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Interpopulation differences in parasite load and variable selective pressures on MHC genes in *Ctenomys talarum*

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We explore potential geographic variation in the pathogen-mediated selective pressures acting on major histocompatibility complex (MHC) loci in the subterranean rodent *Ctenomys talarum*, evaluating the impact of differences in parasite load between 2 populations (Mar de Cobo [MdC] and Necochea [NC]) on immunogenetic variation and selection on MHC genes in this species. Because *Ctenomys* from NC face lower parasite load and presumably weaker pathogen-mediated selection on MHC, we expected to find a weaker correlation between MHC variation and parasite load or immunocompetence, or both, in this population compared to that at MdC. MHC-associated cues are used in other species of rodents as kinship markers to avoid inbreeding, and because kinship structure is less pronounced in NC, we predicted that use of MHC-associated cues in mate choice would be less apparent in this population. We characterized MHC variation in NC as a function of parasite load and immunocompetence and compared our results with previous findings for MdC. The 2 populations were sampled across different, but consecutive, years. Using coinertia analyses, we found a significant positive association between a specific DRB allele and intensity of infection by fleas in NC. We explored the use of MHC-associated cues in mate choice in NC and found support for both the "good-genes" and the "genetic compatibility" hypotheses. As expected, associations between MHC and parasite load or immunocompetence were weaker in NC. Evidence indicated that females in NC selected for males with lower MHC diversity. This suggests that parasite-driven selection acting directly on MHC genes seems to be greater for the population facing higher parasite load. However, parasite-driven selection mediated by mate choice may not only be influenced by levels of parasite diversity in the population but also by characteristics of the mating system.

Key words: immunocompetence, mate choice, major histocompatibility complex (MHC), parasite load, pathogen-mediated selection

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Both physical and biological environments can have profound effects on the diversity, infectivity, and virulence of pathogens (Larsen et al. 2004; Zheng et al. 2004; Morand et al. 2006). As a result, environmental variability may create spatial variation in the intensity of pathogen-driven selection. Given their fundamental role in resistance against parasites and pathogens (Apanius et al. 1997), the genes of the major histocompatibility complex (MHC) are particularly appropriate targets for elucidating the impacts of geographic variation in pathogen communities on the nature and magnitude of selection for immunogenetic diversity (Bonneaud et al. 2006a; Kloch et al. 2010). Specifically, parasite-driven balancing selection is thought to be important in maintaining high levels of variation at these loci, with this selection acting through heterozygote advantage (Doherty and Zinkernagel 1975), rare-allele advantage (Lively and Dybdahl 2000), and spatially or temporally varying parasite pressure (reviewed in Spurgin and Richardson 2010). Whereas under the heterozygote advantage 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), rare-allele

advantage may arise as pathogens adapt to common host genotypes, thereby providing a selective advantage to other resistant host alleles in the population, which are more likely to be rare (Lively and Dybdahl 2000; Eizaguirre et al. 2012b). One predicted evolutionary 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). More specifically, under heterozygote advantage selection, the relationship between heterozygosity at the DRB locus (a well-studied MHC class II locus—Brown et al. 1993; Satta et al. 1994) and parasite load is expected to be negative whereas that between DRB heterozygosity and immune responsiveness should be positive. Under rare-allele advantage selection, associations between parasites and specific DRB alleles are expected; negative associations should indicate greater parasite resistance or immune responsiveness, whereas positive associations may indicate increased susceptibility to infection (e.g., Tollenaere et al. 2008).

Pathogen-driven selection may be mediated by mate choice if individuals are able to use MHC-based phenotypic cues to assess the immunocompetence of potential reproductive partners (Landry et al. 2001; Reusch et al. 2001; Freeman-Gallant et al. 2003), with females favoring males that possess particular MHC alleles, those with diverse MHC genotypes (good-genes hypothesis—Penn and Potts 1999; Eizaguirre et al. 2009), or those whose MHC genotypes differ from that of the female in question (compatibility hypothesis—Neff and Pitcher 2005). Although the pathogen and mate-choice mechanisms of selection are not mutually exclusive (see Penn and Potts 1999), this model provides a useful framework for structuring studies of the selective pressures that maintain variation at these loci. Thus, we evaluated if the distribution of MHC allele frequencies differed between potential sires and random males from the population. According to the good-genes hypothesis (Penn and Potts 1999), possible sires should carry specific MHC alleles with higher or lower average number of amino acid differences between them, and have higher or lower average MHC heterozygosity and distinctive distributions of MHC allele frequencies compared to a sample of randomly assigned males, depending on what genetic combinations confer resistance against pathogens at that moment. Similarly, according to the genetic compatibility hypothesis (Neff and Pitcher 2005), possible sires should share on average fewer or more MHC alleles with the mother and have higher amino acid differences with her.

Parasite diversity within host species may impact the evolution of parasite virulence and infectivity, thus having a key role in the evolution of host–parasite relationships (Rigaud et al. 2010). Several host attributes have been linked to parasite diversity, including population density (Anderson and May 1979; Arneberg 2002) and body size (Morand and Poulin 1998). Spatial variation in these parameters may generate variability in the pathogen-driven selective pressures experienced by conspecifics; over time, this may lead to spatial variability in a host species' behavioral (Freeland 1976; Moore and Wilson 2002) or immunological (Weil et al. 2006) responses to pathogen exposure.

Rodents in the genus *Ctenomys* (tuco-tucos) provide an opportunity to assess the selective processes underlying spatial variation in MHC diversity 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 (transspecies 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 demographic attributes such as social structure and population density (Lacey and Cutrera 2007). Long-term studies in 2 populations of the Talas tuco-tuco (*C. talarum*) from southeastern Argentina have reported marked differences with respect to several demographic characteristics, including animal density and adult sex ratio (Busch et al. 1989; Malizia et al. 1991), degree of polygyny and kin structure (Zenuto et al. 1999a, 1999b; Cutrera et al. 2005), and effective population size (Cutrera et al. 2006). In particular, comparative studies of these 2 demographically distinct populations have demonstrated that number of alleles, heterozygosity, and estimated intensity of selection at 2 class II loci (DRB and DQA) are greater in the population characterized by higher density (Mar de Cobo [MdC]: mean number of individuals/ha $\pm$ *SD*: 57 $\pm$ 6; Necochea [NC]: 15 $\pm$ 4 individuals/ha—see Busch et al. 1989; Malizia et al. 1991; Cutrera and Lacey 2006) and greater parasite exposure (greater parasite prevalence [the percentage of infected individuals in the population], richness, and intensity of infection—Rossin and Malizia 2002; Rossin et al. 2010), as expected if parasite-driven selection maintains MHC polymorphism in this species (Cutrera and Lacey 2006). Detailed analyses of 1 of these populations (MdC) have revealed that specific DRB alleles are associated with parasite load and intensity of humoral immune response (Cutrera et al. 2011), suggesting that individual MHC sequences influence resistance to pathogens. At the same time, field and laboratory studies of animals from this population indicate that females may engage in MHC-associated mate choice (Cutrera et al. 2012), providing another potential source of spatial variation in the selective pressures acting on MHC genes.

This study expands upon our previous analyses of relationships among parasite load, immune response, and genotypic variability in *C. talarum* to explore potential geographic variation in the selective pressures acting on MHC loci in this species. Specifically, by contrasting new information from a 2nd population (NC) of *C. talarum* with data obtained previously for animals at MdC (Cutrera et al. 2011, 2012), we use the pronounced differences in parasite load between these populations to assess how spatial variation in this attribute influences immunogenetic variation and selection on MHC genes in this species. Gastrointestinal parasite load in *C. talarum* at NC is markedly lower than at MdC (Rossin and Malizia 2002; Rossin et al. 2010). Assuming

that increased intensity of parasite infection is associated with enhanced parasite-mediated selection on MHC (reviewed by Charbonnel et al. 2006), we predict that MHC-DRB diversity and evidence of selection upon this locus will be lower, the correlations between matrices of MHC genotypes and those of parasite load or immunocompetence will be weaker, allele divergence will be smaller, and use of MHC-associated cues in mate choice will be less apparent in NC compared to previous findings in MdC. Our comparative analyses of these populations provide important new insights into the role of pathogen-driven selection in shaping intraspecific differences in MHC variation in natural populations of vertebrates.

Materials and Methods

Animal capture and housing.—Talas tuco-tucos (*C. talarum*) are solitary and highly territorial rodents (Busch et al. 1989), with highly genetically structured populations and low vagility (Malizia et al. 1995; Cutrera et al. 2005). DNA fingerprinting suggests that they are polygynous (Zenuto et al. 1999a), with a spatial distribution characterized by the presence of 1 dominant male surrounded by other burrow systems individually occupied by females (Busch et al. 1989). Two populations of this species from Buenos Aires Province, Argentina, were included in this study. Cutrera et al. (2011, 2012) sampled the population at MdC (37°46′S, 57°26′W) in 2006, 2007, and 2008; we subsequently sampled the population at NC (38°33′S, 58°45′W) in 2009 and 2010. The 2 populations are located approximately 150 km apart and are not demographically connected (Mora et al. 2013). Both occur in coastal dune habitats characterized by sandy soils and dominated by *Panicum racemosum*, *Ambrosia tenuifolia*, and *Distichlis scoparia* (see Comparatore et al. 1991, 1992).

The methods used to capture and sample *C. talarum* at NC were the same as those used at MdC (Cutrera et al. 2011), and in both populations animal capture was performed over an area of approximately 2 ha. In brief, animals were captured using plastic tube traps inserted into active burrow entrances (Cutrera et al. 2005). Sample sizes were similar at both sites (MdC, $n = 87$ [Cutrera et al. 2011]; NC, $n = 81$). We captured 23 adult males (mean body mass $\pm$ *SD* = 125.52 $\pm$ 32.77 g) and 28 adult females (111.58 $\pm$ 27.86 g) during the breeding season (August–early December) of 2009. Pregnant females were brought to the laboratory, whereas nursing females were returned to their burrows immediately after capture to avoid distressing pups left in the nest. To assess the possible impact of breeding status on relationships between MHC genotypes and pathogen resistance, we captured an additional 18 adult females (96.95 $\pm$ 15.73 g) and 12 adult males (140.22 $\pm$ 34.43 g) during the nonbreeding season (mid-February–mid-April) of 2010. All individuals were subject to the analyses of parasite load and MHC variability described below. A randomly chosen subset of 42 animals (15 males and 16 females captured in the reproductive season and 6 males and 6 females captured in the nonreproductive season) was used to assess immune response to a novel antigen.

Immediately after release of an animal from a trap, fecal pellets were collected from the trap and fixed in 4% formalin for analyses of endoparasite load. 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. 2011). A single drop of blood was spread onto a microscope slide to produce a thin layer of cells, after which the slide was air dried and fixed in 70% methanol for 10 min to preserve until analysis. All animals were then transported to the Laboratorio de Ecofisiología at the Universidad Nacional de Mar del Plata (Mar del Plata, Argentina).

Housing and sampling procedures were identical to that for *C. talarum* from MdC (Cutrera et al. 2011). Most animals were held in captivity until immune challenge experiments were completed (approximately 4 weeks; see below), after which they were returned to the field and released at the point of capture. Pregnant females were held in captivity until a month after parturition; 11 of 20 pregnant females housed in the laboratory gave birth to litters (mean litter size $\pm$ *SD* = 3.09 $\pm$ 1.45 pups). Immediately prior to release, we collected a tissue sample for genetic analyses from each animal (adults and 1-month-old pups) 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: PICT 0998) as well as the guidelines of the American Society of Mammalogists (Sikes et al. 2011).

Quantification of parasites.—Ectoparasite load was determined by collecting the number of fleas, lice, and mites trapped in an individual's pelage as described in Cutrera et al. (2011). Interindividual comparisons of ectoparasite loads were completed using per-individual counts of the number of parasites of each type and estimates of parasite diversity obtained using H', the Shannon diversity index (Shannon and Weaver 1949), as done by Cutrera et al. (2011).

Endoparasite load was determined by quantifying the number of gastrointestinal parasite eggs and oocysts present in fecal samples collected from the study animals using a modification of the MacMaster flotation technique (Sloss et al. 1994), as described in Cutrera et al. (2011). Interindividual comparisons of endoparasite loads were completed using individual counts of the number of parasite eggs of each species per gram of feces (FEC) and estimates of parasite diversity obtained using H' (Shannon and Weaver 1949), as done by Cutrera et al. (2011). To compare ectoparasite load between the study populations, we assessed the number of ectoparasite species per individual host. Values of ectoparasite species per individual host were compared using a Mann–Whitney rank sum test. Further, we compared the mean number of endoparasite species detected in 1 g of fecal sample (Watve and Sukumar 1995), which represents the mean infracommunity richness (the parasite assemblage found in an individual host—Holmes and Price 1986) using a Mann–Whitney rank sum test. Relative risk (*RR*) analyses were performed between the study populations to assess the risk of infection with a given endo- or ectoparasite in each of the

populations. Estimates of *RR* were generated using Epi Info (Dean et al. 1991) and significance was evaluated using Fisher's exact test. Finally, to quantify the dissimilarity in composition between the parasite communities of NC and MdC, we used an analysis of similarities (ANOSIM) as implemented in PAST (Hammer et al. 2001). Briefly, ANOSIM is a nonparametric test of significant difference between 2 or more groups of sampling units, based on any distance measure (Clarke 1993). Particularly for our data, we used 2 distance measures: the Bray–Curtis distance for data of intensity of parasite infection, and the Jaccard distance for presence–absence data. The test is based on comparing distances between groups with distances within groups. Let r_b be the mean rank of all distances between groups, and r_w the mean rank of all distances within groups. The test statistic R is then defined as:

$$R = \frac{r_b - r_w}{N(N-1)/4}$$

If R is positive (up to 1), this signifies dissimilarity between groups. The 1-tailed significance was computed by permutation of group (population), with 9,999 replicates.

Immune challenge tests.—To quantify differences in immune response to a novel antigen (immune responsiveness, sensu Vinkler and Albrecht [2011]), we used sheep red blood cells (sRBC) to elicit antibody production by the study animals. sRBC are a nonpathogenic antigen known to trigger Th2 and B-lymphocyte–dependent immune responses in mammals and birds (Bacon 1992). The magnitude of response to this antigen 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 and macroparasites—Deerenberg et al. 1997; but see Adamo 2004). Previous research has shown that individual *C. talarum* produce significant antibody titers in response to injection with sRBC, whereas control animals injected with saline solution do not mount a response (Cutrera et al. 2010b). 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 laboratory.

Immunization assays of animals from NC were performed during the breeding and the nonbreeding seasons, following the same protocol used by Cutrera et al. (2011). On day 10 of captivity, the animals were weighed, and all study subjects ($n = 42$) were injected intraperitoneally with sRBC (R3378, 10% suspension, 1.5 µl/g of animal mass; Sigma, Saint Louis, Missouri). Immediately after injection, we collected ~200 µl of blood from the retro-orbital sinus of each animal for use in hemagglutination 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 postinjection (dpi) but that antibody titers were low (Cutrera et al. 2010b). Consequently, animals in this study received a 2nd injection of sRBC at 7 dpi to ensure 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 2nd injection).

To quantify antibody production in response to injection with sRBC, hemagglutination assays were conducted following the protocol of Cutrera et al. (2010a). Antibody titers 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). Results obtained in this study were compared to those obtained for the population of MdC (Cutrera et al. 2011) using a generalized linear–nonlinear model (GLiM) with a Poisson distribution and a logarithmic link function with population of origin as the grouping variable and dpi as the repeated measure, as implemented in the LME4 R package version 1.0–5 (Bates et al. 2013).

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 the ability of an individual 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 because each of these conditions causes similar alterations in neutrophilia or 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 also is associated with these challenges as well as with some endocrine disorders, whereas 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 following standard protocols (Voigt 2000), as described in Cutrera et al. (2011).

Finally, hematocrit (the proportion of blood volume occupied by packed red blood cells) 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 parasite infections (e.g., Soulsby 1987). Hematocrit was measured following the protocol of Cutrera et al. (2011).

Amplification of MHC class II DRB exon 2 and analysis of microsatellite variation.—We assessed variability at the MHC class II DRB locus 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 typically are most subject to balancing selection (Hughes and Hughes 1995). DNA extraction, polymerase chain reaction conditions,

cloning of polymerase chain reaction products, and sequencing were performed according to Cutrera and Lacey (2006) and Cutrera et al. (2011). Allelic and genotypic variability were assessed using Arlequin version 3.11 (Excoffier et al. 2005). Pairwise differences among DRB sequences were assessed at both the nucleotide and amino acid levels using DnaSP version 5.1 (Rozas et al. 2003). DRB alleles whose nucleotide sequences differed but had identical amino acid sequences were considered part of the same allele group (e.g., allele group A). Microsatellite data ($n = 8$) for both populations (see below) were obtained from Cutrera et al. (2006).

Statistical analyses: evidence of selection based on substitution rates.—Rates of non-synonymous (d_N) versus synonymous (d_S) base pair substitutions can be used to identify departures from neutrality and to determine the type of selection that has acted on a locus (Kimura 1983; Ohta 1993). The nature and strength of selection can vary among codons (including codons in the same gene—Anisimova et al. 2001) and thus we used the maximum likelihood approach of Goldman and Yang (1994) to examine values of ω for the codons within the DRB exon 2 for the 2 study populations. ω is equivalent to the d_N/d_S selection parameter of Nei and Gojobori (1986) and captures information regarding both the type and intensity of selection (Goldman and Yang 1994); for codon-specific analyses, ω is preferred because it allows for variation in the selective pressures experienced by different codons (Nielsen and Yang 1998). Estimates of ω were generated according to Yang et al. (2005), using the CODEML subroutine of PAML 3.14 (Yang 2007), as previously described in Cutrera et al. (2010a).

Departures from neutrality.—We tested for departures from neutrality of MHC data obtained in NC using the exact test of Hardy–Weinberg (Guo and Thompson 1992), as implemented in Arlequin version 3.11 (Excoffier et al. 2005). These results were compared with those previously reported for MdC (Cutrera et al. 2011). Additionally, for both study populations Ewens–Watterson tests (Ewens 1972; Watterson 1986) were used to determine if allele frequency distributions were consistent with the effects of balancing selection. Both analyses were conducted using Arlequin version 3.11 (Excoffier et al. 2005). For both study populations we calculated Tajima's D (Tajima 1989) for the DRB exon 2 using DnaSP version 4.0 (Rozas et al. 2003). Because this test is based on the site frequency spectrum, it is influenced by long-term mutational patterns as well as more recent population dynamics (Garrigan and Hedrick 2003). Tajima's D uses the normalized difference between θ_W (estimated from the number of segregating sites) and π (mean number of pairwise differences between sequences) to determine if intermediate-frequency alleles are overrepresented in the population, as would be expected under balancing selection (Tajima 1989). To determine the statistical significance of the values of D, 95% confidence intervals (95% *CI*s) for these statistics were generated from coalescent simulations conducted in DnaSP version 4.0 (Rozas et al. 2003).

Relationships among parasite load, immune status, and MHC genotype.—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 (nonfunctional) 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 ($n = 8$ loci) genotypes for 30 *C. talarum* from the same population that served as the basis for this study.

To explore interactions between pathogen load and MHC variation in tuco-tucos, we quantified genotypic diversity at the class II DRB locus in *C. talarum* as a function of parasite load (a potential agent of selection) and ability to mount an adaptive immune response against a novel antigen (a measure of immune responsiveness). Multivariate analyses were used to explore associations between the genetic, parasitological, and immunological variables as previously described in Cutrera et al. (2011). Coinertia analysis (ACO) is a multivariate ordination method that identifies trends or correlations between multiple sets of data. ACO links 2 independent multivariate analyses by searching for axes that maximize the covariance between rows in distinct data matrices. First, each data matrix is analyzed independently. For this study, 2 data matrices were created: the categorical response (binary) matrix (presence versus absence data), and the continuous matrix. The categorical matrix, which included values for each individual regarding the occurrence (0 = absence, 1 = presence) of each DRB amino acid sequence (allele groups A, B, and C), DRB heterozygosity (0 = homozygote, 1 = heterozygote), season (0 = nonbreeding, 1 = breeding), and sex (0 = female, 1 = male), was analyzed by correspondence analysis (CA). Allele group D was not included in the analyses because it was present in only 1 individual of the sample. The continuous variable matrix included values regarding the abundance of each parasite taxon and Shannon's H' for ecto- and endoparasite diversity, as well as 8 variables thought to provide proxies for immune status or general physiological condition, or both (Davis et al. 2008). The 8 proxy variables used in this matrix were 1) N/L (neutrophil : lymphocyte) ratio, 2) N/T (neutrophil : total leukocyte count) ratio, 3) L/T (lymphocyte : total leukocyte count) ratio, 4) E/T (eosinophil : total leukocyte count) ratio, 5) B/T (basophil : total leukocyte count) ratio, 6) M/T (monocyte : total leukocyte count) ratio, 7) white blood cell counts (standardized per 100,000 erythrocytes [RCs], WC), and 8) body mass. Normality was tested using a Kolmogorov-Smirnov test as implemented in STATISTICA 6.0 (Statsoft, Tulsa, Oklahoma). Data that did not conform to normality were transformed using square-root (WC), $1/x^{0.5}$ (N/L), arcsin (proportion data for E/T and M/T), or $\log_{10}$ (*Eimeria* counts) transformations. 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, measures of general physiological condition, or a combination of these, that may affect interpretations of associations among parasites, genetic variability, and immune status and condition.

In the 2nd step of the ACOs, relationships between the binary and quantitative matrices were estimated from the coinertia (vector correlation) of the matrices, using the *Rv* coefficient (Escoufier 1973), the significance of which was assessed using a random permutation test (array rows permutated 10,000 times, then compared to observed values), as implemented in the ade4TkGUI package for R (Thioulouse and Dray 2007). The 2 matrices were compared by superimposing both categorical and continuous information on the ACO ordination map. Associations between specific variables were detected visually using the vectors for the binary and quantitative variables depicted on the ACO factor map. Vectors pointing in the same direction (relative to the origin) were considered positively associated, whereas vectors pointing in opposite directions were considered negatively associated. Following Haldane (1956), we calculated the *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) and significance was evaluated using Fisher's exact test.

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 hemagglutination analyses were performed. As above, the categorical (binary) matrix included information on DRB amino acid sequence (presence or absence of each allele group), DRB heterozygosity (0 = homozygote, 1 = heterozygote), season (0 = nonbreeding, 1 = breeding), and sex (0 = female, 1 = male) and was analyzed by CA. The continuous matrix included antibody titers at 7 and 14 days post 1st immunization as well as the 8 proxy measures of immune status and general physiological condition described above, with the addition of hematocrit levels. Data for proportions (E/T, N/L, M/T, and WC) that were not normally distributed were transformed as described above.

Analyses of mate choice.—Following Cutrera et al. (2012), we used the genotype of each mother and her pups to infer the genotype(s) of the putative sires for 11 litters, based on previous findings of a lack of multiple paternity of litters in Talas tuco-tucos (Zenuto et al. 1999a). Using these data, we investigated if the number of shared MHC alleles between male and female, the number of amino acid differences between male and female MHC alleles (male–female allele divergence), MHC heterozygosity, and the number of amino acid differences between male MHC alleles differed between potential sires and random males from the population (male allele divergence).

To elucidate whether each female and the putative sire of her pups ("mated pairs" hereafter) were more or less dissimilar than expected under random female choice (genetic compatibility hypothesis) or whether potential sires exhibited particular MHC DRB exon 2 characteristics (good-genes hypothesis), we generated a random model of female choice where we let each female choose randomly for 10,000 times between all males of the respective year to generate a null distribution. Subsequently, the observed values were compared to the simulated values of random female choice using the Simulation (Monte Carlo analysis) and Resample tools available at PopTools version 3.0 (Hood 2008). Specifically, we evaluated whether a) mated pairs shared fewer MHC DRB exon 2 alleles than would be expected under random female choice; b) mated pairs shared fewer MHC DRB exon 2 amino acids than would be expected under random female choice; c) the number of heterozygotes was higher in the sample of potential sires compared to the sample of randomly chosen males; and d) potential sires carried DRB alleles that differed in more amino acids than those of the randomly chosen males. To analyze these 4 predictions, we calculated the sum of shared MHC alleles, shared amino acids between male and female from pairwise combinations of alleles, number of heterozygotes, and shared amino acids between male alleles, respectively. This observed sum was then compared with the distribution of scores generated from 10,000 simulations of 11 random pairings selected from the 35 males and the 11 females captured in the population. We generated 95% *CI*s from the simulated data distribution. If the observed value was contained within the 95% *CI*, the difference between observed and simulated values was considered statistically nonsignificant. Finally, the distribution of allele frequencies of the sample of potential sires was compared with that of the total males captured in the population using a chi-square test. Mean litter size ($\pm$ *SD*) of females from NC (3.09 $\pm$ 1.45 pups) was not significantly different from that reported in Cutrera et al. (2012) for females from MdC (2.88 $\pm$ 1.57 pups; $n = 22$ litters, $t_{33} = -0.387$, $P = 0.701$) thus allowing us to compare the results of the present study with those previously found for MdC without the confounding effect of different litter sizes between populations that could have potentially influenced our different ability to detect, for example, heterozygotes in the sample of putative sires. Throughout the text, results are expressed as mean $\pm$ *SD*.

Results

Parasite abundance and diversity.—We identified eggs and oocysts from 4 different endoparasite taxa in fecal samples collected from the study animals at NC (Table 1). Three of these were gastrointestinal nematodes (*Trichuris pampeana*, *Paraspidodera uncinata*, and *Pudica ctenomydis*); the 4th was an intestinal protozoan (*Eimeria* sp.). *P. uncinata* and *P. ctenomydis* were each present in only 1 of 81 individuals sampled and thus were not included in ACOs. In both seasons (breeding and nonbreeding), *Eimeria* sp. was the most prevalent endoparasite in the study animals, infecting $>$ 90%

Table 1.—A) Median intensity of infection (median fecal egg count [FEC] for endoparasites and median counts for ectoparasites); B) parasite prevalence (percentage of animals infected); C) number of parasite species per individual; and D) relative risk of infection for both study populations of *C. talarum* located at Mar de Cobo (MC, $n = 87$) and Necochea (NC, $n = 81$), Argentina. Interquartile range (25th–75th) intensity values are given in parentheses. In C), asterisks denote statistical differences between populations: Mann–Whitney rank sum test, $** = P < 0.001$. In D), asterisks denote a significantly higher relative risk (*RR*) of infection for animals from MC; relative risk analysis: chi-square test, $* = P < 0.05$, $** = P < 0.001$. The endoparasites quantified were *Eimeria* sp. (Ei), *Trichuris pampeana* (Tp), *Graphidioides subterraneus* (Gs), *Pudica ctenomydis* (Pc), and *Paraspidodera uncinata* (Pu). The ectoparasites quantified were *Eulinognathus* sp. (Eu), *Gyropus* sp. (Gy), laelapids (La), *Poligenis* sp. (Po), and listrophorids (Li).

Population	Ei	Tp	Gs	Pc	Pu	Eu	Gy	La	Po	Li
A) Intensity of infection										
MC	35 (10–126.5)	0 (0–1)	0 (0–1)	0 (0–0)	9 (2–23)	0 (0–1)	Not found	5 (1–11.25)	5 (1–9.25)	0 (0–0)
NC	46 (10.5–127.5)	0 (0–7.75)	Not found	Found once	Found once	Not found	0 (0–0)	Found once	1 (0–2.75)	Found once
B) Prevalence (%)										
MC	91.14	31.64	12.66	21.52	17.72	31.64	0	74.68	89.87	10.87
NC	91.46	54.44	0	1.23	1.23	0	6.09	1.23	2.44	1.23
C) No. parasite species/individual										
			Endoparasites					Ectoparasites		
MC			2.04 ± 0.68**					2.5 ± 0.91		
NC			1.38 ± 0.67					1.03 ± 0.72		
D) Relative risk of parasite infection of animals from MC										
RR	0.88	1.69*	2.23**	2.07**	6.25**	—	—	4.92**	5.55**	1.79*

of individuals (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 chewing lice (*Gyropus* sp.). Of these, fleas were the most prevalent in the breeding and nonbreeding seasons (> 60% of animals infected), and also had the highest mean intensity of infection (Table 1). Laelapid and listrophorid mites were each present in only 1 of 81 individuals sampled and thus were not included in ACOs. The number of ectoparasite species per individual was significantly greater for MdC but the number of endoparasite species per individual was not different between the study populations. Also, animals from MdC had a significantly higher risk of infection by *P. uncinata*, *T. pampeana*, *Gyropus subterraneus*, and *P. ctenomydis* but the risk of infection by *Eimeria* sp. was not significantly different between the study populations. Further, animals from MdC had a significantly higher risk of infection by all of the ectoparasites observed in this study that were shared between the populations, specifically by fleas, laelapid mites, and listrophorid mites. Finally, measures of dissimilarity indicated that parasite community composition differed significantly between MdC and NC (ANOSIM ectoparasites: $R_{\text{Bray–Curtis}} = 0.2862$, $P < 0.0001$; $R_{\text{Jaccard}} = 0.2578$, $P < 0.0001$; ANOSIM endoparasites: $R_{\text{Bray–Curtis}} = 0.1368$, $P < 0.0001$; $R_{\text{Jaccard}} = 0.4428$, $P < 0.0001$).

Response to immune challenge.—All animals from NC injected with sRBC produced detectable antibody titers either at 7 or 14 dpi. There were no significant differences in antibody titers between the study populations (GLiM, $z_1 = 0.173$, $P = 0.862$). In both populations, titers at 14 dpi were significantly higher than those at 7 dpi (GLiM, $z_1 = 6.519$, $P < 0.001$). There was no interaction between "population" and "days postinjection" (GLiM, $z_1 = –0.483$, $P = 0.629$).

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 polymerase chain reaction product. No evidence of chimeric amplification products was detected. After cloning, no more than 2 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, Universidad Nacional de Mar del Plata-CONICET, pers. comm.) revealed no evidence of amplification of more than 1 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.

We detected 5 DRB alleles in the 81 *C. talarum* genotyped during this study, all of which had been previously described (JF799108–JF799112—Cutrera et al. 2011). The mean number of pairwise nucleotide differences among alleles was 5.2 ± 1.61 and mean number of pairwise amino acid differences between alleles was 2 ± 0.96. The 5 DRB alleles identified were assigned to 4 allele groups (named A, B, C, and D—Cutrera et al. 2011) based on shared amino acid sequences. Contrary to what is expected under the divergent allele advantage hypothesis (prediction C), comparisons with previous data on MHC class II DRB exon 2 variability in MdC reported in Cutrera et al. (2011) showed that nucleotide divergence among DRB alleles did not differ significantly between the study populations (Student's $t_{44} = 0.6$, $P = 0.552$), nor did amino acid divergence (Mann–Whitney rank sum test: $T = 267$, $P = 0.401$).

As noted above, the single allele in group D occurred in only 1 individual 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 a higher mean

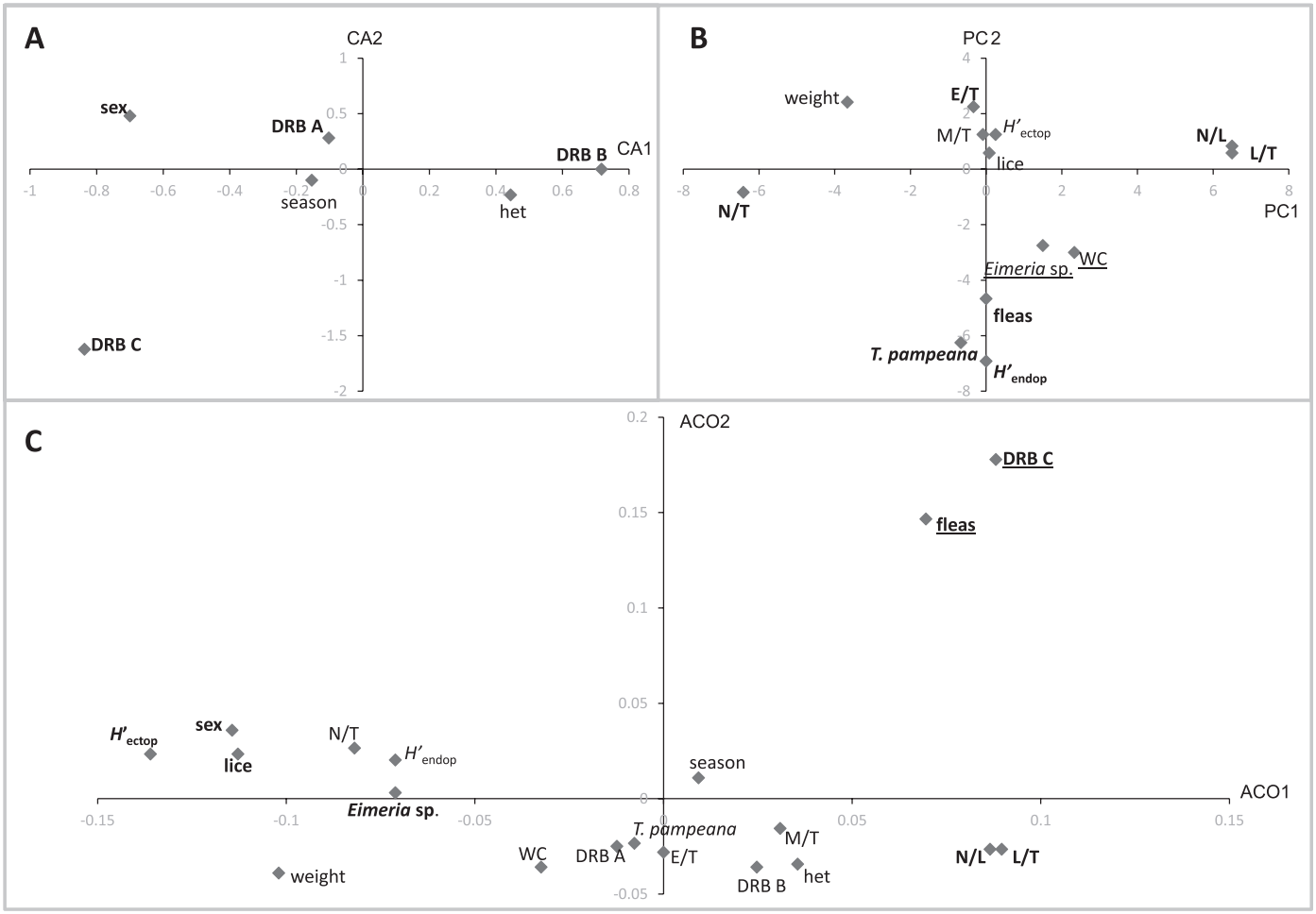

Fig. 1.—Depiction of A) correspondence analysis of the categorical (binary) data matrix, B) principal component analysis of the quantitative data matrix, and C) coinertia analysis relating the categorical and quantitative data matrices for all *Ctenomys talarum* sampled in this study ($n = 81$). Variables are projected on the factor map defined by axes 1 and 2. Variables that substantially structured the data set are in boldface type. Variables shown by relative risk or correlation analyses to be significantly associated with one another are underlined. Diamonds represent the endpoints of vectors for sex, season, DRB genotype, and DRB heterozygosity (het), intensity of parasite infection, diversity (H) of parasite fauna, and 7 measures of leukocyte abundance. DRB allele groups are identified by name. Parasite data reflect fecal egg count values. Blood proxy variables (N/L, N/T, L/T, E/T, B/T, M/T, and WC) are defined in the text.

heterozygosity across the 8 microsatellite loci surveyed than did DRB-homozygous animals (Mann–Whitney *U*-test, $z < 0.01$, $P > 0.99$), suggesting that MHC heterozygosity was independent of microsatellite heterozygosity.

Evidence of selection and departure from neutrality.—Contrary to what was found at MdC ($n = 87$), at NC ($n = 81$) observed heterozygosity at DRB exon 2 (0.622) was not higher than expected under Hardy–Weinberg equilibrium ($He = 0.526$, $P > 0.05$). Ewens–Watterson tests revealed no evidence of selection on the DRB exon 2 in either study population (Watterson's $F_{MdC} = 0.319$, $P = 0.237$, Slatkin's exact test $P = 0.544$, $n = 174$; Watterson's $F_{NC} = 0.503$, $P = 0.339$, Slatkin's exact test $P = 0.454$, $n = 162$). Finally, Tajima's D was significantly positive ($D = 2.69$, $P < 0.05$, $n = 174$) at MdC, as expected under balancing selection, but this was not the case for NC ($D = 0.57$, $P = 0.10$, $n = 162$).

For NC, likelihood-ratio tests revealed no differences in fit between any of the 7 nucleotide substitution models considered (Supporting Information S1 and S2, DOI: 10.1644/13-MAMM-A-120.S1 and DOI: 10.1644/13-MAMM-A-120.S2). In contrast, for MdC, the comparison between models M8a and M8 was significant (Supporting Information S1), suggesting that, for this species, selection may be acting on specific codons within this exon. Specifically, residues 27, 37, 45, and 75 of the DRB exon 2 from MdC were identified as subject to positive selection (Supporting Information S1).

Coinertia analyses: parasites and MHC variability.—Coinertia analysis (ACO) was conducted on data from the 81 individuals screened for ecto- and endoparasites, MHC variability, 8 proxy measures of immune status and condition (N/L, E/T, M/T, L/T, N/T, WC/100,000 erythrocytes, hematocrit, body mass), season, and sex. Only 2 individuals showed presence of basophils and hence B/T was not included in the analyses. In the CA of the categorical response matrix (MHC data plus season and sex [Fig. 1A]), the first 2 axes together accounted for 62.02% of the total variance (CA1 =

31.84%; CA2 = 30.18%). CA1 was structured primarily by DRB allele groups B and C, as well as sex, whereas CA2 was structured primarily by DRB allele groups A and C. MHC heterozygosity and season did not appear to contribute substantially to the structure of this data set. In the PCA of the continuous variable matrix (parasitological plus immune status and condition data), the first 2 axes together accounted for 60.68% of the total variance (PC1 = 34.87%, PC2 = 25.81%; Fig. 1B). The 1st axis was structured primarily by N/L, L/T, and N/T, with N/T acting in opposition to the other 2 variables. The 2nd axis was structured primarily by endoparasite diversity (H'_{endop}), intensity of infection with fleas and *T. pampeana*, in opposition to E/T. Interestingly, based on the first 2 axes, the intensity of infection with *Eimeria* sp. seemed to be positively associated with total white cell counts, and correlation between these 2 variables was significant (Pearson $r = 0.248$, $P = 0.026$).

Coinertia analysis (ACO) did not reveal a significant overall relationship between categorical and quantitative data matrices ($Rv = 0.087$, $P = 0.156$), although this does not exclude the existence of particular associations (see Tollenaere et al. 2008). The first 2 axes of the ACO explained 73.92% of the total variance (ACO1 = 54.53%; ACO2 = 19.39%; Fig. 1C). ACO1 was negatively polarized by diversity of ectoparasites and, to a lesser extent, by sex and abundance of lice, and positively by L/T, N/L, DRB allele group C, and fleas. ACO2 was structured primarily by intensity of infection by fleas and DRB allele group C. Finally, the ACO graphic showed a positive association between DRB group C and intensity of infection by fleas. In fact, individuals with DRB group C alleles had a higher risk of infection by fleas ($RR = 1.48$, $P = 0.05$). However, no other DRB groups yielded significant relationships with either parasite loads or blood parameters. Hence, although 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 one of the parasite taxa considered.

Coinertia analyses: immunocompetence and MHC variability.—We also conducted ACO on the randomly selected subset of 42 individuals 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 titers 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. 2A]), the first 2 axes together accounted for 67.60% of the total variance (CA1 = 35.7%; CA2 = 31.9%). Both axes were structured primarily by genetic variables; CA1 was most strongly polarized by DRB allele groups B and C, whereas CA2 was most strongly polarized by DRB allele group A and, in an opposite direction, MHC heterozygosity and DRB allele group C. Neither sex nor season of capture appeared to contribute substantially to the structure of this matrix. For the quantitative matrix, the first 2 axes of the PCA (antibody titers against sRBC plus immune status and condition data) together accounted for 58.04% of the total variance (PC1 = 39.69%; PC2 = 18.35%; Fig. 2B). PC1 was structured primarily by N/L (neutrophil : lymphocyte) ratio and L/T (lymphocyte : total leukocyte count) ratio, with these variables acting in opposition to N/T (neutrophil : total leukocyte count) ratio. PC2 was structured primarily by antibody titer at 14 dpi, and, to a lesser extent, by antibody titer at day 7 and body mass.

Similar to analyses of the larger data set, ACO of the subset of animals injected with sRBC revealed no significant overall relationship between the categorical and quantitative data matrices ($Rv = 0.124$, $P = 0.138$). The first 2 axes together explained 81.48% of the total variance (ACO1 = 64.45%; ACO2 = 17.03%; Fig. 2C). ACO1 was structured primarily by body mass, sex, and antibody titer at 14 days post initial injection, with both these variables acting in the same direction, and to a lesser extent, ACO1 was structured antibody titer at day 7. ACO2 was positively polarized by DRB allele group C and, negatively, by DRB allele group B. *RR* analyses revealed that animals that carried MHC alleles from group A were not significantly more likely to present white cell counts > median value = 50 ($RR = 0.81$, $P = 0.99$). Also, DRB heterozygotes were not significantly more likely to present lower antibody titers (< median value = 4) 14 dpi ($RR = 1.02$, $P = 0.779$). Finally, none of the allele groups (A, B, or C) were significantly associated with higher antibody titers 14 dpi (A: $RR = 5.82$, $P = 0.161$; B: $RR = 0.5$, $P = 0.44$; C: $RR = 2.38$, $P = 0.629$). Thus, overall, response to injection with sRBC did not appear to be strongly associated with variability at the DRB locus.

Analyses of MHC-associated mate choice.—There were no differences in the number of shared MHC alleles between mated pairs in the field versus the simulated values of randomly assigned males (observed value = 18, simulated 95% *CI* = 14–24; Fig. 3A). However, the number of amino acid differences between MHC alleles of the mated pairs in the field was lower than those of the simulated values of randomly assigned males (observed value = 56, simulated 95% *CI* = 64–96; Fig. 3B). Also, the number of MHC heterozygotes in the sample of potential sires was lower than that in the sample of randomly assigned males in the field population (observed value = 2, simulated 95% *CI* = 3–9; Fig. 3C). Further, the number of amino acid differences between the MHC alleles of potential sires was lower compared to those in the sample of randomly assigned males (observed value = 3, simulated 95% *CI* = 7–28; Fig. 3D). Finally, MHC allele frequency distributions of the potential sires differed from randomly assigned males from the population ($\chi^2_4 = 9.89$, $P = 0.04$).

Discussion

Our findings suggest that parasite-driven selection plays a major role in explaining MHC variation between 2 natural populations of *C. talarum* that face different levels of parasite load (i.e., parasite prevalence and number of simultaneous parasite infections). In accordance with previous results found for these populations (Cutrera and Lacey 2006), selection acting on MHC genes seems to be stronger in MdC, where the

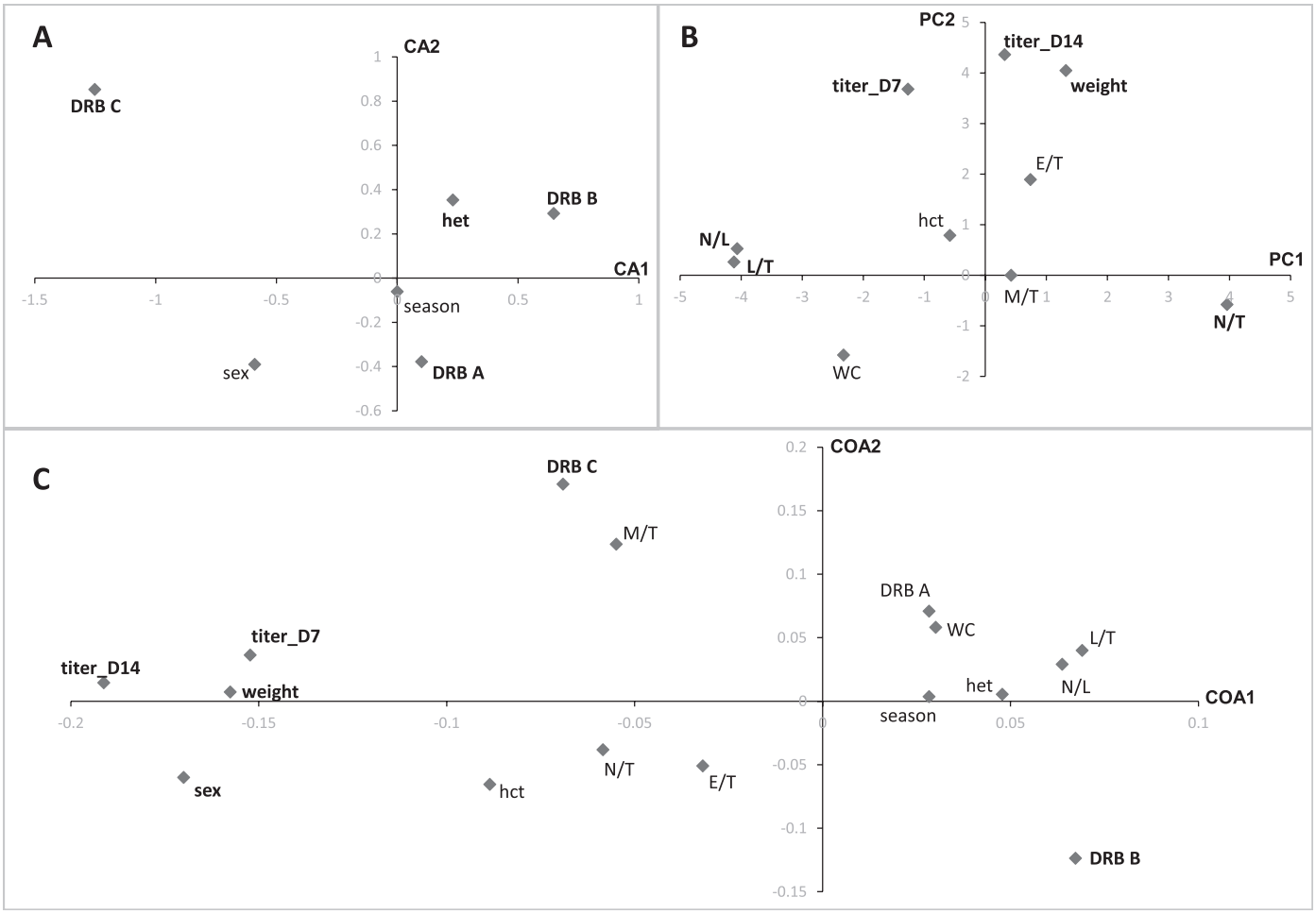

Fig. 2.—Depiction of A) correspondence analysis of the categorical (binary) data matrix, B) principal component analysis of the quantitative data matrix, and C) coinertia analysis relating the categorical and quantitative data matrices for a random sample of *Ctenomys talarum* ($n = 42$) immunized with sheep red blood cells (sRBC). Variables are projected on the factor map defined by axes 1 and 2. Variables that substantially structured the data set are in boldface type. Variables shown by relative risk or correlation analyses to be significantly associated with one another are underlined. Diamonds represent the endpoints of vectors for sex, season, DRB genotype, and DRB heterozygosity (het), intensity of immune response against sRBC at 7 (titer_D7) and 14 (titer_D14) days post initial injection, hematocrit (hct), and 5 measures of leukocyte abundance. DRB allele groups are identified by name, and blood proxy variables (N/L, N/T, L/T, E/T, B/T, M/T, and WC) are defined in the text.

number of simultaneous parasite infections and estimates of relative risk of infection by parasites are generally higher. Specifically, comparisons of nucleotide substitution patterns performed in the present study suggest that positive selection is acting on DRB exon 2 in MdC. Also, a heterozygosity excess and a significantly positive value of Tajima's *D* were only detected in MdC, which is consistent with recent or ongoing balancing selection acting on DRB exon 2, given that the alternative scenario of recent population bottlenecks has been discarded for both populations (Cutrera et al. 2006).

Associations between parasite load and MHC genotype.—Although evidence in favor of the rare-allele hypothesis comes from multiple studies demonstrating associations between specific MHC alleles and resistance or susceptibility to a wide array of infections (e.g., Bonneaud et al. 2006b; Deter et al. 2008; Meyer-Lucht et al. 2010) and also from experimental demonstrations (Eizaguirre et al. 2012b), empirical support for the heterozygote advantage remains less conclusive (reviewed by Spurgin and Richardson 2010; but see Eizaguirre et al. 2012a). In line with these findings, our multivariate analyses revealed a trend of positive association between the DRB allele group C and the intensity of infection by fleas (*Polygenis* sp.) in tuco-tucos from NC (Fig. 1C), but we did not find significant associations between heterozygosity at this locus and either parasite load or diversity. Concomitantly, group C had the lowest allele frequency in the population (0.08), compared to allele groups A (0.62) and B (0.30), which could be related to its negative impact on flea resistance. However, these results should be interpreted with caution because the association between intensity of infection by fleas and the presence of the group allele C could also be a statistical artifact given the low frequency of DRB group C in the population. With respect to immunocompetence, we did not find significant associations between specific DRB alleles or heterozygosity and antibody titers against sRBC (Fig. 2C). Accordingly, we found no significant evidence of excess of DRB heterozygotes in the

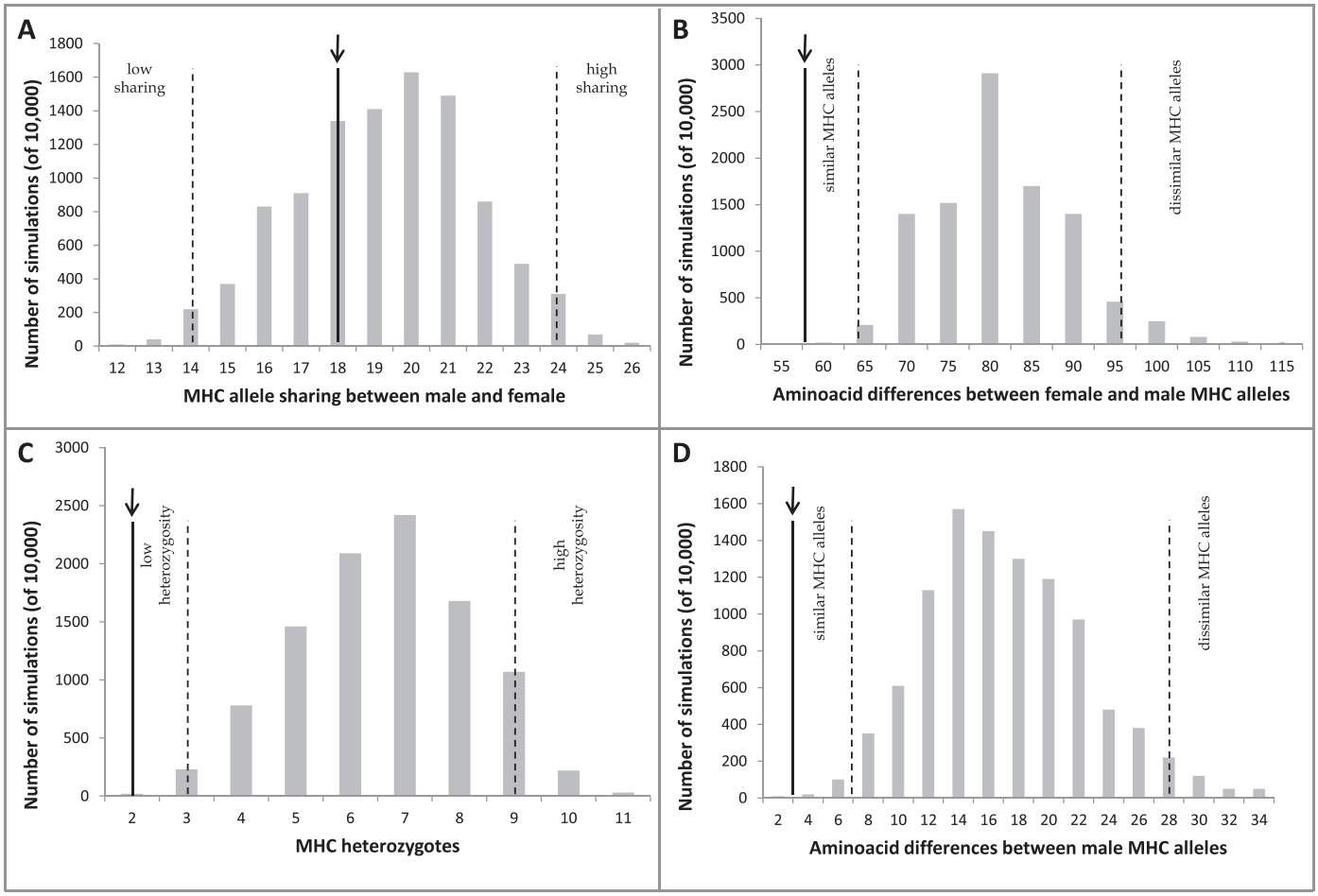

Fig. 3.—Measures of major histocompatibility complex (MHC) allele sharing in 11 known breeding pairs (arrow and bold line) compared with the distribution of values generated from 10,000 simulations of 11 random male–female pairings selected from the same 11 females and 35 males captured in the population. Dashed lines indicate cutoffs for significant departures from random mating. A) Sum of MHC allele-sharing values between male and female. B) Sum of amino acid differences values between male and female MHC alleles. C) Sum of MHC heterozygotes in the male sample. D) Sum of amino acid differences values between male MHC alleles.

population of NC. 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, as previously suggested for *C. talarum* at MdC (Cutrera et al. 2011) as well as other species (De Boer et al. 2004; Meyer-Lucht and Sommer 2005; Deter et al. 2008). Interestingly, in both our study populations, we found positive associations between parasite infection and DRB alleles, such as that between the intensity of infection by *Eimeria* sp. and the presence of DRB alleles of group A in MdC (Cutrera et al. 2011) or that between the intensity of infection by fleas and the presence of the DRB-C allele in NC (Fig. 1C). "Disadvantageous" MHC alleles, positively associated with infection by viruses (Deter et al. 2008), blood parasites (Bonneaud et al. 2006b), and nematodes (Froeschke and Sommer 2005) also have been reported in several other studies, and in some cases these results have been interpreted as evidence of rare-allele advantage (Clarke and Kirby 1966). Finally, on a cautionary note, correlative results between target genes, such as MHC class II DRB locus, and particular phenotypes, such as susceptibility to parasite infection, may arise, at least partly, from the action of the gene itself, or the action of another gene linked to DRB.

Major histocompatibility complex (MHC)–associated mate choice and MHC variation.—Female mate choice may favor males that possess particular MHC alleles or those with diverse MHC genotypes (good-genes hypothesis—Penn and Potts 1999; Eizaguirre et al. 2009) or males that possess MHC genotypes that are compatible with that of the female, whether they are more or less dissimilar (compatibility hypothesis—Neff and Pitcher 2005). Importantly, the choice of MHC-compatible mates might be associated with providing offspring with a more diverse MHC genotype that may resist a broader range of pathogens (Apanius et al. 1997; Fromhage et al. 2009) or avoiding inbreeding (Grob et al. 1998), or both, with MHC genes functioning as kinship markers (Penn and Potts 1998). Evidence supporting the role of MHC in rodent mate choice comes mostly from laboratory experiments (e.g., Roberts and Gosling 2003) as well as some studies conducted in natural or seminatural conditions (e.g., Sommer 2005; Thoß et al. 2011).

However, recent evidence suggests that females may prefer males with intermediate MHC divergence compared to them, which may contribute to avoid negative selection on T cell repertoire size that narrows down the amount of detectable pathogens (Wegner et al. 2003) or to avoid the costs associated with the disruption of coadapted genes (Bonneaud et al. 2006a; Eizaguirre et al. 2009; Kloch et al. 2010).

The results of the present study performed on a population of *C. talarum* located at NC, even though they are based on a limited number of litters ($n = 11$), agree with previous findings for MdC (Cutrera et al. 2012); both studies provide evidence of MHC-associated mate choice in this species. However, contrary to what we expected, patterns of MHC mate-choice preferences in *C. talarum* are in the direction of less MHC variability, a pattern that is more evident at NC and has been reported for other wild rodents, such as the giant jumping rat (*Hypogeomys antimena*—Sommer 2005). As noted by Eizaguirre et al. (2009), mate choice for particular MHC genes or for genetically similar mates could arise if a specific MHC allele confers a strong benefit against a common and virulent pathogen. Accordingly, our results showed that possible sires carried MHC alleles that differed in fewer amino acids (Fig. 3D), were less heterozygous (Fig. 3C), and carried specific MHC alleles in comparison with random males in the population. Further, mated pairs carried MHC alleles that differed in fewer amino acids compared to those of random pairs in the population (Fig. 3A). Assortative mating is expected to lead to purifying selection (Nuismer and Otto 2004), but this may not apply to MHC genes because allele frequencies can vary rapidly between years responding to fluctuating parasite pressures, as proposed by the rare-allele advantage hypothesis (e.g., Westerdahl et al. 2004; Charbonnel and Pemberton 2005; see also Milinski 2006). Interestingly, at NC, where tuco-tucos present lower parasite loads, female tuco-tucos do not seem to prefer MHC heterozygote males, contrary to what was found in MdC, even though this was more difficult to detect in this population given the high heterozygosity observed. Because litter size was comparable in both populations, we can rule out the possibility that our ability to detect heterozygotes in the group of putative sires was lower in NC. Alternatively, in the subterranean habitat occupied by *C. talarum*, characterized by lower parasite loads, a few highly prevalent ecto- and endoparasites, and the use of chemical cues for communication (Zenuto et al. 2004), benefits of MHC-associated mate choice may arise from choosing males that carry locally adapted MHC alleles that provide resistance to these organisms, rather than choosing more MHC-diverse males, as recently reported for sticklebacks (*Gasterosteus aculeatus*—Eizaguirre et al. 2009, 2012a). Finally, although dispersal distances are shorter and kinship genetic structure is more pronounced at MdC (Cutrera et al. 2005), inbreeding avoidance does not seem to be an important mechanism driving MHC-associated mate choice in either of these populations, because we did not find evidence of preferences for MHC-dissimilar mates (this study; Cutrera et al. 2012).

Interpopulation variation in MHC diversity: the role of parasite-mediated selection and MHC-associated mate choice.—As explained earlier in this paper, we compare our results with those previously obtained for *C. talarum* at MdC. Population density, commonly associated with higher pathogen exposure (Anderson and May 1979; Côté and Poulin 1995), is higher at MdC but no other closely related species (which also may increase exposure to parasites—Krasnov et al. 2006) are codistributed with *C. talarum* at this site. On the contrary, population density at NC is lower but the sand dune tuco-tuco (*C. australis*) is found in sympatry with *C. talarum* and shares several taxa of endoparasites with this species (Rossin 2007). Our findings, as well as previous studies of these animals, have revealed that parasite prevalence and species richness generally are higher in MdC, providing an ideal opportunity to assess relationships among parasite load, intensity of parasite-driven selection, and variation at MHC loci. A testable prediction from the parasite-driven selection hypothesis is that populations facing high parasite exposure should have increased MHC variability relative to neutral variation (Goüy de Bellocq et al. 2008). As expected given their differences in parasite load, previous studies have found that MHC allelic variability and heterozygosity are higher at MdC, despite lower variation in microsatellites and introns (Cutrera and Lacey 2006; Cutrera et al. 2006, 2011; this study). If these differences respond to different intensities of parasite-driven selection, we expected to find a higher correlation between MHC genotypic variation and parasite load or immunocompetence, or both, in MdC. Indeed, the correlation between the genetic and the parasitological matrices was significant only at MdC. Further, although a specific DRB allele group at MdC was associated with susceptibility to infection by *Eimeria* sp., resistance to *P. uncinata*, and elevated immunocompetence, at NC a specific DRB allele group was marginally associated only with the intensity of flea infection. Moreover, an excess of DRB heterozygotes was observed only at MdC, which may be consistent with heterozygote advantage or rare-allele advantage (Spurgin and Richardson 2010), suggesting that intensity of parasite-driven diversifying selection at these loci is greater there than at NC.

Considering that MHC-associated mating preferences could provide a moving target to parasites that evade immune recognition (Penn and Potts 1999), we expected to find greater evidence of MHC-associated mate choice in tuco-tucos from MdC. However, we found a stronger pattern of MHC-associated mate choice at NC, although in the direction of lower MHC diversity. In this sense, by using the genotypes of the pups and their mothers to infer the genotypes of the putative sires, we are looking at the outcome of the possible interplay between both female and male choice, which together may have more significant consequences for reproductive success and offspring performance (Drickamer et al. 2003). However, this outcome also may respond to multiple variables that are population-specific, such as monopolization potential (Schwensow et al. 2008), male dominance (Setchell et al. 2010), and female choosiness (Eizaguirre et al. 2009); all these

factors differ between our study populations (Zenuto et al. 1999a, 1999b, 2002) and thus also may have contributed to the observed differences in MHC-associated mate-choice patterns between them.

Finally, our results suggest that parasite-driven selection acting directly on MHC class II DRB locus seems to be greater, as expected, for the population of *C. talarum* in which individuals face higher parasite load. However, due to the great volume of data collected for each population, we were unable to obtain samples simultaneously for both populations and potential differences in parasite communities or tuco-tucos' immunocompetence, or both, between years—sometimes associated with temporal environmental fluctuations—cannot be discarded in our study. Further, although the 2 populations used in our work were sampled across different, but consecutive, years, analysis of previous studies (Rossin and Malizia 2002; Rossin et al. 2010; Cutrera et al. 2011) suggests that the differences reported here between the 2 study sites regarding their parasite communities seem to be stable over time, which makes this variation in parasite diversity a potentially important selective force driving differences in DRB variability between MdC and NC. Specifically, the same differences in parasite load (diversity and species composition) have been reported by several other studies on *C. talarum* at MdC and NC that involved different samplings across a decade (Rossin and Malizia 2002; Rossin et al. 2010; Cutrera et al. 2011). It remains to be explored if the reported differences in DRB variation, associations between DRB alleles, and resistance to parasites and patterns of MHC-associated mate choice are specific to the 2 study populations of *C. talarum* or represent a more general picture of the role of parasite exposure in shaping MHC local variation in natural populations of rodents.

Resumen

Se exploró la variación geográfica potencial en las presiones selectivas mediadas por patógenos sobre los loci de Complejo Principal de Histocompatibilidad (MHC) en el roedor subterráneo *Ctenomys talarum*, evaluando las diferencias en cargas parasitarias entre dos poblaciones (Mar de Cobo [MdC] y Necochea [NC]) con el fin de determinar de qué forma la variación espacial en este atributo influye sobre la variación y selección en los genes de MHC en esta especie. Dado que los tuco-tucos de NC enfrentan menores cargas parasitarias, se esperaba encontrar una correlación más débil entre la variación de MHC y la carga parasitaria/inmunocompetencia en esta población comparada con MdC. También se predijo que el uso de pistas asociadas al MHC en el proceso de elección de pareja fuera menos evidente en NC. Caracterizamos la variación de MHC en NC en función de la carga parasitaria y de la inmunocompetencia y comparamos nuestros resultados con datos previos de MdC. Ambas poblaciones fueron muestreadas a través de años diferentes, pero consecutivos. Utilizando análisis de co-inercia, se encontró una asociación significativamente positive entre un alelo específico de DRB y la intensidad de infección por pulgas en NC. Analizamos el uso de pistas asociadas al MHC en la elección de pareja en NC y encontramos soporte para las hipótesis de "buenos genes" y "compatibilidad genética." De acuerdo a lo esperado, las asociaciones entre MHC y carga parasitaria o inmunocompetencia fueron menos frecuentes en NC, la población en la cual los tuco-tucos enfrentan menores cargas parasitarias. La evidencia indicó que las hembras en NC escogieron a machos con baja diversidad de MHC. Esto sugiere que la selección mediada por parásitos que actúa directamente sobre los genes de MHC parece ser de mayor magnitud en la población que enfrenta mayores cargas parasitarias. Sin embargo, la selección mediada por parásitos a través de la elección de pareja puede no estarúnicamente influida por niveles de diversidad parasitaria en la población sino también por características del sistema de apareamiento.

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Supporting Information

Supporting Information S1.—Results of maximum-likelihood analyses of models of codon evolution for the major histocompatibility complex (MHC) class II DRB in 2 study populations of *Ctenomys talarum*: Mar de Cobo and Necochea.

Found at DOI: 10.1644/13-MAMM-A-120.S1

Supporting Information S2.—Summary of results from likelihood-ratio tests of codon variation at the major histocompatibility complex (MHC) class II DRB (exon 2) in 2 study populations of *Ctenomys talarum*: Mar de Cobo and Necochea.

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Literature Cited

Adamo, S. A. 2004. How should behavioural ecologists interpret measurements of immunity? Animal Behaviour 68:1443–1449.

Anderson, R. M., and R. M. May. 1979. Population biology of infectious disease: part I. Nature 280:361–367.

Anisimova, M., J. P. Bielawski, and Z. Yhang. 2001. Accuracy and power of the likelihood ratio test in detecting adaptive molecular evolution. Molecular Biology and Evolution 18:1585–1592.

Apanius, V., D. Penn, P. Slev, L. R. Ruff, and W. K. Potts. 1997. The nature of selection on the major histocompatibility complex. Critical Reviews in Immunology 17:179–224.

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.

Bacon, L. D. 1992. Measurement of immune competence in chickens. Poultry Sciences Review 4:187–195.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2013. Package LME4: linear mixed-effects models using Eigen and S4. https://

github.com/lme4/lme4/ and http://lme4.r-forge.r-project.org/. Accessed March 2013.

Bonneaud, C., O. Chastel, P. Federici, H. Westerdahl, and G. Sorci. 2006a. Complex MHC based mate choice in a wild passerine. Proceedings of the Royal Society of London, B. Biological Sciences 273:1111–1116.

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

Brown, J. H., et al. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature 364:33–39.

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

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

Charbonnel, N., J. Goüy de Bellocq, and S. Morand. 2006. Immunogenetics of micromammal–macroparasite interactions. Pp. 401–422 in Micromammals and macroparasites: from evolutionary ecology to management (S. Morand, B. R. Krasnov, and R. Poulin, eds.). Springer-Verlag Press, Tokyo, Japan.

Charbonnel, N., and J. Pemberton. 2005. A long-term genetic survey of an ungulate population reveals balancing selection acting on MHC through spatial and temporal fluctuations in selection. Heredity 95:377–388.

Clarke, B., and D. R. Kirby. 1966. Maintenance of histocompatibility polymorphisms. Nature 211:999–1000.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18:117–143.

Comparatore, V., M. Agnusdei, and C. Busch. 1992. Habitat relations in sympatric populations of *Ctenomys australis* and *Ctenomys talarum* (Rodentia: Octodontidae) in a natural grassland. Zeitschrift für Säugetierkunde 57:47–55.

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

Côté , I. M., and R. Poulin. 1995. Parasitism and group size in social animals: a meta-analysis. Behavioral Ecology 6:159–165.

Cutrera, A. P., M. S. Fanjul, and R. R. Zenuto. 2012. Females prefer good genes: MHC-associated mate choice in wild and captive tuco-tucos. Animal Behaviour 83:847–856.

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

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

Cutrera, A. P., E. A. Lacey, and C. Busch. 2006. Intraspecific variation in effective population size in Talar tuco-tucos (*Ctenomys talarum*): the role of demography. Journal of Mammalogy 87:108–116.

Cutrera, A. P., E. A. Lacey, M. S. Mora, and E. P. Lessa. 2010a. Effects of contrasting demographic histories on selection at major histocompatibility complex loci in two sympatric species of tuco-tucos (Rodentia: Ctenomyidae). Biological Journal of the Linnean Society 99:260–277.

Cutrera, A. P., R. R. Zenuto, F. Luna, and C. D. Antenucci. 2010b. 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. Journal of Experimental Biology 213:715–724.

Cutrera, A. P., R. R. Zenuto, and E. A. Lacey. 2011. MHC variation, multiple simultaneous infections and physiological condition in the subterranean rodent *Ctenomys talarum*. Infection, Genetics and Evolution 11:1023–1036.

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

Dean, A. G., J. A. Dean, A. H. Burton, and R. C. Dicker. 1991. Epi Info: a general-purpose microcomputer program for public health information systems. American Journal of Preventive Medicine 7:178–182.

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

Deerenberg, C., V. Arpanius, S. Daan, and N. Bos. 1997. Reproductive effort decreases antibody responsiveness. Proceedings of the Royal Society of London, B. Biological Sciences 264:1021–1029.

Derting, T. L., and M. K. Virk. 2005. Positive effects of testosterone and immunochallenge on energy allocation to reproductive organs. Journal of Comparative Physiology, B. Biochemical, Systemic, and Environmental Physiology 175:543–556.

Deter, J., et al. 2008. Association between the DQA MHC class II gene and Puumala virus infection in *Myodes glareolus*, the bank vole. Infection, Genetics and Evolution 8:450–458.

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

Drickamer, L. C., P. A. Gowaty, and D. M. Wagner. 2003. Free mutual mate preferences in house mice affect reproductive success and offspring performance. Animal Behaviour 65:105–114.

Eizaguirre, C., T. L. Lenz, M. Kalbe, and M. Milinski. 2012a. Divergent selection on locally adapted major histocompatibility complex immune genes experimentally proven in the field. Ecology Letters 15:723–731.

Eizaguirre, C., T. L. Lenz, M. Kalbe, and M. Milinski. 2012b. Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. Nature Communications 3:621–626.

Eizaguirre, C., S. E. Yeates, T. L. Lenz, M. Kalbe, and M. Milinski. 2009. MHC based mate choice combines good genes and maintenance of MHC polymorphism. Molecular Ecology 18:3316–3329.

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

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

Ewens, W. J. 1972. The sampling theory of selectively neutral alleles. Theoretical Population Biology 3:87–112.

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

Freeland, W. J. 1976. Pathogens and the evolution of primate sociality. Biotropica 8:12–24.

Freeman-Gallant, C. R., M. Meguerdichian, N. T. Wheelwright, and S. V. Sollecito. 2003. Social pairing and female mating fidelity predicted by restriction fragment length polymorphism similarity at the major histocompatibility complex in a songbird. Molecular Ecology 12:3077–3083.

Froeschke, G., and S. Sommer. 2005. MHC class II variability and parasite load in the striped mouse (*Rhabdomys pumilio*) in the southern Kalahari. Molecular Biology and Evolution 22:1254–1259.

Fromhage, L., H. Kokko, and J. M. Reid. 2009. Evolution of mate choice for genome-wide heterozygosity. Evolution 63:684–694.

Garrigan, D., and P. W. Hedrick. 2003. Detecting adaptive molecular polymorphism: lessons from the MHC. Evolution 57:1707–1722.

Goldman, N., and Z. Yang. 1994. A codon-based model of nucleotide substitution for protein-coding DNA sequences. Molecular Biology and Evolution 11:725–736.

Goüy de Bellocq, J., N. Charbonnel, and S. Morand. 2008. Coevolutionary relationship between helminth diversity and MHC class II polymorphism in rodents. Journal of Evolutionary Biology 21:1144–1150.

Grob, B., L. A. Knapp, R. D. Martin, and G. Anzenberg. 1998. The major histocompatibility complex and mate choice: inbreeding avoidance and selection of good genes. Experimental and Clinical Immunogenetics 15:119–129.

Guo, S. W., and E. A. Thompson. 1992. A Monte Carlo method for combined segregation and linkage analysis. American Journal of Human Genetics 51:1111–1126.

Haldane, J. B. S. 1956. The estimation and significance of the logarithm of a ratio of frequencies. Annals of Human Genetics 2:309–311.

Hammer, O., D. A. T. Harper, and P. D. Ryan. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4:1–9.

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

Holmes, J. C., and P. W. Price. 1986. Communities of parasites. Pp. 101–157 in Community ecology: pattern and process (J. Kikkawa and D. I. Anderson, eds.). Blackwell Scientific Publications, Oxford, United Kingdom.

Hood, G. M. 2008. PopTools version 3.0.6. http://www.cse.csiro.au/poptools. Accessed September 2012.

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

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge University Press, Cambridge, United Kingdom.

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

Kloch, A., W. Babik, A. Bajer, E. Sinski, and J. Radwan. 2010. Effects of an MHC-DRB genotype and allele number on the load of gut parasites in the bank vole *Myodes glareolus*. Molecular Ecology 19:255–265.

Krasnov, B. R., R. Poulin, and S. Morand. 2006. Patterns of macroparasite diversity in small mammals. Pp. 198–231 in Micromammals and macroparasites: from evolutionary ecology to management (S. Morand, B. R. Krasnov, and R. Poulin, eds.). Springer-Verlag Press, Tokyo, Japan.

Lacey, E. A., and A. P. Cutrera. 2007. Behavior, demography, and immunogenetic variation: new insights from subterranean rodents. Pp. 343–356 in Subterranean rodents: news from underground (S. Begall, H. Burda, and C. Schleich, eds.). Springer-Verlag Press, Tokyo, Japan.

Landry, C., D. Garant, P. Duchesne, and L. Bernatchez. 2001. "Good genes as heterozygosity": the major histocompatibility and mate choice in Atlantic salmon (*Salmo salar*). Proceedings of the Royal Society of London, B. Biological Sciences 268:1279–1285.

Larsen, M. H., N. Blackburn, J. L. Larsen, and J. E. Olsen. 2004. Influences of temperature, salinity and starvation on the motility and chemotactic response to *Vibrio anguillarum*. Microbiology 150:1283–1290.

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

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

Malizia, A. I., R. R. Zenuto, and C. Busch. 1995. Demographic and reproductive attributes of dispersers in two populations of the subterranean rodent *Ctenomys talarum* (tuco-tuco). Canadian Journal of Zoology 73:732–738.

Meyer-Lucht, Y., C. Otten, T. Püttker, R. Pardini, J. P. Metzger, and S. Sommer. 2010. Variety matters: adaptive genetic diversity and parasite load in two mouse opossums from the Brazilian Atlantic forest. Conservation Genetics 11:2001–2013.

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

Milinski, M. 2006. The major histocompatibility complex, sexual selection and mate choice. Annual Review of Ecology, Evolution, and Systematics 37:159–186.

Moore, S. L., and K. Wilson. 2002. Parasites as a viability cost of sexual selection in natural populations of mammals. Science 297:2015–2018.

Mora, M. S., A. P. Cutrera, E. P. Lessa, A. I. Vassallo, A. D'Anatro, and F. J. Mapelli. 2013. Phylogeography and population genetic structure of the Talas tuco-tuco (*Ctenomys talarum*): integrating demographic and habitat histories. Journal of Mammalogy 94:459–476.

Morand, S., and R. Poulin. 1998. Density, body mass and parasite species richness of terrestrial mammals. Evolutionary Ecology 12:717–727.

Morand, S., R. Poulin, and B. R. Krasnov. 2006. Global changes and the future of micromammal–macroparasite interactions. Pp. 617–635 in Micromammals and macroparasites: from evolutionary ecology to management (S. Morand, B. R. Krasnov, and R. Poulin, eds.). Springer-Verlag Press, Tokyo, Japan.

Neff, B. D., and T. E. Pitcher. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Molecular Ecology 14:19–38.

Nei, M., and T. Gojobori. 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Molecular Biology and Evolution 3:418–426.

NIELSEN, R., AND Z. YANG. 1998. Likelihood models for detecting positively selected amino acid sites and applications to the HIV-1 envelope gene. Genetics 148:929–936.

NUISMER, S. L., AND S. P. OTTO. 2004. Host–parasite interactions and the evolution of ploidy. Proceedings of the National Academy of Sciences 101:11036–11039.

OHTA, T. 1993. The nearly neutral theory of molecular evolution. Annual Review of Ecology and Systematics 23:263–286.

PENN, D., AND W. K. POTTS. 1998. MHC-disassortative mating preferences reversed by cross-fostering. Proceedings of the Royal Society of London, B. Biological Sciences 265:1299–1306.

PENN, D. J., AND W. K. POTTS. 1999. The evolution of mating preferences and major histocompatibility complex genes. American Naturalist 153:145–164.

REUSCH, T. H. B., M. A. HÄBERLI, P. B. AESCHLIMANN, AND M. MILINSKI. 2001. Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. Nature 414:300–302.

RIGAUD, T., M.-J. PERROT-MINNOT, AND M. F. J. BROWN. 2010. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. Proceedings of the Royal Society of London, B. Biological Sciences 277:3693–3702.

ROBERTS, S. C., AND L. M. GOSLING. 2003. Genetic similarity and quality interact in mate choice decisions by female choice. Nature Genetics 35:103–106.

ROSSIN, M. A. 2007. Estudio de la fauna endoparasitaria en roedores subterráneos del género *Ctenomys* (Rodentia: Octodontidae) de la Provincia de Buenos Aires. Ph.D. dissertation, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina.

ROSSIN, M. A., AND A. I. MALIZIA. 2002. Relationship between helminth parasites and demographic attributes of a population of the subterranean rodent *Ctenomys talarum* (Rodentia: Octodontidae). Journal of Parasitology 88:1268–1270.

ROSSIN, M. A., A. I. MALIZIA, J. T. TIMI, AND R. POULIN. 2010. Parasitism underground: determinants of helminth infections in two species of subterranean rodents (Octodontidae). Parasitology 137:1569–1575.

ROZAS, J., J. C. SANCHEZ-DEL BARRIO, X. MESSEGUER, AND R. ROZAS. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497.

SATTA, Y., C. O'HUIGIN, N. TAKAHATA, AND J. KLEIN. 1994. Intensity of natural selection at the major histocompatibility complex loci. Proceedings of the National Academy of Sciences 91:7184–7188.

SCHWENSOW, N., M. EBERLE, AND S. SOMMER. 2008. Compatibility counts: MHC-associated mate choice in a wild promiscuous primate. Proceedings of the Royal Society of London, B. Biological Sciences 275:555–564.

SETCHELL, J. M., ET AL. 2010. Odour signals major histocompatibility complex genotype in an Old World monkey. Proceedings of the Royal Society of London, B. Biological Sciences 278:274–280.

SHANNON, C. E., AND W. WEAVER. 1949. The mathematical theory of communication. University of Illinois Press, Urbana.

SIKES, R. S., W. L. GANNON, AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235–253.

SLOSS, M. W., R. L. KEMP, AND A. M. ZAJAC. 1994. Veterinary clinical parasitology. Iowa State University Press, Ames.

SOMMER, S. 2005. Major histocompatibility complex and mate choice in a monogamous rodent. Behavioral Ecology and Sociobiology 58:181–189.

SOULSBY, E. J. L. 1987. Immune responses in parasitic infections: immunology, immunopathology and immunoprophylaxis. CRC Press, Boca Raton, Florida.

SPURGIN, L. G., AND D. S. RICHARDSON. 2010. How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. Proceedings of the Royal Society of London, B. Biological Sciences 277:979–988.

TAJIMA, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

THIOULOUSE, J., AND S. DRAY. 2007. Interactive multivariate analysis in R with the ade4 and the ade4TKGUI packages. Journal of Statistical Software 22:1–14.

THOß, M., P. ILMONEN, K. MUSOLF, AND D. J. PENN. 2011. Major histocompatibility complex heterozygosity enhances reproductive success. Molecular Ecology 7:1546–1557.

TOLLENAERE, C., ET AL. 2008. Multiple parasites mediate balancing selection at two MHC class II genes in the fossorial water vole: insights from multivariate analyses and population genetics. Journal of Evolutionary Biology 21:1307–1320.

VERA, F., C. D. ANTENUCCI, AND R. R. ZENUTO. 2011. Cortisol and corticosterone exhibit differential seasonal variation and responses to acute stress and captivity in tuco-tucos (*Ctenomys talarum*). Genetics and Comparative Endocrinology 170:550–557.

VERA, F., R. R. ZENUTO, AND C. D. ANTENUCCI. 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. Comparative Biochemistry and Physiology, A. Molecular & Integrative Physiology 151:232–238.

VINKLER, M. H., AND T. ALBRECHT. 2011. Handling 'immunocompetence' in ecological studies: do we operate with confused terms? Journal of Avian Biology 42:490–493.

VOIGT, G. L. 2000. Hematology techniques and concepts for veterinary technicians. Iowa State University Press, Ames.

WATTERSON, G. A. 1986. The homozygosity test after a change in population size. Genetics 112:899–907.

WATVE, M. G., AND R. SUKUMAR. 1995. Parasite abundance and diversity in mammals: correlates with host ecology. Proceedings of the National Academy of Sciences 92:8945–8949.

WEGNER, K. M., M. KALBE, J. KURTZ, T. B. H. REUSCH, AND M. MILINSKI. 2003. Parasite selection for immunogenetic optimality. Science 301:1343.

WEIL, Z. M., L. B. MARTIN II, AND R. J. NELSON. 2006. Interactions among immune, endocrine, and behavioural response to infection. Pp. 443–473 in Micromammals and macroparasites: from evolutionary ecology to management (S. Morand, B. R. Krasnov, and R. Poulin, eds.). Springer-Verlag Press, Tokyo, Japan.

WESTERDAHL, H., B. HANSSON, S. BENSCH, AND D. HASSELQUIST. 2004. Between-year variation of MHC allele frequencies in great reed warblers: selection or drift? Journal of Evolutionary Biology 17:485–492.

YANG, Z. 2007. PAML 4: a program package for phylogenetic analysis by maximum likelihood. Molecular Biology and Evolution 24:1586–1591.

YANG, Z., W. S. W. WONG, AND R. NIELSEN. 2005. Bayes empirical Bayes inference of amino acid sites under positive selection. Molecular Biology and Evolution 22:1107–1118.

ZENUTO, R. R., M. S. FANJUL, AND C. BUSCH. 2004. Use of chemical communication by the subterranean rodent *Ctenomys talarum* (tuco-tuco) during the breeding season. Journal of Chemical Ecology 30:2111–2126.

ZENUTO, R. R., E. A. LACEY, AND C. BUSCH. 1999a. DNA fingerprinting reveals polygyny in the subterranean rodent *Ctenomys talarum*. Molecular Ecology 8:1529–1532.

ZENUTO, R. R., A. I. MALIZIA, AND C. BUSCH. 1999b. Sexual size dimorphism, relative testes size and mating system in two populations of *Ctenomys talarum* (Rodentia: Octodontidae). Journal of Natural History 33:305–314.

ZENUTO, R. R., A. I. VASSALLO, AND C. BUSCH. 2002. Comportamiento social y reproductivo del roedor subterráneo solitario *Ctenomys talarum* (Rodentia: Ctenomyidae) en condiciones de semicautiverio. Revista Chilena de Historia Natural 75:165–177.

ZHENG, D., ET AL. 2004. Effect of temperature and salinity on virulence of *Edwardsiella tarda* to Japanese flounder, *Paralichthys olivaceus* (Temminck et Schlegel). Aquaculture Research 35:494–500.

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