

Chapter 4.11

Forest Fragmentation Effects on Functional Genes: Immune Gene Variability (MHC) of *Microcebus murinus* and *Rattus rattus* in the Mandena Forest

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Abstract

Important aspects of ecological monitoring include investigations of changes in the genetic constitution of animals in forest fragments. Habitat fragmentation generates barriers to gene flow by isolating sub-populations, often resulting in a loss of genetic diversity with possible negative effects on fitness parameters. In vertebrates, growing evidence suggests that such genetic diversity is particularly important at the level of the major histocompatibility complex (MHC), because its gene products play an important role in immune functions. Genetic diversity in the MHC is assumed to improve population viability. Here, the genetic variability of the functionally important MHC gene DRB (exon 2) of the endemic lemur *Microcebus murinus*, and the introduced rodent *Rattus rattus* in the littoral forests of southeastern Madagascar is investigated in relation to forest fragmentation. Fourteen different alleles of DRB exon 2 were found in 228 individuals of *M. murinus*, and 13 alleles were found in 58 *R. rattus* samples. Both species indicated high levels of sequence divergence between alleles. Only limited effects of fragmentation on the number of alleles and the gene diversity were observed, but allele frequencies differed in the fragments. Whereas in *M. murinus* F_{ST} -statistics indicated a small but highly significant effect of fragmentation on population differentiation, no such effect was observed in *R. rattus*. This is probably the outcome of the different dispersal abilities of the two species. In both species, significantly more non-synonymous than synonymous substitutions were found in the functionally important antigen recognition and binding sites, indicating selection processes involved which seem to be able to maintain MHC polymorphism in the fragmented populations of *M. murinus* under current habitat conditions.

Résumé

Effets de la fragmentation forestière sur les gènes codants: variabilité des gènes immunes (CMH) de *Microcebus murinus* et *Rattus rattus* dans les fragments forestiers de Mandena. Dans un programme de suivi, il est important de procéder à des investigations sur les changements dans la constitution génétique des animaux rencontrés dans des fragments forestiers. La fragmentation forestière est à l'origine de barrières qui perturbent le flux génétique en isolant des sous populations et se traduit souvent par une perte de la diversité génétique avec d'éventuels effets négatifs sur des paramètres de santé. Chez les vertébrés, il ressort de plus en plus qu'une telle diversité génétique est plus particulièrement importante au niveau du complexe majeur d'histocompatibilité (CMH) car ces gènes jouent un rôle important dans les fonctions immunitaires. On admet que la diversité génétique du CMH augmente la viabilité d'une population. Dans notre cas, la diversité génétique du gène codant DRB (exon 2) qui est important dans le CMH est retenue dans nos analyses de fragmentation forestière pour le lémurien endémique *Microcebus murinus* et le rongeur allogène *Rattus rattus* dans la forêt littorale du sud-est de Madagascar. Quatorze allèles distincts du DRB exon 2 ont été trouvés chez 228 individus de *M. murinus* et 13 allèles distincts dans 58 échantillons de *R. rattus*. Les deux espèces montraient d'importants niveaux de divergence dans les séquences entre les allèles. Les effets de fragmentation n'ont été observés qu'à un niveau limité aussi bien quant au nombre d'allèles qu'en matière de diversité génétique mais les fréquences des allèles différaient dans

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les fragments. Les statistiques F_{ST} calculées pour *M. murinus* indiquaient un niveau faible mais significatif d'effet de la fragmentation sur la différenciation de la population mais aucun effet n'est ressorti pour *R. rattus*. Il s'agit vraisemblablement d'une réponse des différentes capacités de dispersion de ces deux mammifères. Pour les deux espèces, les substitutions non-synonymes étaient sensiblement plus importantes que les substitutions synonymes dans la reconnaissance et la recombinaison des antigènes fonctionnels importants, indiquant ainsi que les processus de sélection impliqués semblent être capables de maintenir le polymorphisme de la CHM dans les populations fragmentées de *M. murinus* dans les conditions actuelles de l'habitat.

Introduction

Many natural populations are threatened not only by a dramatic reduction in total area of available habitat, but also by increasing habitat fragmentation and degradation. This leads to declining population sizes and barriers to gene flow if exchange of individuals between sub-populations is restricted (Wahlberg *et al.* 1996, Meffe and Carroll 1997). Small populations often suffer from reduction of genetic diversity due to genetic drift and inbreeding effects (Dudash and Fenster 2000, Keller and Waller 2002). The loss of genetic variation can lead to the short-term reduction of fitness components such as survival, reproductive output, growth rates, and impaired ability to adapt to long-term changes in the environment (e.g., Lande 1988, Primack 1993, Lacy 1997, Frankham and Ralls 1998). An increasing number of studies indicate that host genetic diversity plays an important role in buffering populations against pathogens and widespread epidemics (Meagher 1999, Cassinello *et al.* 2001, Altizer *et al.* 2003, Spielman *et al.* 2004). Study of the genetic effects of population fragmentation is therefore of central importance for conservation biology (Frankham *et al.* 2002).

Genetic studies of wild animals often employ neutral markers, such as mitochondrial DNA (mtDNA) or microsatellites, to estimate the amount of variation present in both individuals and populations (Avice 2000). However, variation at neutral loci cannot provide direct information on selective processes involving the interaction of individuals with their environment or on the capacity for future adaptive changes (Meyers and Bull 2002), both of which are issues of particular relevance for conser-

vation. In some cases, the time span between the separations of populations might even be too short to leave a signal at neutral loci so that differences between populations are only detectable at genes under selection (Cohen 2002), such as those of the major histocompatibility complex (MHC).

Genes of the MHC encode molecules responsible for the recognition and presentation of foreign antigens in vertebrate genomes. They are the most polymorphic loci in the vertebrate nuclear genome (Klein 1986). The variability of MHC genes is an indicator of parasite and pathogen resistance, which in turn, may influence the long-term survival probability of populations. Especially the region of the molecule responsible for binding antigens, the so-called antigen binding site (ABS, e.g., exon 2 of the DRB gene), shows 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). In particular, the ABS sites display more non-synonymous than synonymous substitutions, which changes the amino acid sequence of the peptide and allows binding of a diverse array of antigens (Brown *et al.* 1988, 1993). Such observations lend strong empirical support to the action of selection processes in the maintenance of diversity at MHC loci. Pathogen pressure is presumed to be the selective force promoting MHC diversity (Sommer 2005). Associations could be demonstrated between pathogen resistance and specific MHC alleles (Harf and Sommer 2005, Froeschke and Sommer 2005, Meyer-Lucht and Sommer 2005, Schad *et al.* 2005), as well as higher parasite resistance of heterozygous relative to homozygous individuals (Froeschke and Sommer 2005). More recently, reproductive mechanisms such as disassortative mating, maternal-fetal interactions, and the use of MHC alleles as olfactory-based kin recognition markers to avoid inbreeding have been suggested as alternative mechanisms maintaining MHC diversity (Sommer 2005). These properties make genes of the MHC among the best candidates for molecular adaptation in vertebrates (Hedrick 1994).

Among the different forest ecosystems in Madagascar, the littoral forests growing on sand along the eastern coast are of particular conservation concern, as they belong to one of the most threatened forest formations of Madagascar, and have only a few forest islands remaining (Ganzhorn *et al.* 2001). The littoral forest in southeastern Madagascar is

especially threatened by degradation, fragmentation, and isolation caused by the expanding human population. In this study, an endemic small lemur (*Microcebus murinus*) and an introduced rodent (*Rattus rattus*) living in littoral forest fragments of southeastern Madagascar were used as model organisms to monitor the effects of fragmentation on immune gene variability (MHC).

The specific aims of this study were to investigate the genetic variability of functionally important MHC genes in *M. murinus* and *R. rattus*, to consider the ratio of synonymous to non-synonymous nucleotide substitutions within and outside the antigen binding site (ABS) for evidence of selection processes, and to compare the impact of forest fragmentation on the MHC constitution and population differentiation of the two species.

Methods

Study species, study site, and DNA sampling

Microcebus murinus is a small (average 60 g), nocturnal, and omnivorous lemur. This arboreal species is widely distributed throughout western, southern, and southeastern Madagascar. It is quite adaptable and occurs not only in largely intact forest but also in degraded and secondary vegetation. Negative effects of habitat fragmentation and degradation on population dynamics of *M. murinus* have been investigated (Ganzhorn and Schmid 1998, Rüdell 2004).

Rattus rattus has an average adult weight of about 100 g in the study area (Ramanamanjato and Ganzhorn 2001). This species was introduced to Madagascar, perhaps as early as the 11th century, and often lives as a human commensal. Subsequent to its introduction, this species spread into the most remote parts of native forests. It can be captured on the ground, as well as in trees (Goodman 1995, Goodman *et al.* 1997).

In the present study, four littoral forest remnants in Mandena were chosen and are indicated as M4/5 (69 ha), M13 (80 ha), M15/16 (188 ha), and M20 (42 ha) (see Figure 5, Vincelette *et al.* Chapter 2.4). The fragments of M4/5 and M15/16 were combined because they are connected by an introduced Australian tree species, *Melaleuca*, which can be used by both study species to disperse between the fragments. Deforested areas on dry sandy soils between the fragments are covered by heath-type vegetation consisting predominantly of *Erica* sp.

Endemic rodent and lemur species have not been recorded in this habitat, but *R. rattus* has been trapped in this vegetation type (Ramanamanjato and Ganzhorn 2001).

For genetic analysis, 228 tissue samples of *M. murinus* (M4/5: n = 37, M13: n = 29, M15/16: n = 136, M20: n = 26) (Schad *et al.* 2004, 2005) and 58 samples of *R. rattus* (M4/5: n = 8, M15/16: n = 38, M20: n = 12) were collected from live, but anaesthetized animals that were trapped in permanent plots between July 1998 and October 2003. A detailed description of site characteristics and trapping procedure are given in Ramanamanjato and Ganzhorn (2001).

Genetic analyses

The genetic analyses have been described previously (Schad *et al.* 2004, 2005). In summary, the PCR amplified MHC DRB exon 2 fragments were subjected to a highly efficient electrophoresis screening technique (SSCP). Individuals can be genotyped, and homozygous and heterozygous animals can be distinguished. At least three examples of all identified alleles were cut from the SSCP gel and re-amplified by PCR prior to cycle sequencing.

Statistical treatment

Allele frequencies and gene diversity were calculated using Arlequin, version 2.000 (Schneider *et al.* 2000). The gene diversity is the probability that two randomly chosen alleles of a sample are different (equivalent to expected heterozygosity, Nei 1987). MEGA3 was used to investigate evidence for balancing selection (Kumar *et al.* 2004). The relative rate of non-synonymous and synonymous substitutions was calculated in the functionally important antigen binding sites (ABS) and outside the antigen binding sites (non-ABS) according to Nei and Gojobori (1986), applying the correction of Jukes and Cantor (1969) for multiple hits. The extent of population subdivision as an effect of forest fragmentation was examined by pairwise F_{ST} (10000 permutations, Wright 1965) using the software package Arlequin, version 2.000 (Schneider *et al.* 2000). Bonferroni corrected significance levels were used for multiple comparisons (Rice 1989, Sachs 1992).

A *Microcebus murinus*

Mimu-1	S	D	V	G	E	Y	R	A	V	T	E	L	G	R	P	D	A	E	Y	W
Mimu-2
Mimu-3	F
Mimu-3	F	Q	I	.	.	.	L
Mimu-5	F	S	.	.	S	.
Mimu-6	I	.	.	.	L
Mimu-7	F	I
Mimu-8	L	.	.	.	P	K	.	.
Mimu-9	I	.	.	.	L
Mimu-10	S
Mimu-12	E	V	G	L	Y	I	A	V	T	E	L	G	R	P	V	A	E	D	R	N
Mimu-13	K	F	L
Mimu-14	F
Mimu-16	L

Position	65	68	70	71	74	78
ABS	*	*	*	*	*	*
Mimu-1	A	A	C	C	G	C
Mimu-2	.	A	.	G	.	.
Mimu-3	.	A	.	G	.	.
Mimu-4
Mimu-5
Mimu-6
Mimu-7
Mimu-8
Mimu-9
Mimu-10
Mimu-12
Mimu-13
Mimu-14
Mimu-16
Mimu-1	N	R	Q	Q	D	I
Mimu-2	.	S	R	.	.	M
Mimu-3	.	S	R	.	.	L
Mimu-4	F	M
Mimu-5	.	.	.	K	F	M
Mimu-6	.	.	.	K	F	M
Mimu-7	.	.	.	K	F	M
Mimu-8	.	S	.	.	.	V
Mimu-9	M
Mimu-10	F	M
Mimu-12	S	Q	K	E	T	L
Mimu-13	.	S	.	K	E	.
Mimu-14	.	S	.	K	E	.
Mimu-16	V

Results

MHC-DRB polymorphism

In 228 individual *Microcebus*, 14 different alleles (*Mimu*-DRB*1 to *Mimu*-DRB*10, *Mimu*-DRB*12 to *Mimu*-DRB*14, *Mimu*-DRB*16) could be resolved by SSCP analysis. Thirteen different alleles (*Rara*-DRB*1 to *Rara*-DRB*13) were found in 58 individual *Rattus* samples (Fig. 1). No more than two alleles were found in any individual, which suggests that in both species a single copy locus had been amplified. Comparisons with other MHC sequences available in GenBank confirmed that all amplified sequences are part of MHC DRB exon 2.

The 14 identified *Mimu*-DRB alleles were based on 71 (41.5%) variable nucleotide positions in a 171 bp sequence. The alleles showed high levels of divergence for an intra-specific comparison, with an average of 23 (13.5%) nucleotide differences (minimum: 9 substitutions, maximum: 45 substitutions) between alleles. All alleles had a unique amino acid sequence, and the absence of stop codons suggested that all sequences encoded functional proteins. The amino acid sequences revealed a high rate of non-synonymous substitutions. Fifty (87.7%) out of 57 amino acids were variable. The number of amino acid differences between alleles varied between 5 (8.8%) and 25 (43.9%) (average 14.3 / 25.1%) (Schad *et al.* 2004, 2005; Fig. 1).

In the 177 bp DRB sequences of *R. rattus*, 72 (40.7%) variable positions were identified. Also, *R. rattus* alleles showed high levels of sequence divergence. Pairwise comparison of nucleotide substitutions per site between alleles ranged from 5 to 47 (average 23.5 / 13.3%). The 13 unique amino acid sequences revealed 33 (55.9%) out of the 59 variable amino acid positions. The pairwise comparison of amino acid substitutions between alleles ranged from 3 (5.1%) to 26 (44.1%) (mean 13.8 / 23.4%; Fig. 1).

Evidence for balancing selection

In *Microcebus murinus*, 15 (88.2%) out of 17 sites predicted to be involved in antigen recognition (Brown *et al.* 1988, 1993) were variable, whereas 16 (40.0%) out of 40 non-ABS sites were polymorph. These variable non-ABS sites were usually located next to an ABS site. The rate of non-synonymous (d_N) and synonymous

(d_S) substitutions was estimated for both ABS and non-ABS amino acid positions (Table 1). For the antigen binding sites, d_N (0.385) is significantly greater than d_S (0.196) and the ratio is 1.96 (Z-test: $p = 0.006$). In the non-antigen binding sites, the ratio between non-synonymous ($d_N = 0.076$) and synonymous ($d_S = 0.128$) is smaller than unity ($d_N/d_S = 0.59$, Z-test: ns). D_N was 5.07 times higher in the antigen binding sites than in the non-antigen binding sites ($t = -18.52$, $p < 0.0001$) (Schad *et al.* 2004, 2005, Table 1).

In *R. rattus*, 14 (82.4%) out of 17 amino acid sites thought to be involved in antigen binding were variable, and 20 (47.6%) of the 42 non-antigen binding sites revealed intra-specific variability. In the ABS, the rate of non-synonymous substitutions (d_N) was 3.48 times higher than the rate of synonymous substitutions (d_S) (Z-test: $p = 0.004$), whereas in the non-ABS, the rate of non-synonymous substitutions did not exceed the rate of synonymous substitutions ($d_N/d_S = 0.82$, Z-test: ns; Table 1). These are clear indications that, in both species, selection processes are able to maintain polymorphism in the functionally important regions of the MHC.

Effects of forest fragmentation

Microcebus murinus populations of all four fragments revealed high amounts of genetic variation in the functionally important MHC DRB exon 2 locus, but the genetic diversity was lower in M4/5 (0.65) compared to the other fragments (genetic diversity: 0.82 - 0.84; Table 2). A small, but highly significant population differentiation was indicated between M4/5 and all other fragments (M4/5 vs. M13: $F_{ST} = 0.07$, $p < 0.001$; M4/5 vs. M15/16: $F_{ST} = 0.07$, $p < 0.001$; M4/5 vs. M20 $F_{ST} = 0.07$, $p < 0.001$, all Bonferroni significant). None of the F_{ST} values are significant once *Mimu*-DRB*3 is excluded from the analysis, which indicates that the population differentiation is caused by the absence of the common allele *Mimu*-DRB*3 in M4/5 (Schad *et al.* 2005; Table 2).

Although the sample size was considerably lower in *R. rattus*, high levels of genetic variation were also found in this species. In contrast to *M. murinus*, the gene diversity was even higher in M4/5 (0.91) than in the other fragments (gene diversity: 0.80 - 0.83). No significant population differentiation between the fragments was indicated (F_{ST} , $p > 0.05$; Table 2).

Table 2. Variability and allele frequencies of MHC class II DRB exon 2 in *Microcebus murinus* (A) (Schad *et al.* 2005) and *Rattus rattus* (B) from the Mandena forest; N = sample size.

A

Fragment	M4-5	M13	M15-16	M20	Total
N	37	29	136	26	228
Gene diversity	0.65 ± 0.06	0.82 ± 0.02	0.84 ± 0.01	0.84 ± 0.02	0.82 ± 0.01
<i>Mimu</i> -DRB*1	0.57	0.29	0.29	0.27	0.33
<i>Mimu</i> -DRB*2	0.12	0.19	0.11	0.21	0.12
<i>Mimu</i> -DRB*3	-	0.19	0.16	0.17	0.14
<i>Mimu</i> -DRB*4	0.03	-	0.01	-	0.01
<i>Mimu</i> -DRB*5	0.05	0.14	0.11	0.12	0.10
<i>Mimu</i> -DRB*6	0.10	0.10	0.12	0.10	0.11
<i>Mimu</i> -DRB*7	-	-	0.01	0.02	0.01
<i>Mimu</i> -DRB*8	-	-	0.01	-	0.01
<i>Mimu</i> -DRB*9	0.03	0.03	0.01	-	0.02
<i>Mimu</i> -DRB*10	0.05	0.05	0.05	0.10	0.06
<i>Mimu</i> -DRB*12	-	-	0.14	-	0.08
<i>Mimu</i> -DRB*13	0.03	-	-	-	0.004
<i>Mimu</i> -DRB*14	0.03	-	-	-	0.004
<i>Mimu</i> -DRB*16	-	-	-	0.02	0.002

B

Fragment	M4-5	M15-16	M20	Total
N	8	38	12	58
Gene diversity	0.91 ± 0.04	0.83 ± 0.02	0.80 ± 0.06	0.85 ± 0.02
<i>Rara</i> -DRB*1	-	0.13	0.04	0.09
<i>Rara</i> -DRB*2	0.13	0.30	0.25	0.27
<i>Rara</i> -DRB*3	0.13	0.16	-	0.12
<i>Rara</i> -DRB*4	0.13	0.11	0.04	0.09
<i>Rara</i> -DRB*5	0.19	0.18	0.38	0.22
<i>Rara</i> -DRB*6	-	0.03	-	0.02
<i>Rara</i> -DRB*7	-	0.03	0.13	0.04
<i>Rara</i> -DRB*8	0.19	0.03	0.08	0.06
<i>Rara</i> -DRB*9	0.13	-	-	0.02
<i>Rara</i> -DRB*10	-	0.03	-	0.02
<i>Rara</i> -DRB*11	0.13	-	-	0.02
<i>Rara</i> -DRB*12	-	0.01	-	0.01
<i>Rara</i> -DRB*13	-	-	0.08	0.02

Discussion

Habitat fragmentation generates barriers to gene flow by isolating sub-populations, often resulting in a loss of genetic diversity due to genetic drift and inbreeding with possible negative effects on fitness parameters (Bijlsma *et al.* 2000). In vertebrates, growing evidence suggests that such genetic diversity is particularly important at the level of the MHC because its gene products play an important role in immune functions. Diversity in the MHC is presumed to improve parasite resistance, and therefore, population viability. MHC variability is believed to be maintained by pathogen-driven selection, mediated either through heterozygous advantage or frequency dependent selection (Sommer 2005).

In this study, the genetic variability of the functionally important MHC gene DRB (exon 2) was investigated in the endemic lemur *Microcebus murinus* and the introduced *Rattus rattus* in relation to forest fragmentation in the littoral forests of southeastern Madagascar. Fourteen different alleles of DRB exon 2 were found in 228 individuals of *M. murinus* and 13 in 58 *R. rattus* samples. Few natural mammal species have been examined for genetic variation at the population level at any MHC locus (Sommer 2005). As a result, there is uncertainty as to what constitutes normal levels of MHC variation (Van den Bussche *et al.* 2002). In populations of three endemic Malagasy rodent species, *Macrotarsomys bastardi*, *Eliurus myoxinus*, and *Hypogeomys antimena*, five to nine DRB alleles were found (Sommer and Tichy 1999, Sommer *et al.* 2002, Sommer 2003). Twenty different MHC class II DRB exon 2 alleles (single DRB loci) were identified in 58 individuals of the mouse *Rhabdomys pumilio* living in the southern Kalahari under natural conditions (Froeschke and Sommer 2005), whereas in 40 sympatrically living gerbils of the species *Gerbillurus paeba*, 34 different DRB exon 2 alleles derived from two DRB loci were detected (Harf and Sommer 2005). In a mainland sample (n = 25) of the mouse *Peromyscus maniculatus*, 16 alleles were recovered (Richman *et al.* 2001). In 146 free-ranging mice of the species *Apodemus flavicollis*, 27 distinct DRB alleles were detected (Meyer-Lucht and Sommer 2005).

DRB locus diversity of primate species has been studied in green monkeys, *Cercopithecus aethiops* (n = 15 individuals, 7 alleles; Rosal-Sánchez *et al.* 1998), cotton-top tamarins, *Saguinus oedipus* (n =

13 captive individuals, 14 alleles; Gyllensten *et al.* 1994), common marmosets, *Callithrix jacchus* (n = 25 captive individuals, 13 alleles; Antunes *et al.* 1998), and owl monkeys, *Aotus nancymae* (n = 15, 14 alleles; Nino Vasquez *et al.* 2000). A recent study on MHC variability in wild gorillas (western gorilla n = 17, mountain gorilla n = 19) revealed 21 different DRB1 exon 2 sequences (Lukas *et al.* 2004). Given the large sample size of this study, DRB diversity of *Microcebus murinus* measured in number of alleles was rather moderate (14 alleles) compared to the primate examples listed above. However, the reported values of the captive populations might be artificially high if they are taken from individuals of different wild populations. In addition, the DRB region has experienced multiple gene duplications and acquired several loci in a number of species, and the orthologous relationships and nomenclature between simian and prosimian DRB genes is not always clear (Go *et al.* 2002). In our *M. murinus* study population in southeastern Madagascar, no individuals were found with more than two alleles, suggesting that only one DRB locus was amplified. However, two different DRB loci were identified in a *M. murinus* population on the western coast of Madagascar about 300 km northwest of Mandena (Schwensow *et al.*, unpubl. data). Differing numbers of DRB alleles in separate populations of the same species is not unusual (Hedrick *et al.* 2001). In addition, in a recent study of 66 lemurs comprising four families, eight genera, and 15 species, a maximum number of only two DRB genes were identified. Lemur DRB genes were clustered and separated from the other primate DRB genes (simians and non-Malagasy prosimians). This suggests that the DRB variations in extant lemur populations have been generated since the divergence of lemurs from other primates. It is postulated that the ancestors of lemurs went through a severe bottleneck when they left the African continent to colonized Madagascar (Go *et al.* 2002).

Only limited effects of fragmentation were observed on the number of alleles and the gene diversity, but allele frequencies differed in the fragments. Whereas, in *M. murinus* F_{ST} statistics indicated a small, but highly significant effect of fragmentation on population differentiation, no such effect was observed in *R. rattus*. This is probably the outcome of the different dispersal capabilities of the two species. In *M. murinus*, the genetic diversity was lower in fragment M4/5 (0.65) compared to the other

fragments (genetic diversity: 0.82 - 0.84). However, in *R. rattus*, the gene diversity was even higher in M4/5 (0.91) than in the other fragments (gene diversity: 0.80 - 0.83). *Rattus rattus* is able to migrate between fragments, even crossing dry and heath-type vegetation. Loss of genetic variation due to fragmentation is commonly observed at neutral or nearly neutral loci. In *M. murinus*, a reduced genetic variability with respect to fragment size was observed in microsatellite and mitochondrial markers in the same study populations (Hapke 2004). In addition, *R. rattus* revealed only five mitochondrial d-loop haplotypes in Mandena (n = 25) (Hingston *et al.* 2005). Balancing selection tends to counteract the effects of genetic drift, and to retard the rate of fixation of alleles. However, this effect on maintaining polymorphism within populations is dependent on the selection intensity and effective population size (Hughes and Yeager 1998). All alleles in both species had a unique amino acid sequence with high levels of sequence divergence between alleles. Polymorphism was highest in the functionally important antigen recognition and binding site (Hughes and Yeager 1998). Significantly more non-synonymous substitutions were found in these positions (Brown *et al.* 1988, 1993). This is considered a clear indication of the selection processes involved (Hughes and Nei 1988, 1989), and is characteristic of proteins with antigen-presenting function (Bergstrom and Gyllensten 1995). This indicates selection processes that seem to be able to maintain MHC polymorphism in the fragmented populations of *M. murinus*, at least under current conditions. The effects of balancing selection and genetic drift on the MHC genetic diversity was investigated in 14 island and two mainland populations of the Australian bush rat *Rattus fuscipes* (Seddon and Baverstock 1999). The results showed higher levels of heterozygosity on two of the islands than would be expected under neutrality, but genetic drift played a dominant role in the majority of island populations. Thus, the strength of selection acting on MHC loci can be insufficient to maintain variation over a long period in small or fragmented populations.

A recent study on the importance of the MHC on the parasite burden of *M. murinus* in the four littoral forest fragments of Mandena, which was also included in the analyses presented here, indicated that specific MHC DRB alleles were associated with parasite resistance or susceptibility (Schad *et al.* 2005). Thus, variation in allele fre-

quencies in the fragments was linked to parasite load, and might influence the long-term survival of animal populations living in small forest parcels. This indicates the maintenance of functional importance of MHC variability in declining or fragmented animal populations, and the role of the MHC in evolutionary ecology and conservation.

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