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Number of MHC alleles is related to parasite loads in natural populations of yellow necked mice, *Apodemus flavicollis*

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ABSTRACT

Hypothesis: Low levels of immune gene variation (major histocompatibility complex) in a population are associated with increased parasite load and infection intensity.

Organism: Different populations of the yellow necked mouse (*Apodemus flavicollis*), a common rodent in European deciduous and mixed forest habitats

Methods: We assessed genetic diversity at selectively neutral, non-coding markers (microsatellites) and adaptive genetic variation at a functionally important part of the immune complex MHC (major histocompatibility complex). We investigated the load with gastrointestinal parasites non-invasively by faecal egg counts and assessed the influence of population genetic variation on parasite burden.

Results: Both neutral and adaptive genetic diversity differed between mice populations. We could not detect an effect of neutral genetic diversity on the parasite burden in a population. Heterozygosity at the MHC did not reveal an effect on the parasite burden either. However, we did identify significant effects of the number of different MHC alleles in a population on parasite burden. Mice populations with a large number of different MHC alleles displayed lower parasite loads than those populations with few different MHC alleles.

Keywords: *Apodemus flavicollis*, gastrointestinal nematodes, genetic variability, major histocompatibility complex, microsatellites, Muridae.

INTRODUCTION

Genetic variability is of paramount importance for the adaptive potential of a population to a changing environment. In particular, it is assumed to be essential for an efficient immune function and pathogen defence (e.g. O'Brien and Evermann, 1988; Hedrick, 2001; Altizer *et al.*, 2003; Reid *et al.*, 2003). The role of genetic variation in buffering host populations against pathogens was emphasized in several studies that revealed associations between low levels of genetic

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diversity and increased pathogen susceptibility (e.g. Liersch and Schmid-Hempel, 1998; Coltman *et al.*, 1999; Meagher, 1999; Cassinello *et al.*, 2001; Acevedo-Whitehouse *et al.*, 2003, 2006; Spielman *et al.*, 2004), a reduced immune reaction (Sanjayan *et al.*, 1996; Reid *et al.*, 2003; Whiteman *et al.*, 2006), and a severe progression of diseases (Dorman *et al.*, 2004; Acevedo-Whitehouse *et al.*, 2005). Moreover, pathogens may spread easily and quickly in genetically uniform host populations (Meagher, 1999; Spielman *et al.*, 2004). In contrast, resistance to pathogens is expected to increase with genetic diversity in a population (O'Brien and Evermann, 1988; Liersch and Schmid-Hempel, 1998; Hedrick, 2001) because high levels of genetic diversity in a population enhance the chance of at least some protective alleles being present and allow some individuals of the host population to deal successfully with co-evolving pathogens.

Routinely in population genetic studies, neutral genetic variation is measured to assess genetic diversity. Significant associations between individual microsatellite heterozygosity and fitness-related traits have been reported in several species (e.g. Coltman *et al.*, 1999; Slate *et al.*, 2000; Amos *et al.*, 2001; Acevedo-Whitehouse *et al.*, 2003). These correlations can be explained by either general or local genetic effects (Hansson and Westerberg, 2002): multilocus heterozygosity at the assessed markers might reflect genome-wide genetic variation or the markers could be in linkage disequilibrium with fitness-relevant loci. Markers that directly affect the host's fitness and therefore reflect adaptive genetic variation are, for instance, immune genes, such as the genes of the major histocompatibility complex (MHC). This gene complex plays a key role in the mammalian immune system in terms of pathogen recognition (Klein, 1986). MHC molecules are cell surface glycoproteins that bind antigens and present them to T-lymphocytes in a process essential for the specific immune response. Recent studies in wild populations confirmed that certain MHC alleles are associated with either increased or decreased parasite load (e.g. Paterson *et al.*, 1998; Froeschke and Sommer, 2005; Harf and Sommer, 2005; Meyer-Lucht and Sommer, 2005; Schad *et al.*, 2005; Axtner and Sommer, 2007; Deter *et al.*, 2008). Typically, the genes of the MHC show an extraordinary high polymorphism, which is thought to be driven by selection processes through pathogens (Doherty and Zinkernagel, 1975).

It has been suggested that, at a state of evolutionary equilibrium between hosts and pathogens, selection through diverse pathogens causes high MHC polymorphism in a species or population, whereas low MHC polymorphism indicates the presence of relaxed pathogenic selection pressure (Wegner *et al.*, 2003; Prugnolle *et al.*, 2005; Goüy de Bellocq *et al.*, 2008). This scenario holds true if hosts and pathogens share a long-term co-evolutionary history. In contrast, in an unbalanced situation – that is, after a recent loss of genetic diversity through, for instance, fragmentation effects – a species with low MHC diversity could have lost resistance alleles or other important parts of its adaptive evolutionary potential. This would facilitate an easy spread of pathogens throughout the population, because most individuals share the same resistance genotype (Meagher, 1999). It has been claimed that the loss of even a single MHC allele may have serious consequences for a population (Hughes, 1991).

In this study, we examined eight wild populations of the yellow necked mouse (*Apodemus flavicollis* Melchior 1834). Specifically, we tested the influence of inter-population genetic variation on parasite burden. We predicted that host populations with high MHC allelic diversity are better armed against parasite infections than populations with low MHC diversity, and hence expected less infected individuals and/or lower infection intensities in MHC diverse populations. Adaptive genetic variation at a functionally important part of the immune complex MHC (MHC class II DRB exon 2) was assessed and neutral genetic diversity was measured using microsatellites.

MATERIALS AND METHODS

Sites and sampling

Mice were trapped at eight sites in and around the city of Hamburg, northern Germany. Sites 1–5 represent public parks in urban areas (1.2–8.6 km from the city centre) with deciduous trees. Sites 6–8 were located in deciduous forest in rural areas (16–34 km from the city centre). Live-trapping was carried out on 1215 trap nights in the summers of the sampling years 2002 and 2004 using Sherman® traps ($3 \times 3.5 \times 9$ inches). Captured animals were anaesthetized for 1–2 min by inhalation of isoflurane (Forene®, Abbott GmbH, Germany), sexed, weighed, measured, and individually marked by ear punches of approximately 3 mm^2. The ear tissue samples were stored in 70% ethanol and used for subsequent genetic analyses. After the sampling procedure, animals were released at their respective trap location. Faeces were collected from each trap and fixed in 4.0% formaldehyde for examination of gastrointestinal nematodes. Traps were thoroughly cleaned after each use. Individuals with a body mass of less than 16 g were classified as juveniles (Jüdes, 1979) and excluded from subsequent analyses to avoid the sampling of family groups.

Genetic analysis

DNA was isolated from ear tissue using the DNeasy™ Tissue Kit (Qiagen®, Hilden, Germany) following the manufacturer's protocol. To assess neutral genetic diversity, we analysed six non-coding microsatellite markers. The loci CAA2A, GTTC4, GCATD7S (Markova *et al.*, 1998), MSAF3, MSAF8, and MSAF22 (Gockel *et al.*, 1997) have previously been described for *A. flavicollis* and were amplified following the authors' protocols. Microsatellite PCR products were genotyped using the CleanGel HyRes Plus System (ETC®, Kirchentellinsfurt, Germany) based on the manufacturer's instructions. We mixed 2–5 μl of the PCR product with 10 μl loading dye (gel buffer, 1% orange G, 1% xylene cyanol, 0.2 M EDTA). Then, 6 μl of each sample were loaded on a HyRes gel in a horizontal-cooling electrophoresis system (Amersham Pharmacia, Freiburg, Germany). The best results were achieved under the following conditions: 45 min at 200 V, 35 mA, 8 W followed by 15 min at 350 V, 50 mA, 20 W and 90–150 min at 500 V, 42 mA, 22 W; all steps were performed at a constant 12°C. After electrophoresis, gels were fixed and silver stained (Plus One DNA Silver Staining Kit, Amersham Pharmacia®, Freiburg, Germany). The patterns visualized were classified to length alleles.

The amplification of the MHC class II DRB exon 2 and single strand conformation polymorphism (SSCP) procedures have been described previously (Meyer-Lucht and Sommer, 2005). At least three independent examples of each identified allele were cut from an SSCP gel and re-amplified before sequencing. Products were sequenced bi-directionally using a dye terminator sequencing kit (Applied Biosystems, Foster City, CA) with an Applied Biosystems® automated sequencer (model 3100) following the manufacturer's instructions. MHC-DRB sequences are available at GenBank (accession numbers AY699757 to AY699771, AY918078 to AY918090).

Parasitological examinations

We focused parasite analyses in *A. flavicollis* on gastrointestinal nematodes due to their high abundance in small mammals (Keymer and Dobson, 1987; Montgomery and Montgomery, 1988; Stefancíková

et al., 1994; Behnke *et al.*, 1999) and their impact on fitness attributes as well as mortality in a wide range of livestock and wild animal species (Gulland, 1992; Tompkins and Begon, 1999; Albon *et al.*, 2002; Stien *et al.*, 2002). Parasite loads were investigated by counting the nematode eggs in the animal's faeces using a modification of the non-invasive McMaster flotation technique (Gordon and Whitlock, 1939; Meyer-Lucht and Sommer, 2005). Faecal egg counts (FEC; number of eggs per gram of faeces) reflect the overall worm burden and their fecundity, which are both influenced by the immune state of the host (Stear *et al.*, 1995, 1997). Faecal egg counts is a widely used approach in field studies as a result of the non-invasive procedure (e.g. Coltman *et al.*, 1999; Cassinello *et al.*, 2001; Ferrari *et al.*, 2004; Seivwright *et al.*, 2004; Froeschke and Sommer, 2005; Harf and Sommer, 2005; Meyer-Lucht and Sommer, 2005; Schad *et al.*, 2005; Schwensow *et al.*, 2007). We used the prevalence of nematodes (infected individuals/examined individuals) and the mean intensity of nematode infection (log-transformed FEC values) per population as variables for parasitism.

Data analysis

Neutral genetic diversity per population was measured as the mean microsatellite MLH [multilocus heterozygosity (Coltman *et al.*, 1999)] and mean microsatellite d^2 [difference in repeat units, averaged over all loci (Coulson *et al.*, 1998)]. Microsatellite F_{ST}-values were calculated using Microsatellite Analyser [MSA (Dieringer and Schlötterer, 2003)]. MHC nucleotide sequences were edited manually and aligned in MEGA 4 (Tamura *et al.*, 2007). MHC genetic diversity was described using the observed heterozygosity and the allelic richness. As the observed number of alleles in a sample is highly dependent on the number of sampled individuals, we calculated the allelic richness corrected for different sample sizes using a rarefaction index implemented in FSTAT (Goudet, 2001). Thereby, the expected number of alleles in each sub-sample is calculated for the number of individuals present in the smallest sample. Observed and expected heterozygosity as well as F_{ST}-values for the MHC marker were calculated by Arlequin 3.0 (Excoffier *et al.*, 2005).

We investigated the effects of genetic diversity for both markers on parasite load by means of generalized linear models (GLMs). Models were fitted for nematode prevalence and mean nematode infection intensity. For prevalence data, logistic regression models were applied with a binomial error distribution and logit link function. For the log-transformed infection intensity data, we used a Gaussian error distribution with an identity link function. Due to the small number of populations ($n = 8$), the four predictors of genetic diversity (microsatellite MLH, microsatellite d^2, MHC heterozygosity, and number of MHC alleles) were included in separate models, each model comprising the sampling year (2002, 2004) and one of the genetic predictors. The proportion of deviance explained by each variable (r^2) was assessed by removing this predictor from the model, comparing the change in deviance to the full model deviance, and testing it for significance. The generalized linear models were performed in R (version 2.7.0) (R Development Core Team, 2008). Figures were generated in Sigma Plot Version 10.0.

RESULTS

Genetic diversity within and among populations

Altogether, 159 individuals from eight populations were genotyped. The study populations differed in their levels of genetic diversity with regard to both genetic markers (Table 1).

Table 1. Nematode parasitism and genetic diversity in eight populations of *A. flavicollis* and in the total sample

		Nematodes		Microsatellites		MHC		
Population	*n*	Prevalence (%)	Mean intensity ± S.D. (FEC, log epg + 1)	Mean MLH	Mean d^2	H_{obs}/H_{exp}	Number of alleles	Allelic richness
1	20	80.0	2.01 ± 1.17	0.996	74.17	0.70/0.86	8	7.56
2	21	66.7	2.23 ± 1.80	1.043	55.87	0.86/0.85	8	7.70
3	20	70.0	1.72 ± 1.32	0.952	22.07	0.90/0.87	9	8.52
4	21	52.4	1.13 ± 1.22	0.987	37.50	0.48/0.71	11	9.32
5	18	61.1	1.45 ± 1.30	0.934	29.17	0.72/0.87	9	8.66
6	16	50.0	1.37 ± 1.46	0.980	25.20	0.94/0.89	12	12.00
7	21	71.4	1.88 ± 1.34	1.025	43.62	0.86/0.85	11	9.52
8	22	45.5	1.15 ± 1.32	1.076	43.20	0.95/0.91	17	14.51
Total	159	62.0	1.62 ± 1.40	1.000	41.43	0.80/0.89	27	12.09

Note: n = sample size, S.D. = standard deviation, FEC = faecal egg count, epg = eggs per gram, H_{obs} = observed heterozygosity, H_{exp} = expected heterozygosity according to Hardy-Weinberg. MHC allelic richness was corrected for sample size.

Of the four predictors describing genetic diversity, mean MLH and mean d^2 were correlated (Spearman's rho = 0.762, $n = 8$, $P < 0.05$). Regarding the microsatellite markers, there was low variation in the mean MLH values among populations but mean d^2 varied notably from 22.07 (population 3) to 74.17 (population 1). In total, 28 different MHC DRB alleles were identified. Of these, two nucleotide sequences translated into the same amino acid sequence and were combined into one allele (*Apfl*-DRB*23a and *Apfl*-DRB*23b). Nucleotide diversity of all *Apfl*-DRB alleles is described in Meyer-Lucht and Sommer (2005). There was a wide range in observed MHC heterozygosity among populations, from 0.48 (population 4) to 0.95 (population 8). Corrected MHC allelic richness varied widely as well, from 7.56 in population 1 to 14.51 in population 8 (Table 1). Differentiation among populations was highly significant in both types of markers (microsatellites: $F_{ST} = 0.059$, $P < 0.001$; MHC: $F_{ST} = 0.0633$, $P < 0.001$). From the four predictors describing genetic diversity, only corrected MHC allelic richness per population was correlated (positively) with the population's geographic distance to the city centre (Spearman's rho = 0.952, $n = 8$, $P < 0.01$).

Parasite load

In 159 *A. flavicollis* individual faecal samples, eight different morphotypes of gastrointestinal nematodes were detected. Five of these were strongyle nematodes, two were from the Trichuridae family (*Capillaria* sp., *Trichuris* sp.), and one belonged to the Oxyuridae family (*Syphacia* sp.). Sixty-two percent of the examined individuals hosted infections of one to three nematode morphotypes; the remaining 38% did not show any infection. The number of different nematode morphotypes in the populations ranged from four to six. Both the strongyle nematodes and those from the family Trichuridae were frequently detected (in 49.7% and 25.2% of all individuals, respectively), whereas *Syphacia* sp. was rarely observed

(in 5.7% of individuals). The sex of the host had no effect on nematode prevalence ($\chi^2_1 = 1.279$; $P = 0.26$) or on infection intensity measured as faecal egg counts (Mann-Whitney U-test, $z = -0.323$, $P = 0.75$). Among the populations, nematode prevalence ranged from 45.5% to 80.0%, and mean infection intensity varied from 1.13 ± 1.22 to 2.23 ± 1.80 (FEC; Table 1). Nematode prevalence and mean nematode infection intensity were correlated (Spearman's rho = 0.782, $n = 8$, $P < 0.05$).

Effects of population genetic diversity on nematode load

Using generalized linear models, we investigated the effects of population genetic diversity on nematode load (Table 2). We calculated models for the influence of each genetic predictor separately. Neutral genetic diversity did not have a significant effect on the nematode load. Neither mean MLH nor mean d^2 had a marked influence on nematode prevalence, as removing these variables from the models did not lead to a significant increase in deviance (MLH: $z_5 = -0.63$, $P = 0.53$; d^2: $z_5 = 1.40$, $P = 0.16$) (Figs. 1a, b; Table 2). Similarly, there were no effects on the intensity of infection (MLH: $t_5 = 0.07$, $P = 0.95$; d^2: $t_5 = 1.58$, $P = 0.18$) (Figs. 2a, b; Table 2). Moreover, for the coding MHC marker, we did not detect an effect of observed MHC heterozygosity on nematode prevalence or intensity of infection (prevalence: $z_5 = 0.04$, $P = 0.97$, Fig. 1c; infection intensity: $t_5 = 0.27$, $P = 0.80$,

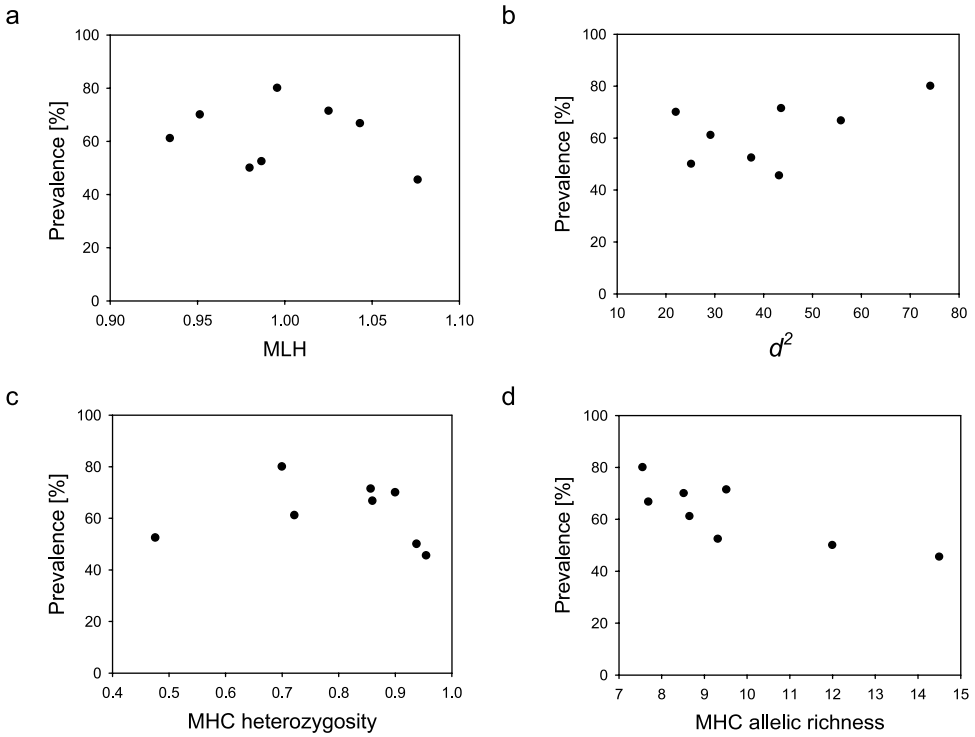

Fig. 1. Nematode prevalence in relation to different measures of genetic diversity in populations of *A. flavicollis*. Microsatellite markers: (a) mean multilocus heterozygosity (MLH), (b) mean averaged differences in repeat units (d^2) per population. MHC markers: (c) MHC heterozygosity, (d) MHC allelic richness per population.

Table 2. Effects of genetic diversity on nematode load in eight populations of *A. flavicollis*, calculated by generalized linear models: full models for (a) nematode prevalence and (b) nematode infection intensity

	$\beta \pm$ S.E.	R^2	P
(a) Nematode prevalence			
Model 1			
Year	−0.034 ± 0.373	0.001	0.93
MLH	−2.278 ± 4.106	0.045	0.53
Model 2			
Year	−0.111 ± 0.336	0.012	0.74
d^2	0.015 ± 0.011	0.229	0.16
Model 3			
Year	−0.145 ± 0.406	0.014	0.72
MHC H_{obs}	0.046 ± 1.312	<0.001	0.97
Model 4			
Year	−0.103 ± 0.342	0.010	0.76
MHC allelic richness	−0.170 ± 0.074	0.606	0.02
(b) Nematode infection intensity			
Model 1			
Year	0.116 ± 0.383	0.018	0.77
MLH	0.304 ± 4.208	0.001	0.95
Model 2			
Year	0.149 ± 0.283	0.036	0.62
d^2	0.013 ± 0.008	0.324	0.18
Model 3			
Year	0.069 ± 0.407	0.006	0.87
MHC H_{obs}	0.352 ± 1.310	0.014	0.80
Model 4			
Year	0.059 ± 0.114	0.023	0.63
MHC allelic richness	−0.128 ± 0.051	0.543	0.05

Note: Year of capture was included in each model. $\beta \pm$ S.E. = the coefficient ± standard error, r^2 = the proportion of variance explained by each predictor. *P*-values were calculated by removing each predictor from the model and testing the change in deviance for significance.

Fig. 2c, Table 2). However, the MHC allelic richness in a population had a significant effect and explained a substantial part of the variation in nematode load. A high number of different MHC alleles in a population significantly decreased the prevalence of nematodes in the population ($z_5 = -2.29$, $P = 0.02$, Fig. 1d). Similarly, a high MHC allelic richness reduced the mean nematode infection intensity in the population, although at the margin of significance ($t_5 = -2.51$, $P = 0.05$, Fig. 2d; Table 2).

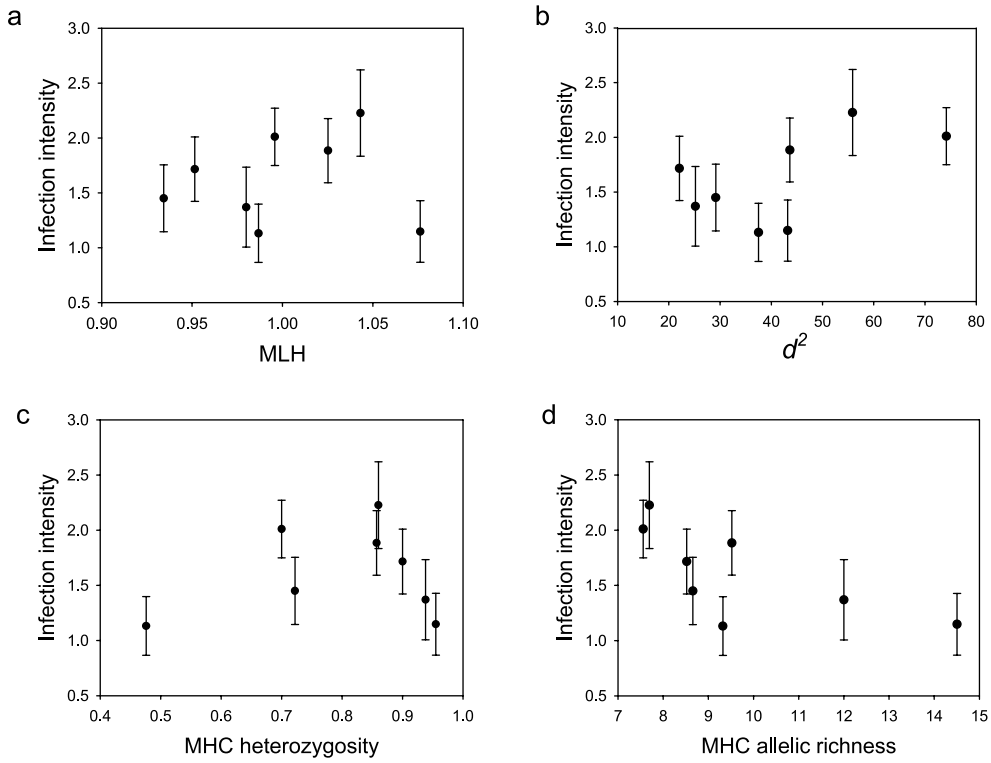

Fig. 2. Nematode infection intensity in relation to different measures of genetic diversity in populations of *A. flavicollis*. Microsatellite markers: (a) mean multilocus heterozygosity (MLH), (b) mean averaged differences in repeat units (d^2) per population. MHC markers: (c) MHC heterozygosity, (d) MHC allelic richness per population. Bars represent the standard errors of the means.

DISCUSSION

The importance of host genetic diversity in pathogen defence was highlighted in several studies, in which mainly single infectious agents were used. For natural populations, these effects are expected to be even stronger due to the presence of a variety of infectious pathogens (Spielman *et al.*, 2004). Here, we studied the importance of genetic diversity for nematode resistance in natural populations of yellow necked mice. We investigated neutral and adaptive genetic variation and tested the influence of inter-population genetic variation on parasite burden. No significant effects of neutral genetic diversity or MHC heterozygosity on parasite burden were detected. However, we observed a significant effect of MHC allelic richness within a population on nematode resistance. Mice populations with a large MHC allele repertoire displayed lower parasite loads.

The number of nematode morphotypes and their prevalence detected in the study populations are within the range of results of other parasitological studies of the genus *Apodemus* [3–7 nematode species, 24–92% helminth prevalence, dominated by nematodes (Stefancíková *et al.*, 1994; Behnke *et al.*, 1999; Abu-Madi *et al.*, 2000)]. In wild rodents, parasite prevalence and intensity are known to underlie spatial and temporal variation, as well as intrinsic factors such as the immune state, sex, and age of the host (Abu-Madi *et al.*, 2000; Behnke *et al.*, 2001,

2005). However, in our data, sampling year had no significant effect on nematode prevalence and intensity.

To date, most studies have examined MHC diversity within single populations (Meyer and Thompson, 2001); few studies (e.g. Prugnolle *et al.*, 2005) have investigated MHC diversity between different populations. Prugnolle *et al.* (2005) examined the relationship between MHC diversity (HLA in humans) in different human populations worldwide and their corresponding pathogen regimes sharing a long-term co-evolutionary history (evolutionary equilibrium between hosts and pathogens). They showed that, besides a strong effect of the human colonization history, MHC diversity is shaped by local pathogen richness. Human populations exposed to a more diverse pathogen array show higher HLA diversity but not higher neutral genetic diversity than those exposed to fewer pathogens. Analogously, in a study of eight stickleback populations (*Gasterosteus aculeatus*), Wegner *et al.* (2003) reported that parasite diversity was positively correlated with MHC *IIB* variation in a population but not with genetic neutral variation. In both studies, populations from very different habitats with different levels of pathogen richness were explored but neither prevalence nor infection intensity were taken into account. In contrast, our study populations originate from similar habitats within the same geographic region and featured very similar parasite communities and diversities. Hence, no association between parasite richness and MHC diversity was detectable (data not shown) but populations differed in prevalence and infection intensity. We assume that in our study populations the pronounced differences in MHC diversity are likely caused by isolation effects in the park areas investigated, as suggested by the association of MHC allelic richness (but no other genetic parameters) with distance to the city. Some host populations seemed to be in an evolutionary unbalanced situation and probably experienced a recent loss of genetic diversity.

An individual itself will not directly benefit from its population's large MHC allele pool in terms of parasite resistance. But constantly challenged by multiple pathogens in the wild, populations with a large MHC allele pool are more likely to harbour individuals that possess protective alleles for each of the different infections. Moreover, several protective alleles in a population will hinder the easy spreading of pathogens because adaptation to diverse resistance genotypes is more complex than in a genetically uniform population. Thus, our findings are in line with our previous findings in *A. flavicollis* and a number of studies of other natural mammal populations, which pointed out the functional importance of specific MHC alleles for resistance or susceptibility to parasite infections in individual-based analyses (Paterson *et al.*, 1998; Froeschke and Sommer, 2005; Harf and Sommer, 2005; Meyer-Lucht and Sommer, 2005; Schad *et al.*, 2005; Axtner and Sommer, 2007; Deter *et al.*, 2008).

Another debated selection mechanism is the 'heterozygote advantage hypothesis' (Doherty and Zinkernagel, 1975). This hypothesis is based on the suggestion that heterozygotes are able to recognize two suites of pathogens, one for each allele, and are therefore favoured due to their ability to resist a broader array of pathogens than homozygotes. Studies supporting the heterozygote advantage hypothesis have usually been carried out under laboratory conditions (Doherty and Zinkernagel, 1975; Penn *et al.*, 2002; McClelland *et al.*, 2003). Few studies on wild populations detected an advantage of MHC heterozygote individuals in terms of parasite infections (e.g. Froeschke and Sommer, 2005; Oliver *et al.*, 2009). For the present study, however, we suggest treating the insignificant effect of MHC heterozygosity on nematode resistance with caution because the small number of observations (eight populations) infers low statistical power. On the other hand, this strengthens the positive result of a significant association between MHC allelic richness and nematode resistance, as it was detectable despite the low

statistical power. Our findings therefore are in line with recent simulations and theoretical models which showed that heterozygote advantage on its own is insufficient to explain the large number of MHC alleles commonly observed (Borghans *et al.*, 2004; De Boer *et al.*, 2004).

An influence of neutral genetic diversity on nematode burden was not observed, although we are aware that six microsatellite loci only partially describe genome-wide variation. Despite being widely used as a surrogate for inbreeding, it was shown that both microsatellite diversity measures (MLH and d^2) are only very weakly correlated with the inbreeding coefficient, even at a high number of typed loci (Balloux *et al.*, 2004; Slate *et al.*, 2004). Therefore, we cannot draw conclusions regarding whether inbreeding depression is present in the populations investigated. There was no correlation between neutral and MHC diversity in our data, which one would expect if genetic drift and inbreeding had predominantly shaped the genetic structure of the populations. It appears that nematode resistance in the studied populations is a matter of genetic variation at loci involved in pathogen defence rather than related to overall genetic diversity. Similarly, Spielman and colleagues (2004) reported that lowered pathogen resistance in *Drosophila* populations was not related to generalized inbreeding effects but to the absence of specific resistance alleles.

In conclusion, our results support the hypothesis that populations with a high MHC allele diversity are better armed against high parasite burdens and highlight the significance of adaptive genetic diversity in the field of conservation genetics. A population with a large MHC allele reservoir is more likely to possess resistance alleles to the multitude of pathogens present in the wild.

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