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ORIGINAL ARTICLE

S. Sommer

Major histocompatibility complex and mate choice in a monogamous rodent

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Abstract A growing number of studies indicate that females can increase the viability of their offspring by gaining direct benefits such as parental care or genetic advantages through selective mating with certain males. Among the best candidates for the genetic basis of mate choice in vertebrates are the genes of the major histocompatibility complex (MHC) because these highly polymorphic genes may increase offspring viability and provide direct cues for mate choice. A free-ranging, pair-living rodent was used as an example to investigate MHC-dependent mate choice in an obligate monogamous species, the Malagasy giant jumping rat *Hypogeomys antimena*. Two possible mechanisms of mate choice were tested. First, mate choice may occur to increase the heterozygosity of MHC genes in the progeny and, second, mates might choose each other according to the degree of dissimilarity of their functional MHC DRB (exon 2) proteins in order to maximise the allelic divergence in their offspring. Analyses of 65 *Hypogeomys* couples failed to confirm associations of mating patterns with the MHC genotype to increase heterozygosity or MHC allelic divergence in the progeny. Also, no evidence for mechanisms to increase the allelic divergence was found in sex-specific analyses where a male or female, respectively, migrated to and was accepted by a territory and burrow holder of the opposite sex. However, the frequency distribution of 0, 1 or 2 new alleles potentially available for the progeny differed significantly when a new male was chosen by a territory-holding female. In contrast to current models, genetically similar instead of dissimilar mates seem to be the preferred choice. This is the first study investigating the role of the MHC in mate selection in an obligate monogamous rodent.

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S. Sommer (✉)
Biozentrum Grindel, Animal Ecology and Conservation,
University of Hamburg,
Martin-Luther-King-Platz 3,
20146 Hamburg, Germany
e-mail: Simone.Sommer@zoologie.uni-hamburg.de
Tel.: +49-40-428384226
Fax: +49-40-428385980

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Introduction

Sexual selection, the preference of certain mating partners over others, is a frequently observed behavioural trait among animals (Darwin 1859; Andersson 1994). In mammals, mate choice may be exercised by either sex, although females are generally choosier than males because they usually invest more in their progeny due to gestation and lactation (Trivers 1972). A growing number of studies indicate that females can increase the viability of their offspring by selective mating with certain males (Promislow et al. 1998). Males might be chosen directly because they offer maternal benefits (e.g. food, territory, shelter) and/or paternal care, or indirectly to increase the genetic quality of the progeny (Andersson 1994). Genetic benefits can be gained either by minimising the risk of genetic incompatibility between maternal and paternal genomes through avoidance of mating with close kin (inbreeding avoidance), or by increasing the genetic diversity (heterozygosity advantage) within the progeny (reviewed by Searcy 1982; Tregenza and Wedell 2000). The importance of heterozygosity as a strong fitness predictor has been indicated by both theoretical and empirical studies (reviewed by Hansson and Westerberg 2002).

Particularly suited as potential candidates for the genetic basis of mate choice in vertebrates are the genes of the major histocompatibility complex (MHC) (Tregenza and Wedell 2000), a multigene family encoding cell-surface glycoproteins (MHC molecules) that present small peptide antigens to T-cells of the immune system (Klein 1986). Certain genes within the MHC constitute the most polymorphic loci known in vertebrates (Klein 1986; Hughes and Hughes 1995). Particularly, the regions of the molecule responsible for binding antigens, the so-called antigen binding sites (ABS), show high levels of variation, not only in the number of alleles but also in the extent of sequence variation between alleles (Hughes and Yeager 1998). These ABS (e.g. of exon 2 of the DRB gene) display more non-synonymous than synonymous substitutions changing the amino acid sequence of the peptide, and thus increasing the ability of binding a diverse array of antigens (Brown et al. 1988, 1993). Such observations lend strong empirical support to the action of selection in the maintenance of diversity at MHC loci, though it is still unclear how natural selection maintains these extreme polymorphisms. The leading hypotheses are selection due to: (1) parasites, either through heterozygosity advantage or frequency-dependent selection ("rare allele advantage hypothesis", "moving target hypothesis"), and (2) disassortative mating preferences (Potts and Wakeland 1990; Brown and Eklund 1994; Potts and Slev 1995; Penn and Potts 1999). These hypotheses are not mutually exclusive. If heterozygotes are able to mount an immune response against a broader array of pathogens than homozygotes, females should mate disassortatively in order to increase the survival capability of their progeny (Brown 1997; Tregenza and Wedell 2000).

The actual cue used in MHC-based mate choice is thought to be based on odour, which allows the animals to distinguish MHC-identity (reviewed in Penn and Potts 1998b, 1999; Yamazaki et al. 1998; Eggert et al. 1999). Mating preferences may arise from MHC-derived odour profiles either directly by the whole MHC proteins in the body fluids (Singh et al. 1987) or indirectly via the parasite load and binding characteristics of the antigen binding sites of the molecules (reviewed in Singh 1998).

Due to the MHC's role in immune defence, two different levels of MHC-based selection mechanisms in mate choice are possible. First, mate choice may occur to increase the heterozygosity of MHC genes in the progeny. This assumes that an individual preferentially mates with partners that differ genetically from itself, without any regard to the nature and quantity of the corresponding differences. This should result in more frequent matings between dissimilar genotypes according to the number of alleles they share than expected by chance alone (Potts et al. 1991; Hedrick 1992; Paterson and Pemberton 1997; Landry et al. 2001). However, MHC mate preference could imply, not only avoidance of mates that share alleles to increase heterozygosity but also a preference for partners that will maximise the divergence between alleles of heterozygotes in the progeny with the benefit of recognising and binding a wide range of different pathogens (Doherty and Zinkernagel 1975; Potts and Wakeland 1990; Landry et al. 2001). This second hypothesis is based on the fact that MHC alleles can be different at the molecular level, but encode the same amino acid sequence due to silent substitutions. The protein would bind the same pathogen or parasite-derived peptides and therefore be functionally similar in terms of immune defence (Rammensee 1995). So far, most studies of MHC-based mating preferences only investigated a possible increase in heterozygosity in the progeny without taking into account the genetic distance between alleles (Landry et al. 2001).

Evidence for MHC-disassortative mating preferences has been found in some species but has failed to be detected in others. It is currently unclear how widespread the phenomenon of reproductive selection at the MHC is among vertebrates (reviewed by Tregenza and Wedell 2000; Penn 2002). To allow general conclusions of the importance of female mate choice based on genetic compatibility, studies of natural populations with different social and mating systems are required.

In this paper, I used a free-ranging, pair-living rodent as an example to investigate the occurrence and mechanisms of MHC-dependent mate choice in an obligate monogamous species (*Hypogeomys antimena*). Within class II DR genes, I focused on the second exon of the beta-chain because it encodes parts of the functionally important peptide binding groove and has been shown to be the most polymorphic region in many class II genes (Ohta 1998; Hughes 1999). In order to investigate whether the heterozygosity of MHC genes in offspring is increased or the divergence between alleles of heterozygotes in the progeny maximised by mating preferences (1), the number of MHC DRB alleles shared between mates without considering the amino-acid composition of the different alleles, and (2) the sum of pairwise amino-acid differences (a) for the whole DRB exon 2 sequence and (b) only for the putative sites involved in antigen recognition and binding (ABS) were compared to random distributions.

Methods

Study species

H. antimena, the Malagasy giant jumping rat, is the largest extant rodent of Madagascar. Adults show no sexual dimorphism in external characters and coloration; they have a head/body length of about 30 cm and weigh ca. 1.2 kg. The species occurs in a specific habitat—coastal dry deciduous forest mixed with baobabs resting on sandy and lateritic soils (Cook et al. 1991). The current distribution of *H. antimena* has recently been reduced to the remaining area of suitable habitat within a geographic range of less than 20×40 km north of the town Morondava near the western coast of Madagascar (Goodman and Rakotondravony 1996; Ganzhorn et al. 2001; Sommer et al. 2002b). *H. antimena* lives in an obligate monogamous mating system (Sommer 1997, 2000, 2003a), a rare social system occurring in only 3% of all mammal species. Pairs usually stay together until one mate dies. The reproduction rate is very low; each couple has only one to two offspring per year, which are born during the rainy season (Dec. to April). Confidence of paternity seems to be very high for males; only 2 extrapair young were found in genetic analyses of 48 parent-offspring trios (4%) (Sommer and Tichy 1999; Sommer 2003a). In *Hypogeomys*, it seems that solitary parents cannot successfully rear offspring. Of 112 family units studied, only 4 consisted of a solitary parent with 1 offspring. Only one of these offspring, which was already about a year old and weighed ca. 1,050 g when its father was killed, survived and dispersed. This may be an indirect evidence for the importance of males' parental care (Sommer 2003a). The herbivorous rodents are strictly nocturnal, and pairs and their offspring spend the day in underground burrows. Burrows are used for many years and are essential for survival and reproduction. After the death of resident animals, vacant burrows are re-occupied by new immigrants. Mates defend an exclusive territory throughout the year irrespective of food abundance or reproductive state. Once female offspring have dispersed from their parental territory and have settled in their own territory, they remain there during the course of their life. After the death of a male mate, the remaining female always keeps the burrow and the territory and a new male immigrates to the site. In the case of death or disappearance of females, the male mate also usually keeps the burrow and territory. However, on a few occasions, it has been documented that a widowed male moves to the territory and burrow of a widowed female (Sommer 1996, 1997, 2000, 2001, 2003a).

Study site and sampling

Field studies were carried out in the 12,500-ha forestry concession of the Centre de Formation de Morondava (C.F.P.F.) in the Kirindy Forest/CFPF (20°03′S, 44°39′E) at the research station of the German Primate Centre (DPZ, Göttingen, Germany), western Madagascar. A detailed description of the area is given in Ganzhorn and Sorg (1996).

Between 1992 and 2000, I trapped all inhabitants of all existing burrow systems (13–22 active burrows) in the 100-ha-sized study area 1–2 times a year (except for 1993). Tomahawk live traps ($51\times19\times19$ cm, Tomahawk, Wisconsin) were set in front of the burrow holes before the nocturnal activity period of the rats started, and were checked at least once every hour after sunset until the animals entered the traps. Captured animals were taken to the camp (distance 1.5 km) and kept in palm huts, protected from disturbances. They were anaesthetised with an intramuscular injection of ketamin hydrochloride and individually marked with a passive integrated transponder (Trovan, Germany). Animals were sexed, weighed, and measured. After disinfection, 2 mm of the tip of the tail or small tissue samples of the ear were taken and preserved in 70% ethanol. The rats were released at their respective trapping site. Since 1992, 186 different individuals of *H. antimena* have been genetically sampled in the study area. Intensive radio-tracking of 15 males, 15 females and 13 offspring (373 h, 3,900 locations) throughout their whole nocturnal activity period during different times of the year provided information on behavioural, ecological and life-history characteristics of the species (Sommer 1996, 1997, 2000, 2001, 2003a, 2003b, 2003c; Sommer and Tichy 1999; Sommer and Hommen 2000).

Genetic analyses

The genetic procedures [primers, PCR and single strand conformation polymorphism conditions (SSCP)] were described in detail in Sommer et al. (2002a) and Sommer (2003b). To summarise, the PCR amplified MHC DRB exon 2 fragments were subjected to SSCP analysis. SSCP analysis relies on the fact that the mobility of a single-stranded DNA molecule in a nondenaturing gel is not only determined by its size, but also by its nucleotide sequence, which governs its three-dimensional structure (Orita et al. 1989). SSCP is one of the most sensitive methods for quickly detecting nucleotide substitutions (Hayashi 1992; Fan et al. 1993; Hongyo et al. 1993; Girman 1996; Law et al. 1996). Single base pair changes should be detected in 99% of 100- to 300-bp fragments (Lessa and Applebaum 1993). Homozygous and heterozygous animals can be distinguished and individuals can be genotyped. All samples were analysed at least twice. In addition to new samples, all known alleles were run as reference on each SSCP gel. In cases where similarity of phenotypes was unclear, equal mixtures of the PCR-product were pooled and compared to reconstruct genotypes. This allowed detection of slight differences in single-strand formations. At least three examples of all identified alleles were cut from the SSCP gel and re-amplified by PCR prior to sequencing. The PCR products were cleaned with a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Cycle sequencing of the PCR products was performed using a dye terminator sequencing kit (Applied Biosystems, Foster City, Calif.) and then analysed by gel electrophoresis with an Applied Biosystems automated sequencer model 3100, following the manufacturer's instructions.

Statistical analyses

Allele frequencies and gene diversity were calculated using Arlequin, ver 2.000 (Schneider et al. 2000). The gene diversity is the probability that two randomly chosen alleles are different in a sample (equivalent to expected heterozygosity; Nei 1987). A phylogenetic tree was constructed in MEGA using the minimum evolutionary criteria (Nei 1991). The inferred phylogenetic relationships were controlled with a bootstrap test using 2,000 replications. MEGA was also used to calculate the pairwise amino-acid distances between alleles.

In order to determine whether the heterozygosity of MHC genes in the progeny is increased by mate choice, the number of MHC DRB alleles shared between mates without considering the nucleotide and amino-acid composition of the different alleles was analysed. This was tested by quantifying the basic similarity statistics M_{xy} based on the numbers of shared MHC alleles (none, one or two) between couples (Blouin et al. 1996; Landry et al. 2001).

Further, behavioural characteristics such as territoriality and long-term use of burrows of *H. antimena* were taken into account: after death of a mate, the remaining sex usually keeps the burrow and territory and a new partner immigrates. Therefore, the number of new alleles potentially available for the progeny was calculated for both cases (new male or new female paired with burrow and territory holder). It was assumed that the resident individual as holder of the limited resource "burrow and territory" might be able to choose the new partner, and the corresponding number of new alleles was calculated from the territory holder's point of view. The difference is illustrated by the following example: if the resident individual has the genotype "aa" and the immigrating individual the genotype "ab", the number of new alleles is "1" from the perspective of the territory holder but "0" from the point of view of the immigrating individual.

A preference for partners that will maximise the divergence between alleles of heterozygotes in the progeny was tested by computing a genotypic distance (AA_{xy}) value for each pair, which corresponds to the sum of all the pairwise amino-acid differences (D) of the four possible alleles (A, a, B, b) carried by mates ($D_{AB}+D_{Ab}+D_{aB}+D_{ab}$) (Landry et al. 2001). The sum of pairwise amino-acid differences were analysed for: (a) the whole DRB exon 2 sequence, and (b) only for the putative antigen binding sites (amino acid positions 9, 11, 13, 28, 30, 32, 37, 38, 47, 56, 58, 60, 61, 65, 68, 70, 71, 74, 78 after Brown et al. 1988, 1993).

The association of each of the above parameters with mate choice was statistically investigated by estimating the likelihood of the values of each observed statistic under random mating. Using the observed allele frequency distributions, I randomly generated 1,000 pairs of unrelated individuals (Blouin et al. 1996) using the Resampling Stats software (vers. 4.1, Simon 1997). The mean value and the observed frequency distribution of each parameter were compared with the values of the random distribution.

Results

MHC polymorphism

A detailed description of the variability of the MHC DRB locus is given in Sommer et al. (2002a) and Sommer (2003b). Briefly, in *Hypogeomys*, 5 DRB-exon 2 alleles (GenBank accessions AJ416074–AJ416078) based on 37 variable nucleotide positions were found with a gene diversity of 0.63. All alleles had a unique amino-acid sequence with high rates of non-synonymous substitutions at the sites predicted to be involved in peptide binding (Brown et al. 1988, 1993). A minimum evolution tree of the alleles is given in Fig. 1. The frequencies of the alleles *Hyan*-DRB-1 to *Hyan*-DRB-5 are 0.54, 0.06, 0.11, 0.05 and 0.24, respectively.

Table 1 Analyses of mating preferences in relation to the MHC genotype. The observed mean value and the frequency distribution of pairs sharing 0, 1 or 2 alleles (M_{xy}) were compared to those expected under random mating. The mean values of shared alleles ($\bar{x}$) were compared by a *t*-test, the frequency distribution by a χ^2-test; *N*=sample size

	All pairs (obs/exp)
Relative frequency of	
sharing 0 alleles	0.25/0.25
sharing 1 allele	0.54/0.58
sharing 2 alleles	0.22/0.17
N	65
$\bar{x}$	0.97/0.92
t (*P*-value)	0.60 (0.55)
χ^2 (*P*-value)	1.02 (0.60)

Mating patterns

Increase of heterozygosity in the progeny

In order to determine whether the heterozygosity of MHC genes in the progeny is increased by mate choice, the observed mean value and frequency distribution of pairs (n=65) sharing 0, 1 or 2 alleles (M_{xy}) were compared to those expected under random mating (Table 1). No significant differences in the mean value and frequency distribution of shared alleles were found ($P \geq 0.05$), thus suggesting that the mating patterns were not associated with the MHC genotype (Table 1).

In order to take behavioural characteristics such as territoriality and long-term use of burrows of *H. antimena* into account, the number and frequency distribution of new alleles potentially available for the progeny were calculated for both cases [new male (n=22) or new female (n=12) paired with burrow and territory holder] and compared to those expected under random mating (Table 2). In both cases, the observed mean values of new alleles potentially available

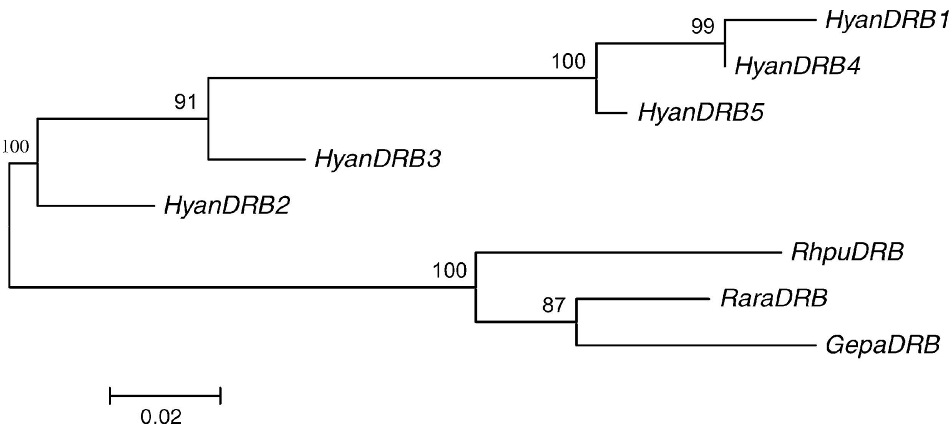

Fig. 1 Minimum evolution tree for the *Hypogeomys*-DRB exon 2 alleles. DRB-exon 2 sequences from three other murid species were used to root the tree (*Gerbillurus paeba*, Harf and Sommer 2005; *Rhabdomys pumilio*, Froeschke and Sommer, 2005; *Rattus rattus*, Hingston and Sommer, unpublished data). The scale bar indicates genetic distance in units of nucleotide substitutions per site. Bootstrap values are based on 2,000 replications

Table 2 Analyses of mating preferences in relation to the MHC genotype. The observed mean value and the frequency distribution of new alleles potentially available for the progeny by male or female choice were compared to those expected under random mating. The mean values of shared alleles ($\bar{x}$) were compared by a *t*-test, the frequency distribution by a χ^2-test; *N*=sample size

	New male paired with resident female (obs/exp)	New female paired with resident male (obs/exp)
Relative frequency of		
gaining 0 new alleles	0.50/0.25	0.42/0.25
gaining 1 new allele	0.32/0.58	0.25/0.58
gaining 2 new alleles	0.18/0.17	0.33/0.17
N	22	12
$\bar{x}$	0.68/0.92	0.92/0.92
t (*P*-value)	1.72 (0.09)	0.02 (0.99)
χ^2 (*P*-value)	8.02 (0.02)	5.54 (0.06)

for the progeny did not differ from the expected values under random mating ($P \geq 0.05$). However, the mean value of new alleles potentially available for the progeny was considerably lower where new males moved to a resident female's territory than vice versa (0.68 vs 0.92, Table 2). Also, the frequency distribution of potentially gaining 0, 1 or 2 new alleles differed significantly (P=0.02) in the category where a new male moved to a territory-holding female; the *P*-value was 0.06 in the opposite case (Table 2). The results suggest the existence of mate preferences in cases where a territory and burrow holder chose a new partner, but the results seem to contradict current models as more similar mates instead of genetically dissimilar mates seem to be preferred.

Increase of divergence between alleles of heterozygotes in the progeny

In order to test the alternative hypothesis that mates might choose each other according to the degree of dissimilarity of their functional MHC DRB-exon 2 protein to maximise the divergence between alleles of heterozygotes in the progeny, the genotypic distances (AA_{xy}) were analysed (Fig. 2). The mean AA_{xy}-values ($\pm$standard deviation) of pairs (n=65) of both the whole DRB exon 2 sequence (all, Fig. 2a) and the antigen binding sites (ABS, Fig. 2b), did not differ from the mean AA_{xy}-values expected under random mating [all: AA_{xy} (obs)=28.3±14.6, AA_{xy} (exp)=28.4±17.0, t=0.08, P=0.93; ABS: AA_{xy} (obs)=10.2±7.1, AA_{xy} (exp)=10.7±8.5, t=0.48, P=0.63]. Also, the comparison of the data where new males were paired with resident females (n=22) to random mating [AA_{xy} (exp) are given above], revealed no significant differences [all: AA_{xy} (obs)=25.50±11.20, t=0.81, P=0.42; ABS: AA_{xy} (obs)=8.68±6.18, t=1.10, P=0.27]. The same results were found comparing data where new females were paired with resident males to the expected AA_{xy}-values [n=12; all: AA_{xy} (obs)=31.08±12.35, t=0.54, P=0.59; ABS: AA_{xy} (obs)=11.33±6.67, t=0.27, P=0.79]. The sum of pairwise amino-acid differences in the mate choice subsamples did not differ from the genotypic distance of the whole sample (*t*-tests: not significant, results not shown). No differences in the observed AA_{xy}-distributions compared to the expected ones under random mating were found (χ^2-tests: not significant, results not shown).

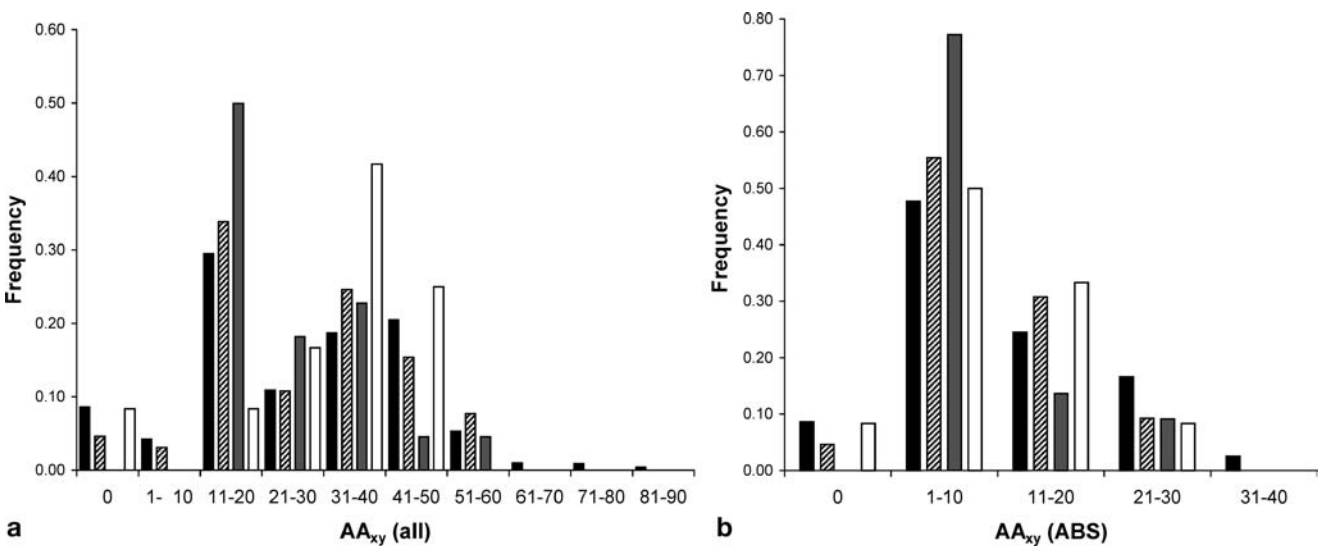

Fig. 2 Frequency distribution of the amino-acid pairwise differences (AA_{xy}) for **a** the whole MHC DRB-exon 2 sequence (all) and **b** the antigen binding region (*ABS*). Distribution under random mating (*black bars*), of all investigated pairs (n=65, *hatched bars*), new male moving to and accepted by a resident female (n=22, *grey bars*) and new female moving to and accepted by resident male (n=12, *white bars*)

Discussion

A growing number of studies indicate that females can increase the viability of their offspring by gaining direct benefits such as parental care or genetic advantages through selective mating with certain males (Promislow et al. 1998). Among the best candidates for the genetic basis of mate choice in vertebrates are the genes of the major histocompatibility complex, because these highly polymorphic genes may both increase offspring viability and also provide direct cues for mate choice (Tregenza and Wedell 2000).

Evidence for MHC-disassortative mating preferences have been found in house mice (*Mus musculus*, Egid and Brown 1989; Eklund 1997; Penn and Potts 1998a), humans (Swiss college students; Wedekind et al. 1995; reproductively isolated population of Hutterites in North America; Ober et al. 1997), Savannah sparrows (*Passerculus sandwichensis*; Freeman-Gallant et al. 2003) and salmon (Landry et al. 2001). The importance of MHC-dependent mate choice and traits under selection is indicated by an increasing number of empirical studies (reviewed by Tregenza and Wedell 2000; Penn 2002). Ober et al. (1998) revealed that human couples sharing either certain MHC alleles or the whole MHC haplotype have significantly elevated rates of foetal loss. MHC-dissassortative mating preferences increase the MHC-heterozygosity in the progeny, which is suspected to confer resistance to infectious diseases. In humans, heterozygosity at MHC loci is associated with increased resistance to hepatitis and HIV infections (Thursz et al. 1997; Carrington et al. 1999). MHC class II-heterozygous male rhesus macaques (*Macaca mulatta*) have a higher reproductive success, which might be associated with higher disease resistance (Sauermann et al. 2001). A study on sexual selection in pheasants (*Phasianus colchicus*) ascertained that females prefer males with larger spurs, and that this sexually selected trait is associated with a particular MHC allele, suggesting that large spurs may advertise a male's genetic resistance (Von Schantz et al. 1989, 1996). Ditchkoff et al. (2001) found that antler development and body mass in male white-tailed deer (*Odocoileus virginianus*) are associated with certain MHC-DRB combinations.

However, other studies, including those on South American Indians and Japanese couples (Hedrick and Black 1997; Ihara et al. 2000), some strains of laboratory mice (Eklund et al. 1991; Beauchamp et al. 1988), semiwild (Manning et al. 1992) and wild female mice (Eklund 1997), Soay sheep (*Ovis aries*, Paterson and Pemberton 1997), African buffalos (*Syncerus caffer*, Wenink et al. 1998) and great reed warblers (*Acrocephalus arundinaceus*, Westerdahl 2004), found no evidence for MHC-dependent mating preferences. For example, in feral Soay sheep, MHC variation is associated with survival and resistance against a parasitic nematode, but neither evidence for a heterozygote advantage (Paterson et al. 1998), nor for a significant role of the MHC in mate choice was found in these species (Paterson and Pemberton 1997). It has been suggested that other factors, such as male-male competition, may determine mating success in these species, leaving little opportunity for female choice. However, the intensity of male-male competition depends on the social system and, as predicted by sexual selection theory, should be more pronounced in polygynous and promiscuous than in monogamous mating systems (summarised in Emlen and Oring 1977; Clutton-Brock 1989). None of the mammal species with missing evidence for MHC-based mating preferences (except for humans) are monogamous. It is unclear how widespread the phenomenon of reproductive selection at the MHC is among vertebrates, especially with respect to the mating system.

The free-ranging, pair-living Malagasy giant jumping rat, *H. antimena*, was used as an example to investigate MHC-dependent mate choice in an obligate monogamous species. Analyses of 65 *Hypogeomys* couples indicated that the mating patterns were not associated with the MHC genotype to increase heterozygosity or to maximise the divergence between alleles of heterozygotes in the progeny. Also, no evidence for mechanisms to increase the divergence between alleles of heterozygotes in the progeny could be found in the two subsamples where a male or female, respectively, migrated to and was accepted by a territory and burrow holder of the opposite sex. However, the mean value of new alleles potentially available for the progeny was considerably lower when new males moved to a resident female's territory than vice versa (0.68 vs 0.92). Also, the frequency distribution of potentially gaining 0, 1 or 2 alleles differed significantly (P=0.02) in the category where a new male moved to a territory-holding female; the P-value was 0.06 in the opposite case. Therefore, in contrast to a number of previous studies (reviewed by Tregenza and Wedell 2000; Penn 2002), more similar mates instead of genetically dissimilar mates seem to be preferred. The results suggest that *Hypogeomys* chooses mates to minimise differences in MHC types. Assortative mating tended to be more pronounced in cases of female choice. Jacob et al. (2002) recently showed that women preferred the smell of male odour donors that had more MHC-allele matches with their own alleles, and thus did not try to maximise MHC heterozygosity by preferring the most dissimilar genotypes. Thornhill and colleagues (2003) found that men preferred the odour of t-shirts worn by MHC-dissimilar women, but women's preferences did not correlate with MHC dissimilarity. This suggests that there might be sex-specific differences in MHC-dependent mating preferences.

The investigated *Hypogeomys* population departed significantly at the DRB exon2 locus from Hardy-Weinberg expectations ($H_{obs/exp}$=0.58/0.75) with a marked deficiency of heterozygotes (Sommer 2003b). Certainly, some assumptions for Hardy-Weinberg equilibrium are not met in fragmented populations, such as an infinite population and neutrality of the markers. Apart from these violations, the observed reduction of heterozygosity could be explained by: (1) the presence of non-amplifying alleles (mutation in the primer binding site), which would cause heterozygous individuals to be counted as homozygous, (2) unconscious sampling of genetically differentiated subpopulations, which causes a Wahlund effect, (3) inbreeding or genetic drift effects due to small population sizes in the isolated fragments, and/or (4) assortative mating or selection against heterozygotes (Hartl and Clark 1989). In *Hypogeomys*, the presence of non-amplifying alleles is improbable as primers were tested in a number of species and proved to be very robust. Also, a Wahlund effect is very unlikely. The study population has been individually marked since 1992. All burrows and territories are known. There is no population subdivision within the area investigated for the purpose of this study. As the remaining geographic range of *Hypogeomys* has recently been restricted to less than 20×40 km in the dry deciduous forest in western Madagascar (Sommer et al. 2002b), inbreeding and genetic drift effects as possible explanations for a heterozygosity deficit cannot be excluded. However, avoidance of inbreeding is considered as an ultimate cause for MHC-dissimilar matings, which were not observed in *Hypogeomys*. In African buffalos, Wenink et al. (1998) explained a reduction in heterozygosity by assortative mating. It was proposed that under some circumstances, choosy females might be expected to avoid the most MHC-dissimilar males: for example, if extreme heterozygosity has the effect of decreasing immunocompetence (Penn and Potts 1999; Reusch et al. 2001; Wegner et al. 2003) or, more generally, if outbreeding depression occurs and the MHC is used as a genetic marker of relatedness (Brown and Eklund 1994). Mating with relatives could favour the survival of rare alleles and could be a strategy to ensure variability within the remaining population (Wenink et al. 1998). However, this is a group selection argument. For such behaviour to be favoured, inbred populations would have to outcompete more outbred populations. In the present *Hypogeomys*-study, the risk of outbreeding depression seems to be very low. Outbreeding is most likely to occur when mates come from ecologically different or isolated populations, which was not the case in this study.

One can argue that the present results could be biased by sample size effects, and the low MHC diversity observed in *H. antimena* compared to other mammalian species (Sommer and Tichy 1999; Sommer et al. 2002a), which, in turn, might have led to limitations in the power of the analyses and/or the possibilities for MHC-dependent mate choice. Whereas the former argument cannot be ruled out, it must be pointed out that, although only five alleles of the functionally important MHC DRB exon 2-locus have been observed in *H. antimena*, all alleles had high levels of both nucleotide and amino-acid divergence. The high rate of non-synonymous substitutions at the sites predicted to be involved in antigen binding indicates the existence of selection processes (Sommer 2003b). Similar levels of low numbers of MHC alleles (DQA, DRB) as in *H. antimena* were found in a sympatric monogamous rodent (*Macrotarsomys bastardi*), whereas a sympatric promiscuous rodent (*Eliurus myoxinus*) revealed high polymorphism in the same loci (Sommer et al. 2002a). *Macrotarsomys bastardi* and *E. myoxinus*, in contrast to *H. antimena*, have a large biogeographic range at the western coast of Madagascar. This might suggest that not only bottleneck effects and limitations of the biogeographic range could influence MHC diversity, but also processes associated with the monogamous mating system as well (Sommer et al. 2002a). Monogamous females depending on male paternal care might be subject to additional selection pressures for female choice compared to polygynous or promiscuous mating systems, which might limit the possibilities of MHC-disassortative matings to increase MHC variability in offspring. In *Hypogeomys*, MHC-dependent mate choice mechanisms might be relegated in priority by the eco-behavioral constraints of this monogamous rodent species, such as the limited resources of burrow and territory, the associated restrictions in dispersal possibilities, or the need for biparental care. In *Hypogeomys*, it seems that solitary parents cannot successfully rear offspring. Studies in the monogamous rodent *Peromyscus polionotus* with extensive biparental care indicated that males are able to choose unfamiliar, distantly related females according to very small differences in their kinship to these potential mates (Ryan and Lacy 2003). Unfortunately, the role of MHC genes in mate choice is not yet known for this species.

In conclusion, it appears that in *Hypogeomys*, more similar instead of genetically dissimilar mates were preferred. Assortative mating tended to be more pronounced in cases of female choice. The results might be explained by different constraints on mate choice driven by different selection pressures in an obligate monogamous mating system. More studies on MHC-based mate choice in relation to different mating systems are required to answer the question of which selective forces are operating, and to determine the strength and generality of preferences and mechanisms.

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