Infection, Genetics and Evolution 11 (2011) 1023–1036

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

MHC variation, multiple simultaneous infections and physiological condition in the subterranean rodent *Ctenomys talarum*

Ana Paula Cutrera [a,*], Roxana Rita Zenuto [a], Eileen Anne Lacey [b]

[a] Laboratorio de Ecofisiología, Departamento de Biología, Universidad Nacional de Mar del Plata - CONICET, C.C. 1245, Mar del Plata, Argentina
[b] Museum of Vertebrate Zoology and Department of Integrative Biology, University of California at Berkeley, 3101 VLSB, Berkeley, CA, 94720-3160, USA

ARTICLE INFO

Article history:
Received 9 September 2010
Received in revised form 28 February 2011
Accepted 24 March 2011
Available online 8 April 2011

Keywords:
MHC
Balancing selection
Immunocompetence
Parasites
SRBC

ABSTRACT

Parasites and pathogens can play a significant role in shaping the genetic diversity of host populations, particularly at genes associated with host immune response. To explore this relationship in a natural population of vertebrates, we characterized Major Histocompatibility Complex (MHC) variation in the subterranean rodent *Ctenomys talarum* (the talas tuco-tuco) as a function of parasite load and ability to mount an adaptive immune response against a novel antigen. Specifically, we quantified genotypic diversity at the MHC class II DRB locus in relation to (1) natural variation in infection by multiple genera of parasites (potential agents of selection on MHC genes) and (2) antibody production in response to injection with sheep red blood cells (a measure of immunocompetence). Data were analyzed using co-inertia multivariate statistics, with epidemiological proxies for individual condition (hematocrit, leukocyte profile, body weight) and risk of parasite exposure (season of capture, sex). A significant excess of DRB heterozygotes was evident in the study population. Co-inertia analyses revealed significant associations between specific DRB alleles and both parasite load and intensity of humoral immune response against sheep red blood cells. The presence of specific DRB aminoacid sequences appeared to be more strongly associated with parasite load and response to a novel antigen than was heterozygosity at the DRB locus. These data suggest a role for parasite-driven balancing selection in maintaining MHC variation in natural populations of *C. talarum*. At the same time, these findings underscore the importance of using diverse parameters to study interactions among physiological conditions, immunocompetence, and MHC diversity in free-living animals that are confronted with multiple simultaneous immune challenges.

1. Introduction

Parasite load is thought to be determined primarily by two factors: exposure to parasites and susceptibility to parasitic infection (Restif and Amos, 2010). The former is influenced by the prevalence of parasites in the host population (e.g. seasonal variation, Abu-Madi et al., 2000) as well as host behavior (e.g. intersexual differences in activity that impact risk of infection, Semple et al., 2002). The latter may be affected by the host's genetic background (see Charbonnel et al., 2006 for a review) and condition (e.g. poor condition may predispose individuals to infection, Beldomenico et al., 2008). Given this multiplicity of factors, it is not surprising that parasite load can vary markedly among individuals, including among conspecifics resident in the same local population. Wild animals cannot pharmacologically control or eliminate parasitic infections (Weil et al., 2006) and thus by undermining host health and reproductive success, parasites are expected to exert strong selective pressures on host immune defenses (Gillespie, 1975).

Selection imposed by parasites and pathogens is thought to play a significant role in shaping the genetic diversity of natural populations, particularly at genes associated with host immune response (reviewed by Charbonnel et al., 2006). The genes of the Major Histocompatibility Complex (MHC) are a fundamental component of the vertebrate immune system (Hedrick and Kim, 2000; Penn et al., 2002). MHC genes code for glycoproteins involved in the recognition and binding of foreign antigens, with presentation of bound antigens to T-lymphocytes eliciting the associated immune response (Klein, 1986). In particular, class II MHC genes recognize and bind peptides produced by extracellular pathogens such as parasites and bacteria (Klein and Horejsi, 1997; Janeway and Travers, 1999). Given the role of MHC genes in the vertebrate immune response, parasite-driven balancing selection is thought to be important in maintaining high levels of variation at these loci, with this selection acting through either heterozygote advantage (Doherty and Zinkernagel, 1975) or rare allele advantage (Lively and Dybdahl, 2000). One predicted evolutionary

* Corresponding author. Tel.: +54 223 4752426x466; fax: +54 223 4753150.
E-mail addresses: acutrera@mdp.edu.ar (A.P. Cutrera), ealacey@berkeley.edu (E.A. Lacey).

1567-1348/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.meegid.2011.03.016

outcome of such pathogen-driven selection is a strong association between MHC variability and patterns of resistance to specific infectious organisms (Spurgin and Richardson, 2010).

In addition to exploring relationships between MHC variation and parasite load, interactions between MHC loci and disease resistance can be assessed by relating MHC variability to the intensity of antibody-mediated immune responses (Bonneaud et al., 2005). Measuring immune activity against a standard challenge with a foreign antigen is thought to provide information regarding immunocompetence (i.e. the magnitude and effectiveness of an animal's immune response, Norris and Evans, 2000) although because the ability of individual immune assays to predict disease resistance depends on the pathogen (e.g. Gross et al., 1980; Adamo, 2004), response to a single antigen should not be interpreted as a proxy for overall resistance to disease. The ability of an individual to mount an adaptive immune response against a novel antigen is in part dependent on its genetically determined capacity to recognize that entity as foreign (Rigby et al., 2002), an ability that, as argued above, may be under parasite-driven selection. This response may be energetically costly (Bonneaud et al., 2003), resulting in physiological trade-offs (i.e. reproduction versus immunity) in affected individuals (Speakman, 2008) and highlighting the importance of exploring the intricate relationships between MHC genes, parasites, immunocompetence and selection in complex environments characterized by diverse host genotypes (Sommer, 2005; Charbonnel et al., 2006). Accordingly, wild vertebrates constitute a promising biological model for exploring the function of MHC genes from ecological and evolutionary perspectives.

Rodents in the genus *Ctenomys* (tuco-tucos) provide an important opportunity to assess the functional significance of MHC variability in naturally occurring populations of vertebrates. These subterranean mammals, which occur from southern Peru to Tierra del Fuego and from the Andes to southeastern Brazil, are characterized by extensive sharing of MHC allele lineages among species (trans-species polymorphism, Klein, 1987), suggesting that balancing selection has played a significant role in shaping MHC diversity in these animals (Cutrera and Lacey, 2007). Within species, selection on MHC genes appears to vary predictably with behavioral and demographic attributes such as social structure and population density (Lacey and Cutrera, 2007). Specifically, comparisons of the group-living colonial tuco-tuco *Ctenomys sociabilis* and the solitary Patagonian tuco-tuco *Ctenomys haigi* have revealed that selection on the class II DQB locus is stronger in the social species (Hambuch and Lacey, 2002), while comparative studies of two demographically distinct populations of *Ctenomys talarum* (talas tuco-tuco) have demonstrated that allelism, heterozygosity and intensity of selection at two class II loci (DRB and DQA) are greater at higher population density (Cutrera and Lacey, 2006). Because both greater social contact and greater population density may increase exposure to pathogens (Anderson and May, 1979; Arneberg, 2002; Coté and Poulin, 1995), these findings are consistent with a possible role for parasite-driven selection in maintaining MHC polymorphism in tuco-tucos.

To explore interactions between pathogen exposure and MHC variation in tuco-tucos more directly, we quantified genotypic diversity at the class II DRB locus in *C. talarum* as a function of (1) parasite load (a potential agent of selection) and (2) ability to mount an adaptive immune response against a novel antigen (a measure of immunocompetence). As noted above, balancing selection may act mainly via heterozygote (Doherty and Zinkernagel, 1975) and/or rare-allele advantage (Lively and Dybdahl, 2000). Under the former, the relationship between heterozygosity at the DRB locus and parasite load/diversity is expected to be negative while that between DRB heterozygosity and immunocompetence should be positive. Under the latter, associations between parasites and specific DRB alleles are expected; negative associations should indicate greater parasite resistance or immunocompetence, while positive associations may indicate increased susceptibility to infection (i.e. Tollenaere et al., 2008). We examined these associations in the context of the physiological and ecological factors that may influence individual-level variability in parasite load and response to immune challenge in talas tuco-tucos. As a result, our findings yield important new insights into the role of pathogen-driven selection in shaping MHC variation in a natural population of vertebrates.

2. Materials and methods

2.1. Animal capture and housing

The talas tuco-tuco (*C. talarum*) is a subterranean rodent that occurs in sand dunes along the Atlantic coast of Buenos Aires Province, Argentina. This species has been studied extensively with regard to its population biology (Busch et al., 1989), mating system (Zenuto et al., 1999), and population genetics (Cutrera et al., 2005). In addition, the parasite fauna of *C. talarum* has been thoroughly characterized (Rossin and Malizia, 2002; Rossin et al., 2004, 2005, 2010). The study population was located at Mar de Cobo, Buenos Aires Province, Argentina (37° 46′ S 57° 27′ W), in coastal dune habitat characterized by sandy soils and dominated by *Panicum racemosum, Ambrosia tenuifolia*, and *Distichlis scoparia*; a detailed description of the study location is provided by Comparatore et al. (1991).

A total of 87 tuco-tucos were trapped at the study site using plastic tube traps that had been inserted into an animal's burrow system. Specifically, 28 adult males (164.8 ± 24.2 g) and 29 adult females (111.1 ± 21.2 g) were captured during the breeding seasons (September–early December) of 2007 and 2008. Of the females captured, 24 were retained for study; 5 lactating females were immediately released back into their burrow systems so as to not deprive dependent young of maternal care. To assess the possible impact of breeding status on relationships between MHC genotypes and pathogen resistance, an additional 22 adult females (109.4 ± 15.2 g) and 13 adult males (153.1 ± 23.2 g) were captured during the non-breeding season (mid February–mid April) of 2008. All individuals were subject to the analyses of parasite load and MHC variability described below. A randomly chosen subset of 44 of animals (both sexes and seasons) was used to assess immune response to a novel antigen.

Immediately following the capture of each study animal, fecal pellets were collected from the trap in which the individual had been caught; pellets were fixed in 4% formalin until analyses of endoparasite load could be conducted. To obtain samples for leukocyte counts, a blood smear was prepared from each animal by making a small incision near the tip of the tail (Vera et al., 2008). A single drop of blood was spread onto a microscope slide to produce a thin layer, then air dried and fixed in 70% methanol for 10 min to preserve the sample until analysis. All animals were then transported to the Laboratorio de Ecofisiología at the Universidad Nacional de Mar del Plata (Mar del Plata, Argentina).

In captivity, the animals were housed individually in plastic cages (25 cm × 32 cm × 42 cm), the bottoms of which were lined with wood shavings. The animals were fed *ad libitum* quantities of a mixture of grasses, alfalfa, lettuce, corn, sweet potatoes, carrots, and sunflower seeds. Temperature and photoperiod in the room housing the animals were strictly controlled (25 ± 1 °C; non-breeding 12L:12D; breeding 14L:10D). The animals were held in captivity until immune challenge experiments were completed (ca. 4 weeks; see below), after which they were returned to the field and released at the point of capture. Immediately prior to release, a non-destructive tissue sample for genetic analyses was obtained from

each animal by removing the distal 1–2 mm of the outer digit of the left-hind foot (Cutrera et al., 2005). All field and laboratory procedures conformed to institutional and national guidelines (Argentine National Council for Scientific and Technological Research: PIP 5670, Argentine Agency for Scientific Promotion: PICT 1992, 2102) as well as the guidelines of the American Society of Mammalogists (Gannon and Sikes, 2007) for the capture, handling, and use of mammals.

2.2. Quantification of parasites

Ectoparasite load was determined by sampling the number of fleas, lice and mites trapped in an individual's pelage. Upon arrival in the lab, each individual was combed in a standardized pattern while the animal was held over a white piece of paper; ectoparasites that fell onto the paper were sorted as to type (e.g., fleas versus lice) and then counted to provide an estimate of ectoparasite load. Because the same combing procedure was employed for all animals, the resulting samples should have provided reliable estimates of the ectoparasites present on each individual. For each tuco-tuco sampled, all ectoparasites collected were fixed in 95% ethanol for subsequent, more precise taxonomic identification. Comparisons of ectoparasite loads were completed using per-individual (1) counts of the number of parasites of each type and (2) estimates of parasite diversity obtained using H, the Shannon Diversity Index (Shannon and Weaver, 1949).

Endoparasite load was determined by quantifying the number of parasite eggs/oocysts present in fecal samples collected from the study animals. Previous analyses of endoparasite load in dissected animals (5 males and 5 females) had revealed that effectively all parasite taxa detected in the digestive tracts of dissected hosts were also present in egg form in fecal samples from those individuals (A.P. Cutrera and R.R. Zenuto, unpubl. data). The only exception was a species of *Taenia* that was not encountered in fecal samples; tuco-tucos are the intermediate host for the larval form of this cestode and therefore do not shed eggs of this parasite in their feces (Rossin et al., 2004). For the remaining endoparasite taxa detected, egg shedding was assessed using a modification of the MacMaster flotation technique (Sloss et al., 1994) employed in multiple studies of endoparasite load in free-living animals (e.g. Harf and Sommer, 2005). Briefly, 1 g of feces (fixed in 4% formalin) was ground up and suspended in 15 ml of a flotation solution of potassium iodide (specific weight: 1.5 g/ml, Schad et al., 2005); suspension in this solution increased the detection of specific types of nematode eggs (Thienpont et al., 1986). Each sample was then divided between 2 MacMaster chambers and each chamber was photographed for subsequent taxonomic identification of parasite eggs (conducted in collaboration with parasitologist A. Rossin). For each egg type, fecal egg counts were estimated per gram of wet feces (eggs/g) by counting all eggs present in the two MacMaster chambers examined per individual. Comparisons of endoparasite loads were completed using individual (1) counts of the number of parasite eggs of each type per g of feces (FEC) and (2) estimates of parasite diversity obtained using H (Shannon and Weaver, 1949).

2.3. Immune challenge tests

To quantify differences in immune response to a novel antigen (immunocompetence), we used sheep red blood cells (SRBC) to elicit antibody production by the study animals. SRBC are a non-pathogenic antigen known to trigger Th2 and B-lymphocyte dependent immune responses in mammals and birds (Bacon, 1992). The magnitude of response to this antigen – which has been shown to be associated with MHC genotype in birds (Bonneaud et al., 2005) – is thought to reflect an individual's ability to mount an acquired immune response to a novel antigen as well as its ability to resist extracellular infections (e.g. bacteria, macroparasites, Deerenberg et al., 1997, but see Adamo, 2004). Previous research has shown that while individual *C. talarum* produce significant antibody titres in response to injection with SRBC, control animals injected with saline solution do not mount a response (Cutrera et al., 2010). Because these studies indicated that a minimum of 10 days is required for the animals to become physiologically accustomed to captivity (Vera et al., 2008), immune challenge tests did not begin until 10 days after animals had been transported to the lab.

On day 10 of captivity, the animals were weighed, after which all study subjects ($n = 44$) were injected intra-peritoneally with SRBC (Sigma R3378, 10% suspension, 1.5 μl/g of animal weight). Immediately after injection, we collected ~200 μl of blood from the retro-orbital sinus of each animal for use in hemoagglutination assays of immune response (see below). Preliminary immune challenge tests using 9 adult *C. talarum* indicated that antibody response to SRBC peaked at 7 days post injection (dpi) but that antibody titres were low (Cutrera et al., 2010). Consequently, animals in this study received a second injection of SRBC at 7 dpi to insure stimulation of a quantifiable immune response (Derting and Virk, 2005); as above, the animals were weighed prior to injection and a blood sample was collected immediately after injection. The animals were again weighed and a final blood sample was collected at 14 days after the initial injection (7 dpi for the second injection).

2.4. Hemoagglutination assays

To quantify antibody production in response to injection with SRBC, hemoagglutination assays were conducted following the protocol of Cutrera et al. (2010). After collection, blood samples were kept at 4 °C until they could be centrifuged at 3000 rpm for 15 min. After centrifugation, the plasma fraction of each sample was heated at 56 °C for 30 min to inactivate the complement proteins (part of the innate immune response; Carrol and Prodeus, 1998) present in blood. Plasma samples were then stored at −20 °C until used in hemoagglutination assays. Assays were conducted in 96-well microplates (Corning Star # 3798) as described by Cutrera et al. (2010). For each assay, 20 μl of heat-inactivated plasma extracted at days 0, 7 or 14 was added to 20 μl of PBS in the first well of the plate; this sample was then serially diluted in PBS (1:2–1:128) to fill the remaining wells in that row of the plate. 20 μl of a 1% suspension of SRBC was then added to each well. The plate was gently agitated for 1 min and then incubated at 37 °C for 1 h. After incubation, the plate was allowed to sit undisturbed at 4 °C for 2 h before visual assessment of agglutination was performed. Antibody titres were expressed as the negative $\log_2$ of the minimum plasma concentration that contained enough antibody to produce visible agglutination of the sample, as described by Eliyahu et al. (2002). To increase the consistency of these data, scoring of assay plates was always performed by the same individual.

2.5. Determination of leukocyte profiles

Leukocyte diversity and abundance were quantified for each study animal. Although leukocyte profiles can provide information on relative stress levels, they do not reflect an individual's ability to mount an immune response (Davis et al., 2008); instead, humoral immune response was explored via SRBC immunization, as described above. Distinguishing stress response from inflammation or disease using total leukocyte counts alone is difficult since each of these conditions causes similar alterations in neutrophilia/lymphopenia patterns (Davis et al., 2008). In contrast, use of relative counts for lymphocytes, neutrophils, monocytes, basophils and eosinophils in conjunction with total leukocyte count allows

discrimination between the effects of infection and those of other stressors. In particular, peripheral eosinophilia is commonly associated with allergies and parasitism; an increase in basophil counts is also associated with these challenges as well as with some endocrine disorders (Voigt, 2000). An increased monocyte count, in contrast, usually indicates a chronic infection or inflammation (Voigt, 2000). Consequently, the occurrence of each of the above leukocyte types was documented during examination of blood smears.

Leukocyte abundance and diversity were quantified following standard protocols (Voigt, 2000). Blood smears prepared in the field (above) were stained with May-Grumwald Giemsa solution and then examined under oil immersion at 100× magnification (Olympus CX 31, Tokyo, Japan). Leukocyte counts were conducted only for the portions of each slide containing a single layer of cells (''wandering technique''; Voigt, 2000). We counted the number of erythrocytes and leukocytes encountered in 30 fields examined during a single visual pass along each slide; using the ratio of erythrocytes to leukocytes, the number of leukocytes detected was standardized to that per 100,000 erythrocytes (Bachman, 2003). To determine the relative abundance of the 5 leukocyte cell types mentioned above, we recorded the number of each type encountered (identifications based on cell morphology as described by Voigt, 2000 and Vera et al., 2008) until a total of 200 leukocytes had been examined. The proportion of each cell type present per 200 leukocytes was determined and the ratio of neutrophils to lymphocytes (N:L) was calculated.

Hematocrit is the proportion of blood volume occupied by packed red blood cells and is considered representative of an animal's overall physiological condition (Hoi-Leitner et al., 2001). Particularly relevant to this study, hematocrit is thought to be affected by ecological conditions and exercise as well as blood parasites (e.g. Soulsby, 1987). Hematocrit was measured for the study animals on day 10 of captivity following standard protocols (Voigt, 2000). Briefly, blood was collected from the retro-orbital sinus in a heparinized capillary tube, after which the sample was centrifuged at 14,000 rpm for 15 min (Cavour VT 1224 centrifuge, Buenos Aires, Argentina). Hematocrit was assessed as the proportion of capillary tube length occupied by packed red blood cells relative to the capillary tube length occupied by all blood components (Abaco CAV 1224). Hematocrit was determined in duplicate for each animal and the resulting values were averaged.

2.6. Genetic analyses

Variability at the MHC Class II DRB locus was assessed for all individuals in this study. Exon 2 of this locus was selected for analysis because it is known to contain the peptide binding regions of the associated MHC molecules, which are the portions of these genes that are typically most subject to balancing selection (Hughes and Hughes, 1995). High molecular weight genomic DNA was extracted from all tissue samples using the DNeasy tissue extraction kit (QIAGEN, Inc., Crawley, West Sussex, United Kingdom). A 270 base pair (bp) fragment of exon 2 was amplified using primers GH46 and GH50 (Scharf et al., 1988). PCR master mixes and reaction conditions were prepared according to Cutrera and Lacey (2006). To characterize allelic diversity at the DRB locus, all MHC amplicons were cloned and sequenced following the protocol of Cutrera and Lacey (2006). In brief, cloning was performed using the TA Cloning Kit with Inv αF- competent cells (Invitrogen, Inc., Carlsbad, California, USA). Amplicons containing inserts of the correct size were cycle-sequenced using the Big Dye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, California, USA), after which cycle-sequencing products were run on an ABI 3730 automated sequencer. Amplicons from 5 to 6 positive clones per individual were sequenced; this number of cloning products was sufficient to determine the genotype of each animal and to avoid reading errors or recombinant sequences generated during PCR amplifications (Bradley and Hillis, 1997). Each new allele detected was sequenced in both directions to confirm diagnostic base pairs changes.

DRB sequences were edited and aligned manually using Sequencher 4.0 (Applied Biosystems, Inc., Foster City, California). Sequences that differed by only a single base pair substitution were considered to be distinct alleles if each variant occurred in (1) multiple individuals and/or (2) multiple cloning products per individual (obtained from at least two different PCR reactions and rounds of cloning). Allelic and genotypic variability were assessed and Hardy–Weinberg equilibrium tests were performed using Arlequin v. 3.1 (Excoffier et al., 2005). Specifically, the method of Guo and Thompson (1992) was used to determine if observed heterozygosity at DRB exon 2 was significantly in excess of that expected based on allele frequencies. Pairwise differences among DRB sequences were assessed at both the nucleotide and amino acid levels using DNAsp v. 5.1 (Rozas et al., 2003). DRB alleles whose nucleotide sequences differed but that had identical aminoacid sequences were considered part of the same allele group (e.g. allele group A).

Prior to exploring relationships among MHC diversity, parasite load, and immunocompetence, we examined whether variation at the DRB locus was correlated with variability at multiple presumptively neutral (non-functional) loci. This was necessary to distinguish between the effects of immunogenetic (DRB) versus genome-wide levels of variability on measures of immune response (Westerdahl et al., 2004; Bryja et al., 2007). For this analysis, we used a Mann–Whitney *U* test to compare levels of microsatellite diversity in DRB-heterozygous versus DRB-homozygous individuals. Data for these analyses were obtained from Cutrera and Lacey (2006), who provided DRB and microsatellite ($N = 12$ loci) genotypes for 30 *C. talarum* from the same population that served as the basis for this study.

2.7. Statistical analyses

Multivariate analyses were used to explore associations between the genetical, parasitological, and immunological variables quantified. Co-inertia analysis (ACO) is a multivariate ordination method that identifies trends or correlations in multiple datasets. ACO links two independent multivariate analyses by searching for axes that maximize the covariance between rows in distinct data matrices. ACO can be applied to datasets in which a large number of variables are measured for relatively few samples (Dolédec and Chessel, 1994; Culhane et al. 2003). ACO can be used to link qualitative and quantitative data sets (Culhane et al., 2003); this type of analysis is particularly useful for highlighting the presence/absence of co-structure between datasets and can be used to identify the best predictors from each dataset for subsequent analysis (Culhane et al., 2003). ACO is robust to correlations between variables within a given matrix, can be applied to all forms of data (Dray et al., 2003), and has been used to analyze MHC-parasite datasets similar to those considered here (Deter et al., 2008; Tollenaere et al., 2008; Schwensow et al., 2010). ACO involves two steps. First, each data matrix is analyzed independently. For this study, two data matrices were created. The categorical response (binary) matrix (presence versus absence data), which included values for each individual regarding the occurrence (0 = absence, 1 = presence) of each DRB aminoacid sequence (allele groups A, B, D), DRB heterozygosity (0 = homozygote and 1 = heterozygote), season (0 = non-breeding, 1 = breeding) and sex (0 = female, 1 = male), was analyzed by correspondence analysis (CA). The continuous variable matrix included values regarding the abundance of each parasite taxon

and Shannon's H for ecto- and endo-parasite diversity, as well as 7 variables thought to provide proxies for immune status and/or general physiological condition (Svensson and Merilä, 1996; Roitt et al., 1998; Nunn et al., 2000; Davis et al., 2008). The 7 proxy variables used in this matrix were (1) N:L (neutrophil:lymphocyte) ratio, (2) E:T (eosinophil:total leukocyte count) ratio, (3) B:T (basophil:total leukocyte count) ratio, (4) M:T (monocyte:total leukocyte count) ratio, (5) white blood cell counts (standardized per 100,000 erythrocytes), (6) body weight, and (7) hematocrit. Normality was tested using a Kolmogorov–Smirnov test as implemented in STATISTICA 6.0 (Statsoft, Tulsa, OK). Data that did not conform to normality were square-root transformed (count data for lice, listrophorid mites, *Trichuris pampeana, Graphidioides subterraneus* and *Pudica ctenomyidis*) or arcsin transformed (proportion data for E:T, B:T and M:T). The values in the continuous variable matrix were analyzed using principal component analysis (PCA), with row weights derived from the CA. The results of the matrix-specific analyses were displayed on factorial maps to visualize distributions for specific DRB alleles, parasites, measures of immune status, and/or measures of general physiological condition that may affect interpretations of associations among parasites, genetic variability, and immune status/condition.

In the second step of the ACO analyses, relationships between the binary and quantitative matrices were estimated from the co-inertia (vectorial correlation) of the matrices, using the R_v coefficient (Escoufier, 1973). A random permutation test (array rows permutated 10,000 times, then compared to observed values) was used to evaluate the statistical significance of R_v. These procedures were implemented in the ade4TkGUI package for R (Thioulouse and Dray, 2007). The two matrices were compared by superimposing both categorical and continuous information on the ACO factor map. Associations between specific variables were detected visually using the vectors for the binary and quantitative variables depicted on the ACO factor map. Both vector length and angle of inclination were used to assess the relative importance of different variables, with longer vectors and smaller angles of inclination being indicative of greater explanatory power. Vectors pointing in the same direction (relative to the origin) were considered positively associated, while vectors pointing in opposite directions were considered negatively associated. Following Haldane (1956), we calculated the relative risk (RR) of parasitic infection or ability to mount an immune response against SRBC that was associated with each of the genetic or physiological variables identified as important by ACO; estimates of RR were generated using Epi Info (Dean et al., 1991).

The same multivariate approach was used to explore relationships among MHC variability, response to injection with SRBC, and our proxies for immunological and physiological condition for the subset of animals for which hemoagglutination analyses were performed. As above, the categorical (binary) matrix included information on DRB aminoacid sequence (presence or absence of each allele group), DRB heterozygosity (0 = homozygote and 1 = heterozygote), season (0 = non-breeding, 1 = breeding) and sex (0 = female, 1 = male) and was analyzed by correspondence analysis (CA). The continuous matrix included antibody titres at 7 and 14 days post first immunization as well as the 7 proxy measures of immune status and general physiological condition described above. Data for proportions (E:T, B:T and M:T) that were not normally distributed were transformed as described above.

Finally, PCA was used to assess if parasite load/diversity was associated with the magnitude of immune response mounted against SRBC (i.e., whether high antibody producers were more parasite resistant). Proportional data (count data for lice, listrophorid mites, *T. pampeana, G. subterraneus* and *P. ctenomyidis*) that were not normally distributed were transformed as described above.

3. Results

3.1. Parasite abundance and diversity

Eggs/oocysts from 5 different endoparasite taxa were identified in fecal samples collected from the study animals. Four of these were gastrointestinal nematodes (*T. pampeana*, *Paraspidodera uncinata*, *G. subterraneus* and *P. ctenomyidis*); the fifth was an intestinal protozoan (*Eimeria* sp.). During the breeding season, *P. uncinata* and the *Eimeria* sp. were the most prevalent parasites in the study animals, with >80% of individuals infected with these organisms. During the non-breeding season, *Eimeria* sp. and *T. pampeana* were the most prevalent parasites in the host population (Table 1). Mean intensity of infection (mean FEC per individual) was greatest for the *Eimeria* sp. (Table 1). The ectoparasites identified included fleas (*Polygenis* sp.), mites (Families Laelapidae and Listrophoridae), and anoplurid lice (*Eulinognathus* sp.). Of these, fleas and laelapid mites were the most prevalent (>70% of

Table 1
Median intensity of infection (median FEC for endoparasites; median counts for ectoparasites; $N = 87$ tuco-tucos examined; (1.A) and parasite prevalence (percentage of animals infected; (1.B) for each sex (M: male, F: female) and season (B: breeding, NB: non-breeding) in the population of *C. talarum* at Mar de Cobo, Argentina (37° 46′ S 57° 27′ W). The endoparasites quantified were *Eimeria* sp. (Ei), *T. pampeana* (Tp), *G. subterraneus* (Gs), *P. ctenomyidis* (Pc), and *P. uncinata* (Pu). The ectoparasites quantified were *Eulinognathus* sp. (Eu), *Poligenis* sp. (Po), laelapid mites (La) and listrophorids (Li). Interquartile range (25th–75th) intensity values are given in parentheses.

(1.A) Intensity of infection

Season	Sex	Ei	Tp	Gs	Pc	Pu	Eu	La	Po	Li
B	M	43.5 (17–120.5)	0 (0–0)	0 (0–0.3)	0 (0–0)	9.5 (1.75–32.5)	0 (0–1)	1.5 (0–7.5)	6 (2–11)	0 (0–0)
	F	17 (2–70.75)	0 (0–0)	0 (0–1)	0 (0–0.25)	5 (1.75–8)	0 (0–1)	3 (1.75–7)	6 (1.75–10.5)	0 (0–0)
NB	M	129 (38–207)	0.5 (0–5.5)	0 (0–0.25)	0 (0–1)	14.5 (7.5–23.25)	0 (0–0.25)	11.5 (8.75–45)	4.5 (2.75–7)	0 (0–0)
	F	17 (2–70.75)	0 (0–0)	0 (0–1)	0 (0–0.25)	5 (1.75–8)	0 (0–1)	1.75 (1.75–7)	1.75 (1.75–10.5)	0 (0–0)

(1.B) Prevalence (%)

Season	Sex	Ei	Tp	Gs	Pc	Pu	Eu	La	Po	Li
B	M	92.8	17.9	25	14.2	82.1	42.8	53.5	89.3	3.6
	F	80	20	30	25	80	30	85	85	0
NB	M	100	50	25	33.3	16.7	25	100	91.7	16.7
	F	94.7	52.6	36.8	21.1	10.5	21.1	84.2	89.5	15.8

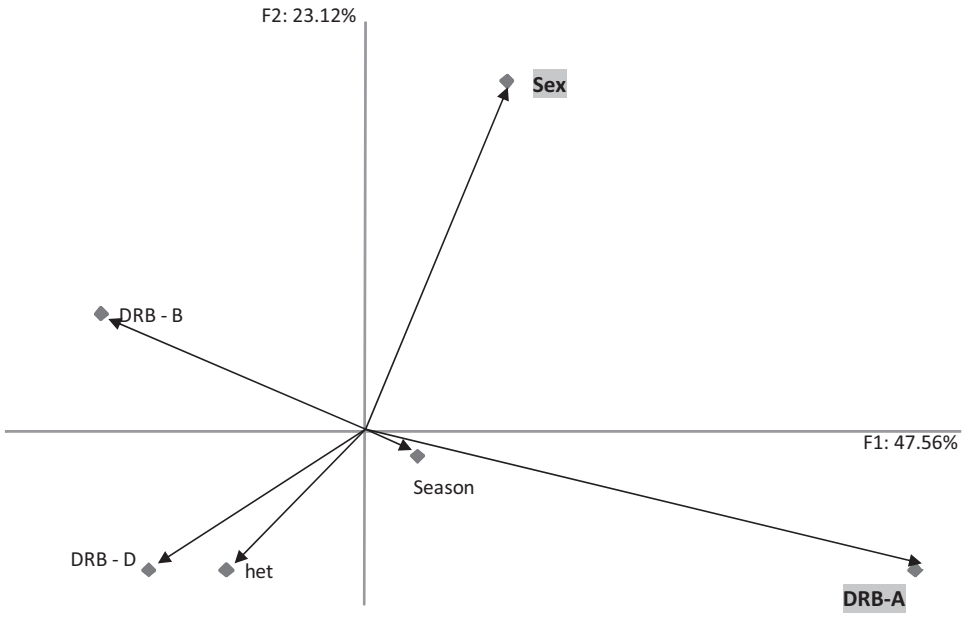

Fig. 1. Depiction of correspondence analysis (CA) of the categorical (binary) data matrix for all *C. talarum* sampled in this study ($n = 87$). Vectors for sex, season, DRB genotype, and DRB heterozygosity (het) are shown; DRB allele groups are identified by name. Variables that substantially structured the data set are highlighted in grey.

individual infected), with laelapid mites having the highest mean intensity of infection (Table 1)

3.2. Response to immune challenge

All animals injected with SRBC produced detectable antibody titres either at 7 or 14 days post injection (dpi). In all cases, titres at 14 dpi (4.59 ± 1.47) were higher than those at 7 dpi (1.43 ± 1.55); this tendency was significant ($t_{43,\ 0.05} = -9.72$, $p < 0.001$).

3.3. DRB variability

As in previous studies of MHC variation in *C. talarum* (Cutrera and Lacey, 2006, 2007), all DNA samples analyzed produced a single, clearly resolved PCR product. No evidence of chimeric amplification products was detected. After cloning, no more than two sequences per individual were obtained, suggesting that only a single copy of the DRB locus was amplified. Additionally, preliminary comparisons of mother-pup genotypes ($n = 35$ families, A.P. Cutrera, unpubl. data) revealed no evidence of amplification of more than one copy of DRB locus. Inspection of the resulting sequences revealed no insertions or deletions and, when translated, no stop codons were evident within the DRB alleles obtained.

Nine DRB alleles were detected in the 87 *C. talarum* genotyped during this study. Observed heterozygosity (0.854) at this locus was significantly higher than expected under Hardy–Weinberg equilibrium (He = 0.756, $p < 0.001$). The mean number of pairwise nucleotide differences among alleles was 4.83 ± 1.35. When translated, the mean number of pairwise amino acid differences between alleles was 1.61 ± 0.78. Based on shared amino acid sequences, the 9 DRB alleles identified were divided into 4 allele groups (Schwensow et al., 2007) that were labeled A, B, C and D (Supplementary data). The mean number of pairwise nucleotide differences among allele groups was 5.83 ± 1.73, with a mean pairwise number of amino acid differences of 2.33 ± 1.12.

The single allele in group C occurred in only one individual (Supplementary data) -and thus this allele and allele group were excluded from analyses of associations among DRB genotypes, parasite load and immune response (Meyer-Lucht and Sommer, 2005). DRB-heterozygous individuals did not have higher mean heterozygosities at microsatellite loci than did DRB-homozygous animals (Mann–Whitney *U* test, $z = 0.053$, $p = 0.958$), suggesting that MHC heterozygosity was independent of microsatellite heterozygosity.

3.4. Co-inertia analyses: parasites and MHC variability

Co-inertia analysis (COA) was conducted on data from the 87 individuals screened for ecto- and endoparasites, MHC variability, the 7 proxy measures of immune status/condition (N/L, E/T, B/T, M/T, WBC/100,000 RC, hematocrit, body weight), season and sex. In the CA of the categorical (binary) response matrix (MHC data plus season and sex, Fig. 1), the first two axes together accounted for 70.68% of the total variance (first axis, F1 = 47.56%; second axis, F2 = 23.12%). F1 was structured primarily by the DRB allele group A while F2 was structured primarily by sex. Neither heterozygosity nor season appeared to contribute substantially to the structure of this data set. In the PCA of the continuous variable matrix (parasitological plus immune status/condition data), the first two axes together accounted for 51.22% of the total variance (F1 = 31.18%; F2 = 20.04%, Fig. 2). The first axis was structured primarily by hematocrit, intensity of infection by *G. subterraneus* and *P. ctenomyidis* and the abundance of fleas, with hematocrit acting in the opposite direction of the other 3 variables. The second axis was structured primarily by intensity of infection by *T. pampeana* and by M/T (relative counts of monocytes), with these two variables acting in opposite directions. Interestingly, with the exception of lice, all hematophagous organisms included in this analysis (*G. subterraneus*, fleas, laelapid mites) were negatively associated with hematocrit.

COA revealed a significant overall relationship between the categorical and the quantitative data matrices ($R_v = 0.128$, $p = 0.001$). The first two axes of the COA explained 81.51% of the total variance (F1 = 53.33%; F2 = 28.18%; Fig. 3). F1 was structured primarily by DRB heterozygosity, abundance of lice, diversity (H) of endoparasites, and hematocrit, with the first variable acting in the opposite direction of the rest. F2 was structured primarily by intensity of infection by *P. uncinata* and *Eimeria* sp. and the DRB allele group A, with a negative relationship between load of *P. uncinata* and DRB allele group A and a positive relationship

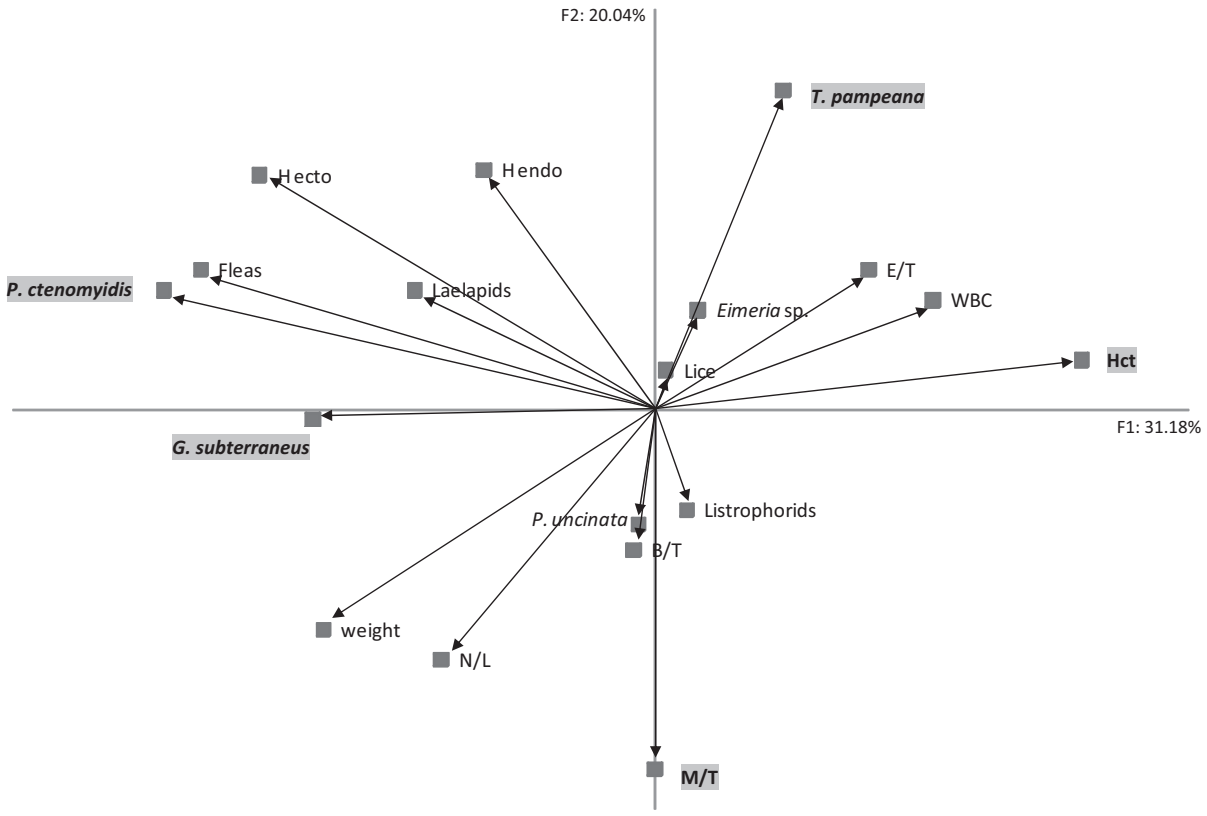

Fig. 2. Depiction of principal component (PA) analysis of the quantitative data matrix for all *C. talarum* sampled in this study (n = 87). Vectors for intensity of parasite infection, diversity (H) of parasite fauna, hematocrit (Hct), and 5 measures of leukocyte abundance are shown. Parasite data reflect fecal egg count values. N/L = neutrophil:lymphocyte ratio, E/T = eosinophil:total leukocyte count ratio, B/T = basophil:total leukocyte count ratio, M/T = monocyte:total leukocyte count ratio, WBC = white blood cell counts (standardized per 100,000 erythrocytes). Variables that substantially structured the data are highlighted in grey.

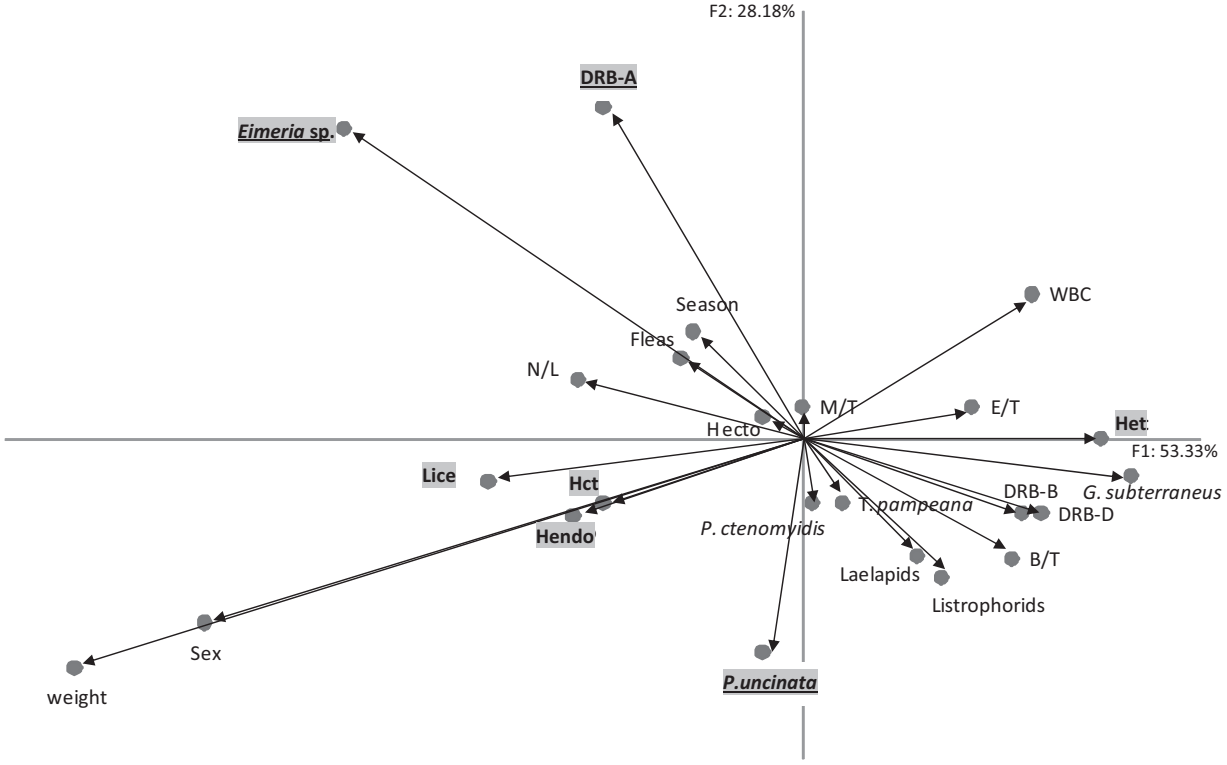

Fig. 3. Results of co-inertia analysis relating the categorical and quantitative data matrices for all *C. talarum* sampled in this study (n = 87). Variable abbreviations are the same as in Figs. 1 and 2. Under strong co-inertia, vectors pointing in the same direction relative to the origin are considered to be positively associated while those pointing in the opposite directions are considered to be negatively associated. Variables that substantially structured the data are highlighted in grey. Variables shown by relative risk analyses to be significantly associated with one another are underlined.

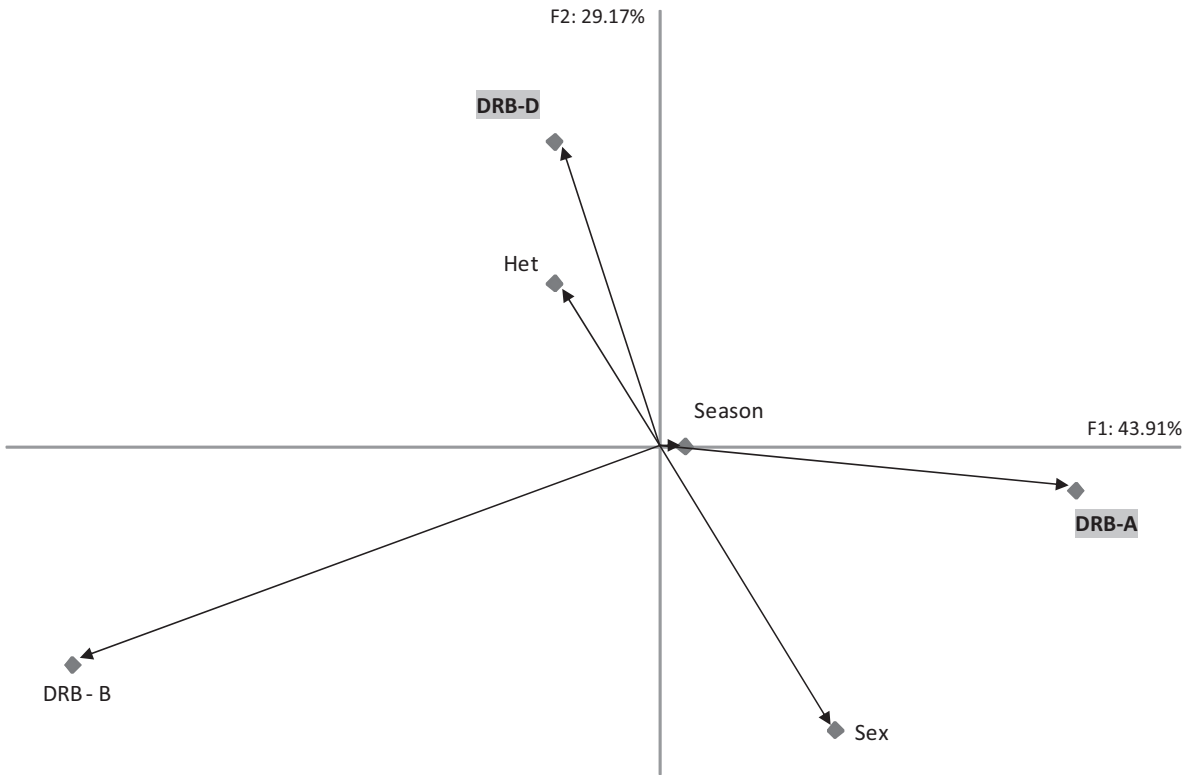

Fig. 4. Depiction of correspondence analysis (CA) of the categorical (binary) data matrix for a random sample of *C. talarum* ($N = 44$) immunized with sheep red blood cells (SRBC). Vectors for sex, season, DRB genotype, and DRB heterozygosity (het) are shown; DRB allele groups are identified by name. Variables that substantially structured the data are highlighted in grey.

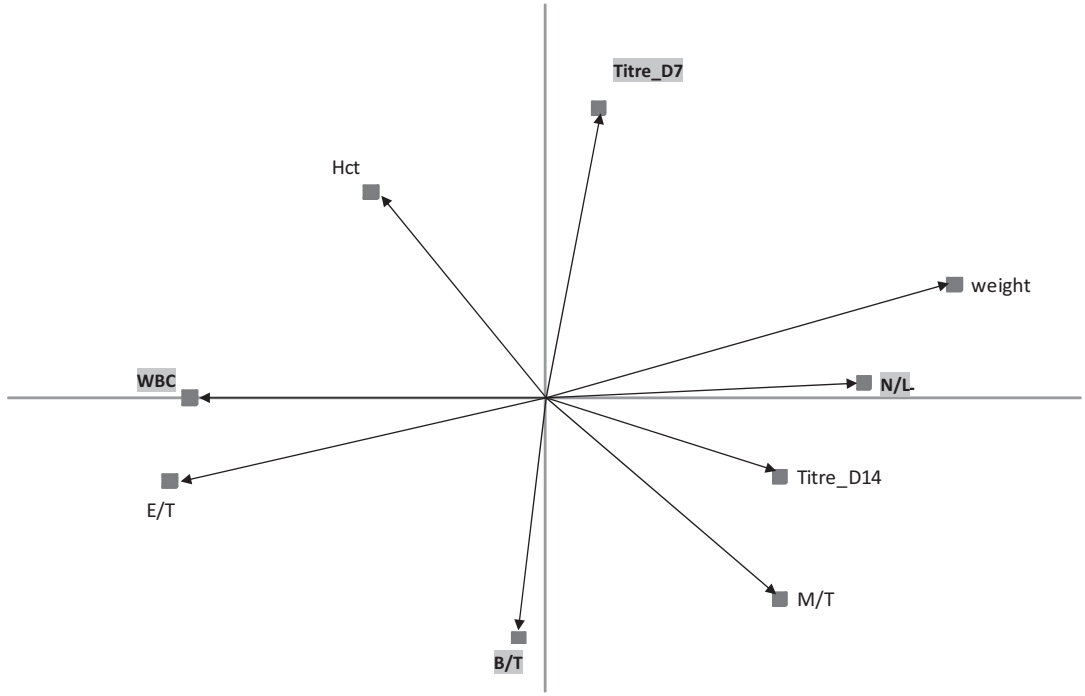

Fig. 5. Depiction of principal component (PA) analysis of the quantitative data matrix for a random sample of *C. talarum* ($N = 44$) immunized with sheep red blood cells (SRBC). Vectors for intensity of immune response against SRBC at 7 (Titre_D7) and 14 (Titre_D14) days post initial injection, hematocrit (Hct), and 5 measures of leukocyte abundance are shown. Parasite data reflect fecal egg count values. N/L = neutrophil:lymphocyte ratio, E/T = eosinophil:total leukocyte count ratio, B/T = basophil:total leukocyte count ratio, M/T = monocyte:total leukocyte count ratio, WBC = white blood cell counts (standardized per 100,000 erythrocytes). Variables that substantially structured the data are highlighted in grey.

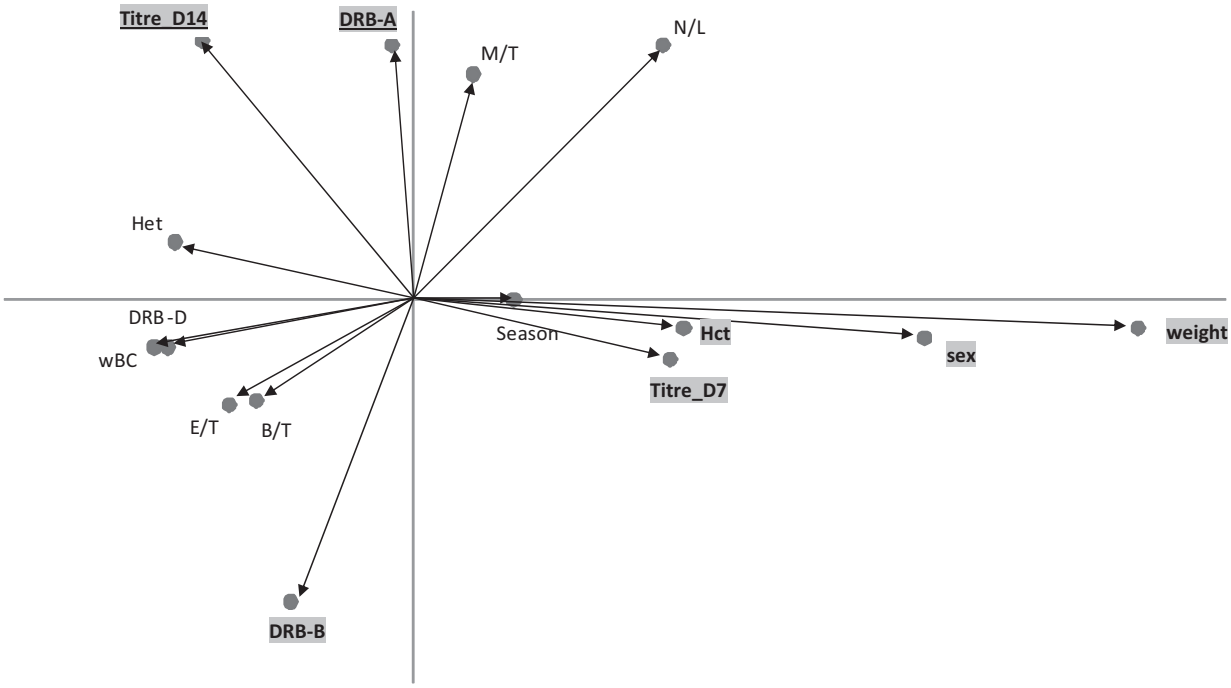

Fig. 6. Results of co-inertia analysis relating the categorical and quantitative data matrices for a random sample of *C. talarum* ($N = 44$) immunized with sheep red blood cells (SRBC). Variable abbreviations are the same as in Figs. 1 and 2. Under strong co-inertia, vectors pointing in the same direction relative to the origin are considered to be positively associated while those pointing in the opposite directions are considered to be negatively associated. Variables that substantially structured the data are highlighted in grey. Variables shown by relative risk analyses to be significantly associated with one another are underlined.

between this allele and *Eimeria* sp. For both axes, the primary variables associated with parasite load were genetic.

Relative risk (RR) analyses revealed that for F1, relationships between DRB genotypes and measure of parasite load were not significant. Neither heterozygous animals nor individuals with DRB alleles in groups B and/or D had a significantly lower risk of infection by *G. subterraneus* than did homozygotes or individuals with DRB group A alleles (RR = 1.26, $p = 0.059$ and RR = 0.83, $p = 0.398$ respectively). Similarly, heterozygous individuals did not have a significantly lower risk of infection by lice (RR = 1.35, $p = 0.49$) or higher diversity of endoparasites (RR = 1.22, $p = 0.44$). In contrast, for F2, individuals with an allele from DRB group A had a significantly lower risk of infection by *P. uncinata* (>30 eggs/gram of feces: RR = 0.45, $p = 0.029$) and a significantly higher risk of being intensely infected by *Eimeria* sp. (>100 oocysts/gram of feces: RR = 3.04, $p = 0.002$). Thus, while heterozygosity at the DRB locus did not appear to be related to parasite load, the occurrence of specific DRB allele groups was associated with risk of infection by two of the parasite taxa considered.

3.5. Co-inertia analyses: immunocompetence and MHC variability

COA was also conducted on the randomly selected subset of 44 individuals (24 males; 20 females) screened for immune response against SRBC; matrices for these animals included all of the variables considered above plus, for the quantitative matrix, measures of antibody titres taken at 7 and 14 days post initial injection. In the CA for the categorical (binary) response matrix (MHC data plus season and sex, Fig. 4), the first two axes together accounted for 73.08% of the total variance (F1 = 43.91%; F2 = 29.17%). Both axes were structured primarily by genetic variables; F1 was most strongly influenced by DRB allele group A while F2 was most strongly influenced by DRB allele group D. As in the preceding analysis of categorical data, neither DRB heterozygosity nor season of capture appeared to contribute substantially to the structure of this matrix. For the quantitative matrix, the first two axes of the PCA (antibody titres against SRBC plus immune status/condition data) together accounted for 55.86% of the total variance (F1 = 31.94%; F2 = 23.92%; Fig. 5). F1 was structured primarily by N/L, (neutrophil:lymphocyte ratio) and WBC (white blood cell counts relative to 100,000 red cells), with these variables acting in opposite directions. F2 was structured primarily by antibody titre at 7 days post injection and B/T (relative counts of basophils), with these variables acting in opposite directions.

In contrast to analyses of the larger data set, COA of the subset of animals injected with SRBC revealed no significant overall relationship between the categorical and quantitative data matrices ($R_v = 0.112$, $p = 0.065$). The first two axes together explained 81.48% of the total variance (F1 = 56.72%; F2 = 24.72%; Fig. 6). F1 was structured primarily by body weight and sex and, to a lesser degree, by hematocrit and antibody titre at 7 days post initial injection, with all of these variables acting in the same direction. F2 was structured primarily by DRB allele groups A and B, with these 2 allele groups acting in opposite directions, and also by antibody titre at 14 days post initial injection. RR analyses revealed that although antibody titre at 7 days post injection was positively associated with body weight, animals that weighed >120 g (typical adult size for animals in this population, Malizia et al., 1991) were not significantly more likely to mount an antibody response than were smaller individuals (RR = 1.45, $p = 0.075$). Also at 7 days post injection, animals with higher than average hematocrit values (population mean = 35 ± 7.43%) were not more likely to show an antibody response (RR = 0.64, $p = 0.13$) nor were males more likely than females to show an antibody response (RR = 1.14, $p = 0.61$). Finally, although antibody titre at 14 days post initial injection appeared to be positively associated with DRB allele group A and negatively associated with allele group B, only the first of these relationships was significant (RR = 1.86, $p = 0.05$; RR = 0.73, $p = 0.29$). Thus, overall, response to injection with SRBC did not appear to be strongly associated with variability at the DRB locus.

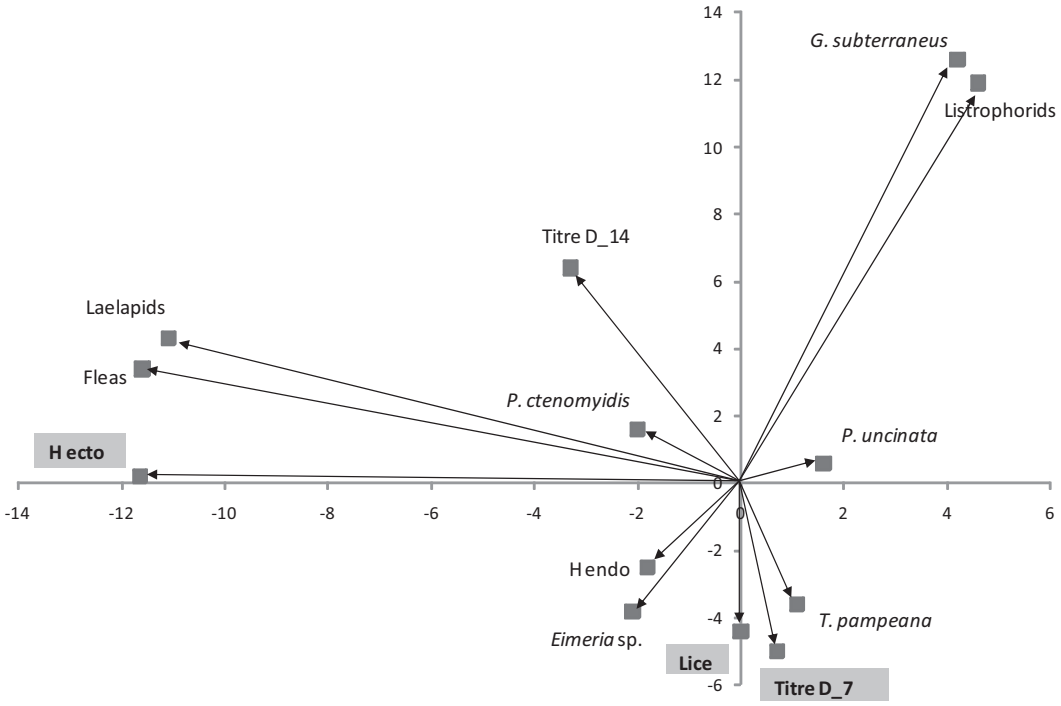

Fig. 7. Depiction of principal component analysis (PCA) of the quantitative data matrix for a random sample of *C. talarum* ($N = 44$) immunized with sheep red blood cells (SRBC). Vectors for intensity of immune response against SRBC at 7 (Titre_D7) and 14 (Titre_D14) days post initial injection, intensity of parasite infection, and diversity (H) of parasite fauna are shown. Parasite data reflect fecal egg count values. Variables that substantially structured the data are highlighted in grey.

3.6. PCA: immunocompetence and parasite load/diversity

The first two axes of the PCA together accounted for 51.15% of the total variance (F1 = 27.85%; F2 = 23.30%; Fig. 7). While F1 was structured primarily by lice counts and antibody titres against SRBC measured 7 days post injection (both variables acting in the same direction), F2 was structured primarily by the diversity of ectoparasites. Thus, overall, response to injection with SRBC did not seem to be strongly associated with measures of parasite load or diversity.

4. Discussion

Our results support a role for parasite-driven balancing selection in maintaining MHC variation in natural populations of *C. talarum*. Our multivariate analyses revealed significant associations between specific DRB allele groups and parasite load, as well as between allele groups and the intensity of humoral immune response against a novel antigen. Not all measures of parasite load and immune response, however, were associated with allelic differences at this locus and DRB heterozygosity was not significantly associated with any measures of parasite load or response to immune challenge. Although these data indicate considerable variability in interactions between MHC genotypes and immunocompetence, the multiple significant relationships detected between DRB allele groups, parasite load, and response to immune challenge suggest that parasite-driven balancing selection is operating on this MHC locus.

4.1. Selective mechanisms operating on MHC variation

Parasite-driven selection may act via rare allele or heterozygote advantage (see Spurgin and Richardson, 2010 for a review). Rare-allele advantage arises as the evolution of new parasite and pathogen phenotypes reduces the relative fitness of common host genotypes, thereby providing a selective advantage to rare host alleles (Lively and Dybdahl, 2000). Evidence in favour of this hypothesis comes from multiple studies demonstrating associations between specific MHC alleles and resistance to viral (e.g., hepatitis, Thursz et al., 1995, Puumala virus, Deter et al., 2008) and bacterial (e.g., tuberculosis, Sousa et al., 2000) infections as well as blood-borne parasites (e.g., malaria, Bonneaud et al., 2006) and helminths (e.g. Tollenaere et al., 2008; Meyer-Lucht et al., 2010). In this study, we found significant associations between DRB allele group A and two endoparasite taxa that were abundant in the population of *C. talarum* examined. At least one of these parasite taxa is known to impact host health: *Eimeria* is an intracellular protozoan known to cause hemorrhagic enteritis in swine (Hill et al., 1985) and chickens (Clare and Danforth, 1989). Thus, these results suggest that the amino acid sequence coded for by DRB allele group A may influence susceptibility to potentially harmful infections, a pattern that is consistent with the rare-allele advantage hypothesis (Spurgin and Richardson, 2010).

Empirical support for the heterozygote advantage hypothesis has been less consistent, leading some authors to suggest that this mechanism cannot account for the high levels of polymorphism that are characteristic of MHC genes (Hedrick and Kim, 2000; De Boer et al., 2004; Stoffels and Spencer, 2008, but see Oliver et al., 2008; Penn et al., 2002; Froeschke and Sommer, 2005; and Spurgin and Richardson, 2010 regarding the paucity of suitable study systems). According to this hypothesis, MHC heterozygous individuals are expected to recognize and therefore to present for destruction a wider variety of antigens than are MHC homozygous individuals (Doherty and Zinkernagel, 1975). If pathogen resistance is over-dominant, then having two distinct alleles at a locus will result in MHC heterozygotes being more fit than the fittest homozygous genotype (Hughes and Nei, 1992). Consequently, heterozygosity is predicted to be negatively associated with pathogen load and diversity (Spurgin and Richardson, 2010). Although we detected a significant excess of DRB heterozygotes in our study population, our multivariate analyses did not reveal significant associations between heterozygosity at this locus and either parasite load or

diversity. Because co-inertia analysis (ACO) can be applied to datasets in which a large number of variables is measured in relatively few samples (Dolédec and Chessel, 1994; Culhane et al., 2003) this approach is particularly useful in studies such us ours in which the ratio of variables to samples is high. Although we cannot rule out the possibility that limited sample sizes contributed to some of our non-significant results (e.g., lack of a significant negative relationship between DRB heterozygosity and parasite diversity), the significant associations detected between DRB allele group A and two endoparasite taxa, indicate that limited statistical power was not a problem for at least some analyses. As noted by Penn (2002) and Spurgin and Richardson (2010), an excess of heterozygotes at MHC loci may also be compatible with rare-allele advantage since heterozygotes may be selected for because they carry rare, resistant alleles rather than because they are heterozygous *per se*. Thus, our results suggest that the presence of specific MHC alleles may play a more significant role in pathogen resistance than heterozygosity at MHC loci (De Boer et al., 2004; Meyer-Lucht and Sommer, 2005; Deter et al., 2008; but see below for further discussion of this issue).

Response to experimental antigen challenge provides an alternative means of exploring the immune consequences of variability at MHC genes (Zhou and Lamont, 2003), particularly if – like SRBC – the antigen employed provides an estimate of resistance to extracellular infections (Deerenberg et al., 1997). Although a single measure of immune activity may not provide a robust picture of immune responses in free-living organisms (Martin et al., 2006; Pedersen and Babayan, 2011), our results suggest that response to injection with SRBC was not strongly associated with measures of parasite load or diversity in our study population. Possible reasons for this lack of relationship include (1) involvement of different immune pathways in resistance to different pathogens (Roitt et al., 1998) and (2) lack of positive correlations among measures of immune resistance (Adamo and Spiteri, 2005). Consequently, both traditional assays and analyses of multiple factors thought to influence immune response should be included in immunological studies of wild animals (Pedersen and Babayan, 2011).

We found that DRB allele group A was significantly associated with production of antibodies against SRBC at 14 days after the first immunization. As noted by Bonneaud et al. (2005), however, associating a specific phenotype (e.g. antibody response to SRBC) with a target gene is difficult because the phenotype in question may be influenced by other loci that are linked to the target gene. Although our experimental design did not allow us to resolve this issue, it is intriguing that DRB allele group A was consistently identified in our analyses as being associated with measures of parasite load and immune response. Because the population of *C. talarum* in Mar de Cobo is characterized by significant kin structure due to limited dispersal, particularly by females (Cutrera et al., 2005), the individuals used in this study were captured over a large area (~5–6 ha) to avoid including related individuals in our sample. Nevertheless, it is possible that inclusion of related animals in our data set may have led to increased sharing of alleles at other loci affecting response to SRBC. While data from additional loci are needed to disentangle fully DRB versus genome-wide effects on immunocompetence (Balloux et al., 2004; Slate et al., 2004), our results support a role for pathogen-driven selection in maintaining MHC variation in *C. talarum*.

4.2. Parasite-driven selection in natural environments

As noted by Oliver et al. (2008), associations between MHC loci and individual pathogens may be context-dependent, arising only under specific conditions that may be difficult to reproduce in the lab (Bernatchez and Landry, 2003; Piertney and Oliver, 2006). As a result, interactions among environment, physiology, pathogen exposure, and MHC diversity may only be fully detectable in studies of free-living animals that are confronted with multiple simultaneous immune challenges and for which multiple types of data are available. Our study illustrates the potential complexity of relationships between these parameters. For example, the animals injected with SRBC also carried gastrointestinal nematodes and protozoans from their natural habitat. Parasite infection can reduce immunocompetence through parasitic manipulation of the host immune response or simply because the parasite consumes host resources, resulting in a reduced ability to mount a response (Cornet and Sorci, 2010). Previous research on *C. talarum* has revealed that mounting a response to SRBC is energetically costly (Cutrera et al., 2010), suggesting that reduction of host resources may make it more difficult for these animals to respond to immune challenges.

Consistent with this (although the results of our risk analyses were not significant) early antibody response against SRBC by the study animals was positively associated with body weight and hematocrit, each of which can be indicators of condition (Boonstra et al., 1998; Toïgo et al., 2006). Hematocrit, in turn, was negatively associated with abundance of several hematophagous parasite taxa (*G. subterraneus*, fleas, laelapid mites), a pattern also observed in other vertebrates (Pilny, 2008; Richner et al., 1993). Early antibody response against SRBC by our study animals was also negatively associated with relative basophil count. Although a relationship between basophil count and specific parasites was not detected in our study, basophilia has been reported in several rodent species following exposure to parasitic infection (Kasugai et al., 1993; Okada et al., 1997) or in association with allergic reactions (Voigt, 2000). Interestingly, DRB allele group A was also associated with response to injection with SRBC, thereby providing a potential link between immune response and genotypic variability. Finally, all *C. talarum* sampled in this study were infected with more than one parasite species. In animals co-infected with multiple parasites, interactions among different parasite species may arise due to direct competition for resources or due to indirect interactions that are mediated via the immune system (Graham, 2008). For example, the negative relationship reported here between *P. uncinata* and the presence of DRB allele group A coupled with the positive relationship between this allele group and the prevalence of *Eimeria* sp. could be the result of competitive interactions between these parasites or cross-effective immune responses between the parasite species (see Telfer et al., 2010). Studies that monitor infection status through time are needed to assess the relative importance of parasite interactions compared with other factors (e.g., host genetic variation and immune condition) in determining the susceptibility of the tuco-tucos to infection.

This series of outcomes underscores both the (1) difficulty of assessing the causality of relationships among MHC genotypes and measures of immunocompetence (Bonneaud et al., 2005) and (2) importance of examining such associations in free-living animals that are subject to multiple immune challenges (Wegner et al., 2003). Considering the variety of potentially interacting parameters, detecting associations among physiological or ecological factors that may influence susceptibility to parasitic infections seems likely to be more challenging than simply documenting a correlation between a specific MHC allele (which determines the ability to recognize and eliminate a particular pathogen) and the abundance of a pathogen. Similarly, detecting an association between MHC heterozygosity and multiple pathogen infections (i.e., parasite diversity) could be difficult (Oppelt et al., 2010). For example, although a negative relationship between MHC heterozygosity and parasite diversity was detected in this study based on co-inertia analyses, relative risk tests did not reveal a significant relationship between these variables. Our observations suggest

that individual *C. talarum* are able to tolerate the endo- and ectoparasites examined here and to survive with few apparent harmful effects, probably due to a significant energetic investment in the activation and maintenance of their immune systems, a cost that may impact other energetically demanding processes such as reproduction. However, further work is required to assess the impacts of more virulent pathogens as well as to determine the fitness consequences of the relationships between parasites and MHC diversity reported here.

C. talarum – like all tuco-tucos – is subterranean, meaning that members of this species spend virtually their entire lives in underground burrows (Nevo, 1999; Lacey et al., 2000). *C. talarum* is characterized by a relatively low richness (number of parasite species, Bush et al., 1990) of gastrointestinal helminths (Rossin and Malizia, 2002), a pattern also evident in other subterranean rodents that has been linked to their typically solitary existence (Nevo, 1999). This reduced parasite fauna may have contributed to the relatively low levels of variability found at the DRB locus in this study relative to studies of other species of wild rodents (e.g. Harf and Sommer, 2005; Froeschke and Sommer, 2005). In contrast, the high prevalence (percentage of infected hosts, Bush et al., 1997) of parasitic infections in *C. talarum* has led Rossin and Malizia (2002) to suggest that burrow systems provide physical conditions (e.g., moisture, low ventilation, protection from UV light) that favour parasite transmission. The more stable physical conditions found in burrows (compared to surface environments, Buffenstein, 2000) may also contribute to sustained levels of parasitic infection across seasons, which may help to explain the minimal effect of season on parasite load in our study population. Finally, the solitary lifestyle of the talas tuco-tuco may result in patterns of infection that differ from those observed in species in which conspecifics frequently interact with one another (Rossin et al., 2010). Thus, the habitat in which *C. talarum* occurs may have multiple, sometimes contradictory effects on selection for MHC diversity in these animals, with different aspects of pathogen exposure (e.g., richness versus prevalence) impacting different facets of immunogenetic variation. Under these conditions, even small changes in an MHC molecule may lead to significant differences in parasite recognition and binding effectiveness (Frank, 2002), with the result that there may be significant variation in disease susceptibility among DRB alleles that, like those in *C. talarum*, differ by only a few aminoacids.

5. Conclusions

Few studies that have demonstrated balancing selection on MHC loci in free-living mammals have also explored the functional consequences of such variation (e.g. Oliver et al., 2008; reviewed by Sommer, 2005). This includes previous studies of MHC variation in tuco-tucos. For example, although interspecific comparisons of the solitary *C. haigi* and the group living *C. sociabilis* revealed that selection on the MHC DQB locus was greater in the latter species (Hambuch and Lacey, 2002), no attempt was made to assess potential interspecific differences in parasite or pathogen loads. Similarly, intraspecific comparisons of MHC variability in 2 demographically distinct populations of *C. talarum* indicated that evidence of balancing selection on the DRB and DQA genes was consistently greater for the more dense, more inbred population (Cutrera and Lacey, 2006) but did not explore the potential functional consequences of this pattern. The current study expands upon these findings to relate patterns of DRB variability in *C. talarum* to differences in parasite load and response to immune challenge. Our study is distinctive in that it simultaneously considered multiple factors that may shape MHC variation in natural populations of mammals. For example, we used both parasite abundance and parasite diversity as measures of the intensity of selection upon MHC loci; our sampling effort was designed to cover an array of possible immune challenges that tuco-tucos may face in their natural habitat while at the same time emphasizing parasite taxa for which data are available regarding effects on hosts (e.g. *Eimeria* sp.: Hill et al., 1985; *G. subterraneus*: Rossin et al., 2005). Additionally, we assessed the potential importance of physical condition on parasite load and immune response, with condition estimated in multiple ways. Finally, we used injection with SRBC to quantify immune response in individuals that were naturally infected with multiple parasites.

Some of the parasite-host relationships reported here were predicted by the models of selection that we considered. Thus, our findings suggest that parasite-driven selection plays an important role in maintaining immunogenetic diversity in our study population, but that the effects of such selection on MHC genotypes vary among parasite taxa and, potentially, with host condition. This study represents a first step towards understanding the relationships among physiological condition, MHC variation and parasite resistance/immunocompetence in free-living tuco-tucos. Future studies will explore the parasite-MHC relationships reported here in greater detail, including use of experimental manipulations to examine interactions between parasite load, MHC genotype, and host response under controlled conditions. Such studies should yield important new insights into the effects of parasite-driven selection on free-living animals that are subject to multiple immune challenges.

Acknowledgments

We would like to especially thank Alejandra Rossin (Laboratorio de Parasitología – UNMdP), Marcela Lareschi (Centro de Parasitología y de Vectores – LP) and Natalia Martino (Museo de Ciencias Naturales Lorenzo Scaglia) for their help with the taxonomic determination of the endoparasites and ectoparasites. We specially thank Camille Bonneaud and two anonymous reviewers for their comments on a previous version of this manuscript. Financial support was provided by CONICET (PIP 5670), Agencia Nacional de Promoción Científica (PICT 1992 and PICT 2102) and Universidad Nacional de Mar del Plata, Argentina (15/E340 EXA392/08).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.meegid.2011.03.016.

References

Adamo, S.A., 2004. How should behavioural ecologists interpret measurements of immunity? Anim. Behav. 68, 1443–1449.
Adamo, S.A., Spiteri, R.J., 2005. Female choice for male immunocompetence: when is it worth it? Behav. Ecol. 16, 871–879.
Abu-Madi, M.A., Behnke, J.M., Lewis, J.W., Gilbert, F.S., 2000. Seasonal and site specific variation in the component community structure of intestinal helminthes in *Apodemus sylvaticus* from three contrasting habitats in south-east England. J. Helminthol. 74, 7–15.
Anderson, R.M., May, R.M., 1979. Population biology of infectious disease: part I. Nature 280, 361–367.
Arneberg, P., 2002. Host population density and body mass as determinants of species richness in parasite communities: a comparative analysis of directly transmitted nematodes of mammals. Ecography 25, 88–94.
Bachman, G.C., 2003. Food supplements modulate changes in leucocyte numbers in breeding male ground squirrels. J. Exp. Biol. 206, 2373–2380.
Bacon, L.D., 1992. Measurement of immune competence in chickens. Poult. Sci. Rev. 4, 187–195.
Balloux, F., Amos, W., Coulson, T., 2004. Does heterozygosity estimate inbreeding in real populations? Mol. Ecol. 13, 3021–3031.
Beldomenico, P.M., Telfer, S., Gebert, S., Lukomski, L., Bennett, M., Begon, M., 2008. Poor condition and infection: a vicious circle in natural populations. Proc. R. Soc. B: Biol. Sci. 275, 1753–1759.
Bernatchez, L., Landry, C., 2003. MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? J. Evol. Biol. 16, 363–377.

Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B., Sorci, G., 2003. Assessing the cost of mounting and immune response. Am. Nat. 161, 367–379.

Bonneaud, C., Perez-Tris, J., Federici, P., Chastel, O., Sorci, G., 2006. Major histocompatibility alleles associated with local resistance to malaria in a passerine. Evolution 60, 383–389.

Bonneaud, C., Richard, M., Faivre, B., Westerdahl, H., Sorci, G., 2005. An Mhc class I allele associated to the expression of T-dependent immune response in the house sparrow. Immunogenetics 57, 782–789.

Boonstra, R., Hik, D., Singleton, G.R., Tinnikov, A., 1998. The impact of predator-induced stress on the snowshoe harecycle. Ecol. Monogr. 68, 371–394.

Bradley, R.D., Hillis, D.M., 1997. Recombinant DNA sequences generated by PCR amplification. Mol. Biol. Evol. 14, 592–593.

Bryja, J., Charbonnel, N., Berthier, K., Galan, M., Cosson, J.-F., 2007. Density related changes in selection pattern for major histocompatibility complex genes in fluctuating populations of voles. Mol. Ecol. 16, 5084–5097.

Busch, C., Malizia, A.I., Scaglia, O.A., Reig, O.A., 1989. Spatial distribution and attributes of a population of *Ctenomys talarum* (Rodentia: Octodontidae). J. Mamm. 70, 204–208.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83, 575–583.

Bush, A.O., Aho, J.M., Kennedy, C.R., 1990. Ecological versus phylogenetric determinants of helminth parasite community richness. Evol. Ecol. 4, 1–20.

Buffenstein, R., 2000. Ecophysiological responses of subterranean rodents to an underground habitat. In: Lacey, E.A., Patton, J.L., Cameron, G.M. (Eds.), Life Underground: Biology of Subterranean Rodents. University of Chicago Press, Chicago, pp. 62–109.

Carrol, M.C., Prodeus, A.P., 1998. Linkages of innate and adaptive immunity. Curr. Opin. Immunol. 10, 36–40.

Charbonnel, N., Gouy de Bellocq, J., Morand, S., 2006. Immunogenetics of macroparasites and small mammals interactions. In: Morand, S., Krasnov, B.R., Poulin, R. (Eds.), Micromammals and Macroparasites: From Evolutionary Ecology to Management. Springer, Tokyo, pp. 401–442.

Clare, R.A., Danforth, H.D., 1989. Major histocompatibility complex control of immunity elicited by genetically engineered *Eimeria tenella* (Apicomplexa) antigen in chickens. Infect. Immun. 57, 701–705.

Comparatore, V.M., Maceira, N.O., Busch, C., 1991. Habitat relations in *Ctenomys talarum* (Caviomorpha: Octodontidae) in a natural grassland. Zeichrift für Saügetier. 56, 112–118.

Cornet, S., Sorci, G., 2010. Parasite virulence when the infection reduces the host immune response. Proc. R. Soc. Lond. B 277, 1929–1935.

Coté, I.M., Poulin, R., 1995. Parasitism and group-size in social animals: a meta-analysis. Behav. Ecol. 6, 159–165.

Culhane, A.C., Perrière, G., Higgins, D.G., 2003. Cross-platform comparison and visualization of gene expression data using co-inertia analysis. BMC Bioinformatics 4, 1–15.

Cutrera, A.P., Lacey, E.A., 2006. Major histocompatibility complex variation in Talas tuco- tucos: the influence of demography on selection. J. Mamm. 87, 706–716.

Cutrera, A.P., Lacey, E.A., 2007. Trans-species polymorphism and evidence of selection on class II MHC loci in tuco-tucos (Rodentia: Ctenomyidae). Immunogenetics 59, 937–948.

Cutrera, A.P., Lacey, E.A., Busch, C., 2005. Genetic structure in a solitary rodent (*Ctenomys talarum*): implications for kinship and dispersal. Mol. Ecol. 14, 2511–2523.

Cutrera, A.P., Zenuto, R.R., Luna, F., Antenucci, C.D., 2010. Mounting a specific immune response increases energy expenditure of the subterranean rodent *Ctenomys talarum* (tuco-tuco): implications for intraspecific and interspecific variation in immunological traits. J. Exp. Biol. 213, 715–724.

Davis, A.K., Maney, D.L., Maerz, J.C., 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct. Ecol. 22, 760–772.

Dean, A.G., Dean, J.A., Burton, A.H., Dicker, R.C., 1991. Epi Info: a general-purpose microcomputer program for public health information systems. Am. J. Prev. Med. 7, 178–182.

De Boer, R.J., Borgans, J.A.M., van Boven, M., Kesmir, C., Weissing, F.J., 2004. Heterozygote advantage fails to explain the high degree of polymorphism of the MHC. Immunogenetics 55, 725–731.

Deerenberg, C., Arpanius, V., Daan, S., Bos, N., 1997. Reproductive effort decreases antibody responsiveness. Proc. R. Soc. Lond. B 264, 1021–1029.

Derting, T.L., Virk, M.K., 2005. Positive effects of testosterone and immunochallenge on energy allocation to reproductive organs. J. Comp. Physiol. B. 175, 543–556.

Deter, J., Bruyja, J., Chaval, Y., Galan, M., Henttonen, H., Laakkonen, J., Voutilainen, L., Vapalahti, O., Vaheri, A., Ribas Salvador, A., Morand, S., Cosson, J.-F., Charbonnel, N., 2008. Association between the DQA MHC class II gene and Puumala virus infection in *Myodes glareolus*, the bank vole. Infect. Genet. Evol. 8, 450–458.

Doherty, C., Zinkernagel, R.M., 1975. Enhanced immunological surveillance in mice heterozygous at the H-2 complex. Nature 256, 50–52.

Dolédec, S., Chessel, D., 1994. Co-inertia analysis: an alternative method for studying species-environment relationships. Freshwater Biol. 31, 277–294.

Dray, S., Chessel, D., Thioulouse, J., 2003. Procrustean co-inertia analysis for the linking of multivariate datasets. Ecoscience 10, 110–119.

Eliyahu, H., Servel, N., Domb, A.J., Barenholz, Y., 2002. Lipoplex-induced hemagglutination: potential involvement in intravenous gene delivery. Gene Ther. 9, 850–858.

Escoufier, Y., 1973. Le traitement des variables vectorielles. Biometrics 29, 751–760.

Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol. Bioinf. Online 1, 47–50.

Frank, S.A., 2002. Immunology and Evolution of Infectious Disease. Princeton University Press, Princeton and Oxford.

Froeschke, G., Sommer, S., 2005. MHC class II DRB variability and parasite load in the striped mouse (*Rhabdomys pumilio*) in the South Kalahari. Mol. Ecol. Evol. 22, 1254–1259.

Gannon, W.L., Sikes, R.S., 2007. Animal Care and Use Committee of the American Society of Mammalogists. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J. Mamm. 88, 809–823.

Gillespie, J.H., 1975. Natural selection for resistance to epidemics. Ecology 56, 493–495.

Gross, W., Siegel, P., Hall, R., Domermuth, C., DuBois, R., 1980. Production and persistence of antibodies in chickens to sheep erythrocytes. Resistance to infectious diseases. Poult. Sci. 59, 205–210.

Guo, S.W., Thompson, E.A., 1992. A Monte Carlo method for combined segregation and linkage analysis. Am. J. Hum. Genet. 51, 1111–1126.

Haldane, J.B.S., 1956. The estimation and significance of the logarithm of a ratio of frequencies. Ann. Hum. Genet. 2, 309–311.

Hambuch, T.M., Lacey, E.A., 2002. Enhanced selection for MHC diversity in social tuco-tucos. Evolution 56, 841–845.

Harf, R., Sommer, S., 2005. Association between MHC Class II DRB alleles and parasite load in the hairy-footed gerbil, *Gerbilluruspaeba*, in the Southern Kalahari. Mol. Ecol. 14, 85–91.

Hedrick, P.W., Kim, T.J., 2000. Genetics of complex polymorphisms: parasites and maintenance of the major histocompatibility complex variation. In: Singh, R.S., Krimbas, C.B. (Eds.), Evolutionary Genetics: From Molecules to Morphology. Cambridge University Press, Cambridge, pp. 204–234.

Hill, J.E., Lomax, L.G., Lindsay, D.S., Lynn, B.S., 1985. Coccidosis caused by *Eimeria scabra* in a finishing hog. J. Am. Vet. Med. Assoc. 186, 981–983.

Hoi-Leitner, M., Romero-Pujante, M., Hoy, H., Pavlova, A., 2001. Food availability and immune capacity in serin (*Serinus serinus*) nestlings. Behav. Ecol. Sociobiol. 49, 333–339.

Hughes, A.L., Nei, M., 1992. Maintenance of MHC polymorphism. Nature 355, 402–403.

Hughes, A., Hughes, M., 1995. Natural selection on the peptide–binding regions of major histocompatibility complex molecules. Immunogenetics 42, 233–243.

Janeway, C.A., Travers, P., 1999. Immunobiology: The Immune System in Health and Disease, 4th ed. Current Biology Ltd., London.

Kasugai, T., Okada, M., Morimoto, M., Arizono, N., Maeyama, K., Yamada, M., Tei, H., Dohmae, K., Onoue, H., Newlands, G.F., 1993. Infection of *Nippostrongylus brasiliensis* induces normal increase of basophils in mast cell-deficient Ws/Ws rats with a small deletion at the kinase domain of c-kit. Blood 81, 2521–2529.

Klein, J., 1986. Natural History of the Major Histocompatibility Complex. Wiley Son, New York.

Klein, J., 1987. Origin of the major histocompatibility complex polymorphism: the trans-species hypothesis. Hum. Immunol. 19, 155–162.

Klein, J., Horejsi, V., 1997. Immunology. Blackwell Science, Oxford.

Lacey, E.A., Patton, J.L., Cameron, G.N., 2000. Life Underground: The Biology of Subterranean Rodent. University of Chicago Press, Chicago.

Lacey, E.A., Cutrera, A.P., 2007. Behavior, demography, and immunogenetic variation: new insights from subterranean rodents. In: Begall, S., Burda, H., Schleich, C. (Eds.), Subterranean Rodents: News From Underground. Springer, Heidelberg, pp. 343–356.

Lively, C.M., Dybdahl, M.F., 2000. Parasite adaptation to locally common host genotypes. Nature 405, 679–681.

Malizia, A.I., Vassallo, A.I., Busch, C., 1991. Population and habitat characteristics of two sympatric species of *Ctenomys* (Rodentia: Octodontidae). Acta Theriol. 36, 87–94.

Martin, L.B., Weil, Z.M., Nelson, R.J., 2006. Refining approaches and diversifying directions in ecoimmunology. Int. Comp. Biol. 46, 1030–1039.

Meyer-Lucht, Y., Sommer, S., 2005. MHC diversity and the association to nematode parasitism in the yellow necked mouse (*Apodemus flavicollis*). Mol. Ecol. 14, 2233–2243.

Meyer-Lucht, Y., Otten, C., Püttker, T., Pardini, R., Metzger, J.P., Sommer, S., 2010. Variety matters: adaptive genetic diversity and parasite load in two mouse opossums from the Brazilian Atlantic forest. Conserv. Genet., doi:10.1007/s10592-010-0093-9.

Nevo, E., 1999. Mosaic Evolution of Subterranean Mammals: Regression, Progression, and Global Convergence. Oxford University Press, Oxford.

Norris, K., Evans, M.R., 2000. Ecological immunology: life history trade-offs and immune defense in birds. Behav. Ecol. 11, 19–26.

Nunn, C., Gittleman, J.L., Antonovics, J., 2000. Promiscuity and the primate immune system. Science 290, 1168–1170.

Okada, M., Nawa, Y., Horii, Y., Kitamura, T., Arizono, N., 1997. Development of basophils in Mongolian gerbils: formation of basophilic cell clusters in the bone marrow after *Nippostrongylus brasiliensis* infection. Lab. Invest. 76, 89–97.

Oliver, M.K., Telfer, S., Piertney, S.B., 2008. Major Histocompatibility Complex (MHC) heterozygote superiority to natural multi-parasite infections in the water vole (*Arvicola terrestris*). Proc. R. Soc. Lond. B: Biol. Sci. 276, 1119–1128.

Oppelt, C., Starkloff, A., Rausch, P., Von Holst, D., Rödel, H.G., 2010. Major histocompatibility complex variation and age-specific endoparasite load in subadult European rabbits. Mol. Ecol., doi:10.1111/j.1365-294X.2010.04766.x.

Pedersen, A.B., Babayan, S.A., 2011. Wild immunology. Mol. Ecol., doi:10.1111/j.1365-294X.2010.04938.x.

Penn, D.J., Damjanovich, K., Potts, W.K., 2002. MHC heterozygosity confers a selective advantage against multi-strain infections. Proc. Natl. Acad. Sci. U.S.A. 99, 11260–11264.

Penn, D.J., 2002. The scent of genetic compatibility: sexual selection and the major histocompatibility complex. Ethology 108, 1–21.
Piertney, S.B., Oliver, M.K., 2006. The evolutionary ecology of the major histocompatibility complex. Heredity 96, 7–21.
Pilny, A.A., 2008. Clinical hematology of rodent species. Veterinary clinics of North America. Exot. Anim. Pract. 11, 522–533.
Restif, O., Amos, W., 2010. The evolution of sex-specific immune defences. Proc. R. Soc. B: Biol. Sci., doi:10.1098/rspb.2010.0188.
Richner, H., Oppliger, A., Christie, P., 1993. Effect of an ectoparasite on reproduction in great tits. J. Anim. Ecol. 62, 703–710.
Rigby, M.C., Hechinger, R.F., Stevens, L., 2002. Why should parasite resistance be costly? Trends Parasitol. 18, 116–120.
Roitt, I.M., Brostoff, J., Male, D.K., 1998. Immunology. Gower Medical Publishing, London.
Rossin, M.A., Malizia, A.I., 2002. Relationship between helminth parasites and demographic attributes of a population of the subterranean rodent *Ctenomys talarum* (Rodentia: Octodontidae). J. Parasitol. 88, 1268–1270.
Rossin, M.A., Malizia, A.I., Timi, J.T., Poulin, R., 2010. Parasitism underground: determinants of helminth infections in two species of subterranean rodents (Octodontidae). Parasitology, doi:10.1017/S0031182010000351.
Rossin, M.A., Malizia, A.I., Denegri, G.M., 2004. The role of the subterranean rodent *Ctenomys talarum* (Rodentia: Octodontidae) in the life cycle of *Taenia taeniaformis*. Vet. Parasitol. 122, 27–33.
Rossin, M.A., Timi, J.T., Malizia, A.I., 2005. *Graphidioides subterraneus* n. sp. (Nematoda: Trichostrongylidae) from the South American subterranean rodent *Ctenomys talarum* Thomas, 1898 (Rodentia: Octodontidae). Parasite 12, 145–149.
Rozas, J., Sanchez-Del Barrio, J.C., Messeguer, X., Rozas, R., 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19, 2496–2497.
Schad, J., Ganzhorn, J.U., Sommer, S., 2005. Parasite burden and constitution of major histocompatibility complex in the Malagasy mouse lemur, *Microcebus murinus*. Evolution 59, 439–450.
Scharf, S.J., Long, C.M., Erlich, H.A., 1988. Sequence analysis of the HLA DR beta and HLA DQ beta loci from the *Pemphigus vulgaris* patients. Hum. Immunol. 22, 61–69.
Schwensow, N., Fietz, J., Dausmann, K., Sommer, S., 2007. Neutral versus adaptive genetic variation in parasite resistance: importance of MHC-supertypes in a free-ranging population. Heredity 99, 265–277.
Schwensow, N., Dausmann, K., Eberle, M., Fietz, J., Sommer, S., 2010. Functional associations of similar MHC alleles and shared parasite species in two sympatric lemurs. Infect. Genet. Evol. 10, 662–668.
Semple, S., Cowlishaw, G., Bennett, P.M., 2002. Immune system evolution among anthropoid primates: parasites, injuries and predators. Proc. R. Soc. B: Biol. Sci. 269, 1031–1037.
Shannon, C.E., Weaver, W., 1949. The Mathematical Theory of Communication. The University of Illinois Press, Urbana.
Slate, J., David, P., Dodds, K.G., Veenvliet, B.A., Glass, B.C., Broad, T.E., Mc Ewan, J.C., 2004. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. Heredity 93, 255–265.
Sloss, M.W., Kemp, R.L., Zajac, A.M., 1994. Veterinary Clinical Parasitology. Iowa State University Press, Iowa.
Sommer, S., 2005. The importance of immune gene variability (MHC) in evolutionary ecology and conservation. Front. Zool. 2, 1–18.
Soulsby, E.J.L., 1987. Immune Responses in Parasitic Infections: Immunology, Immunopathology and Immunoprophylaxis. CRC Press, Boca Raton.
Sousa, A.O., Mazzacaro, R.J., Russel, R.G., Lee, F.K., Turner, O.C., Hong, S., Van Kaer, L., Bloom, B.R., 2000. Relative contributions of distinct MHC class I-dependent cell populations in protection to tuberculosis infection in mice. Proc. Natl. Acad. Sci. U.S.A. 97, 4204–4208.
Speakman, J.R., 2008. The physiological costs of reproduction in small mammals. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 363, 375–398.
Spurgin, L.G., Richardson, D.S., 2010. How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. Proc. R. Soc. Lond. B, doi:10.1098/rspb.2009.2084.
Stoffels, R.J., Spencer, H.G., 2008. An asymmetric model of heterozygote advantage at major histocompatibility complex genes: degenerate pathogen recognition and intersection advantage. Genetics 178, 1473–1489.
Svensson, E., Merilä, J., 1996. Molt and migratory condition in Blue Tits: a serological study. Condor 98, 825–831.
Telfer, S., Lambin, X., Richard, B., Beldomenico, P., Burthe, S., Paterson, S., Begon, M., 2010. Species interactions in a parasite community drive infection risk in a wildlife population. Science 330, 243–246.
Thienpont, D., Rochette, F., Vanparijs, O.F.J., 1986. Diagnosing Helminthiasis by Coprological Examination. Janssen Research Foundation, Beerse, Belgium.
Thioulouse, J., Dray, S., 2007. Interactive multivariate analysis in R with the ade4 and the ade4TKGUI packages. J. Stat. Softw. 22, 1–14.
Thursz, M.R., Kwiatkowski, D., Allsopp, C.E.M., Greenwood, B.M., Thomas, H.C., Hill, A.V.S., 1995. Association between an MHC class II allele and clearance of hepatitis B virus in the Gambia. N. Engl. J. Med. 332, 1065–1069.
Toïgo, C., Gaillard, J.-M., Van Laere, G., Hewison, M., Morellet, N., 2006. How does environmental variation influence body mass, body size, and body condition? Roe deer as a case study. Ecography 29, 301–308.
Tollenaere, C., Bryja, J., Galan, M., Cadet, P., Deter, J., Chaval, Y., Berthier, K., Ribas Salvador, A., Voutilainen, L., Laakkonen, J., Henttonen, H., Cosson, J.-F., Charbonnel, N., 2008. Multiple parasites mediate balancing selection at two MHC class II genes in the fossorial water vole: insights from multivariate analyses and population genetics. J. Evol. Biol. 21, 1307–1320.
Vera, F., Zenuto, R.R., Antinuchi, C.D., 2008. Decreased glucose tolerance but normal blood glucose levels in the field in the caviomorph rodent *Ctenomys talarum*: the role of stress and physical activity. Comp. Biochem. Physiol. A 151, 232–238.
Voigt, G.L., 2000. Hematology Techniques and Concepts for Veterinary Technicians. Iowa State University Press, Iowa.
Wegner, K.M., Reusch, T.B.H., Kalbe, M., 2003. Multiple parasites are driving major histocompatibility complex polymorphism in the wild. J. Evol. Biol. 16, 224–232.
Weil, Z.M., Martin II, L.B., Nelson, R.J., 2006. Interactions among immune, endocrine, and behavioural response to infection. In: Morand, S., Krasnov, B.R., Poulin, R. (Eds.), Micromammals and Macroparasites. Springer, Japan, pp. 443–473.
Westerdahl, H., Hansson, B., Bensch, S., Hasselquist, D., 2004. Between-year variation of MHC allele frequencies in great reed warblers: selection or drift? J. Evol. Biol. 17, 485–492.
Zenuto, R.R., Lacey, E.A., Busch, C., 1999. DNA fingerprinting reveals polygyny in the subterranean rodent *Ctenomys talarum*. Mol. Ecol. 8, 1529–1532.
Zhou, H., Lamont, S.J., 2003. Chicken MHC class I and II gene effects on antibody response kinetics in adult chickens. Immunogenetics 55, 133–140.