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ORIGINAL ARTICLE

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MHC diversity of endemic Malagasy rodents in relation to geographic range and social system

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Abstract The major histocompatibility complex (MHC) encodes a group of closely linked genes that play a central role in the vertebrate immune system. Most natural mammal populations studied so far possess high levels of diversity within the MHC. However, recent investigations in the MHC class II gene *DQA* of the critically endangered Malagasy giant jumping rat (*Hypogeomys antimena*) revealed very low variability compared to other mammalian species. The low genetic variability was confirmed in the present study through analyses of the MHC class II gene *DRB* exon 2. This codes for the antigen-binding site and is therefore considered as one of the most important parts of the MHC. The species' geographic distribution has been reduced recently to a fragmented area of suitable habitat within a geographic range of less than 20×40 km of dry deciduous forest at the west coast of Madagascar. *H. antimena* has some unusual life history characteristics for a rodent species, such as living in obligate monogamy, with pairs staying together until one mate dies, a low reproduction rate (one to two offspring/couple per year), high site and mate fidelity, short dispersal distances, almost no adult migration, and therefore a constant gene pool with very limited gene flow. Current hypotheses usually interpret low MHC polymorphism either as a consequence of reduced selection pressure, bottleneck effects or by constraints of the mating system. To differentiate between these hypotheses, the MHC variability of the *DQA* and *DRB* gene of two additional sympatric, but widely distributed rodent species, *Macrotarsomys bastardi* and *Eliurus myoxinus*, were studied by using universal primers and single-strand conformation polymorphism. The two species differ in their mating systems: *M. bastardi* also lives in pairs but *E. myoxinus* is a promiscuous species. Whereas the investigated MHC class II genes *DQA* and *DRB* had low levels of polymorphism in the pair-living species *H. antimena* and *M. bastardi* (*DQA*: 2 and 3 alleles, *DRB*: 5 and 6 alleles, respectively), higher levels of variation (*DQA*: 11 alleles, *DRB*: 9 alleles) were recorded in the promiscuous species, *E. myoxinus*. Gene diversity was also higher in *E. myoxinus* (*DQA*: 0.85, *DRB*: 0.86) than in the two pair-living species (*DQA*: 0.41–0.45, *DRB*: 0.55–0.63). The results suggest that low MHC variability might not only result from bottleneck effects due to recent declines in population size, but also from a monogamous mating system.

Keywords MHC diversity · Rodents · Geographic range · Social system · Madagascar

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Introduction

The major histocompatibility complex (MHC) encodes a group of closely linked genes that play a central role in the vertebrate immune system. Their products, the MHC molecules (heterodimeric cell surface glycoproteins), control immunological self/non-self recognition by binding foreign peptides and by presenting them to patrolling T lymphocytes (Klein 1986). Variation in antigen binding by alleles in the MHC appears to be a basis for selective pressure through parasitic or pathogen resistance (Edwards and Potts 1996). Selection should favour MHC heterozygotes directly as a result of their increased effectiveness in dealing with a wide variety of pathogens (Nei and Hughes 1991), or indirectly as a result of increased fitness of outbred individuals that are heterozygous across much of their genome, including their MHC loci (Potts et al. 1994). More recently, reproductive mechanisms such a negative assortative mating, maternal-fetal interactions to increase reproductive efficiency,

and the use of the MHC as olfactory-based markers for kin recognition to avoid inbreeding have been suggested as alternative mechanisms maintaining MHC diversity (Hill et al. 1991; Manning et al. 1992; Potts and Wakeland 1993; Brown and Eklund 1994; Hedrick 1994; Edwards and Potts 1996; Penn and Potts 1999). But an animal's ability to discriminate between individuals might be compromised by inbreeding (Nevison et al. 2000). Recent studies have revealed that neutral forces, such as drift, gene flow and inbreeding, might also influence MHC variation [bighorn sheep (*Ovis canadensis*): Boyce et al. 1997; Australian bush rat (*Rattus fuscipes*): Seddon and Baverstock 1999]. To date, studies of natural populations aimed at determining the relative contributions of parasite-driven selection, reproductive selection and neutral forces in the maintenance of MHC polymorphism are still rare (Paterson and Pemberton 1997). The influence of different types of mating systems have not yet been investigated.

Most natural mammal populations that have been studied possess high MHC diversity, both in terms of the number of alleles present and in the extent of sequence variation among alleles (Klein 1986; Potts and Wakeland 1990; Nei and Hughes 1991; Hedrick 1994). Also remarkable are the extremely high levels of heterozygosity that are commonly observed at certain genes within the complex (Hedrick 1994). In contrast, there are a number of species which exhibit low or no detectable polymorphism in MHC genes. These species might possibly suffer from increased susceptibility to epizootics, parasites and diseases (O'Brien et al. 1985; O'Brien and Everman 1988; Hill et al. 1991; Hughes 1991; Lacy 1997).

So far, the following hypotheses have been suggested to explain low MHC polymorphism: (1) reduced selective pressure for polymorphisms due to low incidence and transmission of pathogens in marine environments [elephant seal (*Mirounga leonina*), sei whale (*Balaenoptera borealis*) and fin whale (*B. physalus*): Trowsdale et al. 1989; Slade 1992; but see Hoelzel et al. 1999], (2) nonadaptive loss due to reduced geographic range and small and declining population size (bottleneck effects) [cheetah (*Aconyx jubatus*), Asiatic lions (*Panthera leo persica*), Helgoland Island mice (*Mus musculus*), Scandinavian beaver (*Castor fiber*), moose (*Alces alces*), island population of desert bighorn sheep (*O. canadensis mexicana*): O'Brien et al. 1985; Figueroa et al. 1986; O'Brien and Everman 1988; Yuhki and O'Brien 1990; Ellegren et al. 1993, 1996; Hedrick et al. 2001], (3) solitary or isolated life styles which cause a reduced lateral transfer of pathogens and therefore selection pressure [Swedish moose (*A. alces*), Syrian hamsters (*Mesocricetus auratus*): McGuire et al. 1985; Ellegren et al. 1996] and (4) reduced polymorphism as a consequence of the mating system combined with limited gene flow and limited gene pool (Sommer and Tichy 1999). Species with an obligate monogamous mating system might be subject to different constraints on mate choice driven by different selection pressures than polygynous or promiscuous species. Genetic compatibility, mate choice and patterns of parentage and the role of MHC have been reviewed by Tregenza and Wedell (2000).

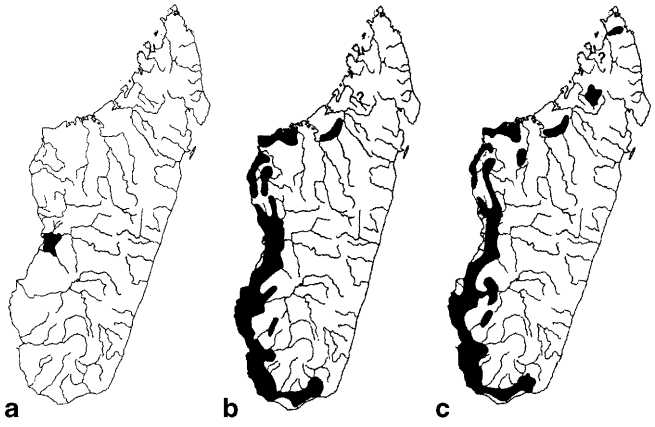

Fig. 1 Geographic range of *Hypogeomys antimena* (**a**), *Macrotarsomys bastardi* (**b**) and *Eliurus myoxinus* (**c**) on Madagascar (modified from Garbutt 1999)

Recent investigations in the levels of polymorphism of the MHC class II gene *DQA* of *Hypogeomys antimena* (Grandidier 1869), the Malagasy giant jumping rat, indicated that this highly endangered endemic rodent species has a very low variability in the amplified gene segment compared with other mammalian species. Only two alleles, two homozygous and one heterozygous genotype, were found in about 200 individuals sampled over the entire actual geographic range (Sommer and Tichy 1999). Palaeontological evidence indicates that only 1,400 years ago, *H. antimena* had a broad geographic range on the island extending from the central highlands to the south. The geographic distribution of *H. antimena* has been reduced recently to a declining fragmented area of suitable habitat within a geographic range of less than 20×40 km, north of the town of Morondava near the west coast of the island (Goodman and Rakotondravony 1996; Sommer and Hommen 2000; Ganzhorn et al. 2001; S. Sommer, A. Toto Volahy, U.S. Seal, unpublished data) (Fig. 1). The species is currently considered as "endangered" according to IUCN criteria but was proposed for upgrading to "critically endangered" in May 2001 (Sommer and Seal 2001).

H. antimena has some unusual life history characteristics for a rodent species, such as living in obligate monogamy. Pair bonds normally last until one mate dies (Sommer 1997, 1998). Male parental assistance is necessary for protecting offspring against predators and successfully rearing offspring. Solitary parents cannot successfully rear an offspring (Sommer 2000, unpublished data). Both sexes are of the same size with a head/body length of about 30 cm and weigh about 1.2 kg. They defend an exclusive territory throughout the year (Sommer 1996, 1997). The reproduction rate is very low: each couple has only one to two offspring per year which are born during the rainy season (December–April) (Sommer 2001). In *Hypogeomys*, confidence of paternity seems to be very high. Only two extrapair young were found in genetic analyses of 48 parent-offspring trios (4% EPP), indicating that *H. antimena* lives not only in socially obligate but also in reproductive monogamy (Sommer and Tichy 1999; S. Sommer, unpub-

lished data). The dispersal distances of offspring are very short. Male offspring move about two territories, female offspring only one territory from the parental territory to the area of own reproduction. Adult migration is very rare. Due to the lifelong mating association, combined with high fidelity and limited gene flow, the gene pool of *Hypogeomys* can be considered as rather constant.

Declines in geographic range and therefore population size and constraints placed by the mating system are often reflected in reduced genetic variability of a population. As knowledge about the original genetic diversity in *H. antimena* is not available, claims about what has been lost cannot be made and comparative studies are necessary to investigate hypotheses explaining the unusual low MHC polymorphism. In the modern range of *H. antimena*, two other endemic rodent species (*Macrotarsomys bastardi*, *Eliurus myoxinus*) occur which also belong to the endemic subfamily Nesomyinae of the family Muridae (Musser and Carleton 1993). In contrast to *H. antimena*, both *M. bastardi* and *E. myoxinus* are widely distributed in the western deciduous forests and in the south-western and southern spiny forests (Fig. 1).

M. bastardi (Milne-Edwards and Grandidier 1898), the western forest mouse, a small gerbil-like mouse, is the smallest member of the subfamily Nesomyinae. Both sexes are similar in size. The head/body length is 80–100 mm, the tail length 100–140 mm and it weighs about 34 g. The life history characteristics of the western forest mouse are quite similar to those of *H. antimena.* It is strictly nocturnal, terrestrial and feeds on seeds, fruits, berries, roots and some plant stems. It also lives in pairs and spends the day in long burrows (up to 1.5 m) that are excavated under large rocks or tree stumps. Home ranges of males and female are similarly sized. Reproductive activity peaks in April and May (but probably occurs throughout the year), and a litter of two or three individuals is born after a gestation period of around 24 days (Petter 1972; Goodman et al. in press; D. Schwab, unpublished data).

E. myoxinus (Milne-Edwards 1885), the western tuft-tailed rat, is a promiscuous species. Males move more than females and have larger home ranges overlapping with several female home ranges. The western tuft-tailed rat has a head/body length of 125–135 mm, a tail length of 140–145 mm and weighs about 80 g. *E. myoxinus* is a more arboreal species and is only occasionally found on the ground. It nests in tree holes. A gestation period of 24 days has been reported (Petter 1972; Goodman et al. in press; D. Schwab, unpublished data).

To investigate hypotheses explaining the unusual low MHC polymorphism, we compared the MHC variability of these three endemic rodent species with respect to their geographic range and social system by using the methods of direct sequencing and single-strand conformation polymorphism (SSCP). SSCP is one of the most sensitive methods for detecting nucleotide substitutions quickly (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. SSCP analysis relies on the fact that the mobility of a single-stranded DNA molecule in a non-denaturing gel is not only determined by its size, but also by its nucleotide sequence, which governs its three-dimensional structure (Orita et al. 1989).

Reduced selective pressure due to low incidence and transmission of pathogens in the marine environment (hypothesis 1) is not applicable for terrestrial mammals to explain low polymorphism. Solitary or isolated life styles which cause a reduced lateral transfer of pathogens and therefore selection pressure (hypothesis 3) is very unlikely because the individuals of all three species live neither isolated nor solitary lives and almost all captured individuals had parasites (D. Schwab, unpublished data; S. Sommer, unpublished data). In addition, family members of *H. antimena* spend the day in close proximity in burrow systems. If the low variability in *H. antimena* is due to the reduced geographic range and small and declining population size (bottleneck effects: hypothesis 2), both the widely distributed species, *M. bastardi* and *E. myoxinus*, should have a higher degree of polymorphism. However, if constraints and selection pressures associated with the monogamous mating system contribute to the low genetic variability in *H. antimena* (mating system: hypothesis 4), similar levels of variability are expected in the pair-living *M. bastardi*.

Materials and methods

Study site and sampling

Field studies to investigate the biology, population ecology, demography and social behaviour of *H. antimena*, *M. bastardi* and *E. myoxinus* were carried out in the 12,500-ha forestry concession of the Centre de Formation de Morondava (CFPF) in the Kirindy Forest/CFPF (20°03′ S, 44°39′ E) at the research station of the German Primate Center (DPZ, Göttingen, Germany), Western Madagascar. The Kirindy forest is the largest remaining forest block of dry deciduous forest within the geographic range of *H. antimena* (Sommer et al., unpublished data). A detailed description of the area is given in Ganzhorn and Sorg (1996).

H. antimena was live-trapped using Tomahawk Live Traps (51×19×19 cm) in front of their burrow holes. The two smaller species were captured with Sherman Traps (7.7×7.7×30.5 cm; Sherman Traps Inc., Tallahassee, Fla.) on a regular grid system (50-m intervals) during their nocturnal activity period. 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 (Sommer 1997). 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 wound stopped bleeding within a few minutes and was completely healed after 3–5 days. Animals were released at their respective trapping sites.

Since 1992, 180 different individuals of *H. antimena* and 75 different individuals of *E. myoxinus* (since 1995) have been sampled. Trapping of *M. bastardi* was more difficult though they were observed in the forest frequently. Only 22 individuals could be trapped. Therefore, we restricted the statistical analyses of the diversity indices of *H. antimena* and *E. myoxinus* to random subsamples (44 non-related individuals), though the statistical software package used is able to account for unbalanced sample design. Furthermore, the number of alleles found in the entire sample of *H. antimena* was not higher than in the random subsample (Sommer and Tichy 1999, unpublished data). Genetic analysis did

not indicate any signs of sample separation and therefore population differentiation within the Kirindy Forest in either species (Schmidt 2001; S. Sommer, unpublished data).

Polymerase chain reaction amplification of the MHC class II gene

All DNA samples were extracted using a QIAamp Tissue Kit, following the "Experienced User Protocol for Mouse Tails" (Qiagen, Hilden, Germany). Intensive testing of several primer pairs designed to conserved regions of the MHC were carried out in the beginning to find primers that amplify variable regions of the same loci in a wide range of species. Genetic variability analysis using universal primers allows for better interpretations of different levels of variability found in diverse taxa. Primers to amplify variable regions of the MHC class II gene *DQA* gene were: SiSo1 (up) 5′-ATTAGGCAGGATCCCAGAACACT-3′ and SiSo2 (dn) 5′-ATATCAGCCACCATGCAGATGA-3′ (Sommer and Tichy 1999) (syntheses: Pharmacia, Freiburg and ARK Scientific, Darmstadt, Germany). They amplify segments of intron 2 and exon 3, coding for the second extracellular domain (α2). The exact location within the *DQA* gene is given in Sommer and Tichy (1999).

Primers to amplify the MHC class II gene *DRB*, exon 2, the most important part of the antigen-binding site were: GH46 (up) 5′-CCGGATCCTTCGTGTCCCCACAGCACG-3′ and GH50 (down) 5′-CTCCCCAACCCCGTAGTTGTGTCTGCA-3′ (Erlich and Bugawan 1990).

The 25-µl reactions contained 1 µl of extracted genomic DNA, 5 mM KCl, 1 mM Tris-HCl (10×buffer: Q BIOgene), 0.2 mM dNTPs (Q BIOgene), 0.5 µM of each primer and 2.5 U Taq-Polymerase (Q BIOgene). Cycling conditions consisted of 35 rounds of 60 s denaturation at 92°C, 60 s annealing at 57–58°C, and 60 s extension at 72°C. A final 10-min extension at 70°C followed the last cycle. PCR was performed on a T-Gradient Thermocycler 96 (Biometra, Göttingen, Germany). For verification of successful amplification, 5 µl of PCR product was visualised in ethidium-bromide-stained 1.5% agarose gels.

Single-strand conformation polymorphism

Polymerase chain reaction (PCR) products were subjected to SSCP analysis. Six microlitres of PCR product was mixed with 10 µl of denaturing loading dye (prepared following the ETC manual; ETC Elektrophoresetechnik, Kirchentellinsfurt, Germany), denatured for 5 min at 95°C, and immediately chilled on ice before loading 6 µl of the mixture on the SSCP gel. Polyacrylamide gels (15%; CleanGel DNA-HP; ETC Elektrophoresetechnik) were prepared following the manufacturer's manual and run on a horizontal cooling electrophoresis system (Amersham Pharmacia Biotech, Freiburg, Germany). Temperature, power and acrylamide concentration affected the running time and had to be optimised. Maximum separation was reached at constant conditions: 200 V, 20 mA, 10 W for 15 min followed by 450 V, 30 mA, 20 W for 4.5 h at 12°C. After separation, the gels were fixed and silver-stained (DNA Plus One Silver Staining Kit; Amersham Pharmacia Biotech). Samples were analysed at least twice. In addition to new samples, all known alleles were run as references 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.

Statistical treatment

For statistical analysis of the genetic variability indices of the three species, the population genetics package Arlequin, Ver 2.000 was used (Schneider et al. 2000). Deviation from Hardy-Weinberg equilibrium was examined through a Markov chain approach which estimates the outcome of an exact test in order to test the null hypotheses (H_o) of "random union of gametes" (Guo and Thompson 1992). Chakraborty's test of population amalgamation (Chakraborty 1990, 1991) was used to test for selective neutrality and population homogeneity and equilibrium. The test contrasts the observed total number of alleles (k) with its expectation based on a Poisson distribution and gives an approximation of the conditional probabilities of observing some number of alleles given the observed homozygosity.

Results

With universal primers designed to amplify regions within the MHC *DQA* and *DRB* gene in diverse mammals,

Table 1 Observed (k) and expected (exp) number of alleles (calculated after Chakraborty 1990), the observed and expected heterozygosities (H), gene diversities and sample sizes at the *DQA* and *DRB* locus for the different species. The number of alleles with a frequency >5% is given in parentheses. Deviations from the Hardy-Weinberg proportions are indicated (*n.s.* not significant, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$)

	Hypogeomys antimena	*Macrotarsomys bastardi*	*Eliurus myoxinus*
Social system	Monogamous	Monogamous	Promiscuous
Distribution	<20×40 km²	Widely distributed	Widely distributed
MHC *DQA* locus			
Number of alleles (k)	2 (2)	3 (3)	11 (7)
Number of alleles$_{exp}$	3.62	2.95	14.75
P (k or more alleles)	0.94	0.60	0.92
H_{obs}	0.43	0.45	0.70
H_{exp}	0.65 n.s.	0.41 n.s.	0.86**
Gene diversity	0.45±0.03	0.41±0.08	0.85±0.02
n	44	22	44
MHC *DRB* locus			
Number of alleles (k)	5 (4)	6 (4)	9 (8)
Number of alleles$_{exp}$	5.92	4.08	12.77
P (k or more alleles)	0.75	0.18	0.95
H_{obs}	0.57	0.68	0.69
H_{exp}	0.75 n.s.	0.71 n.s.	0.89***
Gene diversity	0.63±0.04	0.55±0.08	0.86±0.02
n	42	22	26

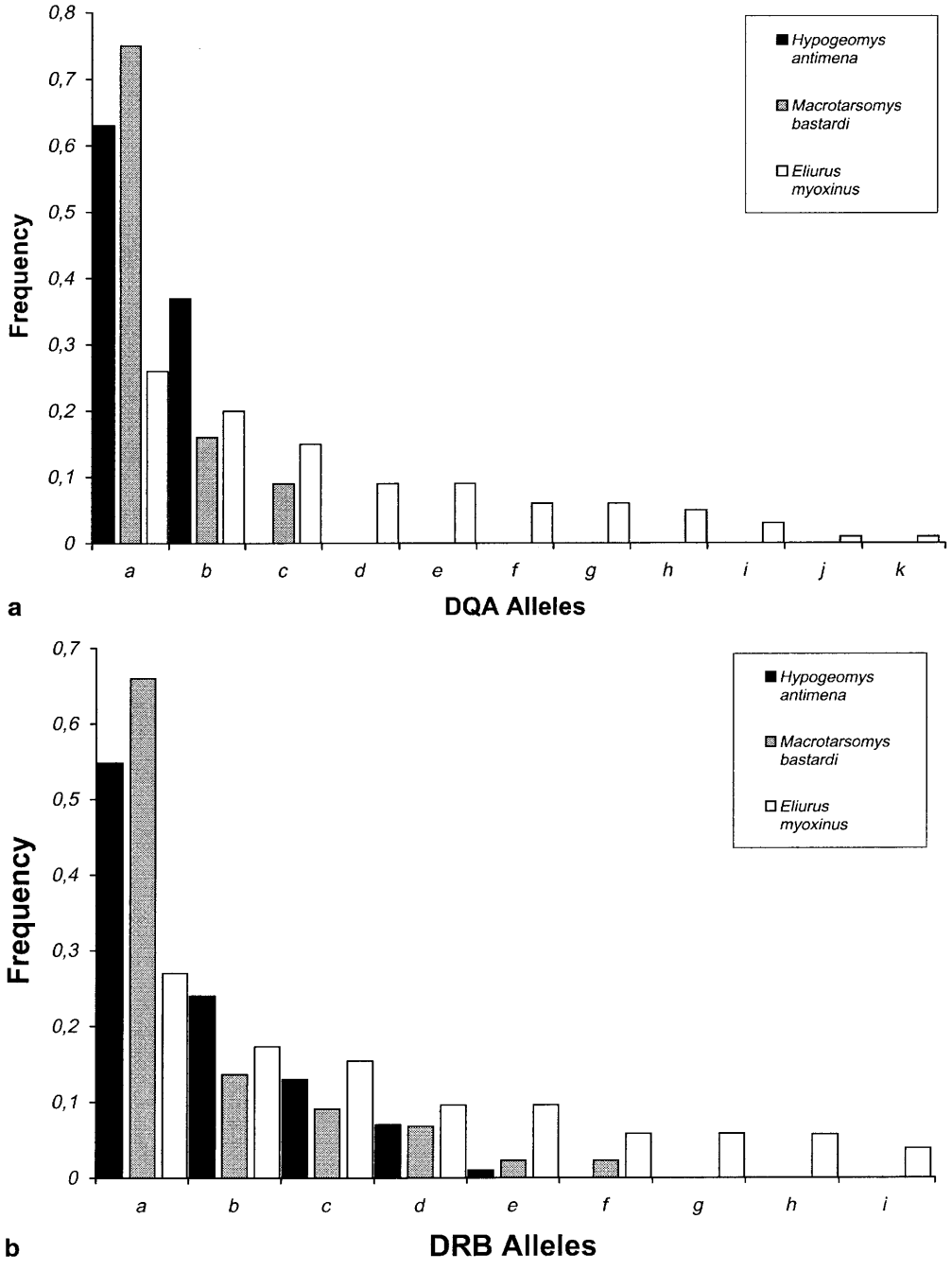

Fig. 2 Allele frequencies of MHC class II *DQA* (**a**) and of MHC class II *DRB* (**b**) of *H. antimena*, *M. bastardi* and *E. myoxinus*

clean amplifications were obtained in all three species. PCR products of the same loci had the same size (*DQA*: ca 200 bp, *DRB* 220 bp) and were in the expected range. About one-third of the investigated *E. myoxinus* samples had multiple SSCP bands in the *DRB* locus. The presence of more than two alleles per individual indicated the presence of an additional *DRB* locus in some individuals which is not unusual in a multigene family such as the MHC (Klein 1986). For comparison, only individuals with the same single *DRB* locus screened as in the two other species were included in the analyses.

The analyses of *DQA* and the *DRB* indicated different levels of genetic variability in the three sympatric rodent species. The differences can be related to the different social systems (Table 1). Whereas the investigated MHC class II genes *DQA* and *DRB* had low levels of polymorphism in the pair-living species *H. antimena* and *M. bastardi* (*DQA*: 2 and 3 alleles, *DRB*: 5 and 6 alleles, respectively), higher levels of variation were recorded in the promiscuous *E. myoxinus* (*DQA*: 11 alleles, *DRB*: 9 alleles). The same results were obtained if rare alleles, with a frequency of less than 5%, were excluded from the analyses (Table 1).

In *M. bastardi*, the expected number of *DQA* alleles (2.95 alleles) calculated from the observed homozygosity (Chakraborty 1990, 1991) was similar to the observed

number (3 alleles). The expected number of *DRB* alleles (4.08 alleles) were even lower than the observed number of alleles (6 alleles) but was similar to the number of frequent alleles (4 alleles, >5% frequency). In *H. antimena*, the expected number of *DQA* (3.62 alleles) and *DRB* alleles (5.92 alleles) was higher than the observed allele numbers (*DQA*: 2, *DRB*: 5 alleles). The number of alleles remained low even when considering all samples of *Hypogeomys* (Kirindy/CFPF: 180 samples; entire remaining geographic range: 236 samples; Sommer and Tichy 1999, unpublished data). In the promiscuous species, *E. myoxinus*, where the highest number of *DQA* and *DRB* alleles were observed, the expected numbers of alleles were even higher (*DQA*: 14.75, *DRB*: 12.77 alleles) (Table 1). The frequencies of the MHC class II *DQA* and *DRB* alleles found with SSCP analyses are shown in Fig. 2.

The gene diversity, defined as the probability that two randomly chosen alleles are different in the sample (Nei 1987), was higher in *E. myoxinus* (*DQA*: 0.85, *DRB*: 0.86) than in the two pair-living species (*DQA*: 0.41–0.45, *DRB*: 0.55–0.63). Furthermore, the observed heterozygosity levels of the *DQA* gene in *H. antimena* and *M. bastardi* were much lower than in *E. myoxinus* (43–45% versus 70%; Table 1). The observed differences in the heterozygosity levels of the *DRB* gene were not as pronounced as in *DQA*. This might be due to the deficit in heterozygosity which was highly significant for both gene segments in *E. myoxinus*. If the expected heterozygosity levels of the *DQA* and *DRB* gene were considered, higher levels are expected in the promiscuous than in the monogamous species.

Discussion

Understanding the relative contribution of different forces influencing genetic variation is a prime goal in population and conservation genetics, behavioural ecology and sociobiology. In general, the effects of genetic drift, gene flow and inbreeding on levels of genetic variability and the ability to maintain genetic variation in natural populations are related to the number of individuals constituting a population. However, a species' life history, breeding system and dispersal patterns can also affect its genetic characteristics (e.g. Sugg et al. 1996; Balloux et al. 1998; Storz 1999). Furthermore, the presence of selection processes and heterozygosity advantage might retard the rate of allele fixation (Robertson 1962; Nevo et al. 1997). Finally, balancing selection (Hughes and Yeager 1998; reviewed in Richman 2000) and reproductive mechanisms (reviewed in Jordan and Bruford 1998; Penn and Potts 1999) which are thought to act at MHC loci have been suggested to counteract the effects of genetic drift by maintaining polymorphism within populations.

Despite the usually abundant MHC diversity found in most natural mammal populations (Klein 1986; Potts and Wakeland 1990; Nei and Hughes 1991; Hedrick 1994), there are a number of species in which MHC genes exhibit low or no detectable polymorphism. The hypotheses proposed to explain limited MHC polymorphism consider reduced selection specifically at MHC loci on the one hand, and the influence of drift, gene flow and inbreeding on the other. In the present study, we assessed the effects of processes associated with reduced population size and different mating systems on the levels of polymorphism in the MHC class II *DQA* and *DRB* genes in three sympatric Malagasy rodent species differing in their geographic range and social system.

Whereas the investigated MHC class II genes *DQA* and *DRB* had a lower number of alleles in the pair-living species *H. antimena* and *M. bastardi* (*DQA*: 2 and 3 alleles; *DRB*: 5 and 6 alleles, respectively), higher allele numbers (*DQA*: 11 alleles, *DRB*: 9 alleles) were recorded in the promiscuous species *E. myoxinus*. As was to be expected, the differences were more pronounced in the *DQA* than in the *DRB* gene. The investigated exon 2 of *DRB* has been suggested to be one of the most important parts of the MHC. It codes for the antigen-binding site. Peptides are bound as straight, extended chains that project out of both ends of the site (Brown et al. 1993). Thus, it might be under higher selection pressure for maintenance of polymorphism than other parts of the MHC. Furthermore, all investigated diversity indices revealed higher levels of MHC variability in the promiscuous *E. myoxinus*, than in the pair-living species *M. bastardi* and *H. antimena*. Unfortunately, no comparative data are yet available for the MHC variability of other taxa in relation to different mating systems.

The differences in the MHC variability of *E. myoxinus* and *M. bastardi*, two species with broad geographic ranges, indicate that bottleneck effects (hypothesis 2) cannot be the only explanation for the reduced polymorphism in *H. antimena*. The results support the hypothesis that low MHC variability is not only influenced by the effects of genetic drift and inbreeding due to recent declines in population size, but also by the monogamous mating system (hypothesis 4; Sommer and Tichy 1999).

Social monogamy is common in birds but rare among mammals. Less than 3% of all mammal species live in this puzzling mating system which seems to contradict sexual selection theory (Darwin 1871; Kleiman 1977). Obligate social monogamy is usually associated with extensive male parental care (Kleiman and Malcolm 1981), and is thought to have evolved because male care is required for successful rearing of offspring (Clutton-Brock 1989; but see Komers 1996). In *Hypogeomys*, males are required for successful rearing of the offspring (Sommer 2000, unpublished data). The occurrence of young sired outside the observed pair bond, a behavioural strategy quite common in monogamous birds and thought to increase gene flow and genetic variability (Birkhead and Møller 1992), is very rare in this rodent species (Sommer and Tichy 1999; S. Sommer, unpublished data). Unfortunately, no comparative long-term data on the extent of pair-bonding and genetic monogamy in *M. bastardi* are available yet.

Different constraints on mate choice driven by different selection pressures might be found in species with an obligate monogamous mating system than in polygynous or promiscuous species. Females are generally choosier than males (reviewed by Tregenza and Wedell 2000). Monogamous females depending on male parental care might be subject to additional selection pressures for female choice compared to other mating systems. To test this idea, studies on MHC-based mate choice in relation to different mating system are required.

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