHC-diverse offspring, that are able to resist a broader range of pathogens (Apanius *et al.*, 1997; Fromhage *et al.*, 2009). Finally, mate choice for MHC dissimilarity (disassortative mating) may provide several, nonexclusive, fitness benefits: preventing inbreeding and increasing genome-wide genetic diversity (Brown & Eklund, 1994); increasing the ability of offspring to resist pathogens through either heterozygote advantage (Zuk, 1990) or the production of offspring that are dissimilar to the parents (Penn & Potts, 1999); or giving offspring an optimal number of MHC alleles for parasite resistance (allele counting) (Nowak *et al.*, 1992; Reusch *et al.*, 2001; Wegner *et al.*, 2003; Forsberg *et al.*, 2007) (but see Borghans *et al.*, 2003).

Support for MHC-based mate choice hypotheses was first obtained from studies of laboratory mice (Yamazaki *et al.*, 1976). More recently, evidence that the MHC influences mate choice has come from studies of fish, birds and mammals (review in Piertney & Oliver, 2006). However, few studies have examined MHC-associated mate choice in nonmodel species living in natural, or semi-natural, populations (Piertney & Oliver, 2006). Of the studies that exist, some have found evidence for choice for MHC-dissimilar mates (Landry *et al.*, 2001), some that females choose males to achieve an intermediate, and optimally resistant, level of MHC diversity in their offspring (Milinski *et al.*, 2005; Bonneaud *et al.*, 2006), and still other studies found no influence of the MHC on mate choice at all (Paterson & Pemberton, 1997; Ekblom *et al.*, 2004; Westerdahl, 2004). These studies suggest that MHC-associated mate choice may occur in some species, but not in others, and that the exact strategies employed may differ between species (Piertney & Oliver, 2006). Furthermore, most studies of MHC-associated mate choice have failed to include expression analyses, and it remains to be seen whether the MHC sequences studied actually produce functional molecules for pathogen resistance (Knapp, 2007).

The role of the MHC in human mate choice is particularly controversial. Initial studies suggested that MHC dissimilarity plays a role in human mate choice (Ober *et al.*, 1997), and experiments suggest that this phenomenon may be mediated via odour (Wedekind *et al.*, 1995; Wedekind & Füri, 1997; Jacob *et al.*, 2002). However, other studies found no influence of MHC dissimilarity on human mate choice (Hedrick & Loeschcke, 1996; Hedrick & Black, 1997; Ihara *et al.*, 2000; Chaix *et al.*, 2008). This controversy extends to nonhuman primates. A study of group-living rhesus macaques (*Macaca mulatta*) found no evidence of mate choice for MHC dissimilarity, although MHC-heterozygous males enjoy increased reproductive success (Sauermann *et al.*, 2001). However, female choice for both MHC dissimilarity and within-male MHC diversity, as well as for males with higher genome-wide heterozygosity, has been reported for socially monogamous fat-tailed dwarf lemurs (*Cheirogaleus medius*) (Schwensow *et al.*, 2007a) and solitary foraging grey mouse lemurs (*Microcebus murinus*) (Schwensow *et al.*, 2008).

We investigated the influence of MHC genotype on patterns of reproduction in the mandrill (*Mandrillus sphinx*, Cercopithecinae). Mandrills live in large multimale, multi-female groups (Abernethy *et al.*, 2002), and are moderately seasonal breeders (Setchell & Wickings, 2004). The potential for male–male contest to monopolize access to individual receptive females is thus high, and mandrills have a polygynous mating system, with strong sexual dimorphism (Setchell *et al.*, 2001) and high reproductive skew in favour of the alpha male (Charpentier *et al.*, 2005a). Nevertheless, female mandrills are able to mate with multiple males during a single receptive period (Setchell, unpublished observations), and express precopulatory mate choice (Setchell, 2005). Female mandrills gain little in the way of direct benefits from males and females choice is likely, therefore, to be driven by the potential indirect (genetic) benefits that a sire may provide. Both inbreeding and the reduction of genome-wide heterozygosity have deleterious consequences for individual fitness (Charpentier *et al.*, 2005b, 2006) meaning that mate choice for nonrelatives and/or genetically complementary individuals would produce more heterozygous, fitter progeny. However, the relatively tight control that dominant males appear to have over both mating and paternity may reduce the ability of females to reproduce with nondominant males of their choice, as proposed for Soay sheep (Paterson & Pemberton, 1997). We also test for the possibility that

within-male MHC diversity, or the possession of particular MHC types, confer a reproductive advantage on males, via either superior competitive ability (intrasexual selection), or via female choice for such males.

We genotyped a large population of mandrills for a highly variable group of MHC class II loci known as MHC-DRB genes. These genes encode proteins that are directly involved in the immune response and are under strong positive selection pressure, with the peptide-binding region containing significantly more nonsynonymous than synonymous changes (Abbott *et al.*, 2006), suggesting that this area of the genome is under balancing selection. We also demonstrated that many of the MHC sequences we identified via genomic DNA analysis are expressed. Next, we compared genetic and demographic characteristics of the sire of each individual offspring with all the potential sires available when the individual was conceived, to address four specific questions: (1) Do mandrills choose genetically dissimilar mates to avoid inbreeding? (2) Do mandrills mate disassortively based on MHC genotype? (3) Do males with greater overall genetic diversity, or greater within-male MHC diversity, experience greater reproductive success? (4) Do specific MHC genotypes influence male reproductive success? We found that the probability that a given male sired increased as pedigree relatedness decreased, and overall genetic dissimilarity and MHC dissimilarity with the mother increased. Reproductive success also increased with male microsatellite heterozygosity and within-male MHC diversity. These effects were apparent despite the strong influence of dominance rank on male reproductive success.

Methods

Study population

We studied a large, semi-free-ranging population of mandrills, at the Centre International de Recherches Médicales, Franceville (CIRMF), Gabon, established in 1983/4, when 15 wild founder were released into a 6.5-ha naturally rain-forested enclosure (see Setchell *et al.*, 2005b for details of the colony). The date of birth is recorded for all individuals born into the colony, whereas the age of founder animals was approximated using dental estimates when the animals arrived at CIRMF and their previous history. Daily observations are made of female reproductive status, births, injuries and disappearances. Male rank is determined on the basis of avoidance behaviours; the identity of the top-ranking (alpha) male is unambiguous. Paternity skew is concentrated in alpha males, and beta males do not sire more offspring than other subordinate males (Setchell *et al.*, 2005a), so we limit comparisons to alpha vs. nonalpha males.

Group sizes ranged from 15 in 1983/1984 to a maximum of 104 animals in 2002, corresponding to smaller groups observed in the wild (Rogers *et al.*, 1996). How the situation in the colony relates to wild mandrills is currently unknown, but it seems likely that the restricted conditions of the CIRMF colony represent an extreme, but not totally un-natural, situation (Setchell *et al.*, 2005b).

Microsatellite genotyping and paternity

We extracted DNA for genetic analyses from blood samples obtained during annual captures of the colony. We genotyped up to 10 microsatellite loci for 14 founder animals and 205 offspring born into the colony between 1983 and 2002. We obtained an accurate assignment of paternity for 193 (94%) of 205 offspring (for details of methods and paternity assignment criteria, see Charpentier *et al.*, 2005a).

MHC genotyping

We conducted MHC-DRB genotyping for 155 of the study population (insufficient DNA was available for the remaining individuals). We PCR amplified MHC-DRB sequences using primers known to amplify all MHC-DRB sequences in species ranging from humans to New World monkeys and analysed products using denaturing gradient gel electrophoresis and direct sequencing (Abbott *et al.*, 2006). We amplified DNA samples from each individual multiple times and repeated all genotyping experiments to ensure that any sequence found in one individual would also be detected in all other individuals in the population.

The MHC-DRB region in Old World primates frequently experiences expansion and contraction through gene duplication and deletion, respectively (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, therefore, focus on the number of different sequences possessed by an individual as a measure of MHC diversity, without making any assumptions about the number of loci involved (see also Málaga-Trillo *et al.*, 1998; Aeschlimann *et al.*, 2003; Ekblom *et al.*, 2004, 2008).

To determine whether the mandrill MHC sequences produce functional molecules for pathogen resistance, we examined patterns of expression using cDNA analysis for a subset of seven mandrills chosen to represent all known Masp-DRB loci and lineages. We calculated the number of amino-acid differences between each pair of MHC sequences as an estimate of genetic dissimilarity (Landry *et al.*, 2001), because MHC sequences may differ in nucleotide composition but be functionally similar in terms of immune defence if the proteins they encode bind the same peptides (Rammensee, 1995; Sidney *et al.*, 1995). We also used MHC-DRB sequences to determine MHC-DRB supertypes. These are groups of MHC-DRB

sequences that share peptide-binding motifs and are therefore functionally similar (Doytchinova & Flower, 2005), and have been shown to be biologically relevant in studies of both human and nonhuman primates (Southwood *et al.*, 1998; Trachtenberg *et al.*, 2003; Schwensow *et al.*, 2007b). We identified variable amino acid positions, presumed to represent the peptide-binding region, using phylogenetic analysis of MHC sequences in MEGA 4 (Tamura *et al.*, 2007). We then used PAML 4 (Yang, 2007) to identify positively selected sites (PSS). Finally, we identified supertypes by analysing the chemical specificities of these PSS in GENESIS version 1.7.2 (Sturn *et al.*, 2002), following Doytchinova & Flower (2005).

Relatedness and reproduction

To determine whether reproduction was biased towards unrelated partners, we estimated the overall genetic similarity between the genotypes of two individuals as:

R_{ped}	A relatedness coefficient calculated using the colony pedigree (R_{ped} in mother–son and father–daughter pairs is 0.5, full-siblings 0.5, half-siblings 0.25, etc.).
R_{QG}	Microsatellite allele sharing, calculated as the Queller-Goodnight index (Queller & Goodnight, 1989) using RELATEDNESS (version 5.0.8; available from http://www.gsoftnet.us/GSoft).

We also classified R_{ped} as > 0.25 (i.e. father/daughter dyads and half-siblings) and < 0.25 for some analyses ($R_{<> 0.25}$).

MHC-dissassortative mating

To determine whether reproduction was biased towards partners with dissimilar MHC genotypes, we calculated three measures of MHC dissimilarity for each potentially reproductive dyad:

MHC_{diff}	The number of MHC sequences that differed between the male and female. This was highly and significantly correlated with the number of MHC sequences shared and the number of MHC sequences unique to the male so we report only results for $MHC_{diff.}$
AA_{diff}	Amino acid sequence dissimilarity, calculated as the mean number of pairwise amino acid differences between the sequences of the dyad.
S_{diff}	The number of MHC supertypes that differed between the male and female.

Male genotype and reproduction

To determine whether reproduction was biased towards males that were more genetically diverse, possessed higher MHC diversity, or possessed particular MHC supertypes, we described the genotype of a potential sire as follows:

IR_{male}	Internal relatedness (IR, Amos *et al.*, 2001). The more an individual is genetically diverse, the more IR is negative. Although measures of heterozygosity based on small number of neutral markers may not accurately reflect genome-wide heterozygosity (Balloux *et al.*, 2004; Slate *et al.*, 2004), we have previously shown that our measure of IR is a good measure of genome-wide inbreeding in this population (Charpentier *et al.*, 2005b).
MHC_{male}	Number of MHC sequences possessed.
AA_{male}	MHC sequence diversity, calculated as the mean number of amino acid differences between all MHC sequences.
S_{male}	Number of supertypes possessed.
S1 to S13	The presence/absence of individual MHC supertypes.

Statistical analyses

We conducted statistical analyses at the level of the individual offspring, asking the following question: 'Based on the potential sires available, their genetic similarity to the female, and their individual genetic characteristics, which male sired the offspring?' Potential sires were any adolescent (4–9 years) or adult male (> 9 years, Setchell *et al.*, 2006) present in the group at the time that the mother conceived. Our microsatellite dataset contained 193 offspring, 51 potential sires (1–113 potential offspring per sire, mean 46 ± 5), 17 actual sires, (1–42 true offspring per sire, mean 11.4 ± 3) and 42 mothers (1–15 offspring per female, mean 4.6 ± 0.7). The MHC dataset contained 180 offspring, 40 potential sires (1–109 potential offspring per sire, mean 45 ± 5), 15 actual sires (1–42 true offspring per sire, mean 12 ± 3.2) and 34 mothers (1–15 offspring each, mean 5.3 ± 0.8). The same potential sires and mothers appeared several times in our dataset. However, the number and identity of potential sires available differed for each offspring born to an individual female because female mandrills conceive approximately one infant per year (Setchell *et al.*, 2005b) and potential sires differed across breeding seasons. Thus, although a potentially reproducing dyad could appear more than once in the dataset, the set of alternative potential sires (i.e. the 'choice' of sire available) for a given female was different for each of her offspring.

Our dependent variable ('decision') took the value 1 when a given male was identified as the sire of the offspring; and 0 for all other potential sires present in the group at the time of conception. This decision variable does not follow a binomial distribution because only one potential sire scored '1' for each offspring, whereas all other scored '0'. To resolve this problem, we used conditional logit regression models (multinomial discrete choice: MDC procedure, type = clogit, SAS version 9) to investigate the influence of different variables on the probability that a given male sired. The MDC procedure analyses models where the choice set consists of multiple alternatives, in this case multiple potential sires for each offspring. The model takes into account the number of

sires available to sire that particular individual. It also takes into account the identity of the males, and therefore their repetition throughout the dataset. However, the model does not consider the fact that some mothers contributed more than one offspring to the dataset. To evaluate pseudo-replication due to the multiple contributions of some mothers, we also conducted a binomial analysis considering the mother's identity as a random effect. This analysis failed to account for the fact that only one male can successfully sire each offspring (see above), but the results were very similar to those found using MDC, strengthening our conclusions.

R_{ped} and R_{QG} were significantly correlated with one another, as were the three estimates of MHC dissimilarity (MHC_{diff}, AA_{diff} and S_{diff}) (Table S1). We, therefore, performed separate analyses with each of these measures to address the questions 'Does overall genetic dissimilarity influence reproduction?' (two analyses, using R_{ped} and R_{QG}) and 'Does MHC dissimilarity influence reproduction' (three analyses, using MHC_{diff}, AA_{diff} and S_{diff}).

Within-male MHC diversity was collinear with other potential explanatory variables (Table S1). We, therefore, addressed the question 'does male genotype influence reproduction?' by including MHC_{male} and AA_{male} in separate analyses, but did not attempt to draw conclusions regarding the relative influence of genetic diversity and male heterozygosity on reproduction.

In each analysis, we included the age, dominance status (alpha vs. nonalpha) and IR of the potential sire, as these are known to influence the probability that a male reproduced (Charpentier *et al.*, 2005b). The correlation between age and dominance status was very low ($R^2 = 0.08$), meaning that the two covariates could be included in the same analyses without problems of colinearity. Measures of within-male MHC diversity were not significantly related to male dominance rank (GENMOD procedure with binomial distribution, some males are included twice because they were both alpha and nonalpha during the study, IR: $n = 60$, $\chi^2 = 1.25$, $P = 0.263$; MHC_{male}: $n = 49$, $\chi^2 = 0.78$, $P = 0.378$; AA_{male}: $n = 49$, $\chi^2 = 0.83$, $P = 0.361$; S_{male}: $n = 49$, $\chi^2 = 0.148$, $P = 0.224$).

We used Akaike's information criteria (AIC) to measure and compare the goodness of fit of statistical models. Where variables significantly influenced reproduction, we calculated odds ratios as the exponential function of the conditional logit estimate.

Results

MHC genotyping

We identified 34 different Mandrill sphinx Masp-DRB sequences in 155 individual mandrills (Table S2). Sequences were deposited in GenBank (accession numbers: DQ103715–DQ103732, DQ103734–DQ103746, EU693911–EU693914). Each individual mandrill possessed 1–7 sequences (those possessing a single sequence were homozygous for that sequence). The seven individuals used for cDNA analysis possessed a total of 16 different *Masp-DRB1*03, 1*04, 3, 5, *W* and *6* sequences. We identified 15 cDNA *Mhc-DRB* sequences in these individuals, suggesting that most (15/16) of the mandrill MHC-DRB sequences were expressed and possibly functional. The one sequence that was undetected using cDNA had a 1-bp deletion, which would disrupt the sequence reading frame and render it incapable of making a functional protein. This sequence was removed from subsequent analyses. Fourteen sequences were assigned to the *Masp-DRB1*03, 1*04, 3, 5* and **W* loci and lineages, representing all loci and lineages known to exist in mandrills. Unexpectedly, the additional expressed sequence (Masp-DRB6*0403) was assigned to the *-DRB6* locus, typically a nonfunctional pseudogene in other primates (Klein & O'hUigin, 1995).

Each nucleotide sequence resulted in a unique amino acid sequence, with the exception of one pair (*Masp-DRB*W301* and *-DRB1*0402*), which differed in nucleotide sequence but encoded the same amino acid sequence. Of 75 amino acid positions, 59 were variable and sequences differed at a mean of 18.3 ± 0.2 sites. Supertype analysis identified 11 MHC-DRB supertypes, containing 1–6 sequences each (Table S2). Of these, S2 was composed only of -DRB6 sequences. We conducted all subsequent analyses both with and without this supertype, because our cDNA study identified one DRB6 sequence that appeared to be expressed.

Patterns of reproduction

The range of variation in values for the various genetic variables investigated is presented in Table S3. The age, rank (alpha vs. not alpha) and IR of a potential sire all significantly influenced which male sired an individual offspring (Table 1). Alpha males sired 148 offspring

Table 1 The influence of male age, rank and relatedness to the mother on the probability that a given male sired an offspring.

Analysis	Parameter	d.f.	Estimate ± SE	*t*-value (t_{192})	Approx. $P > \|t\|$
1	Age	1	1.45 ± 0.28	5.10	< 0.0001
AIC = 368	Age^2	1	−0.06 ± 0.01	−4.61	< 0.0001
	Rank	1	2.56 ± 0.20	12.93	< 0.0001
	R_{ped}	1	−1.72 ± 0.85	−2.02	0.043
	IR	1	−3.85 ± 0.87	−4.40	< 0.0001
2	Age	1	1.42 ± 0.28	5.01	< 0.0001
AIC = 369	Age^2	1	−0.06 ± 0.01	−4.52	< 0.0001
	Rank	1	2.57 ± 0.20	12.98	< 0.0001
	R_{QG}	1	−1.05 ± 0.58	−1.82	0.069
	IR	1	−3.37 ± 0.88	−3.83	0.0001

Results of MDC procedure, conditional logit estimates. Analyses were conducted separately for R_{ped} and R_{QG} due to colinearity.

(76%), whereas nonalpha males sired 45 (see also Charpentier *et al.*, 2005a). Alpha males were 18 times more likely to sire a given offspring than nonalpha males, older males were more likely to sire than younger males, and male IR was negatively related to the chances of siring, confirming previous results that showed that males with high microsatellite heterozygosity have higher reproductive success in this colony (Charpentier *et al.*, 2005b).

Relatedness and reproduction

The range of values for R_{ped} and R_{QG} are presented in Table S3. R_{ped} significantly influenced the probability of reproduction, which decreased as relatedness increased (Table 1). R_{QG} showed a nonsignificant trend towards the same effect, but AIC values for the two models were very similar (368 vs. 369, Table 1). Replacing the continuous R_{ped} variables with a cut-off point at $R = 0.25$ made very little improvement to the fit of the model (estimate ± SE: 1.21 ± 0.45, $t_{179} = 2.67$, $P = 0.008$, AIC 365).

MHC-disassortative mating

Mothers possessed 2–7 MHC sequences ($n = 34$, mean 3.9 ± 0.2), and potential sires possessed 2–6 sequences ($n = 40$, mean 4.0 ± 0.2). Both mothers and potential sires possessed 2–6 MHC supertypes (mothers mean 3.6 ± 0.2, potential sires mean 3.9 ± 0.1). The range of values for the various measures of MHC dissimilarity in a dyad is presented in Table S3. The probability of reproduction by a given sire increased as MHC_{diff} and AA_{diff} increased (Table 2, Fig. 1). In each case, the probability of reproduction increased by 17% for each additional MHC sequence or amino acid position that differed (odds ratio 1.17). However, the probability of reproduction did not increase significantly with S_{diff} (Table 2). When we added a quadratic effect of MHC_{diff} to the model, we found no significant influence on the probability of reproduction (estimate ± SE: −0.01 ± 0.02, $t_{179} = -0.71$, $P = 0.48$), and thus no evidence of choice for intermediate MHC diversity in offspring. AIC was lowest (by a small margin) for the model with AA_{diff}, suggesting that this was the best predictor of reproduction among the MHC variables that we tested.

When we included R and MHC_{diff} in the same model, MHC_{diff} remained a significant influence on reproduction with R_{QG} and $R_{<>0.25}$ and showed a tendency to do so with R_{ped}, whereas the influence of R was nonsignificant in each case (Table 3). Adding R to the model with MHC_{diff} increased the AIC minimally (Table 3). This suggests that the influence of MHC dissimilarity on reproduction may be stronger than that of overall genetic dissimilarity. However, R and MHC_{diff} were collinear (Table S1), which increases uncertainty in the coefficient estimates. To circumvent this problem, we examined only dyads where $R < 0.25$ (excluding father/daughter dyads and half-siblings) and found that MHC_{diff} was no longer a significant influence on reproduction (Table 3). Nevertheless, this analysis excludes the least MHC-dissimilar dyads, meaning that we cannot distinguish between the two influences definitively. When we included the variable $R_{<>0.25}$ in the same model as MHC_{diff}, only MHC_{diff} was a significant influence on reproduction (Table 3).

Table 2 The influence of MHC dissimilarity and male genotype on the probability that a given male sired an offspring.

Analysis	Parameter	d.f.	Estimate ± SE	*t*-value (t_{179})	Approx. $P > \|t\|$
1	MHC_{diff}	1	0.17 ± 0.07	2.33	0.020
AIC = 301	MHC_{male}	1	−0.10 ± 0.15	−0.70	0.49
	Age	1	1.56 ± 0.41	3.85	0.0001
	Age^2	1	−0.06 ± 0.02	−3.69	0.0002
	Alpha (0/1)	1	2.64 ± 0.23	11.26	< 0.0001
	IR	1	−3.90 ± 1.10	−3.53	< 0.0001
2	AA_{diff}	1	0.16 ± 0.06	2.67	0.008
AIC = 298	Age	1	1.60 ± 0.42	3.85	0.0001
	Age^2	1	−0.06 ± 0.02	−3.66	0.0002
	Alpha (0/1)	1	2.66 ± 0.23	11.34	< 0.0001
	IR	1	−3.67 ± 1.04	−3.53	0.0004
3	AA_{male}	1	0.07 ± 0.03	2.01	0.044
AIC = 302	Age	1	1.60 ± 0.41	3.88	0.0001
	Age^2	1	−0.06 ± 0.02	−3.70	0.0002
	Alpha (0/1)	1	2.65 ± 0.23	11.52	< 0.0001
	IR	1	−3.28 ± 1.04	−3.14	0.002
4	S_{diff}	1	0.10 ± 0.08	1.24	0.22
AIC = 306	S_{male}	1	0.07 ± 0.17	0.39	0.70
	Age	1	1.51 ± 0.41	3.71	0.0002
	Age^2	1	−0.06 ± 0.02	−3.49	0.0005
	Alpha (0/1)	1	2.63 ± 0.22	11.70	< 0.0001
	IR	1	−3.98 ± 1.08	−3.68	0.0002

Results of MDC procedure, conditional logit estimates. Analyses were conducted separately due to colinearity of the different estimates of MHC dissimilarity. Conducting supertype analyses without S2 (because S2 comprised only *-DRB6* sequences, which may be nonfunctional) did not alter the significance of our results.

Male genotype and reproduction

The range of values for the various measures of within-male MHC diversity is presented in Table S3. MHC_{male}, AA_{male} and S_{male} were not significantly related to IR_{male} (Table S1) suggesting that neutral heterozygosity and adaptive MHC variability were not linked in these males. AA_{male} significantly influenced the probability that a male sired a given offspring (Table 2, Fig. 2) with a 7% increase in the probability of reproduction for each additional amino acid position that differed. However, there was no significant influence of either MHC_{male} or S_{male} on the probability of reproduction (Table 2). This suggests that the amino acid sequence diversity of the MHC genotype of the male was more important in reproduction than the simple number of sequences or

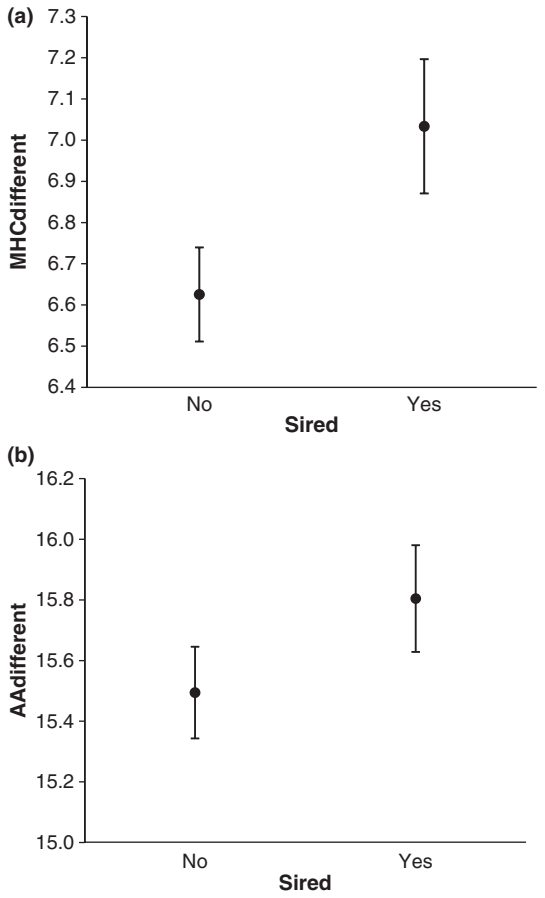

Fig. 1 Influence of MHC dissimilarity on whether reproduction occurred. Figure compares mean ± SE MHC_{diff} (a) and AA_{diff} (b) for the sire of each offspring with the mean value for nonsires for each individual offspring (n = 180 offspring).

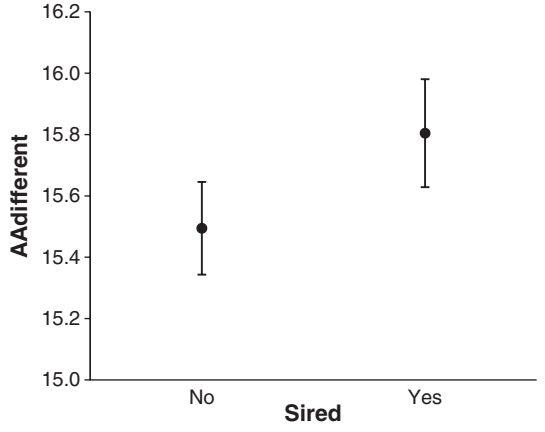

Fig. 2 Influence of AA_{male} on whether reproduction occurred. Figure compares the mean ± SEM AA_{male} for the sire of each offspring with the mean value for all the nonsires of that offspring (n = 180 offspring).

Table 3 Comparing the influence of overall genetic dissimilarity and MHC dissimilarity on the probability that a given male sired an offspring.

Analysis	Parameter	d.f.	Estimate ± SE	t-value (t_{179})	Approx. $P > \|t\|$
1	R_{ped}	1	−0.83 ± 1.06	−0.78	0.43
AIC = 303	MHC_{diff}	1	0.12 ± 0.07	1.70	0.09
	Age	1	1.57 ± 0.41	3.83	0.0001
	Age^2	1	−0.06 ± 0.02	−3.67	0.0002
	Rank	1	2.66 ± 0.23	11.53	< 0.0001
	IR	1	−3.72 ± 1.05	−3.55	0.0004
2	R_{QG}	1	−0.35 ± 0.68	−0.52	0.60
AIC = 302	MHC_{diff}	1	0.13 ± 0.06	2.11	0.03
	Age	1	1.54 ± 0.40	3.80	0.0001
	Age^2	1	−0.06 ± 0.02	−3.64	0.0003
	Rank	1	2.67 ± 0.23	11.58	< 0.0001
	IR	1	−3.58 ± 1.06	−3.39	0.0007
2	$R_{<> 0.25}$	1	0.13 ± 0.40	0.32	0.748
AIC = 302	MHC_{diff}	1	0.15 ± 0.07	2.30	0.021
	Age	1	1.54 ± 0.40	3.81	0.0001
	Age^2	1	−0.06 ± 0.017	−3.64	0.0003
	Rank	1	2.68 ± 0.23	11.54	< 0.0001
	IR	1	−3.63 ± 1.05	−3.46	0.0005

Results of MDC procedure, conditional logit estimates. Analyses were conducted separately for R_{ped} and R_{QG} due to colinearity.

supertypes he possessed. However, the P-value for AA_{male} was close to 0.05 (0.044), and given that we also tested two other measures of within-male MHC diversity (MHC_{male} and SS_{male}) this may represent a type 1 error. There was no significant influence of the possession of individual supertypes on the probability that a male sired (Table S4).

Discussion

We genotyped a large population of mandrills for MHC-DRB, and demonstrated that many of the MHC sequences we identified via genomic DNA analysis are expressed. Together with previous results showing significantly higher rates of nonsynonymous than synonymous substitutions within the mandrill DRB (Abbott *et al.*, 2006), this suggests that the MHC sequences are capable of providing resistance to pathogens, and thus might be the foundation of MHC-associated mate choice. However, expression is not proof of functionality. For example, although different MHC loci are expressed in the bank vole, only one is under positive selection and associated with parasite resistance, whereas another expressed MHC locus is not under selection (Axtner & Sommer, 2007). We are currently investigating the association between specific MHC sequences and parasite resistance in our study population.

The nature of our large dataset, which involves reproduction over multiple years for a long-lived species and colinearity between measures of genetic similarity,

poses a problem for statistical analyses. However, using the best statistical models currently available, we found that pedigree relatedness, overall genetic dissimilarity, MHC dissimilarity (number of different MHC sequences and amino acid difference) and male genotype (overall genetic diversity and MHC amino acid diversity) all influenced reproduction in this mandrill colony. The influence of MHC dissimilarity on reproduction appeared to be stronger than that of overall relatedness (*R*), which was only borderline significant. However, this pattern may still be driven by females simply avoiding brothers/fathers as mates, or low fertilization success if these males do inseminate a female, because when we excluded closely related dyads (who are also least MHC dissimilar) from our analyses, we found that MHC dissimilarity was no longer significant.

Given the polygynous mating system, strong sexual dimorphism and high male reproductive skew that occur in mandrills, it is quite surprising that other genetic factors also predict which male reproduces. Male rank was by far the strongest influence on reproduction in males, with alpha males being 18 times more likely to sire any given offspring. The nature of our study population limits our power to draw general conclusions on MHC-associated mate choice in wild mandrills, because female choice in our study population is limited to natal males (although these may not related to the female). However, it is interesting to note that findings of MHC-associated mate choice in humans are also from small or isolated populations with little or no migration to introduce genetic variation (Ober *et al.*, 1997; Chaix *et al.*, 2008), situations analogous to the mandrill colony studied here, suggesting that MHC-associated mate choice may be stronger, or easier to detect, under such conditions.

Despite the limitations of the colony environment, our results are broadly similar to those found in previous studies of strepsirrhine primates living in very different social systems: in fat-tailed dwarf lemurs MHC supertype dissimilarity (but not sequence or amino acid dissimilarity) significantly influenced reproduction, and specific supertypes were also associated with male reproductive success (Schwensow *et al.*, 2007a). In grey mouse lemurs sires were more dissimilar to the mother at the level of amino acid sequences, and had more MHC supertypes (but fewer MHC sequences) than randomly assigned males, but no specific supertypes influenced reproduction (Schwensow *et al.*, 2008). In the only other study of MHC-associated mate choice in a nonhuman anthropoid, male rhesus macaques heterozygous at the MHC-DQB1 locus were found to have greater reproductive success than homozygous males, but MHC dissimilarity did not influence mate choice (Sauermann *et al.*, 2001). Our results suggest that MHC-associated mate choice may be widespread across the order primates, although the exact patterns observed differ between species. Moreover, our results are the first to demonstrate a reproductive advantage associated with MHC dissimilarity (and possibly MHC diversity measured as amino acid diversity) in a polygynous species with high levels of male–male competition, and suggest that MHC-associated mate choice may be more widespread across different mating systems than previously thought (Paterson & Pemberton, 1997).

Dissassortative mating

Choosing a genetically dissimilar reproductive partner may serve two functions: as a mechanism to avoid inbreeding (Grob *et al.*, 1998; Jordan & Bruford, 1998); or to increase MHC diversity in offspring, improving their ability to recognize and react to a broader range of pathogens, and rendering them fitter than less diverse individuals (Doherty & Zinkernagel, 1975). Our results suggest that the influence of MHC dissimilarity on reproduction was stronger than that of overall genetic dissimilarity, and that mandrills aim to ensure MHC diversity in their offspring. This would result in offspring that were able to respond to a broader range of antigens than less MHC diverse individuals (Doherty & Zinkernagel, 1975). Such pathogen resistance may be particularly important in mandrills, which live in tropical rainforest, and can form very large groups in the wild (Abernethy *et al.*, 2002). Both wetter environments (McGrew *et al.*, 1989) and larger group sizes (Davies *et al.*, 1991) have been shown to lead to higher rates of parasite infection in primates, and annual rainfall is also positively related to immune system parameters, suggesting that primates living in wetter habitats have evolved to combat a higher risk of disease infection (Semple *et al.*, 2002). Finally, we found no evidence that mandrills choose for an intermediate level of MHC diversity to ensure optimal parasite resistance in their offspring (e.g. Wegner *et al.*, 2003), suggesting that they are choosing for maximum MHC diversity, rather than an intermediate level.

These findings raise the question of how female mandrills select genetically complementary mates. As noted above, we cannot rule out 'standard' inbreeding avoidance of close kin as opposed to finer-scale discrimination among genotypes. Mandrills are female philopatric (Setchell, 1999), and the best indicator of pedigree relatedness of a potential mate may be whether he was born into the same group. However, mandrills live in very large groups in deep rain-forest (Abernethy *et al.*, 2002) and this information may not necessarily be available to females. Moreover, MHC dissimilarity was a stronger predictor of which male sired a given offspring than pedigree relatedness. MHC-disassortative mating requires comparison of the MHC genotype of potential mates with the chooser's own genotype. Both pre- and post-copulatory mechanisms of female choice may play a role here. Female mandrills are able to express mate choice at the precopulatory level (Setchell, 2005). The possibility that primates employ self-referent phenotype

matching has attracted renewed attention recently (Widdig *et al.*, 2001), and mandrills appear to able to discriminate paternal kin from nonkin, despite their polygynandrous mating system (Charpentier *et al.*, 2007). The mechanism underlying this phenomenon remains unknown, but it may occur via visual, olfactory, acoustic, or behavioural cues (Widdig, 2007). In this context, it is striking that both male and female mandrills possess a sternal gland which produces a glandular secretion (Feistner, 1991). If genetic similarity at the MHC is reflected in similar odour profiles, then olfaction may play a role in the assessment of mate compatibility, as demonstrated for both rodents and humans (review in Penn, 2002).

Female mandrills mate with multiple males during their fertile phase (Setchell, unpublished observations) and genetic compatibility may be determined at the post-copulatory level via selective fertilization and/or selective abortion (Zeh & Zeh, 2003; Ziegler *et al.*, 2005). MHC molecules are known to be expressed on the surface of spermatozoids (Paradisi *et al.*, 2000), and mouse oocytes are able to select sperm based on MHC genotype (Wedekind *et al.*, 1996) suggesting that selective fertilization may potentially account for the observed patterns of reproduction. MHC-associated post-copulatory mate choice has been suggested for grey mouse lemurs, where no difference was found in the MHC genotype of mated and nonmated males in the vicinity of a receptive female, but sires were more dissimilar to the mother at the MHC than randomly assigned males (Schwensow *et al.*, 2008).

Male genotype

Reproduction in the mandrills was also influenced by the genetic characteristics of potential sires, in terms of both neutral (microsatellite) heterozygosity (see also Charpentier *et al.*, 2005b) and MHC amino acid sequence diversity. These results suggest that individual genetic characteristics in mandrills may be linked to male vigour and we are currently investigating whether any or all of microsatellite heterozygosity, MHC diversity, and the possession of particular supertype are linked to better condition or reduced susceptibility to disease. Higher levels of microsatellite heterozygosity are known to bring general fitness advantages (review in Hansson & Westerberg, 2002), for example via increased metabolic efficiency (Mitton *et al.*, 1993), and this is true for our study population (Charpentier *et al.*, 2005a,b, 2006). Increased MHC diversity may also allow a male to resist a greater variety of parasites (review in Penn, 2002). These results may thus reflect intrasexual competition, with MHC diversity conferring superior competitive ability on particular males. However, our analyses included the influence of male dominance rank as a separate variable, implying that intersexual selection (mate choice) may also be occurring for genetic characteristics.

Males are unable to pass on heterozygosity at specific loci (Brown, 1997; Mays & Hill, 2004) and heterozygous males have therefore been thought to confer direct, rather than indirect, fitness benefits on their offspring (Partridge, 1983). However, heterozygous males also sire offspring that are themselves more heterozygous, on average (Mitton *et al.*, 1993), and possess more rare alleles than homozygotes, which can be inherited by offspring (Apanius *et al.*, 1997), suggesting that females may also receive indirect benefits from genetically diverse mates. Indeed, a recent theoretical model has shown that directional mating preferences for heterozygous males can evolve and be maintained in the absence of direct fitness benefits (Fromhage *et al.*, 2009). Genome-wide heterozygosity has been suggested to act as a marker of MHC diversity (Aparicio *et al.*, 2001; Acevedo-Whitehouse *et al.*, 2003), such that mate choice for males signalling general genetic diversity leads to choice for MHC-diverse mates. However, the reverse has also been suggested: that MHC diversity may act as a marker of genome-wide heterozygosity (Penn & Potts, 1999). In this context, it is interesting that we found no significant relationship between neutral heterozygosity and adaptive MHC diversity in males, suggesting that the two measures of diversity are independent in mandrills, and that females would be unable to use one as a marker for the other.

If the increased reproductive success enjoyed by genetically diverse males is due to increased vigour in these males, which is preferred by females, then this raises the question of how heterozygosity and MHC diversity are signalled to females. Male mandrills possess a suite of secondary sexual ornaments, including bright red coloration on the face, rump and genitalia (Hill, 1970), and females prefer to mate with redder males (Setchell, 2005). If sexually selected traits signal the possession of 'good genes' (Zahavi, 1975; Hamilton & Zuk, 1982; Brown, 1997) in mandrills, in the form of genome-wide or MHC diversity, then males with more exaggerated ornaments should also be genetically more diverse. Condition dependent secondary sexual traits have been shown to correlate positively with overall genetic diversity in a variety of other species, including birds (Aparicio *et al.*, 2001; Foerster *et al.*, 2003; Marshall *et al.*, 2003), fish (Müller & Ward, 1995; Sheridan & Pomiankowski, 1997; van Oosterhout *et al.*, 2003), and invertebrates (Aspi, 2000). At the level of the MHC, male pheasants with particular MHC genotypes have larger spurs, a trait which is known to influence female choice (von Schantz *et al.*, 1997). Certain MHC genotypes are also associated with antler size and body size in white-tailed deer (*Odocoileus virginianus*) (Ditchkoff *et al.*, 2001). Antler size in this species is also related to helminth abundance, suggesting that antlers honestly advertise the possession of good genes for parasite resistance (Ditchkoff *et al.*, 2001).

In conclusion, we demonstrate both sexual selection for genetic complementarity (MHC dissimilarity) and directional selection for good genes (genome-wide

heterozygosity and MHC diversity) in a primate species living in large multi-male, multi-female groups. This implies that female mandrills employ a combination of mate choice strategies, as the male with the best genes may not be the most genetically compatible mate for every female. For example, they may switch between the two mate choice strategies according to the available diversity of males (Roberts & Gosling 2003), or employ a hierarchical, nested model of mate choice, in which they choose the most compatible male from the subset of males possessing good genes (Mays & Hill, 2004). Our results are the first to demonstrate mate choice for genetic dissimilarity in a species characterized by high reproductive skew among males, and suggest that MHC-associated mate choice can occur even where male–male competition is intense. Finally, our results concern an isolated population with no migration to introduce genetic variation, a situation analogous to those in which MHC-associated mate choice has been found in humans, suggesting that MHC-associated mate choice may be especially important in such populations.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Results of Spearman correlation analyses between the variables considered for potentially reproductive dyads.

Table S2 Supertype designations identified using cluster analysis of physiochemical identities.

Table S3 Range of variation in values for the various genetic variables investigated in the context of mate choice.

Table S4 The influence of individual MHC supertypes on the probability that a given male sired an offspring.

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