American Genetic Association

Journal of Heredity, 2017, 628–639
doi:10.1093/jhered/esx054
Original Article
Advance Access publication June 9, 2017

OXFORD

Original Article

Selection on MHC in a Context of Historical Demographic Change in 2 Closely Distributed Species of Tuco-tucos (*Ctenomys australis* and *C. talarum*)

Ana Paula Cutrera and Matías Sebastián Mora

From the Instituto de Investigaciones Marinas y Costeras, CONICET – Universidad Nacional de Mar del Plata, Funes 3350, Mar del Plata (7600), Argentina.

Address correspondence to A.P. Cutrera at the address above, or e-mail: acutrera@mdp.edu.ar.

Received September 7, 2016; First decision November 17, 2017; Accepted June 7, 2017.

Corresponding Editor: Robert C Fleischer

Abstract

Selection necessarily acts within the same current and historical demographic framework as neutral evolutionary processes, and the outcome of the interplay between these forces may vary according to their relative strength. In this study, we compare the variation at a major histocompatibility complex (MHC) locus (DRB exon 2), typically subject to strong diversifying selection, and mitochondrial diversity (D-loop) across populations encompassing the entire distribution of 2 species of South American subterranean rodents: *Ctenomys australis* and *C. talarum* (tuco-tucos). Although these species are parapatric along most of their distribution, historically they have followed distinct demographic trajectories associated with sea level changes during the Quaternary. We surveyed 8 populations of *C. australis* and 15 of *C. talarum*, from which we analyzed 70 and 212 D-loop haplotypes and 91 and 346 DRB genotypes, respectively. Both species have gone through a recent demographic expansion; however, the signal of this process only encompasses the entire distribution of one of the species: *C. australis*. While balancing selection on MHC in *C. talarum*-enhanced DRB diversity at the local level compared to D-loop, although not promoting divergence among populations, in *C. australis* local diversifying selection may have driven higher population differentiation at DRB than at D-loop. Our findings reinforce the idea that the relative strength of selection acting on MHC genes varies spatially and temporally within and among species, even between species using the same macrohabitat and exposed to similar immune challenges.

Subject area: Population structure and phylogeography
Keywords: balancing selection, DRB exon 2, D-loop, subterranean rodents

Immune defenses play an essential role in parasite and pathogen resistance, and thus understanding variation in these traits is key to integrate organism biology, ecology, and evolution (Adelman 2014). The major histocompatibility complex (MHC) is a fundamental component of the vertebrate immune system (Penn *et al.* 2002); they code for glycoproteins involved in the recognition and binding of foreign antigens, for their presentation to T-lymphocytes, which elicit the associated immune response (Klein 1986). In particular, class II MHC genes bind peptides produced by extracellular pathogens such as parasites and bacteria (Klein and Horejsi 1997; Janeway and

© The American Genetic Association 2017. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

Downloaded from https://academic.oup.com/jhered/article-abstract/108/6/628/3865731/Selection-on-MHC-in-a-Context-of-Historical
by guest
on 15 September 2017

Travers 1999). Given the role of MHC alleles in recognizing specific pathogen peptides, 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). Temporal and geographic heterogeneity of the parasite community may also create opportunities for variation in the magnitude of pathogen-mediated selection, thus contributing to maintenance of MHC variation in host populations (Hedrick 2002).

However, selection necessarily acts within the same current and historical demographic framework as neutral evolutionary processes, and while in some cases MHC variation correlates with that of neutral markers (Berggren *et al.* 2005; Babik *et al.* 2008; Biedrzycka and Radwan 2008; Miller *et al.* 2010; Vásquez-Carrillo *et al.* 2014), other studies have not found such association (Bryja *et al.* 2007). In some of these cases, balancing selection was able to outweigh the effects of genetic drift, even in severely reduced populations (Aguilar *et al.* 2004; Oliver and Piertney 2012), leading to similar levels of MHC allelic variation among populations (Koutsogiannouli *et al.* 2009; Tobler *et al.* 2014). On the contrary, increased MHC divergence has been documented among populations relative to neutral markers, which may be the consequence of diversifying local selection as a result of spatial variation in pathogen-mediated regimes (Ekblom *et al.* 2007; Alcaide *et al.* 2008; Malé *et al.* 2012).

Rodents of the genus *Ctenomys* (tuco-tucos) provide an opportunity to assess the selective and neutral processes underlying spatial variation in MHC diversity in naturally occurring populations of mammals. These subterranean mammals, which occur from southern Peru to Tierra del Fuego and from the Andes to southeastern Brazil (Reig *et al.* 1990; Lessa and Cook 1998; Castillo *et al.* 2005), have shown 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 over the history of this genus (Cutrera and Lacey 2007). Further, comparisons of 2 parapatric populations, one of *Ctenomys talarum* versus another of *C. australis*, suggested that demographic history (i.e., recent expansion) can substantially influence genetic structure at MHC loci (Cutrera *et al.* 2010). Within species, selection on MHC genes appears to vary predictably with demographic attributes such as social structure and population density (Lacey and Cutrera 2007). Detailed analyses of two populations of *C. talarum* have revealed that specific DRB alleles are associated with parasite load and intensity of humoral immune response (Cutrera *et al.* 2011), suggesting that particular MHC alleles can influence resistance to pathogens, but more so in the more dense population, with higher parasite exposure (Cutrera *et al.* 2014). At the same time, field and laboratory studies of animals from this species 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, considering that there is evidence that suggests that the strength of polygyny may vary between populations of tuco-tucos (Zenuto *et al.* 1999a, 1999b, 2003).

This study expands on our previous assessment of the MHC variation in 2 parapatric species of subterranean rodents of the genus *Ctenomys* that had experienced concordant or different demographic histories, depending of the geographical location considered (Cutrera *et al.* 2010; Mora *et al.* 2007, 2013a). Although their distributions are sympatric in one portion in the south-east of the Buenos Aires Province, ecologically, these species occupy slightly different microhabitats. *C. australis* is restricted to sandy friable soils of permanent dunes on the coast of the Buenos Aires province, Argentina, whereas *C. talarum* occupies interdune and inland habitats characterized by harder, more humid, and more vegetated soils (Vassallo 1993). Related to this, previous work using neutral genetic variation of mitochondrial DNA control region has demonstrated that while *C. australis* has experienced a recent and gradual demographic expansion (Mora *et al.* 2006), *C. talarum* shows a stable demographic history in the northern and inland populations, but presents signs of a recent and abrupt population expansion in the southeastern distributional range (Mora *et al.* 2007, 2013a). This study aims to expand the geographic scope of the previous work performed by Cutrera *et al.* (2010), in which only one population of each species was included, missing the complexity of the demographic history of these subterranean rodents over their entire distribution, particularly that of *C. talarum* (Mora *et al.* 2013b). To test the contrasting hypotheses about the role of balancing versus directional selection on MHC loci in *C. australis* and *C. talarum*, and to further our understanding of their history, this study compares MHC (DRB exon 2) and mtDNA (D-loop) variation over the entire distribution of *C. australis* (8 populations) and almost the complete distribution of *C. talarum* (15 populations). Specifically, if DRB exon 2 is subject to balancing selection, variation at this gene is expected to be higher within a population, and differentiation among populations to be lower compared with variation at mtDNA. Alternatively, if DRB is subject to different selective local regimes, this locus is expected to show higher population divergence among populations compared with mtDNA. However, if the recent demographic expansion experienced by *C. australis* and the southern populations of *C. talarum* has a strong effect on MHC, as it does on mtDNA, we expect to find a correlation in the patterns of differentiation at these two genetic regions.

Materials and Methods

Sampling

This study included sampling sites from the entire distributional range of *C. australis* (from Necochea to Punta Alta, see Figure 1), and sampling locations from almost the entire distribution of *C. talarum*, which is composed of 2 continuous coastal geographic ranges (the southern coastal distribution from Necochea to Pehuén Có, and the northeastern coastal distribution from Magdalena to Mar de Cobo localities along the Atlantic coast; Figure 1), and for some small inland populations (e.g., Sierra de la Ventana; Figure 1). Individuals were live-trapped as described by Mora *et al.* (2006, 2007, 2013a) and a tissue sample (toe snip, preserved in 95% ethanol for subsequent DNA extraction) was obtained, after which they were released back inside the same burrow system from where they had been originally captured. All parts of this study concerning the handling of animals in the field followed guidelines of the American Society of Mammalogists (Sikes *et al.* 2011).

Genomic DNA was isolated following a protocol modified from Miller *et al.* (1988), involving treatment with sodium dodecyl sulphate and digestion with proteinase-K, NaCl precipitation of proteins, and subsequent isopropylic alcohol precipitation of DNA, as described by Mora *et al.* (2006, 2007, 2013a).

Mitochondrial DNA Data

We used 70 sequences of 401 bp of the mitochondrial control region (D-loop) of *C. australis* generated by Mora *et al.* (2006) and 212 D-loop sequences (438 bp) of *C. talarum* generated by Mora *et al.* (2007, 2013a) available at Genbank (Accession numbers:

Downloaded from https://academic.oup.com/jhered/article-abstract/108/6/628/3865731/Selection-on-MHC-in-a-Context-of-Historical
by guest
on 15 September 2017

DQ416717–DQ416740 for *C. australis*; and JX297345–JX297358, JX297361–JX297368, JX297370–JX297372, EF531719.1–EF531750.1 for *C. talarum*).

MHC Data

We amplified the MHC class II DRB exon 2 from a total of 57 tissue samples of *Ctenomys australis* and 174 of *C. talarum* collected by Mora *et al.* (2006, 2007, 2013a: Table 1). Plus, we used 34 DRB exon 2 sequences from *C. australis* and 172 of *C. talarum* generated by Cutrera *et al.* (2007, 2010, 2011: Table 1) and available at Genbank (Accession numbers: *C. australis*: EU035205, GQ497462–GQ497470 and *C. talarum*: JF799108–JF799116). Therefore, a total of 91 samples of *C. australis*, which encompassed 8 geographically defined populations (Figure 1), and 346 samples of *C. talarum*, categorized in 15 populations (Figure 1), were used for this study. This represents the entire distribution of *C. australis* and almost the complete distribution of *C. talarum*, with the exception of some small inland populations from the Buenos Aires province, Argentina (Figure 1; see also Mora *et al.* 2013a).

MHC Amplification

We assessed variability at the MHC class II DRB locus for all the individuals mentioned above. 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, 2014). Specifically, amplicons from 6 positive clones per individual were sequenced; this number of cloning products was enough to determine the genotype of each animal and to avoid reading errors or recombinant sequences generated during the PCR amplifications. Each new allele detected was sequenced in both directions to confirm diagnostic base-pair changes. Those sequences that differed by only a single base-pair substitution were considered distinct alleles if each variant occurred in multiple cloning products per individual (obtained from at least 2 different PCRs and rounds of cloning or both).

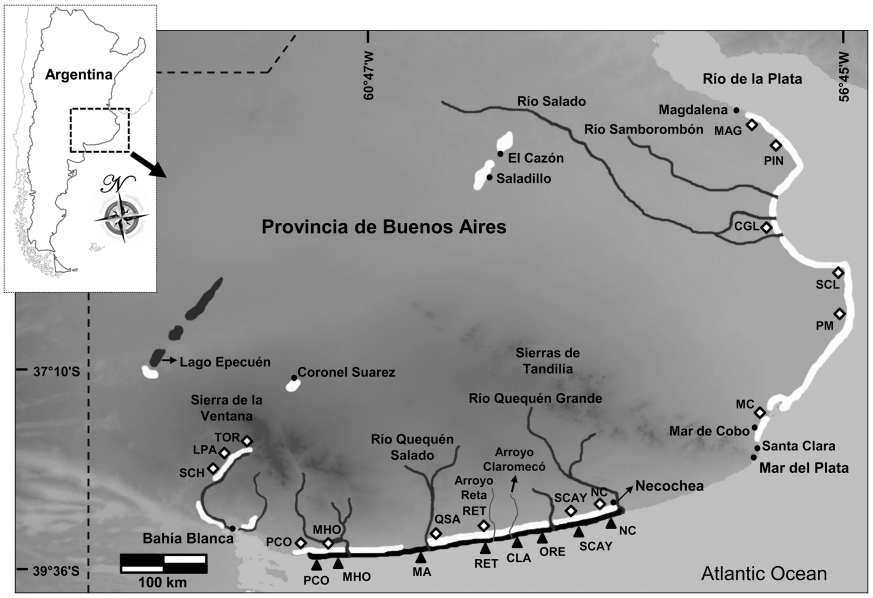

Figure 1. Geographical distribution of the sampled populations of *Ctenomys talarum* and *Ctenomys australis* in Buenos Aires Province, Argentina. The areas in dark gray represent the current distribution of *C. talarum* (see Mora *et al.* 2007), whereas areas in black represent the current distribution of *C. australis* (Mora *et al.* 2006). The most important rivers and streams in the area are also shown. Abbreviations and coordinates of sampling localities for *C. talarum* (diamonds) and *C. australis* (triangles) are indicated as follows: MAG, El Destino (35°08′S 57°23′W); PIN, Punta Indio (35°20′S 57°11′W); CGL, Cerro de la Gloria (35°51′S 57°26′W); SCL, San Clemente (36°19′S 56°45′W); PM, Punta Médanos (36°52′S 56°42′W); MC, Mar de Cobo (37°46′S 57°26′W); NC, Necochea (38°37′S 58°50′W); SCAY, Balneario San Cayetano (38°43′S 59°26′W); RET, Reta (38°53′S 60°19′W); QSA, Río Quequén Salado (38°54′S 60°28′W); SCH, Arroyo Sauce Chico (38°19′S 62°34′W); LPA, Estancia La Paloma (38°09′S 62°23′W); TOR, Tornquist (38°03′S 62°15′W); MA, Balneario Marisol (38°55′S 60°34′W); PCO, Pehuén Có (39°0′S 61°34′W); MHO, Monte Hermoso (38°59′S 61°14′W); CLA, Claromecó (38°50′S 59°58′W); and ORE, Orense (38°48′S 59°43′W).

Table 1. Number of sequences analyzed (*n*), source, year of collection, and reference to original studies in which they were published for the first time for MHC Class II DRB exon 2 and mitochondrial DNA control region (D-loop) for both study species: *Ctenomys australis* and *C. talarum*

	Locus	*N*	Sequence source	Year of sample collection	Reference
C. australis	DRB	57	Tissue	2002–2006	This study
		34	Genebank	2003	Cutrera *et al.* 2010
	D-loop	70	Genebank	2002–2006	Mora *et al.* 2006
C. talarum	DRB	174	Tissue	2003–2008	This study
		172	Genebank	2006–2010	Cutrera *et al.* 2010, 2011, 2014
	D-loop	212	Genebank	2003–2008	Mora *et al.* 2007, 2013b

Downloaded from https://academic.oup.com/jhered/article-abstract/108/6/628/3865731/Selection-on-MHC-in-a-Context-of-Historical
by guest
on 15 September 2017

Characterizing Variation at MHC and mtDNA

To obtain an estimate of the relative variability at the DRB exon 2, the number of MHC alleles was compared to the number of D-loop haplotypes (Seddon *et al.* 2001; Berggren *et al.* 2005). MHC allelic richness (A) was estimated using rarefaction to standardize A to the smallest population size in each species as performed by Fstat (Goudet 2001) while ARLEQUIN version 3.11 (Excoffier *et al.* 2007) was used to calculate MHC observed (Ho) and expected (He) heterozygosity. Number of segregating sites, haplotype diversity, and nucleotide diversity were calculated for mtDNA and MHC data for each species and population using DnaSP version 5 (Librado and Rozas 2009).

Evidence of Selection Based on Substitution Rates

Rates of nonsynonymous (d_N) to synonymous (d_S) substitutions are routinely used to identify departures from neutrality and to identify the type of selection that has acted on a given locus (Kimura 1983; Ohta 1993). ω is equivalent to the d_N/d_S selection parameter of Nei and Gojobori (1986). Simply, values of $\omega < 1$, $= 1$ or >1 mean negative purifying selection, neutral evolution, and positive selection, respectively. However, the ratio averaged over all sites and lineages is almost never >1, since it is unlikely that positive selection will affect all sites over prolonged time. Thus, for codon-specific analyses, ω is preferred because it allows for variation in the selective pressures experienced by different codons (Nielsen and Yang 1998). To identify codons subject to positive selection and to determine if the nature of selection varied among codons in the same exon, observed values of ω were compared to 5 model distributions of nucleotide substitutions using the CodeML subroutine of PAML, version 3.14 (Yang 1997), as previously described in Cutrera *et al.* (2010). The 5 distributions examined were M1a (nearly neutral), which assumes both conserved sites (i.e., sites under purifying selection, $0 < \omega < 1$) and selectively neutral sites ($\omega = 1$) among codons for the same protein; M2a (positive selection), which adds a third class of sites with ω as a free parameter (thus allowing for sites with $\omega > 1$); M3 (discrete), which estimates the proportion of conserved, neutral, and unrestricted codons from the data; M7 (beta), which does not allow for positively selected sites ($0 < \omega < 1$); and M8 (beta and ω), which adds an additional site class to the beta model to account for sites under positive selection (for details on models M1a and M2a, see Yang *et al.* 2005). Likelihood-ratio tests (Yang *et al.* 2005) were used to compare model M1a with M2a and model M7 with M8. According to Yang *et al.* (2005), when alternative models M2a and M8 suggest the presence of codons with $\omega > 1$, this can be interpreted as evidence of positive selection. Bayesian posterior probabilities were used to identify codons that appeared to be conserved versus neutral or subject to positive selection (Nielsen and Yang 1998).

Departures from Neutrality

To detect departures from neutrality, Hardy–Weinberg tests were used to determine whether observed heterozygosities at the DRB locus were significantly in excess of those expected under equilibrium conditions in each population. Also, Ewens–Watterson tests (Ewens 1972; Watterson 1986; Garrigan and Hedrick 2003) were applied per population to determine whether DRB allele frequency distributions were consistent with those expected under balancing selection. These analyses were conducted using ARLEQUIN version 3.11 (Excoffier *et al.* 2007). As an alternative means of detecting departures from neutrality, Tajima's *D* (Tajima 1989) and Fu's F_S (Fu 1997) tests of neutrality for MHC and mtDNA data were performed globally and for each population using 10 000 iterations as performed in ARLEQUIN 3.11 (Excoffier *et al.* 2007). Because these tests are based on the site frequency spectrum, they are influenced by long-term mutation patterns as well as more recent population dynamics (Garrigan and Hedrick 2003). Significant negative values of Tajima's *D* and Fu's F_S are indicative of an excess of low frequency mutations, relative to expectations under the standard neutral model (strict neutrality of variants, constant population size, and lack of population subdivision), and are consistent with demographic expansion and/or purifying selection.

Phylogeographical Analyses

In order to infer hierarchical population structure, analyses of molecular variance (AMOVAs) were performed considering both genetic distances between mtDNA haplotypes/MHC alleles and their frequencies, using ARLEQUIN version 3.11 (Excoffier *et al.* 2007). For both data sets (neutral and adaptive) AMOVAs were performed for the 2 study species 1) across sampling localities as independent units, and 2) across different regions, to test the effect of major natural barriers and geographical proximity on the partitioning of the genetic variance, using previous information regarding the phylogeography of both species (Mora *et al.* 2006, 2007; see Table 3). Haplotype networks for mtDNA haplotypes and MHC alleles were built using the median-joining algorithm defined by Bandelt *et al.* (1999) in NETWORK 4.5.1, which essentially uses a maximum parsimony approach to search for the shortest, least complex network from a given data set. Networks were then edited manually using Microsoft Office Excel v. 2007.

Pairwise and overall F_{ST} values for DRB exon 2 and D-loop were estimated using ARLEQUIN version 3.11 (Excoffier *et al.* 2007) and their significance was evaluated using 100 000 permutations. Standardized pairwise values for DRB exon 2 and D-loop were also estimated using AMOVA routines implemented in GenAlEx version 6.5 (Peakall and Smouse 2012); the estimation of standardized F_{ST} values was done following Meirmans (2006). This approach is recommended when levels of genetic variation obtained from molecular markers with different mutation rates and/or comparisons between species are used in the analysis (see Supplementary Material). We assessed whether pairwise F_{ST} values were correlated with geographic distances between populations using Mantel tests. Also, to test whether population differentiation at DRB was similar to differentiation at D-loop irrespective of geographic distance, we used partial Mantel tests where pairwise F_{ST} for MHC data was correlated with pairwise F_{ST} for mtDNA data, while controlling for geographic distance (Ekblom *et al.* 2007). Ordinary and partial Mantel tests were performed using PASSage v. 2 (Rosenberg and Anderson 2011).

Historical Demography

Mismatch distribution analyses allowed us to differentiate between populations that have been demographically stable over time (according to an "equilibrium model" with constant long-term effective population size) from those that have experienced recent demographic or range expansion, departures from strict neutrality or a combination of these factors (Harpending *et al.* 1998; Schneider and Excoffier 1999; Ramos-Onsins and Rozas 2002). We employed parametric bootstrapping as implemented in ARLEQUIN 3.11 to test the goodness of fit of the observed mismatch distribution to that expected under the sudden and spatial expansion model using the sum of squared deviations (SSD) statistic.

Downloaded from https://academic.oup.com/jhered/article-abstract/108/6/628/3865731/Selection-on-MHC-in-a-Context-of-Historical
by guest
on 15 September 2017

In addition, population change through time was inferred using Bayesian skyline plots (BSPs) as implemented in BEAST 1.5.4 (Drummond and Rambaut 2007). This approach includes the uncertainty in the genealogy by using Markov chain Monte Carlo integration under a coalescent model, providing information about effective population sizes through time (Drummond *et al.* 2005). The program was run for 6×10^7 iterations and sampled every 10 000 steps under a relaxed lognormal molecular clock with uniformly distributed priors. In our analyses, the first 10% of the iterations were discarded to allow for burn-in. The best-fit substitution model for the data was estimated in jModelTest (Posada 2008). To assess the robustness of parameter estimates, 4 independent chains were run with identical settings. Log-files were analyzed in Tracer 1.4.8 (Drummond and Rambaut 2007), and effective sample sizes were used to evaluate Markov chain Monte Carlo convergence within chains. These analyses were performed globally in both species and for the most important macroregions in *C. talarum*. To obtain estimates of absolute times of demographic events suggested by BSPs, we used the substitution rate (and node ages) previously estimated for the mtDNA control region for the genus *Ctenomys*, reported in Mora *et al.* (2013a), which is 4.41×10^{-8} per site per year.

Data availability

In accordance with the *Journal of Heredity* data archiving policy, we have deposited the MHC sequences in Genbank (accessions KY906252- KY906266 for *C. talarum* sequences and KY906267-KY906279 for *C. australis* sequences).

Results

Variation and Evidence of Selection at MHC

For *C. australis*, some of the populations showed greater variation at D-loop than at MHC class II DRB exon 2, whereas others showed the opposite pattern (Table 2). Patterns of D-loop variation were not associated with geographic location: the lowest level of genetic variability was presented in CL (Hd = 0.15 and Nd = 0.0004), and the highest in ORE (Hd = 0.9 and Nd = 0.0088), both populations are only 20 km apart from each other (Table 2). For *C. talarum*, the majority of the populations surveyed showed higher variability at the DRB exon 2 compared to D-loop. Specifically, higher number of DRB alleles, greater number of segregating sites, and higher haplotype and nucleotide diversity were observed in almost all of the *C. talarum* populations of this study (Table 2).

Table 2. Number of allelic variants, V ratio (D-loop/DRB exon 2), DRB allelic richness (A), number of segregating sites (S), haplotype diversity (Hd), and nucleotide diversity (Nd) calculated by population for *C. australis* and *C. talarum* are shown

Pop	Variants	Vratio	A	S	Hd	Nd	Ho	He	Fs	D
C. australis				D-loop/DRB exon 2						
PCO (8/9)	5/4	1.25	3.250	7/12	0.86/0.63	0.0055/0.021	**0.222**	0.627	−0.90	−0.79/2.29
CLA (13/14)	2/5	0.40	3.992	1/11	0.15/0.69	0.0004/0.011	**0.714**	0.698	−0.53	−0.15/0.13
SCAY (7/5)	4/3	1.33	3.000	5/2	0.81/0.65	0.0049/0.0028	1.000	0.644	−0.13	−0.10/0.22
RET (7/8)	4/9	0.44	6.609	7/14	0.71/0.89	0.0035/0.011	0.875	0.892	−0.70	−1.50/−1.09
MHO (7/5)	4/1	4.00	1.000	3/0	0.81/0	0.0028/0			−1.22	−0.3/---
ORE (5/6)	4/4	1.00	3.818	8/5	0.9/0.68	0.0088/0.006	**0.833**	0.742	−0.04	−0.44/−0.31
MA (12/10)	4/6	0.67	4.746	7/12	0.71/0.76	0.0044/0.015	0.900	0.763	0.64	−0.92/0.36
NC (11/34)	7/10	0.70	2.324	7/26	0.82/0.25	0.0064/0.003	0.265	0.274	***−2.26***	0.08/−2.68
Total	24/23			19/31						
Overall	0.83/0.72					0.0055/0.015			***−27***	***−1.33***/−0.76
C. talarum				D-loop/DRB exon 2						
CGL (18/17)	4/6	0.67	4.152	8/9	0.63/0.8	0.0077/0.013	0.765	0.795	3.37	1.54/1.86
PIN (24/25)	3/3	1	2.747	2/6	0.58/0.56	0.0021/0.01	0.640	0.564	1.25	1.65/**2.58**
TOR (13/13)	3/5	0.60	3.888	3/9	0.3/0.75	0.0010/0.013	**0.923**	0.748	−0.69	−1.65/1.41
SCL (18/18)	4/7	0.57	4.764	2/9	0.54/0.78	0.0015/0.014	0.833	0.783	−1.05	0.33/1.84
LPA (11/11)	2/5	0.40	4.367	1/9	0.18/0.78	0.0004/0.012	**0.910**	0.784	−0.41	−1.13/0.96
MAG (9/9)	4/4	1	3.856	12/7	0.75/0.76	0.0136/0.012	0.778	0.758	3.13	1.64/1.86
PM (18/18)	1/9	0.11	4.852	0/11	0/0.78	0/0.011	0.500	0.779–	---/0.37	
SCH (5/5)	1/5	0.20	5.000	0/9	0/0.8	0.0/0.016	1.000	0.800---	---/1.65	
RET (22/24)	7/8	0.88	2.658	8/11	0.77/0.75	0.0046/0.014	**0.792**	0.754	−0.96	−0.59/1.61
PCO (10/10)	8/3	2.67	1.556	7/9	0.96/0.43	0.0046/0.009	0.300	0.426	***−5.04***	−0.77/0.007
MHO (8/8)	8/2	4	4.571	17/9	1/0.13	0.013/0.004	0.125	0.125	**−3.46**	−1.04/**−2.15**
QSA (7/7)	3/5	0.60	4.621	4/8	0.52/0.78	0.0026/0.012	1.000	0.780	0.26	−1.43/1.12
SCAY (9/9)	5/6	0.83	4.162	6/8	0.72/0.78	0.003/0.012	**0.778**	0.784	−1.78	**−1.72**/1.45
MC (90)	9/11	0.82	2.633	10/13	0.81/0.76	0.0045/0.012	**0.855**	0.756	**−2.45**	−0.76/1.18
NC (82)	5/5	1	5.074	7/11	0.7/0.53	0.0041/0.008	**0.622**	0.528	−0.33	−0.77/0.23
Total	58/26			63/17						
Overall					0.95/0.84	0.017/0.015			***−24.4***	−0.85/1.45

The number of individuals per population sequenced for each molecular marker is given between parentheses besides the population name (*n* D-loop/*n* DRB exon2). The total and overall values of these parameters are also presented. Observed (Ho) and expected (He) heterozygosity are shown. For Ho values, those presented in bold are significantly different to values expected under Hardy–Weinberg equilibrium ($P < 0.05$). Values of Fu's Fs and Tajima's D are presented (note that Fu's Fs were calculated only for D-loop sequences). Numbers in bold are statistically significant ($P < 0.05$) as well as those presented in bold and italics ($P < 0.01$). ---: Statistic not estimated. Abbreviations are detailed in Fig. 1.

Downloaded from https://academic.oup.com/jhered/article-abstract/108/6/628/3865731/Selection-on-MHC-in-a-Context-of-Historical
by guest
on 15 September 2017

For *C. australis*, likelihood-ratio tests revealed no differences in fit between any of the 5 nucleotide substitution models considered (see Supplementary Table S1). In contrast, for *C. talarum*, models that allowed for the inclusion of positive selection (M2a and M8) had a significantly better fit than simpler models (M1a and M7), suggesting that, for this species, selection may be acting on specific codons within this exon. Specifically, residues 19, 27, 37, 45, and 75 of the DRB exon 2 from *C. talarum* were identified as subject to positive selection (Supplementary Table S1). For *C. australis*, Hardy–Weinberg (H-W) tests revealed evidence of significant excess of MHC heterozygotes in 2 out of the 8 populations surveyed, while 1 population showed a significant deficit of heterozygotes (Table 2). For this species, no significant departures from neutrality were detected by Ewens–Watterson (E-W) tests. For *C. talarum*, H-W tests revealed evidence of significant excess of MHC heterozygotes in 5 out of the 15 populations surveyed, while only 1 population showed a significant deficit of heterozygotes (Table 2); E-W tests revealed evidence of balancing selection in only 1 population (MAG: F = 0.284, Watterson's F, P = 0.054, Slatkin's exact test, P = 0.033).

For *C. australis*, overall values of Fu's *Fs* and Tajima's *D* calculated for D-loop were significantly lower than zero. Accordingly, Tajima's *D* for DRB exon 2 in this species was negative, but not significant (Table 2). For *C. talarum*, while overall values of Fu's *Fs* and Tajima's *D* calculated for D-loop were also negative, Tajima's *D* values for DRB exon 2, although not significant, were positive (Table 2). Specifically, in 9 of the 15 populations, Tajima's *D* for D-loop were negative while in 14 populations out of 15 these values for DRB exon 2 were positive. Despite this latest tendency, only Fu's *Fs* statistics was significantly positive at a global level for *C. talarum*, and both Tajima's *D* and Fu's *Fs* were significantly negative for *C. australis*.

Phylogeographical Structure

C. australis did not show significant apportionment of the genetic variance among regional groups using the neutral marker (D-loop) and considering localities assembled into groups separated by major rivers (Quequén Salado), forestations, and streams (Cristiano Muerto and Sauce Grande; see AMOVAs in Table 3; Figure 1). Thus, population differentiation in this species does not seem to be related to the presence of major natural barriers in the landscape. Although DRB exon 2 showed high population differentiation when the western populations (Pehuén-Co [PCO], Monte Hermoso [MHO], and MA) and the most eastern populations (NC) were divided from the central populations (SCAY, CLA, ORE, and RET), the greatest population differentiation was found without clustering populations into particular regions (Table 3). For this molecular marker, it seems that differentiation among localities in *C. australis* has no apparent regional pattern: isolation among local populations has been more significant than the effect of the major natural barriers considered in this study. Finally, overall F_{ST} values for D-loop and DRB were 0.27 and 0.56, respectively, and, for all levels of apportionment of the genetic variance, the Φ_{ST} values were greater for the exon 2 of DRB than for D-loop (Table 3), suggesting that the MHC variation in *C. australis* seems to be more geographically structured than the neutral variation. For *C. talarum*, we found the opposite pattern: neutral variation was more geographically structured than MHC variation. Specifically, overall ΦF_{ST} for D-loop and DRB were 0.79 and 0.22, respectively. Also, at all levels explored, the Φ_{ST} values were greater for D-loop than for DRB. For both markers, however, there was a pronounced differentiation between the 2 populations situated at PCO and MHO and the rest of the populations. Haplotype networks built for both species showed similar results to those of AMOVA, regarding the geographic apportionment of the variation at MHC and D-loop loci (see Supplementary Figure S1).

Table 3. Hierarchical analysis of molecular variance (AMOVA), using a square matrix of pairwise genetic distance between D-loop haplotypes and DRB alleles for *C. australis* (A) and *C. talarum* (B)

A. Source of variation (*C. australis*)	Regions defined	Φ_{CT}	P	Φ_{ST}	P
No clustering of subpopulations into regions	[PCO] [MHO] [MA] [NC] [SCAY] [CLA] [ORE] [RET]			0.56	<0.001
				0.27	**<0.001**
Among 2 macroregions	[PCO MHO] [MA NC SCAY CLA ORE RET]	0.35	0.11	0.66	<0.001
		0	**0.82**	**0.24**	**<0.001**
Among 3 macroregions limited by the most important natural barriers (Quequén Salado River and forestations)	[PCO MHO MA] [NC] [SCAY CLA ORE RET]	0.5	<0.05	0.6	<0.001
		0	**0.9**	**0.25**	**<0.001**
Among 5 regions limited by major natural barriers (major rivers, streams, highlands)	[PCO MHO] [NC] [SCAY] [CLA ORE] [RET MA]	0.03	0.4	0.56	<0.001
		0.12	**0.1**	**0.28**	**<0.001**
B. Source of variation (*C. talarum*)	Regions defined	Φ_{CT}	P	Φ_{ST}	P
No clustering of subpopulations into regions	[MAG] [PIN] [CGL] [SCL] [PM] [MC] [NC] [SCAY] [RET] [QSA] [SCH] [LPA] [TOR] [MHO] [PCO]			0.22	<0.001
				0.79	**<0.001**
Among 2 macroregions	[MAG PIN CGL SCL PM MC NC SCAY RET QSA SCH LPA TOR] [MHO PCO]	0.30	<0.05	0.43	<0.001
		0.63	**<0.001**	**0.9**	**<0.001**
Among 6 regions limited by major natural barriers (major rivers, streams, highlands)	[MAG PIN CGL] [SCL PM MC] [NC SCAY] [RET QSA] [SCH, LPA, TOR] [MHO PCO]	0.12	<0.05	0.23	<0.001
		0.46	**<0.001**	**0.8**	**<0.001**
Among 3 macroregions	[MAG PIN CGL SCL PM MC] [NC SCAY RET QSA MHO PCO] [SCH LPA TOR]	0	0.65	0.22	<0.001
		0.18	**<0.05**	**0.8**	**<0.001**
Among 4 macroregions	[MAG PIN CGL SCL PM MC] [NC SCAY RET QSA] [MHO PCO] [SCH LPA TOR]	0.08	<0.05	0.24	<0.001
		0.45	**<0.001**	**0.82**	**<0.001**

The fixation indexes (Φ statistics) are shown. Significance of Φ statistics was tested by 16 000 permutations according to Excoffier et al. (2007). Regions defined are presented between brackets. Adaptive variation (DRB exon 2) and neutral variation (D-loop) are presented in black and bold, respectively.

Downloaded from https://academic.oup.com/jhered/article-abstract/108/6/628/3865731/Selection-on-MHC-in-a-Context-of-Historical
by guest
on 15 September 2017

For *C. australis*, no significant relationship was found between pairwise F_{ST} values of D-loop and geographic distance (Mantel test: $r = -0.18$, $P = 0.77$). However, a significant positive relationship was observed between pairwise F_{ST} values of DRB exon 2 and geographic distance (Mantel test: $r = 0.53$, $P < 0.05$). As expected given these results, no significant relationship was found between pairwise F_{ST} values of D-loop and those of DRB (Partial Mantel correlation test: $r = -0.19$, $P = 0.74$). On the contrary, for *C. talarum*, neither D-loop pairwise F_{ST} values nor DRB exon 2 pairwise F_{ST} values were significantly correlated with geographic distance (Mantel test: $r_{D\text{-}loop} = 0.12$, $P = 0.21$; $r_{DRB} = 0.01$, $P = 0.37$). However, a significant positive correlation was found between pairwise F_{ST} values of D-loop and those of DRB (Partial Mantel correlation test: $r = 0.39$, $P < 0.01$). Population pairwise F_{ST} values for D-loop and DRB for both species are reported as Supplementary Data (Supplementary Tables S2 and S3).

Historical Demography

Mismatch distribution of *C. australis* was unimodal, as expected for populations that have undergone a population expansion in the recent past (Figure 2). In accord with this, neither SSD nor Harpending's Raggedness index (HRI) showed significant departures from a population expansion for *C. australis* considering both models, a sudden and geographic expansion (see Supplementary Table S4). The mismatch distribution of *C. talarum* was not clearly multimodal at a global level, suggesting a lack of constant population size and/or sustained subdivision for a long period of time, such that the SSD between observed and expected mismatch distributions did not show significant departures from a sudden or spatial expansion model (Figure 2; Supplementary Table S4).

Further, SSD and HRI did not present significant departures neither from a sudden nor from a geographic expansion models in the regions defined for *C. talarum* (Supplementary Table S4).

BSPs were performed 1) over the entire distribution range of both species and 2) considering partial demographic reconstructions for southern coastal distribution (excluding MHO and PCO), southern coastal distribution (including MHO and PCO), northern coastal distribution, and inland distribution for *C. talarum* (see Figure 3). The demographic scenario inferred by the BSP showed populations expansions for both species. Although *C. australis* registered a gradual and steady increment in their effective population size since 40 000 years B.P. until present, an abrupt population expansion since approximately 20 000 years B.P. until present was estimated for *C. talarum*. Population expansion in *C. talarum* seems to be related to a population size increment in the southern current distribution from Necochea to Quequén Salado River. On the contrary, inland and northern distributional ranges of this species seem to have been stable over time, at least during the Late Quaternary. *C. talarum* appears to have occupied the sand dune habitat in the southern distribution in the recent past, in the Late Pleistocene to Early Holocene limit (Figure 3).

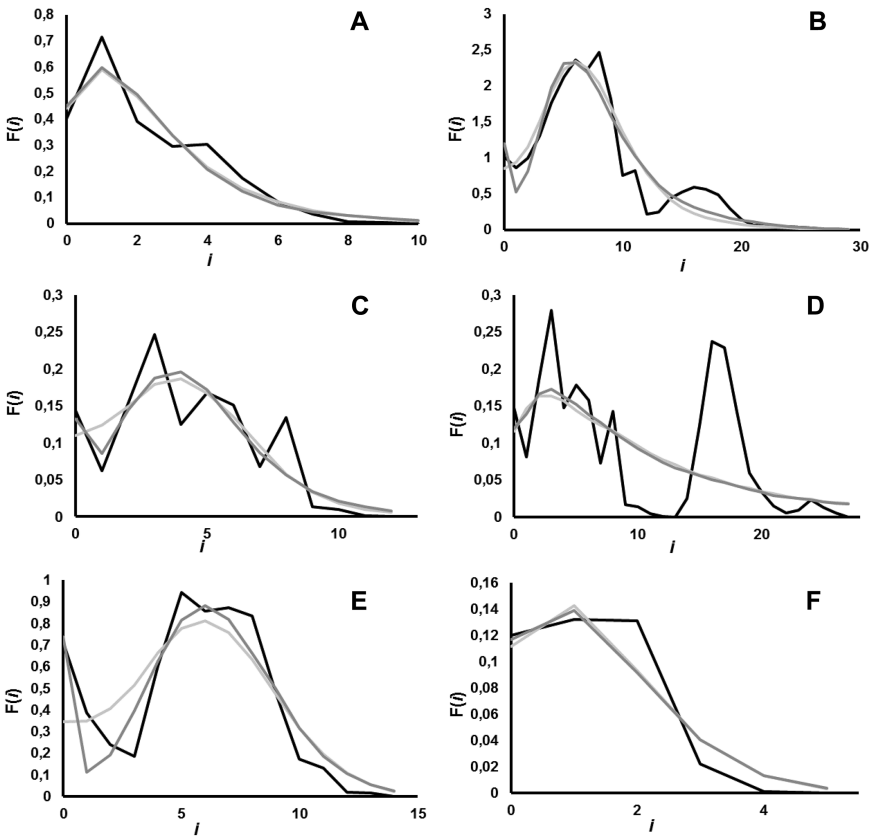

Figure 2. Observed and expected global mismatch distributions for *C. australis* and *C. talarum* (**A** and **B**, respectively). For *C. talarum*, mismatch distributions from the southern coastal distribution (excluding MH and PCO, **C**), from the complete southern coastal distribution (including MH and PCO, **D**), from the northern coastal distribution (**E**), and inland distribution (**F**) are also shown. The black line represents the observed distribution; the light gray line represents the theoretical expected distribution under a sudden population expansion model, the dark gray line represents the theoretical expected distribution under a spatial expansion model, following Schneider and Excoffier (1999). F(*i*) indicates frequency, *i* denotes pairwise nucleotide differences.

Downloaded from https://academic.oup.com/jhered/article-abstract/108/6/628/3865731/Selection-on-MHC-in-a-Context-of-Historical
by guest
on 15 September 2017

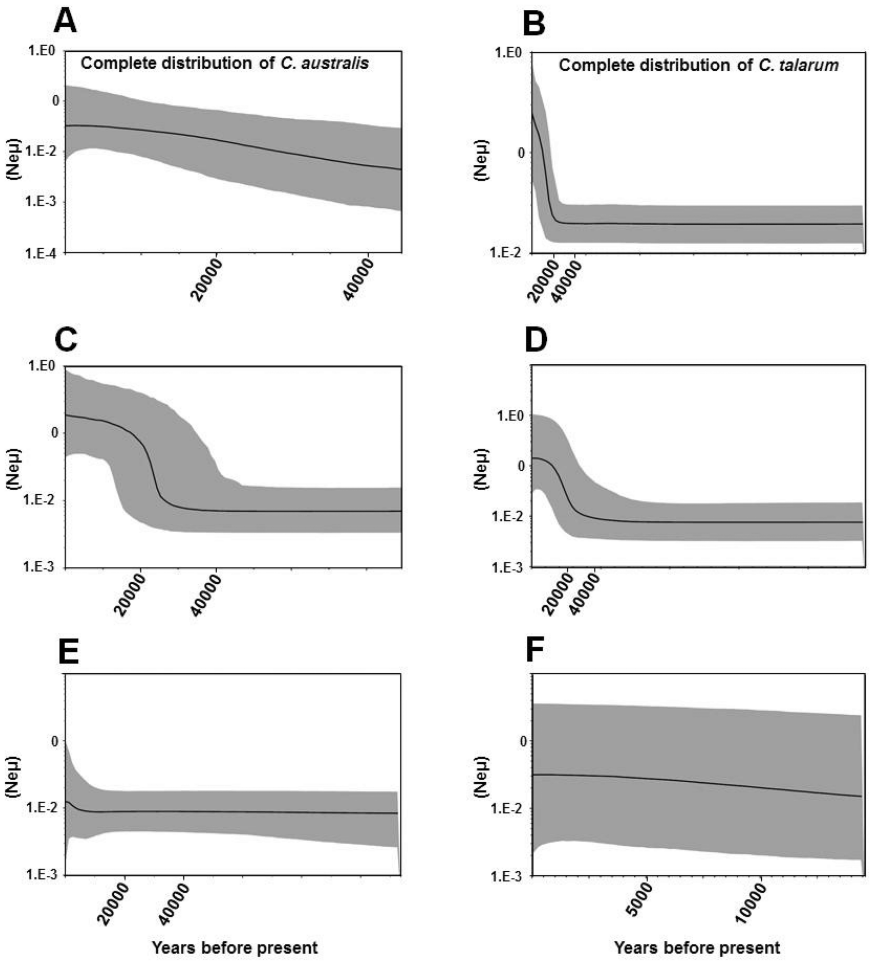

Figure 3. Bayesian skyline plots showing the complete reconstruction of female effective population size fluctuations (based on mtDNA data) throughout time in *C. australis* (**A**) and in *C. talarum* (**B**, complete distribution) during the Pleistocene–Holocene period. **C**, **D**, **E**, and **F** show partial demographic reconstructions for the southern coastal distribution (excluding MH and PCO), the complete southern coastal distribution (including MH and PCO), the northern coastal distribution, and the inland distribution of *C. talarum,* respectively. Black lines represent the median estimations and gray shading represents the upper and lower 95% credible intervals. The *x*-axes of these figures were estimated using a mutation rate of 4.41 per site per year estimated by Mora *et al.* (2013a) for the genus *Ctenomys*.

Discussion

It is well known that the markedly high polymorphism observed at MHC loci is the result of the action of positive selection (Garrigan and Hedrick 2003). However, selection operates necessarily against a given demographic background, historical, or contemporary, and therefore the preeminence of this force against stochastic processes (gene flow, genetic drift) may vary, depending on the strength of selection and population size (Maruyama and Nei 1981). Our findings demonstrate that while selection is most likely the predominant force that contributes to shape the low differentiation among populations at MHC loci in *C. talarum*, the pattern seems to differ for *C. australis*, for which the local divergence at MHC among populations seems to be greater in comparison to D-loop. Overall, our results partially agree with a previous study that compared MHC and D-loop variation in only 2 sympatric populations of *C. australis* and *C. talarum* (Cutrera *et al.* 2010). At the same time, our findings contribute to shed new light into the interplay between demography and selection over larger geographic distances and deeper time scales for these 2 species of subterranean rodents.

Comparing the Phylogeography of *C. australis* and *C. talarum*: Historical Demographic Scenarios

Our study species showed different patterns of population structure based on neutral variation; while *C. talarum* presented a strong phylogeographical signal on mtDNA variation, *C. australis* showed only a slight population differentiation. As previously suggested by Mora *et al.* (2006), lack of clear differentiation among regional groups of *C. australis* suggests that putative geographical barriers do not markedly affect the existing pattern of genetic structure. One reason is that important barriers in the landscape (e.g., streams and rivers) do not prevent dispersal among localities, possibly because of the small size or transient status of these barriers, such as water streams. On the other hand, if these species did not occupy their current distributions for a sufficiently long time (under the hypothesis of recent expansion), the effect of barriers could not be detected yet. In our study, global departures from both mutation–drift and migration–drift equilibriums were observed for mtDNA in both species. However, *C. talarum* showed a dual pattern: while no departures from mutation–drift equilibrium were detected in the northern and inland regions, disequilibrium was observed in the southern

Downloaded from https://academic.oup.com/jhered/article-abstract/108/6/628/3865731/Selection-on-MHC-in-a-Context-of-Historical
by guest
on 15 September 2017

distribution range (see also Mora *et al.* 2013a). For all *C. talarum* populations, pairwise estimates of population differentiation (F_{ST}'s) suggest low level of gene flow; these high levels of population fragmentation, limited gene flow, and the existence of isolated small-sized demes have been associated with an intense action of genetic drift in the species (Mora *et al.* 2013a).

Phylogeographical Signal on MHC: Relative Roles of Selection and Historical Demography in *C. australis* and *C. talarum*

Differentiating between the effects of demographic history and selection is problematic and remains an important challenge to studies investigating the processes underlying variation at functional loci (Nielsen 2005). Comparing the current distribution of variation at neutral and adaptive markers is essential to understand the processes that shape the polymorphism of genes subject to selection (Schierup *et al.* 2000). For example, continuously distributed populations may be genetically structured if gene flow is limited or if they are under diversifying local selection (Hudson *et al.* 1992; Congdon *et al.* 2000). Specifically for our study, while it was clear that all measures of variation—including number of allelic variants, number of segregating sites and haplotype and nucleotide diversity—were generally higher for DRB exon 2 compared to D-loop in most populations of *C. talarum*, as expected if balancing selection was the predominant force acting on MHC, the pattern was less evident for *C. australis*. Although both species seem to have undergone demographic population expansions in the Late Quaternary, this demographic change seems to have had a different impact in these species. Although an abrupt population increment was clearly observed in *C. talarum* in a restricted geographical region in the last 25 000 years, a gradual and steady increase has been reported for the entire distributional range of *C. australis* since 40 000 years B.P. Therefore, the demographic expansion undergone by *C. australis* encompassed the entire distribution of this species, while for *C. talarum* the sign of expansion is restricted to the Southern region of its distribution. However, the sign of recent population expansion following repeated cycles of change in habitat availability associated with sea level fluctuations in the southern populations of *C. talarum* has not erased the high levels of polymorphism observed at a global level in MHC loci. In particular, the relative level of D-loop variation in comparison to the MHC variation was 1–9 times lower in 10 out of the 15 populations surveyed of *C. talarum*, whereas for *C. australis* 4 out of 8 populations showed greater, although much more moderate, D-loop variation compared to DRB. For *C. talarum*, these findings are in agreement with what is expected within a population for genes under balancing selection: DRB exon 2 shows higher variation than the neutral marker analyzed (in this case, D-loop). Lower differentiation between populations is expected for genes subject to balancing selection in comparison to neutral loci (Klein *et al.* 1998; Schierup *et al.* 2000). Our results support this prediction for *C. talarum*, but not for *C. australis*. Specifically, AMOVA results for *C. talarum* showed that differentiation at DRB exon 2 was lower when compared with D-loop for all levels of apportionment of genetic variation explored. However, this pattern does not seem to hold for *C. australis* populations, for which population differentiation at mitochondrial DNA was not associated with the presence of major landscape barriers. The absence of a pattern of isolation by distance (IBD) for D-loop in *C. australis*, based on the results of the Mantel test, further supports this scenario. Contrary to this, a significantly positive relationship was found between DRB F_{ST}s and geographic distance in this species, suggesting that MHC variation may be under the action of local pathogen-mediated selection, in which populations that are spatially closer may have more similar pathogen communities. This pattern has also been described in *C. australis* for other traits that may evolve under selection, such as allozymes and skull morphology, which also show an IBD pattern (Mora *et al.* 2013b), despite the strong signal of expansion verified for mtDNA. For *C. talarum*, neither of the markers used showed a global pattern of IBD, probably reflecting the fact that different regions have experienced distinct demographic trajectories coupled with very restricted gene flow in this species, as was previously reported by Mora *et al.* (2013b), who showed that evidence of IBD was present in some regions of *C. talarum* distribution but not in others.

Regarding the strength of selection, we found that maximum-likelihood analyses of patterns of codon-based substitutions, which are less sensitive to demography and are able to detect long-term selection, provided evidence of positive selection acting on DRB exon 2 in *C. talarum*, but not in *C. australis*. Specifically, models that incorporated the action of positive selection fitted the data significantly better than simpler models in *C. talarum*, and several aminoacidic residues of the DRB exon 2 were identified as subject to positive selection, 4 of which (27, 37, 45, and 75) had already been identified in a previous study of DRB variation conducted in a single population in this species (Cutrera *et al.* 2011).

As an alternative means of detecting departures from neutrality, we used Fu's *Fs* and Tajima's *D* tests; however, for both of these analyses, rejection of the "standard neutral model" (Rosenberg and Nordborg 2002) does not necessarily imply acceptance of a selective alternative. Rejection of the neutral model may be caused by non-selective processes, such as a population bottleneck, that imitate the effects of balancing selection maintaining several alleles or classes of alleles over a long time (Kreitman 2000), or population expansion, which can simulate the outcomes of selective sweeps and selection against slightly deleterious mutations (Bertorelle and Slatkin 1995; Harpending *et al.* 1998). Therefore, applying these tests to functional and neutral markers in the same population is essential to tease apart the effects of selection versus purely demographic factors. Paleontological as well as population genetic data support the hypothesis that changes in sea level during the Quaternary made available areas of sand dune habitat for *C. australis* (Isla 1998; Isla *et al.* 2001), facilitating a geographic and demographic expansion of this species (Mora *et al.* 2006, 2007). Fu's *Fs* and Tajima's *D* values for D-loop reflected this historical scenario: they were negative in all but one of the *C. australis* populations analyzed; however, Tajima's D values were negative for DRB exon 2 in only 3 of the 7 populations analyzed in this species. On the contrary, although most *Fs* and *D* values were negative for mitochondrial genetic data in *C. talarum*, *D* values for MHC were positive in all of the populations except one, suggesting that balancing selection is the predominant force shaping MHC variation in this species, considering that the alternative demographic hypothesis (population bottleneck) is not supported by the results obtained with the neutral marker.

Evidence of balancing selection acting on MHC in *C. talarum* comes from previous studies conducted at a local scale, which documented extensive polymorphism at genes of this complex (Cutrera and Lacey 2006; Cutrera *et al.* 2010), as well as associations between MHC variation and resistance/susceptibility to common parasite infections for the species (Cutrera *et al.* 2011). Parasite-mediated balancing selection can weaken the influence of demographic history on MHC genes, as reported for bank voles (Malé *et al.* 2012) and brown hares (Koutsogiannouli *et al.* 2009). For *C. talarum*, however,

Downloaded from https://academic.oup.com/jhered/article-abstract/108/6/628/3865731/Selection-on-MHC-in-a-Context-of-Historical
by guest
on 15 September 2017

lower DRB differentiation among populations is accompanied by higher levels of DRB diversity compared to D-loop within populations, suggesting that even though MHC diversity may be driven by local host–parasite interactions (see Lion and Gandon 2015 for a review), these do not strongly promote population divergence, a pattern that has also been recently described in fish (*Poecilia mexicana*, Tobler *et al.* 2014). In fact, Cutrera and Lacey (2007) reported the persistence of very similar, or even the same, MHC variants for longer periods than expected under neutral evolution across *Ctenomys* species, resulting in a pattern of "trans-species polymorphism", which is considered another evidence of the action of balancing selection on these loci (Klein 1987). Although demographic changes associated with a postglacial expansion seem to notably influence DRB diversity in *C. australis*, much more so than in *C. talarum*, the role of selection cannot be ruled out. Besides the results of the Mantel test, which showed higher population differentiation at DRB than at D-loop, we also found that one of the alleles widely spread across populations of *C. australis*, Ctau-DRB01, is shared with other 4 species of *Ctenomys* (see Cutrera and Lacey 2007). This suggests that selection has contributed to shape MHC variation in *C. australis* as well. Therefore, although diversity at DRB exon 2 seems to be comparable between *C. australis* and *C. talarum*, patterns of population apportionment of such variation seem to differ substantially between these species, supporting distinct roles of local versus balancing selective regimes in shaping MHC structure in these species.

Conclusions

The relative influence of selection, genetic drift, and migration on MHC diversity has been explored in small mammals, with a focus on European species that have suffered demographic changes associated with glacial refugia and postglacial expansions, such as European hedgehogs (*Erinaceus concolor* and *E. europaeus*, Berggren *et al.* 2005), water voles (*Arvicola terrestris*, Bryja *et al.* 2007), brown hares (*L. europaeus*, Koutsogiannouli *et al.* 2009), and bank voles (*Myodes glareolus*; Malé *et al.* 2012). Lack of or weak spatial MHC structure among populations has been reported in comparison with neutral markers in brown hares (Koutsogiannouli *et al.* 2009) as well as in water and bank voles (Bryja *et al.* 2007; Malé *et al.* 2012), while the opposite pattern was observed in hedgehogs, in which demographic changes have been more influential on MHC than balancing selection (Berggren *et al.* 2005). Fluctuations in population size in cyclic populations of rodents and the strong genetic drift associated with these changes has also contributed to shape MHC diversity in water voles (Oliver and Piertney 2012), although these events are much less influential in the montane vole (*Microtus montanus*) MHC genes, despite the large fluctuations in population size documented for the species (Winternitz *et al.* 2014). Therefore, our findings and those described above support the idea that the relative strength of selection acting on MHC genes varies spatially and temporally within and among species, even between species that inhabit the same macrohabitat and may be exposed to similar immune challenges, as is the case for *C. talarum* and *C. australis*. The different selective models proposed to maintain the high levels of MHC diversity observed in natural populations have been extensively reviewed (Apanius *et al.* 1997; Hughes and Yeager 1998; Hedrick 2002; Piertney and Oliver 2006), with a focus on the benefits for pathogen recognition and resistance that high MHC diversity entails. More recently, the importance of preserving MHC diversity in conservation strategies has been emphasized, due to its role in disease resistance (Vásquez-Carrillo *et al.* 2014) and the severe consequences that a bottleneck may have on variation at these loci (Sutton *et al.* 2011). Our findings reinforce the need to study simultaneously the role of selection as well as historical demographic changes in modeling MHC diversity in natural populations of mammals.

Supplementary Material

Supplementary data are found at *Journal of Heredity* online.

Funding

This work was supported by CONICET (PIP 0272), Agencia Nacional de Promoción Científica (PICT 2349) to A. P. C. and Universidad Nacional de Mar del Plata (EXA 798/16) to M. S. M.

Acknowledgments

We thank F. Mapelli for assistance with sample collection in the field, the Evolutionary Genetics Lab, and the Lacey Lab at the Museum of Vertebrate Zoology, University of California at Berkeley for assistance during collection of MHC data.

References

Adelman JS. 2014. Immune systems: linking organisms, populations, and evolution through disease. In: Martin LB, Ghalambor CK, Woods H, editors. *Integrative organismal biology*. Hoboken, New Jersey: Wiley Blackwell. p. 327.

Aguilar A, Roemer G, Debenham S, Binns M, Garcelon D, Wayne RK. 2004. High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. *Proc Natl Acad Sci USA*. 101:3490–3494.

Alcaide M, Edwards SV, Negro JJ, Serrano D, Tella JL. 2008. Extensive polymorphism and geographical variation at a positively selected MHC class II B gene of the lesser kestrel (*Falco naumanni*). *Mol Ecol*. 17:2652–2665.

Apanius V, Penn D, Slev PR, Ruff LR, Potts WK. 1997. The nature of selection on the major histocompatibility complex. *Crit Rev Immunol*. 17:179–224.

Babik W, Pabijan M, Radwan J. 2008. Contrasting patterns of variation in MHC loci in the Alpine newt. *Mol Ecol*. 17:2339–2355.

Berggren KT, Ellegren H, Hewitt GM, Seddon JM. 2005. Understanding the phylogeographic patterns of European hedgehogs, *Erinaceus concolor* and *E. europaeus* using the MHC. *Heredity (Edinb)*. 95:84–90.

Bertorelle G, Slatkin M. 1995. The number of segregating sites in expanding human populations, with implications for estimates of demographic parameters. *Mol Biol Evol*. 12:887–892.

Biedrzycka A, Radwan J. 2008. Population fragmentation and major histocompatibility complex variation in the spotted suslik, *Spermophilus suslicus*. *Mol Ecol*. 17:4801–4811.

Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol*. 16:37–48.

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

Castillo AH, Cortinas MN, Lessa EP. 2005. Rapid diversification of South American tuco-tucos (*Ctenomys*; *Rodentia*, Ctenomyidae): contrasting mitochondrial and nuclear intron sequences. *J Mammal*. 86:170–179.

Congdon BC, Piatt JF, Martin K, Friesen VL. 2000. Mechanisms of population differentiation in marbled murrelets: historical versus contemporary processes. *Evolution*. 54:974–986.

Cutrera AP, Lacey EA. 2006. Major histocompatibility complex variation in talas tuco-tucos: the influence of demography on selection. *J Mammal*. 87:706–716.

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

Cutrera AP, Fanjul MS, Zenuto RR. 2012. Females prefer good genes: MHC-associated mate choice in wild and captive tuco-tucos. *Anim Behav*. 83:847–856.

Downloaded from https://academic.oup.com/jhered/article-abstract/108/6/628/3865731/Selection-on-MHC-in-a-Context-of-Historical
by guest
on 15 September 2017

Cutrera AP, Lacey EA, Mora MS, Lessa EP. 2010. Effects of contrasting demographic histories on selection at major histocompatibility complex loci in two sympatric species of tuco-tucos (*Rodentia*: Ctenomyidae). *Biol J Linnean Soc*. 99:260–277.

Cutrera AP, Zenuto RR, Lacey EA. 2011. MHC variation, multiple simultaneous infections and physiological condition in the subterranean rodent *Ctenomys talarum*. *Infect Genet Evol*. 11:1023–1036.

Cutrera AP, Zenuto RR, Lacey EA. 2014. Interpopulation differences in parasite load and variable selective pressures on MHC genes in *Ctenomys talarum*. *J Mammal*. 95:679–695.

Doherty PC, Zinkernagel RM. 1975. Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature*. 256:50–52.

Drummond AJ, Rambaut A, Shapiro B, Pybus OG. 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol Biol Evol*. 22:1185–1192.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol*. 7:214.

Ekblom R, Saether SA, Jacobsson P, Fiske P, Sahlman T, Grahn M, Kålås JA, Höglund J. 2007. Spatial pattern of MHC class II variation in the great snipe (*Gallinago media*). *Mol Ecol*. 16:1439–1451.

Ewens WJ. 1972. The sampling theory of selectively neutral alleles. *Theor Popul Biol*. 3:87–112.

Excoffier L, Laval G, Schneider S. 2007. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol Bioinform Online*. 1:47–50.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*. 147:915–925.

Garrigan D, Hedrick PW. 2003. Perspective: detecting adaptive molecular polymorphism: lessons from the MHC. *Evolution*. 57:1707–1722.

Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9. 3):485–486.

Harpending HC, Batzer MA, Gurven M, Jorde LB, Rogers AR, Sherry ST. 1998. Genetic traces of ancient demography. *Proc Natl Acad Sci USA*. 95:1961–1967.

Hedrick PW. 2002. Pathogen resistance and genetic variation at MHC loci. *Evolution*. 56:1902–1908.

Hudson RR, Slatkin M, Maddison WP. 1992. Estimation of levels of gene flow from DNA sequence data. *Genetics*. 132:583–589.

Hughes AL, Hughes MK. 1995. Natural selection on the peptide-binding regions of major histocompatibility complex molecules. *Immunogenetics*. 42:233–243.

Hughes AL, Yeager M. 1998. Natural selection at major histocompatibility complex loci of vertebrates. *Annu Rev Genet*. 32:415–435.

Isla FI. 1998. Holocene coastal evolution of Buenos Aires. *Quatern South Am Antarctic Pen* 11:297–321.

Isla FI, Cortizo LC, Turno Orellano HA. 2001. Dinámica y evolución de las Barreras Medanosas, Provincia de Buenos Aires, Argentina. *Rev Brasil Geomorfol*. 2:73–83.

Janeway CA, Travers P. 1999. *Immunobiology: the immune system in health and disease*. 4th ed. London: Current Biology.

Kimura M. 1983. Rare variant alleles in the light of the neutral theory. *Mol Biol Evol*. 1:84–93.

Klein, J. 1986. Natural history of the major histocompatibility complex.New York: Wiley.

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

Klein J, Sato A, Nagl S, O'huigin C. 1998. Molecular trans-species polymorphism. *Ann Rev Ecolog Syst*. 29:1–21.

Klein J, Horejsi V. 1997. Autoimmunity and autoimmune diseases. In: Klein J, Horeysi V, editors. *Immunology*. 2nd ed. Boston: Blackwell. p. 651–658.

Koutsogiannouli EA, Moutou KA, Sarafidou T, Stamatis C, Spyrou V, Mamuris Z. 2009. Major histocompatibility complex variation at class II DQA locus in the brown hare (*Lepus europaeus*). *Mol Ecol*. 18:4631–4649.

Kreitman M. 2000. Methods to detect selection in populations with applications to the human. *Annu Rev Genomics Hum Genet*. 1:539–559.

Lacey EA, Cutrera AP. 2007. Behavior, demography, and immunogenetic varia- tion: new insights from subterranean rodents. In: Begall S, Burda H, Schleich C, editors. *Subterranean rodents: news from underground*. Heidelberg: Springer. p. 343–356.

Lessa EP, Cook JA. 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, *Rodentia*: Octodontidae) suggests an early burst of speciation. *Mol Phylogenet Evol*. 9:88–99.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*. 25:1451–1452.

Lion S, Gandon S. 2015. Evolution of spatially structured host-parasite interactions. *J Evol Biol*. 28:10–28.

Lively CM, Dybdahl MF. 2000. Parasite adaptation to locally common host genotypes. *Nature*. 405:679–681.

Malé PJG, Martin JF, Galan M, Deffontaine V, Bryja J, Cosson JF, Michaux J, Charbonnel N. 2012. Discongruence of Mhc and cytochrome b phylogeographical patterns in *Myodes glareolus* (Rodentia: Cricetidae). *Biol J Linnean Soc*. 105:881–899.

Maruyama T, Nei M. 1981. Genetic variability maintained by mutation and overdominant selection in finite populations. *Genetics*. 98:441–459.

Meirmans PG. 2006. Using the AMOVA framework to estimate a standardized genetic differentiation measure. *Evolution*. 60:2399–2402.

Miller HC, Allendorf F, Daugherty CH. 2010. Genetic diversity and differentiation at MHC genes in island populations of tuatara (*Sphenodon* spp.). *Mol Ecol*. 19:3894–3908.

Miller SA, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 16:1215.

Mora MS, Lessa EP, Kittlein MJ, Vassallo AI. 2006. Phylogeography of the subterranean rodent *Ctenomys australis* in sand-dune habitats: evidence of population expansion. *J Mammal* 87:1192–1203.

Mora MS, Lessa EP, Cutrera AP, Kittlein MJ, Vassallo AI. 2007. Phylogeographical structure in the subterranean tuco-tuco *Ctenomys talarum* (Rodentia: Ctenomyidae): contrasting the demographic consequences of regional and habitat-specific histories. *Mol Ecol*. 16:3453–3465.

Mora MS, Cutrera AP, Lessa EP, Vassallo AI, D'Anatro A, Mapelli FJ. 2013a. Phylogeography and population genetic structure of the Talas tuco-tuco (*Ctenomys talarum*): integrating demographic and habitat histories. *J. Mammal*. 94:459–476.

Mora MS, Kittlein MJ, Vassallo AI, Mapelli FJ. 2013b. Diferenciación geográfica en caracteres de la morfología craneana en el roedor subterráneo *Ctenomys australis* (Rodentia: Ctenomyidae). *Mastozoologia Neotropical*. 20:75–96.

Nei M, Gojobori T. 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Mol Biol Evol*. 3:418–426.

Nielsen R. 2005. Molecular signatures of natural selection. *Annu Rev Genet*. 39:197–218.

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

Ohta T. 1993. An examination of the generation-time effect on molecular evolution. *Proc Natl Acad Sci USA*. 90:10676–10680.

Oliver MK, Piertney SB. 2012. Selection maintains MHC diversity through a natural population bottleneck. *Mol Biol Evol*. 29:1713–1720.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. *Bioinformatics*. 28:2537–2539.

Penn DJ, Damjanovich K, Potts WK. 2002. MHC heterozygosity confers a selective advantage against multiple-strain infections. *Proc Natl Acad Sci USA*. 99:11260–11264.

Piertney SB, Oliver MK. 2006. The evolutionary ecology of the major histocompatibility complex. *Heredity (Edinb)*. 96:7–21.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Mol Biol Evol*. 25:1253–1256.

Ramos-Onsins SE, Rozas J. 2002. Statistical properties of new neutrality tests against population growth. *Mol Biol Evol*. 19:2092–2100.

Reig OA, Busch C, Contreras J, Ortells M. 1990. An overview of evolution, systematics, population biology and molecular biology in Ctenomys. In: Nevo E, Reig OA, editors. *Biology of subterranean mammals*. New York: Allan Liss. p. 71–96.

Downloaded from https://academic.oup.com/jhered/article-abstract/108/6/628/3865731/Selection-on-MHC-in-a-Context-of-Historical
by guest
on 15 September 2017

Rosenberg MS, Anderson CD. 2011. PASSaGE: pattern analysis, spatial statistics and geographic exegesis. Version 2. *Methods Ecol Evol*. 2:229–232.

Rosenberg NA, Nordborg M. 2002. Genealogical trees, coalescent theory and the analysis of genetic polymorphisms. *Nat Rev Genet*. 3:380–390.

Schierup MH, Vekemans X, Charlesworth D. 2000. The effect of subdivision on variation at multi-allelic loci under balancing selection. *Genet Res*. 76:51–62.

Schneider S, Excoffier L. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics*. 152:1079–1089.

Seddon JM, Santucci F, Reeve NJ, Hewitt GM. 2001. DNA footprints of European hedgehogs, *Erinaceus europaeus* and *E. concolor*: Pleistocene refugia, postglacial expansion and colonization routes. *Mol Ecol*. 10:2187–2198.

Sikes RS, Gannon WL. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J Mammal*. 92:235–253.

Sutton JT, Nakagawa S, Robertson BC, Jamieson IG. 2011 Disentangling the roles of natural selection and genetic drift in shaping variation at MHC immunity genes. *Mol Ecol*. 20:4408–4420.

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

Tobler M, Plath M, Riesch R, Schlupp I, Grasse A, Munimanda GK, Setzer C, Penn DJ, Moodley Y. 2014. Selection from parasites favours immunogenetic diversity but not divergence among locally adapted host populations. *J Evol Biol*. 27:960–974.

Vásquez-Carrillo C, Friesen V, Hall L, Peery MZ. 2014. Variation in MHC class II B genes in marbled murrelets: implications for delineating conservation units. *Anim Conserv*. 17:244–255.

Vassallo AI. 1993. Habitat shift after experimental removal of the bigger species in sympatric *Ctenomys talarum* and *Ctenomys australis* (Rodentia: Octodontidae). *Behaviour*. 127:247–263.

Watterson GA. 1986. The homozygosity test after a change in population size. *Genetics*. 112:899–907.

Winternitz JC, Wares JP, Yabsley MJ, Altizer S. 2014. Wild cyclic voles maintain high neutral and MHC diversity without strong evidence for parasite-mediated selection. *Evol Ecol*. 28:957–975.

Yang J, Su AI, Li WH. 2005. Gene expression evolves faster in narrowly than in broadly expressed mammalian genes. *Mol Biol Evol*. 22:2113–2118.

Yang Z. 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. *Comput Appl Biosci*. 13:555–556.

Zenuto RR, Lacey EA, Busch C. 1999a. DNA fingerprinting reveals polygyny in the subterranean rodent Ctenomys talarum. *Mol Ecol*. 8:1529–1532.

Zenuto RR, Malizia AI, Busch C. 1999b. Sexual size dimorphism, testes size and mating system in two populations of Ctenomys talarum (Rodentia: Octodontidae). *J Nat Hist*. 33:305–314.

Zenuto RR, Vitullo AD, Busch C. 2003. Sperm characteristics in two populations of the subterranean rodent Ctenomys talarum (Rodentia: Octodontidae). *J Mamm*. 84:877–885.

Downloaded from https://academic.oup.com/jhered/article-abstract/108/6/628/3865731/Selection-on-MHC-in-a-Context-of-Historical
by guest
on 15 September 2017