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MHC class II *DRB* diversity, selection pattern and population structure in a neotropical bat species, *Noctilio albiventris*

J Schad[1], DKN Dechmann[1,2,3], CC Voigt[1] and S Sommer[1]
[1]*Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany;* [2]*Max Planck Institute of Ornithology, Vogelwarte Radolfzell, Radolfzell, Germany and* [3]*Smithsonian Tropical Research Institute, Balboa, Ancon, Republic of Panama*

Genes of the major histocompatibility complex (MHC) have a crucial role in the immune response of vertebrates, alter the individual odour and are involved in shaping mating preferences. Pathogen-mediated selection, sexual selection and maternal–fetal interactions have been proposed as the main drivers of frequently observed high levels of polymorphism in functionally important parts of the MHC. Bats constitute the second largest mammalian order and have recently emerged as important vectors of infectious diseases. In addition, Chiroptera are interesting study subjects in evolutionary ecology in the context of olfactory communication, mate choice and associated fitness benefits. Thus, it is surprising that they belong to the least studied mammalian taxa in terms of their MHC diversity. In this study, we investigated the variability in the functionally important MHC class II gene *DRB*, evidence for selection and population structure in the group-living lesser bulldog bat, *Noctilio albiventris*, in Panama. We found a single expressed, polymorphic *Noal-DRB* gene. The substitution pattern of the nucleotide sequences of the 18 detected alleles provided evidence for positive selection acting above the evolutionary history of the species in shaping MHC diversity. Roosting colonies were not genetically differentiated but females showed lower levels of heterozygosity than males, which might be a sign that the sexes differ in the selection pressures acting on the MHC. This study provides the prerequisites for further investigations of the role of the individual MHC constitution in parasite resistance, olfactory communication and mate choice in *N. albiventris* and other bats.
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Introduction

Genes of the major histocompatibility complex (MHC) play an essential role in the adaptive immune response of vertebrates and are crucial for the understanding of the influence of natural selection on genetic diversity in wild populations (Bernatchez and Landry, 2003). MHC-encoded cell surface glycoproteins present antigens of intracellular (MHC class I genes) and extracellular (MHC class II genes) origin to T cells that trigger the appropriate immune response (Klein, 1986; Janeway and Travers, 2002; Kumanovics *et al*., 2003). Class II-derived molecules are heterodimers of two associated polypeptide chains, an α- and a β-chain. The β-chain consists of an antigen-binding $\beta 1$-domain, an immunoglobulin-like $\beta 2$-domain and a transmembrane domain. Certain amino-acid positions of the $\beta 1$-region, the so-called antigen-binding sites (ABS, encoded by exon 2), show high levels of genetic variation (Brown *et al*., 1993) with a higher rate of non-synonymous (d_N, amino-acid altering) over synonymous (d_S, silent) nucleotide substitutions (Hughes and Nei, 1988, 1989). According to neutrality theory (Kimura, 1977), the rate of synonymous nucleotide substitution is predicted to be larger than the rate of non-synonymous substitutions because a change of the amino-acid composition is more likely to be deleterious. The pattern of an elevated rate of non-synonymous substitutions at ABS is considered as clear evidence for 'positive Darwinian selection' (Hughes and Nei 1988, 1989) shaping genetic variation (reviewed in Hughes, 2007). Others used the term 'historical positive selection' (Hedrick, 1999) or 'positive selection over evolutionary time scale' (Bernatchez and Landry, 2003) to describe this observation. This polymorphism enables the immune system to recognise an extensive range of pathogens and is therefore crucial for the immunological fitness of individuals and, thus, animal populations (Edwards and Potts, 1996; Bernatchez and Landry, 2003). The high levels of polymorphism at MHC class II loci found in most vertebrate species are thought to be maintained by different forms of balancing selection, including heterozygosity advantage (Doherty and Zinkernagel, 1975) and rare allele advantage (also called negative frequency-dependent selection; Takahata and Nei, 1990; Slade and McCallum, 1992) as well as selection that varies in space and time (Hill, 1991; Hedrick, 2002). Distinguishing between the different forms of balancing selection in natural populations is difficult, as they are not mutually

Correspondence: Dr S Sommer, Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Street 17, Berlin D-10315, Germany.
E-mail: sommer@izw-berlin.de
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exclusive and may operate together with other selective and neutral forces (Sommer, 2005; Piertney and Oliver, 2006; Spurgin and Richardson, 2010).

Genes of the MHC are known to alter the individual body odour (for example, Penn and Potts, 1998; Milinski, 2006; Kwak *et al*., 2009) and may therefore be involved in mate choice decisions (for example, Wedekind *et al*., 1995; Chaix *et al*., 2008; Ilmonen *et al*., 2009), thus promoting genetic structure within and among social groups (Chesser, 1990; Kundu and Faulkes, 2004; Matocq and Lacy, 2004; Cutrera and Lacey, 2006). Sexual selection processes may not only be supported in direct mate choice situations but also through postcopulatory mechanisms including maternal–fetal interactions (for example, Wedekind *et al*., 1996; Ober and Van der Ven, 1997). Several studies have already highlighted that reproduction among MHC-dissimilar mates is favoured (for example, primates: Schwensow *et al*., 2008; and humans: Chaix *et al*., 2008; Ilmonen *et al*., 2009); mostly because a genetically diverse offspring genotype is advantageous in the defence against pathogens (Parham and Otha, 1996; Penn and Potts, 1999). Yet, the debate is still controversial. In some populations, MHC-based mate choice was not affirmed (humans: Hedrick and Black, 1997; Chaix *et al*., 2008; and ruminants: Patterson and Pemberton, 1997). Some studies have suggested that an intermediate rather than the highest level of MHC diversity is optimal (Wegner *et al*., 2003; Woelfig *et al*., 2009).

Bats are not only the second largest mammalian order but also the most gregarious of all mammals. Some bat colonies harbour several million individuals. Thus, social communication among bats can be complex, involving not only visual and acoustical but also olfactory signals (McCracken and Wilkinson, 2000; Altringham and Fenton, 2003). Many bat species produce distinct odours from a variety of glands (for example, Quay, 1970; Dapson *et al*., 1977; Voigt and von Helversen, 1999; Scully *et al*., 2000; Caspers *et al*., 2009). Pilot studies have shown that bats use odours for kin and individual recognition (Gustin and McCracken, 1987; Bouchard, 2001; Safi and Kerth, 2003) and during male–female interactions (Voigt and von Helversen, 1999; Bouchard, 2001). Recently, it has been discovered that bats are reservoir hosts and potentially important vectors of many infectious diseases (Calisher *et al*., 2006; Wong *et al*., 2007; Field, 2009; Wibbelt *et al*., 2010). Given the combination of these facts, it becomes apparent that the study of bat immunogenetics is of fundamental relevance not only for understanding the mechanisms underlying the zoonotic potential of bats but also to shed light on the link between MHC, pathogen resistance and olfaction-based mate choice in mammals.

In the present study, we examined the MHC class II *DRB* region of a neotropical bat species, the lesser bulldog bat, *Noctilio albiventris*, in Panama. *N. albiventris* lives in social groups of so far unknown relatedness. Besides acoustical signals, group members also communicate among each other via volatile compounds from the so-called oily spots in the subaxillary region. In addition, males possess inguinal pockets next to the scrotum that provide a distinct odour, particularly during the reproductive period (Studier and Lavoie, 1984; Brook and Decker, 1996). In summary, *N. albiventris* harbours morphologically distinct scent-producing organs, suggesting that volatiles are of key importance not only for individual communication but also for mate choice.

Our specific aims were: (1) to design reliable primers to characterise MHC class II *DRB* exon 2 diversity in *N. albiventris*, using a genome walking approach that enables the amplification of specific DNA fragments in situations where the sequence of only one primer is known (modified from Ko *et al*., 2003), (2) to examine evidence for selection acting on MHC in *N. albiventris*, that is, a higher rate of non-synonymous over synonymous substitutions in ABS would indicate positive selection acting above the evolutionary history of the species and (3) to investigate whether subpopulations, roosting colonies and the sexes differ in their genetic population structure. Differences in the MHC constitution of subpopulations and roosting colonies could indicate local adaptation as a result of recent selection events, whereas differences between sexes could indicate MHC-related sexual selection mechanisms.

Materials and methods

Study site and sampling

Our main study site was the village Gamboa (09.07° N and 079.41° W) in Panama. Bats were caught with mist nets (see Dechmann *et al*., 2009) as they emerged at dusk from their roost during 2006–2008. Individuals caught from the same daytime roost were assumed to be from the same colony. A second study site was Barro Colorado Island (BCI; 09.10° N and 079.51° W) situated 15 km away from Gamboa, where animals foraging above water were caught in mist nets set up along the boat docks of a marina (Figure 1). Bats from the two sites were considered separate subpopulations as their foraging areas did not overlap (Dechmann *et al*., 2009). Bats were sexed, aged and body measurements taken. From all 215 bats, we collected a 4-mm skin sample from the wing membrane using a sterile biopsy punch (Worthington Wilmer and Barrat, 1996). Skin samples were stored in 96% ethanol until DNA isolation.

For optimal primer design and to prove expression of MHC alleles, a liver sample was collected from an euthanised male *N. albiventris*. The liver sample was preserved in RNA later (Sigma-Aldrich, Steinheim, Germany) and stored at −20 °C until subsequent analysis.

Genomic DNA and RNA isolation and complementary DNA library construction

Genomic DNA (gDNA) was extracted using the DNeasy Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. RNA was isolated from the liver using the RNeasy Kit (Qiagen) that includes a DNase treatment according to the manufacturer's instructions. The quantity was determined by measuring the absorbance at 260 nm and purity (that is presence of contaminants) was assessed by the ratio at 260/280 nm, which should be ~1.8 for DNA and ~2.0 for RNA (Nanotrop, Peqlab, Erlangen, Germany). Up to 5 μg of total RNA were used to construct a first strand complementary DNA (cDNA) library. For reverse transcription, we included 5 μg Oligo(dT)12–18 Primer (Invitrogen, Karlsruhe, Germany) and 200 U of Revert

Aid H-Minus M-MulV reverse transcriptase (Fermentas, St Leon-Rot, Germany) together with 20 U of a ribonuclease inhibitor (RiboLock, Fermentas) as instructed in the manufacturer's protocol. cDNA was checked spectrophotometrically (Nanotrop) and by electrophoresis on a 1.5% agarose gel. Reproducibility was ensured by two independent repeats of the whole procedure.

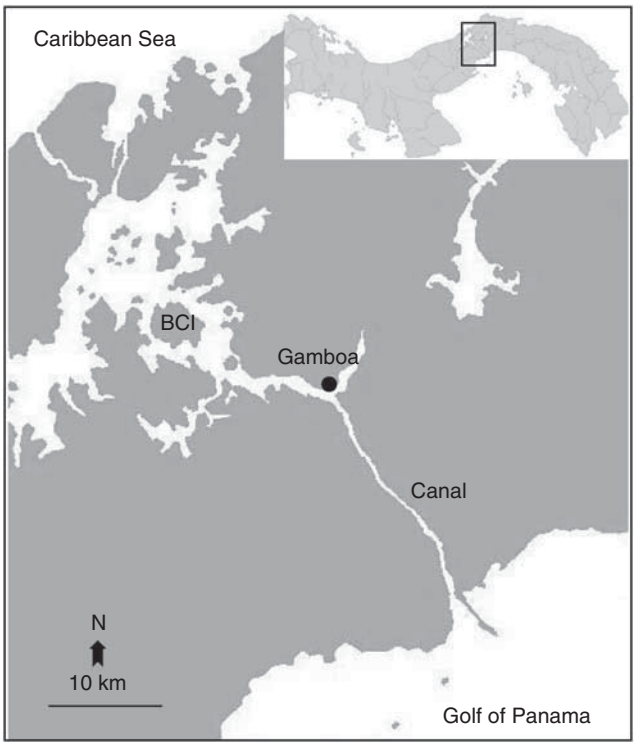
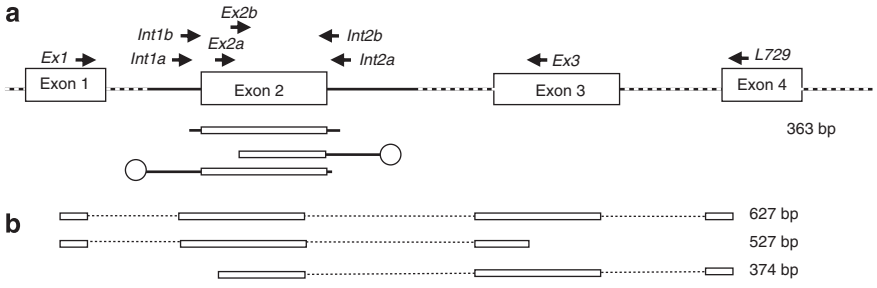


Figure 1 Map of the study sites Gamboa and BCI in Panama (modified from the Smithonian Tropical Research Institute mapserver).

Primer design using cDNA and vectorette PCR

An MHC class II *DRB* cDNA alignment of different mammalian species was constructed by a BLAST search (http://www.ncbi.nlm.nih.gov) of the GenBank database. Primers complementary to conserved parts of exon 1 (*Ex1*) and exon 3 (*Ex3*) were designed (Figure 2). They were used together with primer L729 situated in exon 4 (Bowen *et al*., 2004, Figure 2) in different combinations to amplify *DRB* sequences from the cDNA of *N. albiventris*. PCR amplification was carried out in a total reaction volume of 20 µl, including 20 ng cDNA, 1 U of proofreading polymerase (HotStar HiFidelity Polymerase, Qiagen), 1 × HotStar HiFidelity PCR buffer, 0.3 mM deoxynucleotid triphosphates (dNTPs) and 0.4 µM of each primer (Sigma-Aldrich, Steinheim, Germany). Thermocycling started with an initial denaturation step at 96 °C for 10 min, followed by 35 cycles of denaturation at 96 °C for 45 s, annealing at 58 °C for 45 s and elongation at 72 °C for 2 min. An additional 15 min elongation step followed the last cycle. PCR products were purified (Cycle pure Kit, Peqlab) and cloned using the pCR4-TOPO TA cloning Kit (Invitrogen). At least 23 recombinant clones per primer combination were amplified using the vector primers T7for and M13rev, sequenced using BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and analysed on ABI PRISM 310 Automated Genetic Analyzer (Applied Biosystems). All primer combinations amplified the same two MHC class II *DRB* alleles in *N. albiventris*. These *Noal* sequences were used to design the species-specific primers *Ex2a* and *Ex2b* (Figure 2) binding to conserved sites of exon 2 (inferred from the



Primer		Sequence 5' to 3'
JSex1-*DRB*	*Ex1*	GCTCCYGGATGRCAGCTCTGA
JSex3-*DRB*	*Ex3*	AGAGCAGACCAGGAGGTTGTG
DRB-L729	*L729*	ACTCAMCATCTTGCTCTG
JSN2-*DRB*	*Ex2a*	GAGTGTCATTTCTMCAAYGGGAC
JSN2R-*DRB*	*Ex2b*	GTGCGCTTCGACAGCRACGT
JSi2N-*DRB*	*Int2a*	CACACGCACGTACACAAGTACACA
JSi2A-*DRB*	*Int2b*	CCGCCCCGCCGCGCTCAC
JSi1N-*DRB*	*Int1a*	GGCGCCCGGCCTGGCCGACGTC
JSi1N2-*DRB*	*Int1b*	CAGCTGCRTSACGGTGGTTCCTG

Figure 2 Positions and sequences of PCR primers to amplify the indicated fragments of the MHC class II *DRB* gene in *N. albiventris* based on gDNA (**a**) and cDNA (**b**). Boxes symbolise exons, dark lines mark introns and dotted lines mark not sequenced parts of the introns. Open circles indicate positions of ligated vectorettes (see Materials and Methods for details). The primer *DRB*-L729 is taken from Bowen *et al*. (2004).

established cDNA alignment and available sequences of the bat *Saccopteryx bilineata* (GenBank accession numbers: EF533888-EF533900)), which were used for the subsequent first vectorette PCR assay.

Vectorette PCR is a method to amplify DNA fragments of interest, where the sequence information is only available on one side, and is described in detail by Ko *et al*. (2003). We constructed vectorette libraries with gDNA of five individuals. Up to 5 μg of gDNA, because of available template of each animal, were digested with restriction enzymes *Eco*RI, *Xap*I, *Fsp*, and *Csp*I (Fast Digest, Fermentas). In total, 1–5 μg gDNA were digested by 1–5 U restriction enzyme in a total volume of 50 or 100 μl depending on the amount of gDNA. Double-stranded vectorettes (1 μM) consisting of vect53 and vect57TTAA (*Eco*RI and *Xap*I) or vect53 and vect57AT (*Fsp* and *Csp*I) (Ko *et al*., 2003) were ligated to the sticky ends of the digested gDNA using 2 U of T4DNA ligase per 1 μg digested gDNA (Rapid DNA Ligation Kit, Fermentas).

Vectorette PCR assays consisted of two PCRs. The first PCR was followed by a second nested PCR to avoid false positives. A step-down scheme was always applied, using HotStar *Taq* Master Mix (Qiagen) according to the user's manual in a total reaction volume of 25 μl with the following conditions: initial activation of HotStar *Taq* and denaturation at 95 °C for 14 min, followed by 5 cycles of denaturation at 95 °C for 60 s, annealing at 67 °C for 60 s and elongation at 72 °C for 2 min, five cycles with annealing at 63 °C, followed by 15 cycles denaturation at 95 °C for 45 s, annealing at 60 °C for 45 s and extension at 72 °C for 120 s, 15 cycles with annealing at 57 °C and a final extension at 72 °C for 10 min. Amplification products were checked on a 1.5% agarose gel and purified with cycle Pure Kit (Peqlab).

The first vectorette PCR assay was performed with the primer *Ex2a* and the vectorette primer *C20* (Ko *et al*., 2003), and the nested vectorette PCR included the nested primer *Ex2b* in combination with the nested vectorette primer *B21* (Ko *et al*., 2003). The resulting intron 2 sequences allowed the design of *Noctilio*-specific intron 2 primers (*Int2a* and *Int2b*) (Figure 2). These were used to amplify intron 1 sequences in another vectorette PCR assay. Finally, according to the intron 1 sequences, the primers *Int1a* and *Int1b* were developed. *Int1a* and *Int2a* were used to amplify the whole 270 bp MHC *DRB* class II exon 2 and partial introns (45 bp in intron1, 48 bp in intron2, Figure 2) and were applied to genotype all 215 *N. albiventris* individuals.

Genotyping and allele identification

Amplification was performed in 25 μl volumes containing 0.4 μM of each primer (Sigma-Aldrich), 1 × reaction buffer (10 mM Tris HCl, 50 mM KCl, 0.1% Triton x-100, 0.2 mg ml^{-1} bovine serum albumin, MP Biomedicals, Heidelberg, Germany). 0.2 mM of each dNTP and 1 U *Taq* polymerase (MP Biomedicals). Step-down PCR was performed as follows: initial incubation at 95 °C for 5 min, followed by 5 cycles of denaturation at 95 °C for 10 s, annealing at 67 °C for 10 s and elongation at 72 °C for 30 s, five cycles with annealing at 64 °C and 25 cycles denaturation at 95 °C for 10 s, annealing at 60 °C for 10 s and elongation at 72 °C for 30 s, final extension was at 72 °C for 3 min. Amplicons were genotyped by single strand confirmation polymorphism on a polyacrylamide gel as described in Schad *et al*. (2004). Allele identification was done by excising the single strands from the gel matrix and diluting them in distilled water. Thereafter a reamplification of diluted single strands with primers *Int1a* and *Int2a* was performed before sequence analyses as described above. An autonomous amplicon with primer *Int1b* and *Int2b* of each individual was directly sequenced to confirm the individual single strand confirmation polymorphism pattern.

Statistical analyses

We edited and aligned nucleotide sequences manually using MEGA 4.0 (Tamura *et al*., 2007). We also used this programme for calculating the *P*-distance of amino-acid sequences as a measurement of functional MHC class II *DRB* divergence (Nei and Kumar, 2000) and for analysing the relative rates of non-synonymous (d_N) and synonymous (d_S) base pair substitutions according to Nei and Gojobori (1986), applying the Jukes–Cantor correction for multiple hits (Jukes and Cantor, 1969). Calculations were applied for all sites and separately for putative ABS and non-ABS, assuming functional congruence to human ABS of the *HLA-DR1* (*DRA/DRB1**0101) molecule (Brown *et al*., 1993). The d_N/d_S ratios of all sites as well as for ABS and non-ABS separately were compared with an implemented *Z*-test (Nei and Kumar, 2000) to test for positive selection.

We identified species-specific positively selected sites (PSS) with maximum-likelihood analysis using CODEML (included in PAML version 3.15 software package; Yang, 1997; Yang, 2007) and compared these PSS with the human ABS. First, we fitted models with different assumptions of selection patterns to the sequence data. We used the models M7 (β) and M8 (β and ω) as described in Yang *et al*. (2007). M7 served as a null model, where the ω-ratio varies according to the β- distribution and does not allow positive selected sites ($0<\omega<1$). M8 adds a class of sites to account for the possible occurrence of PSS ($\omega>1$). The models were compared using a likelihood ratio test by calculating the likelihood difference $2\Delta l = 2(l_1 - l_0)$ and then compared with a χ^2-distribution, with the degrees of freedom equal to the difference in the number of estimated parameters (Yang and Bielawski, 2000). In the next step, after likelihood ratio test provided evidence for positive selection, the Bayes empirical Bayes method (Yang *et al*., 2005) integrated in CODEML was used to identify the sites under positive selection with the cutoff posterior probability set at $P_b = 95\%$.

Allele frequencies, observed and expected heterozygosity, and deviation from Hardy–Weinberg expectations were estimated using the software ARLEQUIN version 3.0 (Excoffier *et al*., 2005). Pairwise F_{ST} based on haplotype frequencies were calculated to infer population subdivision (10 000 permutations, Wright, 1951, 1965). Allelic richness (*R*) as a measure of the number of alleles independent of sample size was estimated using the rarefaction method as implemented in FSTAT version2.9.3 (Goudet, 2001).

The χ^2-tests were used to compare the number of heterozygote individuals between the sexes. Differences in the mean individual amino-acid distance of males and females were investigated by analysis of variance (ANOVA). Calculations were always two-tailed with

significance level at $\alpha = 0.05$ and performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Bonferroni corrected significant levels were applied for multiple comparisons (Rice, 1989).

Results

Capture and sampling

In total, we caught 215 individuals (91 males and 123 females), 185 in Gamboa (59 males and 108 females) and 29 in BCI (22 males and 7 females). All of them were genotyped for the MHC class II *DRB* gene. In Gamboa, we caught bats from seven roosts. We included only colonies with more than 10 individuals in statistical analyses (colony A: $N = 52$, 22 males and 30 females; colony B: $N = 74$, 20 males and 54 females; colony C: $N = 27$, 13 males and 14 females; and colony D: $N = 14$, 4 males and 10 females).

MHC class II *DRB* diversity

Using cDNA of one male *N. albiventris*, all primer combinations amplified the same two MHC class II *DRB* alleles differing only in fragment length (628, 527 and 376 bp, respectively, Figure 2). We designed species-specific intron primers (*Int1a* and *Int2a*) for amplification of the whole MHC class II *DRB* exon 2 (270 bp) of *N. albiventris* flanked by short intron sequences with a total length of 363 bp (Figure 2) by means of the vectorette PCR approach based on gDNA. No indels or stop codons were found and no more than two alleles per individual were amplified, suggesting that a single *DRB* locus was expressed. We named this locus *MhcNoal-DRB* according to the established MHC nomenclature (Klein *et al*., 1990; Ellis *et al*., 2006). The nucleotide and deduced amino-acid sequence showed high homology with human (84%), canine (83%), equine (83%) and other mammalian class II *DRB* genes.

We detected 18 different alleles of *Noal-DRB* in the 215 individuals of *N. albiventris* (GenBank accession numbers: HM347941-HM347958). In the nucleotide sequences, we observed 71 (26.3%) variable positions and the alleles differed by 2 to 40 (average 26.3 ± 2.9) nucleotide positions. All alleles had a unique amino-acid sequence, whereas 38 (43%) out of 89 amino acids were polymorphic. They differed by 1 to 26 (average 17.6 ± 2.5) amino-acid positions.

The most common allele *Noal-DRB**10 occurred at a frequency of 0.255 followed by *Noal-DRB**02 (0.175) and *Noal-DRB**04 (0.117). The remaining alleles occurred in 2 to 36 individuals with a frequency ranging between 0.005 and 0.089 (Table 1). Observed heterozygosity (0.902) was higher than expected (0.871) and did not deviate from Hardy–Weinberg expectations (Table 2). The individual *Noal-DRB* exon 2 distance of an individual based on amino-acid sequence ranged from 0 (homozygote) to 0.287 with an average of 0.177 ± 0.078 (Table 2).

Evidence for historical selection

Two approaches were used to test for historical positive selection acting on the examined exon 2 of *Noal-DRB* locus. First, the averaged rates of non-synonymous (d_N) and synonymous (d_S) base pair substitutions of all sequences were calculated for all sites and separately for putative ABS and non-ABS, assuming functional congruence to human ABS of the *HLA-DR1* molecule (Brown *et al*., 1993). Non-synonymous substitutions occurred at a significantly higher rate than synonymous ones ($d_N/d_S = 2.90$, *Z*-test, $P = 0.004$) especially in the regions that code for ABS ($d_N/d_S = 3.14$, *Z*-test, $P = 0.002$; Table 3). Second, we observed a significantly higher log likelihood estimate ($2\Delta l = 59.67$, d.f. $= 2$, $P < 0.0001$) for model M8 (positive selection) than for its corresponding null model M7 (no positive selection). The Bayes empirical Bayes approach under model M8 inferred 17 significant sites (PSS) to be under positive selection with the cutoff posterior probability set at 95%. In total, 14 of these sites were congruent with predicted ABS of the human *HLA-DR1* β-chain (Brown *et al*., 1993). The other

Table 1 Allele frequencies of MHC class II *DRB* alleles in the whole population and in subpopulations, roosting colonies and sexes

Level	*All*	*Subpopulations*		*Roosting colonies*				*Sexes*	
Category		*BCI*	*Gamboa*	*A*	*B*	*C*	*D*	*Females*	*Males*
N	215	29	185	52	74	27	14	123	91
*NoalDRB**01	0.040	0.069	0.035	0.048	0.027	0.056	0.036	0.024	0.060
*NoalDRB**02	0.175	0.172	0.176	0.154	0.182	0.296	0.072	0.175	0.176
*NoalDRB**03	0.009	—	0.012	0.010	0.014	—	—	0.012	0.006
*NoalDRB**04	0.117	0.138	0.113	0.135	0.095	0.056	0.143	0.106	0.124
*NoalDRB**05	0.044	0.017	0.049	0.087	0.054	0.019	—	0.045	0.044
*NoalDRB**06	0.040	0.069	0.035	0.039	0.020	0.074	0.072	0.049	0.026
*NoalDRB**07	0.005	—	0.005	0.010	0.007	—	—	0.004	0.006
*NoalDRB**08	0.042	0.034	0.043	0.087	0.027	0.019	0.036	0.033	0.055
*NoalDRB**09	0.054	0.034	0.057	0.029	0.081	0.037	0.036	0.057	0.050
*NoalDRB**10	0.255	0.190	0.264	0.183	0.297	0.259	0.464	0.293	0.209
*NoalDRB**11	0.028	0.034	0.027	0.039	0.027	—	—	0.012	0.050
*NoalDRB**12	0.088	0.172	0.076	0.087	0.061	0.074	0.071	0.081	0.100
*NoalDRB**13	0.028	—	0.032	0.019	0.014	0.037	0.071	0.029	0.022
*NoalDRB**14	0.005	0.017	0.003	—	0.007	—	—	0.004	0.006
*NoalDRB**15	0.005	—	0.008	—	0.007	0.019	—	0.008	-
*NoalDRB**16	0.007	—	0.007	0.010	0.014	0.019	—	0.008	0.011
*NoalDRB**17	0.044	0.017	0.049	0.058	0.054	0.037	—	0.053	0.033
*NoalDRB**18	0.014	0.034	0.012	0.010	0.013	—	—	0.008	0.022

Abbreviations: BCI, Barro Colorado Island; MHC, major histocompatibility complex; *N*, sample size.

Table 2 MHC class II *DRB* exon 2 variability in *N. albiventris* in the whole population and in subpopulations, roosting colonies and sexes

Level	N	*Number of alleles*	R	H_{obs}	H_{exp}	*Individual distance*
All	215	18		0.902	0.871	0.177 ± 0.005
Category						
Subpopulation						
BCI	29	13	13	0.931	0.886	0.189 ± 0.013
Gamboa	185	18	13.6	0.817	0.868	0.175 ± 0.006
Roosting colonies						
A	52	16	11.1	0.981	0.901	0.190 ± 0.009
B	74	18	10.4	0.838	0.856	0.164 ± 0.010
C	27	13	10.1	0.889	0.839	0.166 ± 0.016
D	14	9	9.0	0.857	0.767	0.171 ± 0.022
Sexes						
Females	123	18	17.2	0.854	0.856	0.164 ± 0.008
Males	93	17	17.0	0.967	0.888	0.195 ± 0.006

Abbreviations: BCI, Barro Colorado Island; H_{exp}, expected heterozygosity; H_{obs}, observed heterozygosity; Individual distance (± s.e.), individual amino-acid distance between alleles; *N*, sample size; *R*, allelic richness adjusted to the smallest sample size per level.

Table 3 Non-synonymous (d_N) and synonymous (d_S) substitutions (± s.e.) as well as their ratio in ABS and non-ABS assuming concordance with the human *HLA-DR1* molecule (Brown *et al*., 1993)

Region	N	d_N	d_S	d_N/d_S	P
ABS	25	0.353 ± 0.077	0.112 ± 0.044	3.139	0.002
Non-ABS	64	0.053 ± 0.013	0.034 ± 0.018	0.942	NS
All	89	0.124 ± 0.021	0.055 ± 0.018	2.904	0.004

Abbreviations: ABS, antigen-binding sites; *N*, number of codons in each category; NS, not significant; *P*, probability ($\alpha \leqslant 0.05$) that d_N and d_S are different using a *Z*-test.

three sites were located in close proximity within one to two amino-acid positions to the human ABS (Table 4, Figure 3). In the *Noal-DRB* sequences, all but one (61 W) of certain amino-acid positions, which are conserved in human *HLA-DR1* molecules (Brown *et al*., 1993; Stern *et al*., 1994), were also conserved presenting the identical amino acids. At position 61, tryptophan was replaced by leucine in six *Noal* alleles (33.3%) and identified as PSS (Table 4, Figure 3).

Population structure and MHC

We observed no genetic differentiation between the two subpopulations of Gamboa and BCI ($F_{ST} = 0.003$, $P = 0.23$). Allele frequencies of the two subpopulations are shown in Table 1. Numbers of alleles were similar after correction for differences in sample size. Observed heterozygosity was high in both subpopulations without a significant deviation from Hardy–Weinberg expectations (Table 2).

All four roosting colonies with more than 10 individuals captured in the village of Gamboa showed high levels of heterozygosity and allelic richness (*R*) ranging from 9 to 11.1. Observed heterozygosity exceeded the expected value in all but one colony (colony B) and did not deviate significantly from Hardy–Weinberg equilibrium (Table 2). Pairwise F_{ST} statistics showed a slight differentiation between colonies A, C and D but this

Table 4 Identification of species-specific PSS by likelihood analysis in MHC class II *DRB* exon 2 amino-acid sequences of *N. albiventris*

PSS	*Mean* ω	P_b *(ω > 1)*	*Distance to ABS*
9	4.561 ± 0.496	1.000**	0
11	4.561 ± 0.496	1.000**	0
12	4.490 ± 0.693	0.982*	0
13	4.556 ± 0.514	0.999**	0
16	3.908 ± 1.434	0.836	2
18	4.353 ± 0.950	0.948	4
28	4.464 ± 0.740	0.976*	0
34	3.633 ± 1.604	0.768	4
37	4.552 ± 0.526	0.998**	0
47	3.460 ± 1.677	0.724	0
57	4.561 ± 0.496	1.000**	0
60	3.733 ± 1.530	0.793	1
61	4.512 ± 0.643	0.988*	1
63	4.462 ± 0.747	0.975*	0
66	4.334 ± 0.970	0.944	0
67	4.524 ± 0.604	0.991**	0
70	4.560 ± 0.493	1.000**	0
71	4.561 ± 0.496	1.000**	0
74	4.561 ± 0.496	1.000**	0
76	3.581 ± 1.650	0.755	2
77	4.529 ± 0.592	0.992**	1
78	4.559 ± 0.503	1.000**	0
86	4.561 ± 0.496	1.000**	0
88	3.646 ± 1.731	0.778	0
92	4.545 ± 0.549	0.996**	2

Abbreviations: ABS, antigen-binding sites; Distance to ABS, amino acids to nearest human ABS of the HLA-DR1 *β*-chain (Brown *et al*., 1993); Mean ω (± s.e.), ratio of non-synonymous and synonymous substitutions at PSS and its probability (P_b ($\omega > 1$)) using a cutoff posterior probability of 95%; PSS, positively selected sites, numbers indicate the amino-acid positions in the *β1*-domain.
**$\alpha \leqslant 0.01$, *$\alpha \leqslant 0.05$.

significance was lost after Bonferroni correction ($\alpha' \leqslant 0.008$; Table 5).

We found no significant differentiation within males and females when comparing colonies (males: range of F_{ST}: 0.001–0.026, *P*-values not significant; females: F_{ST}: < 0.001–0.039, *P*-values not significant; Bonferroni corrected significance level $\alpha' \leqslant 0.008$). We found no

```
Position        9  11  13   16 18      26 28  30    34    37          47      52  55   57    61 63   66    70    74   76 78   81    85 86 88 90 92
ABS          ---*-****- ---------- *-*-*----- -**-------- -*-------- -**------- **--***--*- --*-------* *****-----
ConsSites    ---------- ---------- ---------- ---------- ------*--* -----*---- ---------- -----**--- ----------
PSS          ---*-***-- *-*------- --*-----*- -*-------- -*-------- -*--***-*-- **--***--*- ***------- *-*---*--
NoalDRB*01   HFLYQTTSEC YFSNGTERVR FLDRYFYNRE EYVRFDSDVG EYRAVTELGR PIAKDWNGQE AILERKRAAV EVYCKHNYAV FDGFLVHRQ
NoalDRB*02   ...F.MS... ..T......Q L.Q..I.... .......... .F........ .S.EHL.A.. DA..QL.... .T........ S.....Q..
NoalDRB*03   ...Q.ALY.. H......... ..L..I..Q. .FL....... .F........ .F.EHL.A.. T....R..E. .T........ S.....Q..
NoalDRB*04   ...Q.GST.. H.T....... YVQ..I.... .......... .F........ .D..Y..A.. ....DA..R. .A........ S........
NoalDRB*05   .......... .......... .......... .......... .......... .D..Y..... .F.....GQ. .A........ .........
NoalDRB*06   ...S.AS... H.T....... ..Q..I.... .F........ .F........ .......S.. ....QM..Q. DTV......G .........
NoalDRB*07   .......... .......... .......... .F........ .......... .D..Y..... .....R..E. .T........ S........
NoalDRB*08   .......... .......... .......... .......... .......... .S..Y..A.. DFM.QR.... .T........ .........
NoalDRB*09   ...F...... .......... .......... .......... .F........ .S..Y..A.. DFM.QR.... .T........ L.R......
NoalDRB*10   ...S.AS... ..T....... ..Q..I.... .F........ .F........ .G.EHL.A.. .F..QM..Q. DTV....... S........
NoalDRB*11   ...L.MSF.. H.A....... L.E....... ..L....... .......... .S.EHL.A.. T...DE..Q. DTV....... S........
NoalDRB*12   ...F.FKP.. H.T...D... ........G. .......... .......... LS.EH..S.. DV..DA.... .......... S.....Q..
NoalDRB*13   ...H.MS... .......... ..Q..I..G. .D........ VF.....G.. .D.EY..S.. ....QM..Q. DTV....... .....Q..
NoalDRB*14   .......... .......... .......... .......... .......... .V..Y..A.. ....QM.... .T........ S.....Q..
NoalDRB*15   ...F.MS... H.T......Q L.Q..I.... .F........ .F........ .G.EHL.A.. ....QM..Q. DTV......A I........
NoalDRB*16   .......... .......... .......... .......... .......... .D..Y..... .F..QM..Q. DTV....... .........
NoalDRB*17   ...S.AS... H.T....... ..Q..I.... .F........ .F........ .G.EHL.A.. .F..QM..Q. DTV....... S........
NoalDRB*18   ...S.AST.. H.T....... YVQ..I..Q. .VL....... .F........ .E.EH..S.. ....DA.... .T.......G .........
HLADRB1*0101 R..W.LKF.. H......... L.E.CI..Q. .S........ .......... .D.EY..S.K DL..QR.... DT..R..... GES.T.Q.R
```

Figure 3 Amino-acid sequence variation of 18 MHC class II *DRB* exon 2 alleles of *N. albiventris* and the human *HLA-DRB1**0101 sequence. Dots mark identity with the top sequence. Numbers indicate the amino-acid positions of the *β1*-domain, * signify the amino-acid positions of ABS and conserved sites (ConsSites) of the human *HLA-DR1 β*-chain (Brown *et al*., 1993; Stern *et al*., 1994). PSS indicates species-specific positive selected sites identified by CODEML (Yang *et al*., 2005).

Table 5 Pairwise differentiation between roosting colonies using conventional F-statistic based on haplotype frequencies (Wright, 1965)

Colony	*A*	*B*	*C*	*D*
A		NS	NS	0.010
B	0.006		NS	NS
C	0.012	0.002		0.031
D	0.036	0.013	0.036	

Abbreviation: NS, not significant.
F_{ST} values are provided below the diagonal and corresponding *P*-values above diagonal. Bonferroni corrected significance level $\alpha' \leqslant 0.008$.

significant differentiation between males and females within colonies either ($F_{ST}A = 0.009$, $P = 0.87$; $F_{ST}B = 0.001$, $P = 0.44$; $F_{ST}C = 0.007$, $P = 0.54$; $F_{ST}D = 0.030$, $P = 0.82$; and $F_{ST}All = 0.002$, $P = 0.87$).

However, males and females differed significantly in their individual amino-acid distance (ANOVA: $F = 8.48$, $P = 0.004$, d.f. = 1, Figure 4, Table 2). Furthermore, the observed heterozygosity of males was significantly higher than in females (overall: $\chi^2 = 7.73$, d.f. = 1, $P = 0.005$). The analyses were not significant when colonies were analysed separately (A: $\chi^2 = 0.81$, $P = 0.36$; B: $\chi^2 = 2.54$, $P = 0.11$; C: $\chi^2 = 3.13$, $P = 0.07$; and D: $\chi^2 = 0.93$, $P = 0.33$). In males, the observed heterozygosity exceeded the expected value in the overall sample as well as in all colonies analysed separately but did not deviate significantly from Hardy–Weinberg equilibrium (overall: $H_{obs} = 0.97$, $H_{ex}P = 0.89$; A: $H_{obs} = 1.00$, $H_{ex}P = 0.91$; B: $H_{obs} = 0.96$, $H_{ex}P = 0.89$; C: $H_{obs} = 1.00$, $H_{ex}P = 0.88$; D: $H_{obs} = 1.00$, $H_{ex}P = 0.89$; and BCI: $H_{obs} = 1.00$, $H_{ex}P = 0.89$). In females, the observed heterozygosity was always lower than in males but close to the expected value in the overall sample as well as in two out of four colonies (overall: $H_{obs} = 0.85$, $H_{ex}P = 0.86$; A: $H_{obs} = 0.96$, $H_{ex}P = 0.90$; B: $H_{obs} = 0.83$, $H_{ex}P = 0.86$; C: $H_{obs} = 0.78$, $H_{ex}P = 0.79$; D: $H_{obs} = 0.75$, $H_{ex}P = 0.75$; and BCI: $H_{obs} = 1.00$, $H_{ex}P = 0.92$; Table 2).

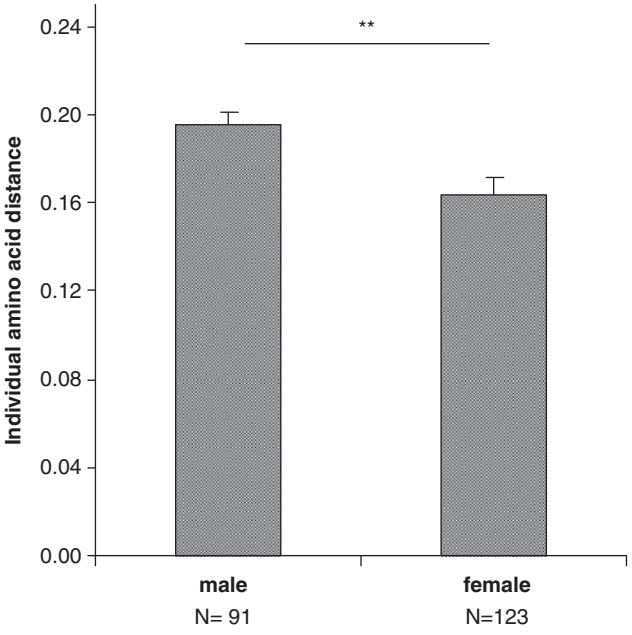


Figure 4 Mean (± s.e.) amino-acid distance between individual MHC class II *DRB* exon 2 alleles of *N. albiventris* in males and females. ANOVA: $F = 8.48$, d.f. = 1, $^{**}P = 0.004$; *N*, sample size.

Discussion

To date, not much is known about the genetic structure and polymorphism of the MHC in bats (Meyer and Brunner, 2007) mainly because of a lack of sequence data for primer design. This is astonishing given the size of the order, the importance of olfactory signals for social communication in bats and their zoonotic relevance as reservoir hosts for many pathogens. Therefore, our primary aim was to determine MHC class II *DRB* exon 2 diversity in the lesser bulldog bat, *N. albiventris*. Further, we tested for evidence of positive selection acting above the evolutionary history of the species.

And third, we were interested whether local adaptation and sexual selection may shape the contemporary genetic structure of *N. albiventris* in a Central American population.

MHC class II *DRB* diversity

Well-designed primers are essential for population genetic analyses. In this study, cDNA analyses followed by a vectorette PCR approach offered the possibility of intron mapping. Species-specific primers binding to conserved segments of the flanking introns of *N. albiventris* were designed to amplify the whole *DRB* exon 2. The reliability of the resulting species-specific sequence patterns of the locus of interest is very high because the incidence of non-amplifying alleles can be neglected. This might turn into a problem when exon primers are used to amplify parts of polymorphic genes. The occurrence of non-amplifying alleles would preclude the use of data for many population genetic purposes because they can seriously bias population genetic analyses (Dakin and Avise, 2004; Cummings *et al*., 2010).

We found evidence for a single expressed MHC class II *DRB* locus in *N. albiventris* with moderate allelic variability of 18 alleles detected in 215 individuals. This allelic variability is within the range of MHC class II *DRB* polymorphism of other mammalian species (for example, Sommer, 2005). The only other *DRB* gene studied in a bat, *Saccopteryx bilineata*, revealed a rather low allelic variability (11 alleles in 85 individuals; Meyer and Brunner, 2007). However, this might be an underestimate of the actual variability because preliminary studies on RNA and DNA with species-specific developed primers revealed evidence for at least five *DRB* loci in *Saccopteryx bilineata* (Schad *et al*., unpublished data). In general, the occurrence of different MHC class II genes (*DP*, *DO*, *DM*, *DQ* and *DR*) is conserved in mammals. However, the number of functional α- and β-genes is highly variable because of species-specific local duplication events. Yet, it can even vary between individuals of the same species (see Kumanovics *et al*., 2003). The *DRB* is the most widely studied and usually the most diverse class II gene, not only with respect to high allelic richness but also in terms of gene duplications (for example, Doxiadis *et al*., 2000; Bowen *et al*., 2004; Babik *et al*., 2005; Schwensow *et al*., 2007). We cannot rule out that other MHC class II genes may provide a higher variability in bats as it is described, for instance, in the cetacean MHC class II *DQB* gene (Baker *et al*., 2006). As a future task, it will be necessary to investigate other loci to evaluate MHC diversity in bats more comprehensively.

Evidence for historical selection

We found a higher rate of non-synonymous versus synonymous nucleotide substitutions over the entire sequence and especially in the putative ABS but not in non-ABS (Brown *et al*., 1993). Such elevated d_N/d_S ratios are a widely accepted sign for historical positive selection in polymorphic MHC genes (Hughes and Nei 1988, 1989; Nei and Kumar, 2000; Nielsen, 2001; Hughes, 2007). They indicate positive selection on ABS acting above the evolutionary history of the species (Hedrick, 1999; Bernatchez and Landry, 2003; Sommer, 2005). We subsequently confirmed these results in a species-specific analysis where different codon evolution models were compared by maximum-likelihood analyses (Yang and Bielawski, 2000; Yang, 2007). This method has been suggested as a powerful tool with a high likelihood of detecting effects of positive selection (Wong *et al*., 2004). Our data fitted best with the model incorporating positive selection and 17 species-specific PSS were identified by the Bayes empirical Bayes analysis. A total of 14 were congruent with human ABS of the *HLA-DR1* β-chain. Three PSS were situated outside the human ABS and some of the human ABS were not identified as PSS in *Noal-DRB*. Comparable results have been reported from other species (Kundu and Faulkes, 2004; Schwensow *et al*., 2007; Babik *et al*., 2008; Meyer-Lucht *et al*., 2008). In general, high congruence of PSS with human ABS is assumed to demonstrate homologous functionality of the molecule. Contrarily, human ABS that are not identified as PSS might be not involved in the antigen recognition and binding in the respective species. Furthermore, in most species investigated so far additional PSS have been reported. These findings suggest species-specific selection pressures acting on *MHC* genes because of a different pathogen exposure. In addition, certain amino-acid residuals of *HLA-DR1* molecules are highly conserved and involved in universal hydrogen bond of antigens (61 W, 81 H and 82 N) or are responsible for the stability of *DR1* heterodimers in building salt bridges between the dimers (52 E and 55 R) (Brown *et al*., 1993; Stern *et al*., 1994). Also in the *Noal-DRB* sequences, all of these positions, except position 61, were conserved indicating similar conserved functionality of the molecules.

Population structure and MHC

Mating behaviour as a correlate and driver of social structure has been suggested in addition to pathogens as another main subject of selection on MHC loci in natural populations (Hambuch and Lacey, 2002; Kundu and Faulkes, 2004; Cutrera and Lacey, 2006). Animals form social groups and colonies in response to cooperative interactions as well as mating tactics. Furthermore, patterns of genetic subdivision are also shaped by the extent and nature of philopatric behaviour (Travis *et al*., 1995; Sommer *et al*., 2002; Solomon, 2003; Cutrera and Lacey, 2006). We examined the genetic structure of three different levels of social formations or units (subpopulation, roosting colonies and sexes) in our population of *N. albiventris*. We found no genetic differentiation between the two subpopulations (Gamboa and BCI, separated by 15 km), indicating the presence of gene flow at a larger spatial scale. Both subpopulations showed similar levels of polymorphism at the *Noal-DRB* locus. Observed heterozygosity was high in the two subpopulations and the allele frequencies and the number of alleles were similar after correcting for differences in sample sizes. This implies an equivalent selection pressure maintaining diversity at the *Noal-DRB* locus in both subpopulations in the recent past.

To investigate social structure based on roosting habits, we compared four colonies in the village of Gamboa, all located in the range of 1.5 km^2. All colonies showed similar levels of genetic variation (heterozygosity, allelic richness and allele frequencies). F-statistics revealed only limited effects of subdivisions. The colony D showed a slight differentiation compared with the

colonies A and C (not significant after Bonferroni correction), which might be rather the result of missing rare alleles because of small sample size than an effect of population structure based on roosting habits. Dechmann *et al*. (2009) distinguished social groups of females (2–5 individuals) by the fact that they emerged simultaneously from a roost. We could not find genetic differences between male and female members of different colonies. The composition of colonies might vary in time, indicating a fission–fusion society rather than stable associations. Neutral markers would offer the possibility to gain insights into population dynamic processes like kin relationships of social groups of females and roosting colonies. Thus, further ecological studies on demographic structure as well as genetic analyses adding neutral markers will help to fully understand the social system of this species.

We tested for gender-specific differences in the MHC constitution to assess the occurrence of sexual selection. We found no population differentiation between males and females. However, in the overall sample, males showed a significantly higher heterozygosity rate and also a higher individual amino-acid distance than females. In males, the observed heterozygosity exceeded the expected value in the overall sample as well as in all within-colony comparisons. In females, the observed heterozygosities were always lower than in males but were almost identical with the expected ones in the overall sample and in two out of four colonies. As in both sexes observed and expected heterozygosities did not significantly deviate from Hardy–Weinberg expectations, it remains unclear whether the difference between sexes is because of a higher heterozygosity rate in males or a lower heterozygosity rate in females. An increased heterozygosity rate in males could indicate balancing selection in form of a heterozygote advantage and a deficit in heterozygote females could suggest a reduced selection pressure to maintain diversity in females. It might be that the selection intensity in the investigated *Noctilio* population is not strong enough to detect significant deviations from Hardy–Weinberg expectations. It is well known that levels of allelic diversity in relation to sample size have an effect on the statistical power to detect significant deviations from Hardy–Weinberg expectations (Seddon and Ellegren, 2004). We did not find small-scale population structure that would offer a likely explanation for this gender-specific difference. Male-based long-distance dispersal is unlikely as it would promote the occurrence of new alleles in the male population, raising the overall polymorphism at the MHC locus that we did not observe. A methodological error because of DNA quality differences between the sexes seems also to be unlikely because collection and treatment of samples have been the same throughout the study. Although direct female choice for heterozygote males would lead to heterozygote offspring in general including daughters, it cannot explain the sex-specific bias in heterozygosity. Currently, the differences in heterozygosity in males and females is most likely due to MHC-mediated postcopulatory mechanisms (for example, caused by myotic drive, gametic selection and maternal–fetal interactions) or by sex-specific survival differences that result in an increased rate of MHC heterozygote males or a deficit in heterozygote females.

The possibility of the existence of postcopulatory mechanisms resulting in sex-specific differences in MHC heterozygosity was reported only in a few studies so far. Dorak *et al*. (2002) found an increased heterozygosity for MHC class II *DRB* lineages in newborn male babies and suggested that negative selection of homozygotes might be restricted to male offspring only. A deficit in MHC homozygosity in newborn males was also observed in mice (Hamilton and Hellstrom 1978) and rats (Palm 1969, 1970, 1974). Some studies in humans investigated the compatibility at different *HLA* loci between mothers and infants and observed differences in the sex ratio assuming a different fetal loss in males and females. The results are heterogeneous and differ between *HLA* loci (Ober *et al*., 1987; Astolfi *et al*., 1990, 1996). In all of these studies, the underlying mechanisms have not been investigated. The ongoing discussion has been reviewed by Fernandez *et al*. (1999) and Ziegler *et al*. (2005). The overall conclusion is that the MHC is critical for numerous aspects of mammalian reproduction concerning spermatogenesis (Ziegler *et al*., 2002, 2005), a sperm-selective egg-cumulus complex (Wedekind *et al*., 1996; Rülicke *et al*., 1998; Eisenach and Giojalas, 2006), and viability and development of the foetus (Gill, 1992; Ober *et al*., 1987; Astolfi *et al*., 1990; Wedekind *et al*., 1996; Ziegler *et al*., 2005). However, in all these processes the selective forces might act because of MHC-linked genes, for example, olfactory receptor genes, transcription factors and others rather than by the MHC itself (Ho *et al*., 1990; Gill, 1992; Ziegler *et al*., 2002, 2005; Eisenach and Giojalas, 2006). To the best of our knowledge, sex-specific differences in offspring survival based on MHC heterozygosity have not been reported yet. But they have been investigated using a microsatellite-based measure of outbreeding (mean d^2) in a few species. In the bat *Rhinolophus ferrumequinum*, outbreeding was positively associated with significantly increased survival in male offspring only (Rossiter *et al*., 2001). The authors postulate that outbreeding at the microsatellite markers reflects immunocompetence, which in turn influences mortality. They also suggest that characterisation of MHC loci may provide a suitable test for their hypothesis. Even so, in the red deer (*Cervus elaphus)* a contrary pattern was found as male offspring survival was negatively associated with outbreeding at microsatellite markers (Coulson *et al*., 1999). In mice, a reduced survivorship was reported for inbred adult males but not for females, most likely as a consequence of aggressive interactions of males in the defence of territories (Meagher *et al*., 2000). Ongoing studies might reveal the mechanisms that have contributed to the sex-biased diversity pattern in the investigated population of *N. albiventris*. In addition, neutral markers would provide more detailed information about social structure, dispersal behaviour and gene flow of both sexes.

In this first study on the MHC class II variability of the lesser bulldog bat, we detected high genetic variation and evidence for historical positive selection acting on a single expressed *Noal-DRB* locus. The polymorphism at the antigen-binding region of the molecule is considered as the precondition to cope with a variety of pathogens. No population differentiation between subpopulations, roosting colonies and sexes was observed, but males revealed a significantly higher heterozygosity rate and genetic variability in terms of the genetic distance

between the individual MHC alleles than females. We are aware that at this state of the investigation no conclusions on the underlying mechanisms can be made but our data can provide the basis for further research on the role of the MHC constitution in host–pathogen interactions, individual body odours and sexual selection in a highly interesting bat species.

Conflict of interest

The authors declare no conflict of interest.

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