underlying the increased SCE rates in lymphocytes of alcoholic subjects.

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Selection for class II *Mhc* heterozygosity by parasites in subterranean mole rats

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Abstract. Mhc organization and polymorphism have previously been studied[26] in the four chromosomal species of the *Spalax ehrenbergi* superspecies in Israel, serologically, and at the DNA, RFLP and sequence levels of class I and class II genes. Here we demonstrate that the observed heterozygosity of *Mhc* class II genes $P\alpha_1$ with 11 alleles, and $Q\beta$, with at least 14 alleles, is positively and significantly correlated with infectivities of ectoparasites (gamasid mites)[17] and endoparasites (helminths)[18]. *Mhc* heterozygosity is highest in the most infected area, which is in the most humid-warm region of the superspecies range, or where two zoogeographic regions overlap. We conclude that the evolutionary forces responsible for the *Mhc* class II two-gene polymorphisms include selection for increased heterozygosity as a defense strategy against ecto- and endoparasite infections.
Key words. Mhc; heterozygosity; parasites; natural selection; subterranean mole rats; *Spalax ehrenbergi.*

The major histocompatibility complex, *Mhc*[1], is among the most complex gene clusters so far known, reflecting a very long and involved evolutionary history. *Mhc* covers about one thousandth of the mammalian genome, 3800 kb in man[2], including two major gene classes (I and II) of cell surface glycoproteins with different but related functions of immunoregulation[1–4]. The function of the *Mhc* genes is to control the recognition of foreign and self proteins by T lymphocytes. Class II *Mhc* molecules present foreign peptides to helper T lymphocytes[5–8]. Helper T cell receptors recognize foreign protein-derived peptides only when these are associated with self class II *Mhc* molecules. A given T cell receptor is both peptide-specific and *Mhc*-restricted. The *Mhc* controls the specificity of the immune response against pathogens including viruses, bacteria and other parasites. It also contributes to the susceptibility to over 40 different autoimmune diseases[9], in which the body's immune system attacks self proteins.

Subterranean mole rats of the *S. ehrenbergi* superspecies in Israel represent an active case of ecological speciation[10–16]. The superspecies comprises four chromosomal species ($2n = 52$, 54, 58 and 60), displaying progressive stages of late chromosomal speciation. Their adaptive radiation in Israel from the Lower Pleistocene to Recent times is closely associated with fossoriality and increasing aridity, i.e., with distinct climatic diversity: $2n = 52$, cool-humid (north); $2n = 54$, cool-semidry (north-east); $2n = 58$, warm-humid (center); and finally, $2n = 60$, warm-dry, in the southern part of the range[13].

The ecological speciation of *S. ehrenbergi* into increasingly arid environments involves climatically coadapted genomic and organismal adaptations[16].

Earlier, we studied ectoparasite[17] and endoparasite[18] infections in the *S. ehrenbergi* superspecies. Sixty-five breeding nests of *S. ehrenbergi* were collected from 23 localities throughout the range and processed for nidicolous arthropods[17]. A total of five species of fleas and 53 species of gamasid mites were collected. The analysis is based on 31 gamasid species that appear in more than a single nest. Their distribution in the investigated area was analyzed in relation to that of the chromosomal species of *S. ehrenbergi*. It was observed that the Palaearctic species reach their southern limit of distribution on Mount Hermon and some on the Golan Heights in the region of $2n = 54$. This observation led to a separate analysis of the Hermon subregion and the rest of the $2n = 54$ region. The highest species diversity of gamasid mites was observed on Mt. Hermon, where two zoogeographical regions overlap, and in the range of 2n = 58, which is characterized by a humid-warm climatic regime (table).

The helminth fauna of the four chromosomal species of the *S. ehrenbergi* superspecies in Israel was also studied[18]. In the examination of 153 hosts, five species of adult helminths and one larval nematode were recovered, namely: *Heligmonina nevoi* n.sp. (Wertheim and Durrette-Desset)[19]; *Trichuris muris* (Schrank, 1788); *Ganguleterakis spalaxi* Kozlov and Yangolenko 1962/63; *Gongylonema longispiculum* Schultz, 1927 and *Paranoplocephala* sp. The helminth *Heligmonina nevoi*, the most prevalent, was recovered from all climatic regions and chromosomal species, while the other helminth species occurred in the northern and central regions in two or three *Spalax* species only. A correlation was found between climatic conditions and the distribution of the helminths. In the case of helminths, the percentage of infected mole rats was the measure of infectivity used in the correlation.

The *Mhc* of the mole rat was designated *Smh* for *Spalax* major histocompatibility[20-26]. The *Smh* class II region consists of two gene families, P and Q, instead of the four families (P,O,Q,R), found in all mammals studied hitherto. The *Spalax* P family comprises at least four β and three α genes or gene fragments[24]. RFLPs of two *Smh* class II genes ($P\alpha_1$ and $Q\beta$) were studied in the superspecies range in Israel. Polymorphisms were found in both genes, $P\alpha_1$ with 11 alleles and $Q\beta$ with at least 14 alleles[25].

We tested RFLPs by $P\alpha_1$ and $Q\beta$ *Mhc* probes in 121 mole rats comprising 13 populations and 4 chromosomal species of the *Spalax ehrenbergi* superspecies in Israel. High molecular weight genomic DNA was extracted from the kidneys. Five different six-base recognition site restriction endonucleases were used: *Eco*RI, *Hin*dIII, *Bst*EII, *Kpn*I and *Bam*HI. Two probes of *S. ehrenbergi* extracted from a *Spalax* genomic library[23] were used: a 4-kb probe containing most of the $P\alpha_1$ gene, and a 5.2-kb probe containing almost the entire $Q\beta$ gene[23]. The data were analyzed first in terms of allele fragment frequencies by scoring the bands directly from the autoradiograph, thus identifying directly the homozygotes and the heterozygotes.

Observed heterozygosity was calculated by scoring directly from gels the proportion of heterozygous individuals per population and per species. H was calculated in a previous study[25] for each restriction enzyme separately, and then averaged for each locus. In the present study each animal heterozygous in at least one restriction enzyme has been considered heterozygous in the relevant locus, hence the higher H values in the present study. Both procedures are legitimate. The present procedure follows the estimation of H in classical population genetics. Here we demonstrate that mite and helminth infections correlate positively with the heterozygosity of the two class II *Smh* genes.

The results, given in the table and figure 1, show that the percentages of helminth and gamasid mite infections increase with the climatic index, i.e., with both humidity and temperature. The Mt. Hermon data were excluded from the gamasid climatic correlation, because nonclimatic, zoogeographical evidence explains the high species diversity in this subregion. Infections of helminths and mites were positively correlated with the humidity – temperature index ($r = 0.973$, $p = 0.014$; and $r = 0.813$, $p = 0.094$, respectively, using one-tailed test).

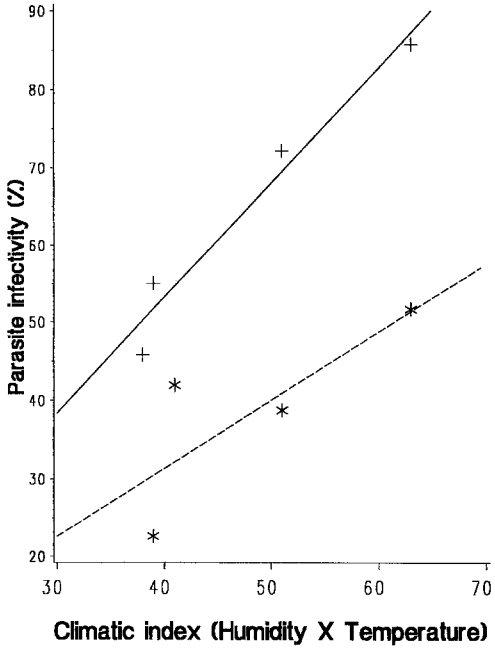

Figure 1. Infectivity of helminth and gamasid mites in the *Spalax ehrenbergi* superspecies as a function of climate. Dashed line and stars represent gamasid mites, and continuous lines and plus signs represent helminths.

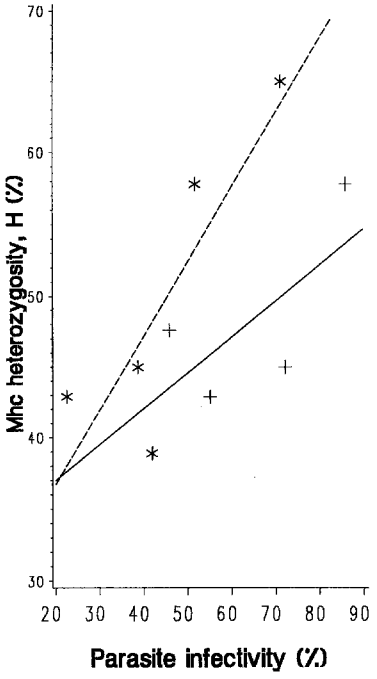

Figure 2. *Smh* heterozygosity as a function of gamasid mites and helminth infectivity. See legend in fig. 1.

Figure 2 shows that *Smh* heterozygosity, *H*, correlates positively and significantly with helminth and mite infections ($r = 0.684$, $p = 0.158$; and $r = 0.849$, $p = 0.034$, respectively). The combined probability is $p < 0.05$ (Fisher's method[27]). Thus, *H* was highest in the most infected regions, which coincide either with the most humid-warm regime, occupied by the $2n = 58$ species, or with Mt. Hermon, where two zoogeographical regions overlap, thus enriching the gamasid load[17] (table).

Mhc loci are known to be highly polymorphic in humans, mice, and other mammals, with heterozygosity as high as 80–90%[1], even at individual amino acid sites[28]. High levels of polymorphism and heterozygosity, as well as high numbers of *Smh* genes (> 65 in class I alone!) have recently been described in mole rats of the superspecies *Spalax ehrenbergi*[20–26]. It is noteworthy, however, that limited polymorphism of both classes of *Mhc* genes was described in four different species of the Balkan mole rat of the superspecies *Spalax leucodon*[29]. This contrasting result, based on a relatively small sample size, needs further examination.

Four different hypotheses, and a fifth one presented here, have been proposed to explain this high degree of *Mhc* polymorphism[30]. These include: 1) a high mutation rate[31]; 2) gene conversion or interlocus genetic exchange[32–34]; 3) overdominant selection[30,35,36]; 4) frequency-dependent selection[37,38]; and 5) spatiotemporal ecological selection (here expressed by parasites), as presented in this study. These five hypotheses are not mutually exclusive and can separately or combinatorially contribute to the high level of *Mhc* diversity. However, only the last two hypotheses can explain the *differential* levels of *Smh* heterozygosity found here. The significant correlations of *Smh* heterozygosity with ecogeography, climate and the level of parasite infection, rule out in *S. ehrenbergi* hypotheses 1) and 2) as an explanation of the observed correlation. Likewise, we found excess, deficiency, and expected frequencies of *H*, reflecting over-, under-, and no dominance in fitness of heterozygotes in different populations. By elimination, we are left with hypotheses 4) and 5), which are interrelated and complementary forms of balancing selection. Previously, comparisons to neutrality expectations of the extent and pattern of single-locus and two-locus variation in the *HLA* in humans, strongly suggested that selection is important at these loci, as indicated by prevalent nonrandom associations (gametic phase disequilibria) in this region, and DNA sequence levels[30,38–42]. Heterozygosity at individual amino acid sites was found to be extremely high for *HLA*-A and B genes in humans; this is explicable by balancing selection[39].

In the present study, we demonstrated that heterozygosity at the two class II loci $Q\beta$ and $P\alpha_1$ is non-random, and the level of *H* is highest at the foci of high ecto- and endoparasite infection. This pattern eliminates random

Chromosomal species of the *Spalax ehrenbergi* complex and their climatic, pathogenic, and *Smh* heterozygosity data

Chromosomal species (2n)	Climatic index Humidity × January temperature	Infectivity Gamasid mites; species no. %	Helminths, % of mole rats infected	*Smh* class II genes ($P\alpha_1 + Q\beta$) Heterozygosity (*H*)
52	392	7 (22.6)	55.0	0.429
54*	387	–	45.8	0.476
54**	419	13 (41.9)	–	0.389
(54) Hermon	180	22 (71.0)	–	0.650
58	638	16 (51.6)	85.7	0.578
60	511	12 (38.7)	72.1	0.450
Total		31 (100)		
Mean				0.483
References	12	17	18	25 (see text)

*Used for correlation with helminth data. **Without Hermon population, due to its unique zoogeographical position[17], used for correlation with gamasid data.

genetic drift, neutrality and migration (see correlations in results) as explanatory models, and strongly suggests that various forms of natural balancing selection (e.g. frequency-dependent and spatiotemporal variation in the level of parasite infection) play an important role in the varied *Smh* patterns of heterozygosity. The patterns of *H* found here deviate significantly from neutrality expectations, and are consistent with a selection model.

High individual heterozygosity and high population polymorphism at the *Smh* region seem to safeguard survivorship under stresses of variable and high pathogenicity. Interestingly, allozyme heterozygosity increases with climatic unpredictability towards the desert in *S. ehrenbergi* and in 13 other unrelated genera involving 21 species of plants, invertebrates and vertebrates in Israel tested on average for 27 enzyme loci[42]. By contrast, *Smh* heterozygosity increases with climatic predictability in warm-humid regions where high and variable pathogenicity, hence parasite *unpredictability* is highest. Viability selection presumably acts on *Smh* variants through differential effects on resistance to other important and diverse pathogens in addition to those demonstrated here (which might only be carriers of other pathogens, i.e., viruses, bacteria and protozoans, and also of other yet unidentified acarines). In both cases of allozymes and *Smh*, however, various forms of balancing natural selection appear to be the major deterministic evolutionary forces causing differential heterozygosity, hence, increase in fitness. These ideas are supported experimentally[43], and theoretically[44]. However, experimental, functional and sequence studies in *Spalax* are needed to identify and establish directly a priori, rather than a posteriori as demonstrated here, the importance of differential high levels of *Smh* heterozygosity and different variants at the amino acid, protein and DNA levels in the control of the immune response against pathogens and diseases.

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