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Assessing micro-macroparasite selective pressures and anthropogenic disturbance as drivers of immune gene diversity in a Neotropical wild cat

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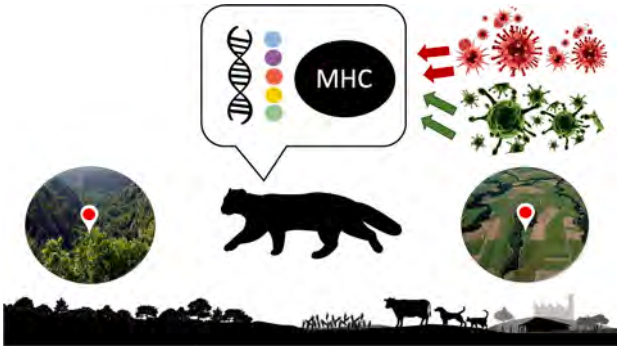
HIGHLIGHTS

- We hypothesize diversity at MHC is partly maintained by pathogen-driven selection.
- We integrated MHC diversity, landscape anthropogenic disturbance and infection data.
- Guignas in human-dominated landscapes (pathogen exposure) have higher MHC genetic diversity.
- Guignas infected with macroparasites (pathogen load) have higher MHC genetic diversity.
- Insights into changing eco-evolutionary dynamics that human activities disturb.

GRAPHICAL ABSTRACT



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ABSTRACT

Anthropogenic environmental change is reducing available habitat for wild species, providing novel selection pressures such as infectious diseases and causing species to interact in new ways. The potential for emerging infectious diseases and zoonoses at the interface between humans, domestic animals, and wild species is a key global concern. In vertebrates, diversity at the major histocompatibility complex MHC is critical to disease resilience, and its study in wild populations provides insights into eco-evolutionary dynamics that human activities alter. In natural populations, variation at MHC loci is partly maintained by balancing selection, driven by

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Chile
Genetic diversity

pathogenic selective pressures. We hypothesize that MHC genetic diversity differs between guigna populations inhabiting human-dominated landscapes (higher pathogen pressures) versus more natural habitats (lower pathogen pressures). We predict that MHC diversity in guignas would be highest in human-dominated landscapes compared with continuous forest habitats. We also expected to find higher MHC diversity in guignas infected with micro and macro parasites (higher parasite load) versus non infected guignas. We characterized for the first time the genetic diversity at three MHC class I and II exons in 128 wild guignas (*Leopardus guigna*) across their distribution range in Chile (32–46° S) and Argentina, representing landscapes with varying levels of human disturbance. We integrated MHC sequence diversity with multiple measures of anthropogenic disturbance and both micro and macro parasite infection data. We also assessed signatures of positive selection acting on MHC genes. We found significantly higher MHC class I diversity in guignas inhabiting landscapes where houses were present, and with lower percentage of vegetation cover, and also in animals with more severe cardiorespiratory helminth infection (richness and intensity) and micro-macroparasite co-infection. This comprehensive, landscape-level assessment further enhances our knowledge on the evolutionary dynamics and adaptive potential of vertebrates in the face of emerging infectious disease threats and increasing anthropogenic impacts.

1. Introduction

Anthropogenic destruction of wildlife habitat and human encroachment is threatening global biodiversity and ecosystem function, providing novel selection pressures such as infectious diseases and causing species to interact in new ways (Cunningham et al., 2017). Rapid human population expansion has caused more people to live in close contact with wildlife, livestock, and pets, which in turn creates increasing opportunities for diseases to transmit among them (Kerr et al., 2020; Leifels et al., 2022). The potential for emerging infectious diseases and zoonoses at this interface is a key global concern for the welfare of both human and animal populations (Cunningham et al., 2017). The 'One Health' unifying perspective aims to balance and optimize the health of people, animals and the environment, with the objective to prevent, predict, detect, and respond to global health threats (WHO, 2017) such as the COVID-19 pandemic (Leifels et al., 2022) or avian influenza virus (H5N1) outbreaks (Vreman et al., 2023). Therefore, understanding environmental factors shaping interactions between animals, humans and their pathogens is important to guide disease surveillance and inform management efforts and public health policy (Leifels et al., 2022).

In vertebrates, disease resilience is critically underpinned by diversity at the major histocompatibility complex (MHC). Hence, investigating patterns of MHC diversity of wild populations on the boundary of human activities can inform the eco-evolutionary dynamics that human activities disturb. MHC is the most polymorphic gene family described in vertebrates, responsible for the direct involvement in mounting adaptive immune responses (Kumánovics et al., 2003; Piertney and Oliver, 2006; Radwan et al., 2020). Peptides encoded by MHC genes are cell-surface glycoproteins that form the first line of host defense against pathogens, binding and presenting antigenic peptides to T-cell receptors to trigger the appropriate pathogen-specific immune response (Klein and Sato, 2000a, 2000b; Fleischer et al., 2022). MHC class I genes code for molecules that display intracellular peptides (e.g., viruses), whereas MHC class II molecules present extracellular peptides (e.g., bacteria, helminths) (Piertney and Oliver, 2006; Neefjes et al., 2011). MHC diversity determines the breadth of antigens that an individual is capable of responding to, and ultimately influences fitness, long-term survival and population extinction risk (Ollier et al., 2001; Quinnell et al., 2003; Sommer, 2005; Radwan et al., 2010).

The exceptionally high variation found in MHC loci is maintained by balancing selection driven mainly by pathogen selective pressures (Jeffery and Bangham, 2000; Aguilar et al., 2004; Sommer, 2005; Eizaguirre et al., 2012). Pathogen-driven selection operates when specific alleles, which confer enhanced protection to a pathogen, are favored (Radwan et al., 2020). Several mutually nonexclusive mechanisms have been proposed: heterozygosity advantage (HA) and rare-allele advantage or negative frequency-dependent selection (NFDS) (Radwan et al., 2020). Selection pressures differ among different environments within the range of a species, thereby an allele may be favored in one environment but not in another (Spurgin and Richardson, 2010). Two contrasting scenarios have been proposed to explain the relationship between MHC diversity and parasite burden: Populations with low MHC diversity would either be under relaxed selection pressure when exposed to low micro-macroparasite diversity ('Evolutionary equilibrium'), or there was a recent loss in MHC diversity leading to a lack of resistance alleles and increased micro-macroparasite burden ('Unbalanced situation') (Meyer-Lucht et al., 2010). Studies in a range of taxonomic groups have found a positive correlation between MHC diversity and parasite richness or load (fish, Wegner et al., 2003; humans, Prugnolle et al., 2005; rodents, Goüy de Bellocq et al., 2008; lizards, Radwan et al., 2014).

In the Felidae, higher levels of MHC diversity are found in exons 2 and 3 of MHC class I, and exon 2 of MHC class II-DRB, because these regions include the functionally important antigen binding sites (ABS), amino acid positions that recognize and interact directly with foreign antigens (O'Brien and Yuhki, 1999; Smith and Hoffman, 2001; Plasil et al., 2022). Diversity at MHC loci and the role of positive selection have been documented in several felid species (e.g., domestic cat, tiger, cheetah, and other non-domestic Felidae cat species; Yuhki and O'Brien, 1990, 1997, O'Brien and Yuhki, 1999, Pokorny et al., 2010, Castro-Prieto et al., 2011a, Serieys et al., 2015). Selection pressure imposed by viruses on MHC genes was recorded in free-ranging Namibian cheetahs (*Acinonyx jubatus*), where high genetic differentiation at MHC class I was observed between two populations that differed in the level of exposure to viruses (Castro-Prieto et al., 2012). This result was attributed to cheetah populations inhabiting unprotected areas bordering towns and cities, associated with higher human population densities, higher (non-vaccinated) feral and domestic cat and dog densities and thus higher exposure to viral pathogens and higher contact opportunities with cheetahs.

The small (~2 kg) wild cat guigna (*Leopardus guigna*), occupies a restricted distribution in central and southern Chile and a narrow strip of land in southwestern Argentina, closely associated with temperate rainforests of southern South America (Napolitano et al., 2014). Guignas use habitats with high-density vegetation cover >0.4 m high, including all vegetation types (henceforth "vegetation cover") (Sanderson et al., 2002; Acosta-Jamett and Simonetti, 2004). This species can adapt to human-dominated areas using vegetation corridors and dense understory to move across fragmented landscapes (Sanderson et al., 2002; Acosta-Jamett and Simonetti, 2004; Gálvez et al., 2013), even using remnant forest strips along vineyard borders (García et al., 2021). Threats to guignas include land-use change, habitat loss and fragmentation, direct persecution as retaliation for poultry depredation, attacks by domestic dogs, and pathogens transmitted by domestic cats and dogs (Sanderson et al., 2002; Gálvez et al., 2013; Mora et al., 2015; Napolitano et al., 2015b; Sacristán et al., 2019a, 2020, 2021a, 2021b; Sieg et al., 2020; Ortega et al., 2021). The species is currently classified by the IUCN Red List as Vulnerable (Napolitano et al., 2015a). Guigna populations inhabiting fragmented human-dominated landscapes were

found to have reduced genetic diversity (based on neutral microsatellite loci and mitochondrial DNA) and increased dispersal (Napolitano et al., 2015b). The propensity for guignas to increase dispersal in human-dominated landscapes may lead to higher probabilities of pathogen transmission among guignas and domestic carnivores from nearby human dwellings (López-Jara et al., 2021; Sacristán et al., 2019b, 2021a, 2021b; Sieg et al., 2020; Busch et al., 2021). Low genetic diversity has been described for other fragmented wildlife populations (Radespiel and Bruford, 2014), which may render these populations particularly susceptible to infectious diseases, resulting in epidemics that have the potential to cause local extinctions (Meli et al., 2010). Given that deforestation and human encroachment into wild habitats are increasing worldwide and including in the Chilean temperate rainforest (Wilson et al., 2005; Echeverría et al., 2006), subsequent contact and exposure of wild animals with domestic pathogens might be an increasing threat. In Chile, pathogen infection and prevalence in wild guignas have been significantly associated with human landscape alteration and the presence of domestic carnivores (Sacristán et al., 2021a, 2021b; Ortega et al., 2021). In this context, assessing genetic diversity in these populations at functional genes responsible for the adaptive immune response may inform the mechanisms by which this species is adapting to changing environments.

Here we study variation at MHC class I and class II loci in free-ranging guigna across its distribution in Chile and Argentina, comparing its diversity among landscapes with varying levels of anthropogenic disturbance. We also integrated MHC diversity with both micro and macro parasite infection data (parasite load). Assuming pathogen-driven selection pressures maintain MHC genetic diversity, we assessed whether micro and macro parasite infection and human landscape alteration (as a proxy for domestic animal presence and higher parasite exposure) are imposing differential selection pressures upon host immune-genes. We hypothesized that MHC genetic diversity differs between guigna populations inhabiting human-dominated landscapes with higher pathogen pressures and those inhabiting more natural habitats with lower pathogen pressures (i.e., the 'Evolutionary equilibrium' scenario). It is likely that guigna populations in closer proximity to humans and their domestic carnivores (dogs and cats) and livestock are at greater risk for pathogen exposure. Specifically, we predicted that MHC diversity of guigna populations would be highest in human-dominated landscapes compared with continuous forest habitats. We also expected to find higher MHC diversity in guignas infected with micro and macro parasites (higher parasite load) versus non infected guignas. We also evaluated evidence of positive selection (i.e., both balancing and directional selection; Hedrick, 2007) acting on MHC genes in the studied populations.

2. Methods

2.1. Study area

The study area encompassed the entire current distribution range of guigna, including the two described subspecies, *Leopardus guigna tigrillo* and L. *g. guigna*, a range of bioclimatic regions (Mediterranean and Temperate macrobioclimates (Luebert and Pliscoff, 2018)) in central and southern Chile (32°-46°S) and a small strip of land in southwestern Argentina (Napolitano et al., 2014) (Fig. 1). Sample sites included a gradient of landscapes varying in anthropogenic disturbance, from continuous native forest with no human presence (no feral dogs or cats), to human-dominated landscapes with higher human densities (fragments of remnant forest surrounded by a matrix of agriculture and livestock activities and households with domestic cats and dogs) (maximum 6 households/ha). Guignas were sampled across five geographic regions: Central Chile (Valparaíso, Metropolitan, O'Higgins, Maule and Ñuble regions; from 32° to 37°S), Southern Chile (Bío-Bío, Araucanía, Los Ríos and Los Lagos regions; 37–43°S), Chiloé Island (41–43°S), Extreme southern Chile (Aysén region; 43–48°S) and

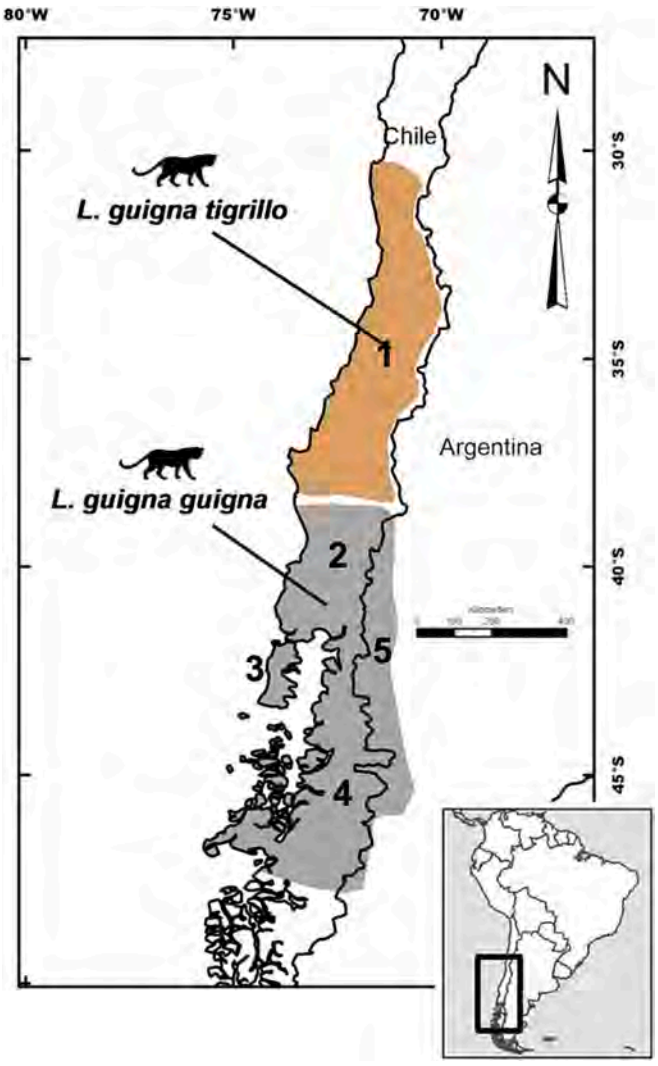

Fig. 1. Study area showing guigna complete distribution range in Chile and Argentina and geographic groups sampled. Brown area = *L. g. tigrillo* subspecies distribution. Grey area = *L. g. guigna* subspecies distribution. 1. Central Chile (*L. g. tigrillo*), 2. Southern Chile (*L. g. guigna*), 3. Chiloé Island (*L. g. guigna*), 4. Extreme southern Chile (*L. g. guigna*), and 5. Argentina (*L. g. guigna*).

Argentina (39–46°S, west of 70°W). Geographic groups are based on previous guigna population genetic and phylogeographic baseline information across the range (Napolitano et al., 2014).

2.2. Sample collection

Between 2008 and 2018, free-ranging guignas were sampled either (i) using tomahawk-like live traps (whole blood samples), (ii) after being received injured in wildlife rescue and rehabilitation centers (whole blood samples), (iii) by conducting complete necropsies on road-killed animals or euthanized at WRRC (tissue samples: liver, kidney or muscle), or (iv) from pelts found in local communities or confiscated (skin fragments). Guigna captures and tissue collection were conducted using established methods (Napolitano et al., 2015b; Sacristán et al., 2021b), following handling and supervision protocols within bioethical and animal welfare frameworks, with permission from the Chilean Agriculture and Livestock Service (SAG) (capture permits 814/13 2008, 109/9 2009, 1220/22 2010, 1708/26 2010, 7624/2015, 2288/2016, 2185/2017, 4072/2018) and the approval of an Animal Ethics Committee (Institute of Ecology and Biodiversity, resolution 20 November 2015).

Felids have relatively wide home ranges and are territorial (adult

males maintain exclusive intraspecific territories; female home ranges occur within male home ranges), therefore sampled individuals in this study are probably residents (with the exception of dispersing subadults). Thus, the movement of resident adults from a natural to a human-altered area, or vice versa, is not expected to be frequent.

For each guigna, sex, age range (estimated from dentition (Crowe, 1975); juvenile, adult), date and GPS location of collection/capture were recorded. Samples were stored frozen at −20 °C until molecular analysis.

2.3. DNA extraction and amplicon sequencing

Total DNA was extracted using a commercial kit (DNeasy Blood & Tissue kit, Qiagen®), following the instructions from the manufacturer. DNA quantification was performed using the Qubit™dsDNA HS Assay Kit and a Qubit 3.0 fluorometer. Samples were normalized to a DNA concentration of 5 ng/μL. Next-generation sequencing libraries and bioinformatic analyses were performed in the AUSTRAL-*omics* core research facility at Universidad Austral de Chile (Valdivia, Chile). MHC exons were amplified using the specific primers a1-F, a1-R (class I exon 2) and a2-F, a2-R (class I exon 3) described in Sachdev et al. (2005), primers 2F, 2R (class II-DRB exon 2) described in Castro-Prieto et al. (2011a), and DRB219m-F, DRB61a-R (class II-DRB exon 2) described in Yuhki and O'Brien (1997). Additionally, sequences were complemented with the design described in Fadrosh et al. (2014) containing a linker sequence optimized for sequencing on Illumina, an index sequence and a heterogeneity spacer. Library construction followed the Illumina protocol 16S Metagenomic Sequencing Library. Amplicon sequencing was performed using 2 × 250-bp paired-end sequencing on an Illumina MiSeq sequencer (Illumina, San Diego, CA).

2.4. Genetic analysis

Raw sequences were filtered according to their quality (q-value>33), using PRINSEQ v0.20.4 (Schmieder and Edwards, 2011). Paired-end reads were assembled using PANDASeq v2.9 (Masella et al., 2012). Sequences were demultiplexed and variant depths were quantified among amplicons, along with removal of barcodes and priming sequences in jMHC v1.0.471 (Stuglik et al., 2011).

Sequences were checked with AmpliCHECK (Amplicon Sequencing CHECKing tool) v8 (Sebastian et al., 2016) which performs an analysis retrieving length, coverage and frequency of the most abundant variants in each amplicon, to explore sources of possible artifacts and set appropriate clustering and filtering parameters. We removed individuals with fewer than 50 reads and variants with fewer than 10 reads, and set thresholds of 100 minimum amplicon depth and 3 % minimum per amplicon frequency (PAF).

Sequences were then analyzed using AmpliSAS (Amplicon Sequencing ASsignment tool) v8 (Sebastian et al., 2016). For MHC class II-DRB exon 2, for which we amplified loci using 2 sets of primers, we analyzed this data additionally with AmpliCOMBINE v8 (Sebastian et al., 2016) and AmpliCOMPARE v8 (Sebastian et al., 2016).

Multiple sequence alignments were conducted using CLUSTAL W algorithm in Geneious prime v2022.1.1 (Biomatters, Auckland, New Zealand). MHC nucleotide sequences were converted to their respective amino acid sequences with Geneious prime. To characterize the genetic diversity of MHC, we estimated descriptive diversity indices among nucleotide sequences with DNAsp (5.1 (Librado and Rozas, 2009) and MEGA v5 (Tamura et al., 2011).

We explored evidence of positive selection across entire MHC exons using a gene-wide likelihood ratio test using BUSTED v4.1 (Branch-site Unrestricted Statistical Test for Episodic Diversification) (Murrell et al., 2015) in Datamonkey (Weaver et al., 2018). We also explored a site-by-site test for positive selection using software MEME (Mixed Effects Model of Evolution) (Murrell et al., 2012) in Datamonkey. MEME employs a mixed-effects maximum likelihood approach to test the hypothesis that individual sites have been subject to episodic positive or diversifying selection. MEME aims to detect sites evolving under positive selection on a proportion of branches (Murrell et al., 2012). We then compared sites under selection with putative antigen recognition sites according to domestic cat MHC (Smith and Hoffman, 2001; Yuhki et al., 2008).

Positive selection was further investigated through the ratio of nonsynonymous to synonymous substitution rates (ω = dN/dS). Positive selection is implicated when ω > 1 (diversity at the amino acid level is favored, likely due to the fitness advantage provided by the mutations). Large dN/dS ratios suggest that adaptive genetic variations have been generated and fixed at a high rate. Purifying selection is implicated when ω < 1 (most amino acid changes are deleterious and, therefore, are selected against).

2.5. Landscape analyses

To describe landscape features associated with MHC diversity in guigna, for each sample location we generated a circular buffer area with QuantumGIS 2.14® (QGIS Development Team), corresponding to the mean home range of the species for females (170 ha) and males (446 ha), according to sample sex (Dunstone et al., 2002; Sanderson et al., 2002; Schüttler et al., 2017).). Within each buffer area, we quantified the following biologically relevant landscape variables: (1) percentage of vegetation cover (Hansen et al., 2013) (proportion (%) of remnant vegetation cover within the buffer, including all vegetation types >0.4 m high: native forest, thicket, exotic pine plantations if present), (2) presence/absence of houses (as a proxy for the presence of domestic dogs and cats), (3) number of houses (continuous effect associated with degree of exposure), (4) house density (houses/ha; number of houses/buffer area), (5) distance to the nearest house, and (6) a categorical variable land use with three levels: (i) complete vegetation cover (e.g. continuous native forest) (100 % vegetation cover within the buffer, including all vegetation types >0.4 m high), (ii) partial vegetation cover (e.g. fragments of remnant forest surrounded by a matrix of agriculture and/or livestock activities) (1–99 % vegetation cover within the buffer, including all vegetation types >0.4 m high), (iii) no vegetation cover (e.g. agricultural or developed area; Beltrami et al., 2021) (0 % vegetation cover within the buffer, for all vegetation types >0.4 m high). GIS layers were obtained from the Ministerio de Bienes Nacionales website. QGIS 2.14® was used to extract the attribute values of landscape variables from spatial analysis.

We tested collinearity among predictors by running bivariate Pearson correlations among pairs of variables using a threshold of $r > 0.70$ (p-value <0.05). No collinearity among predictors was found. To address spatial autocorrelation in our data, we conducted a Global Moran's I test using ArcGIS Pro. The result was not statistically significant (Moran's index = 0.38, z-score = 0.46, p-value = 0.64) suggesting there is no spatial clustering of data.

2.6. Micro-macroparasite infection

To understand selection processes acting on MHC loci under natural conditions in guignas, we studied the relationship between individual MHC genotype and micro and macro parasite infection. To investigate associations between MHC diversity and microparasite infection status we used data from previous studies assessing feline leukemia virus (FeLV) (21/102, 20.6 % observed prevalence) and feline immunodeficiency virus (FIV) (3/102, 3.0 %) (Mora et al., 2015; Sacristán et al., 2021a), Carnivore protoparvovirus-1 (13/98, 13.3 %) (Sacristán et al., 2021b), hemoplasma (24/102, 23.5 %) (Sacristán et al., 2019a) and feline paramyxovirus (11/35, 31.4 %) (Sieg et al., 2020) infection in guignas. We recorded infection as PCR-positive individuals with successfully sequenced amplicons.

We also studied possible associations among MHC diversity and macroparasites (gastrointestinal (GI) and cardiorespiratory (CR)

parasitism) in guigna using infection data from a previous study (Acuña et al., 2020). The data were collected by direct analysis of the organs (i.e., adult helminth detection). At the individual level, we used information on helminth infection status (being infected or not), intensity of infection (number of parasites/individual), and parasite species richness of helminths. These counts reflect the overall worm burden and worm fecundity, influenced by the host immune system (Meyer-Lucht et al., 2010). This dataset included 33 guignas, 81.8 % positive for helminth endoparasites. In the studied animals, 14 parasite species were identified, with intensity of infection ranging from 0 to 152, and 76 % rate of multiparasitism (Acuña et al., 2020).

We evaluated possible associations among MHC diversity (response variable) and our predictors of co-infection status, the number of different micro and/or macroparasite simultaneously infecting the host, including the 5 microparasites assessed: FeLV, FIV, hemoplasma, Carnivore protoparvovirus-1 and feline paramyxovirus, in addition to the 14 macroparasite (helminth) species identified. Overall, 33 % (29/88) of the screened guignas were co-infected with between two and eight different micro and/or macroparasites.

2.7. Statistical analysis

We explored associations among genetic diversity at the three MHC exons, Class I exon 2 (C1-E2), Class I exon 3 (C1-E3), Class II exon 2 (C2-E2), and the study predictor variables. For all models, our response variable was the number of MHC alleles per individual. Separate models were fitted for each MHC exon. All statistical analyses were performed in R v4.2.3 (R Core Team, 2023) through RStudio v2022.07.2 (RStudio Team, 2020) or the interface implemented in Infostat (Di Rienzo et al., 2019) with a statistical significance threshold of $\alpha < 0.05$. We attempted to fit generalized linear models (GLMs) with Poisson distribution and log function, but the models did not converge. We visually examined the distribution of our response variable (Fig. S1), and decided to proceed with linear models (LM, Gaussian link), which converged successfully. Our data composition is unbalanced: sample sizes vary substantially among variables due to the opportunistic nature of data collections. Therefore, in order to best facilitate comparisons and inferences across our hypotheses and across the study as a whole, we favored taking a standardized approach for all analyses using simple bivariate modelling.

Prior to investigating our main predictors of interest (landscape and parasite variables), we first determined whether it was necessary to include additional covariates of sampling year, sex, geographic group (1–5: Central Chile, Southern Chile, Chiloé Island, Extreme southern Chile and Argentina), the categorical variable 'Island versus Continent' (Chiloé Island compared to the other four groups), and subspecies as predictors of immune gene diversity ($n = 125$; Tables 3, S3). We found no important effects of year, sex, geographic group, or subspecies (see Section 3 Results), so these variables were not included in subsequent modelling. We did find a difference in MHC diversity between Chiloé Island versus continent (see Results), and so considered this comparison in our further analyses.

To determine whether landscape and anthropogenic disturbance can predict MHC diversity, we examined the predictors of percentage vegetation cover, presence/absence of houses, number of houses (log transformed), distance to the nearest house and land use (complete, partial or no vegetation cover). For each datapoint (i.e., guigna), our predictor variables were calculated within the individual circular buffers (see Section 2.5. Landscape analyses) of the animal's capture/sampling site ($n = 125$; Tables 3, S7).

For microparasites, our predictors were infection status (PCR-positive/negative) of FeLV ($n = 84$), Carnivore protoparvovirus-1 ($n = 85$), hemoplasma ($n = 83$) and feline paramyxovirus ($n = 31$) (Table S8). FIV was not included in further analysis due to its low observed prevalence in our dataset (3.0 %). For macroparasites (helminths), we tested whether there was an effect of infection status (infected/not infected) and intensity of infection (number of parasites/individual) both overall and separately for GI and CR helminths, and also of overall parasite species richness, on MHC diversity ($n = 29$; Tables 3, S8). We also tested the effect of the predictor co-infection, the number of all micro and/or macroparasites infecting individual guignas, on MHC diversity. For co-infection models we only included animals that were screened for all 5 microparasites and helminths ($n = 28$; Tables 3, S8).

3. Results

A total of 139 free-ranging guignas were sampled. Eleven samples failed to pass QC at sequencing or filtering steps, and were removed from our dataset. The 128 guignas included in subsequent analysis included 54 whole blood samples (tomahawk-like live traps, $n = 43$; WRRC, $n = 11$), 45 tissue samples (liver, kidney or muscle), and 29 skin fragments (pelts). A total of 46 females and 66 males (no sex available for 16 animals), and 50 adults and 18 juveniles were sampled (no age available for 60 animals). Guignas were sampled in landscapes with complete vegetation cover (29/128, 23 %), partial vegetation cover (88/128, 68 %), and no vegetation cover (11/128, 9 %) land use categories.

3.1. Genetic diversity

After filtering, we obtained 492,913 reads for MHC class I exon 2 (216 bp), 256,682 reads for MHC class I exon 3 (202 bp) and 2,003,151 reads for MHC class II-DRB exon 2 (238 pb). Per individual, we obtained a mean of 554 reads (± 349; min 10; max 3134) for class I exon 2, 632 reads (± 320; min 10; max 4354) for class I exon 3, and 988 reads (±557; min 10; max 6962) for class II exon 2. From all sampled guignas, for MHC Class I exon 2 (216 pb, $n = 73$) we found a total of 18 alleles, average 5.9 alleles per individual (3 min, 9 max; SD = 1.4), a minimum of 5 *co*-amplifying loci and 51 polymorphic sites. For MHC Class I exon 3 (202 bp, $n = 81$), we observed a total of 12 alleles, average 3.7 alleles per individual (1 min, 6 max; SD = 1.19), a minimum of 3 *co*-amplifying loci and 30 polymorphic sites. For MHC Class II-DRB exon 2 (238 bp, $n = 125$) we observed a total of 26 alleles, average 5.03 alleles per individual (2 min, 10 max; SD = 1.63), minimum 5 *co*-amplifying loci and 109 polymorphic sites (Table 1).

MHC class II-DRB exon 2 had higher genetic diversity than MHC class I exons 2 or 3, in terms of polymorphic sites, nucleotide diversity and average number of differences between sequences, even when considering diversity retrieved with only one set of primers (Tables 2, S1, S2). For MHC class II-DRB exon 2, we retrieved more alleles using two sets of primers combined compared to just one (Tables S1, S2).

Alleles were more or less evenly distributed across geographic groups for all of the exons (Fig. 2). Private alleles for MHC class I exon 2 were found only in the South group ($n = 2$), for MHC class I exon 3 only in the Central ($n = 2$) and South groups ($n = 1$) and for MHC class II-DRB in all geographic groups (range 1–4) (Table 2).

Genetic diversity was relatively similar among geographic groups,

Table 1
Overall genetic diversity for each studied MHC exon in guignas across populations.

MHC exon (base pairs)	*n*	Total # alleles	Average # alleles per individual (min-max)	Min. number of co-amplifying loci	# polymorphic sites
Class I exon 2 (216)	73	18	5.90 (3–9)	5	51
Class I exon 3 (202)	81	12	3.70 (1–6)	3	30
Class II-DRB exon 2 (238)	125	26	5.03 (2–10)	5	109

Table 2
Genetic diversity among haplotypes/alleles for each studied MHC exon per geographic group. *L. g. tigrillo* subspecies = Central population; *L. g. guigna* subspecies = South, Chiloé Island, Extreme south and Argentina populations. Abbreviations: *n* = sample size; A = average number of alleles per individual; SD = standard deviation of number of alleles per individual; max A = maximum number of alleles per individual; min A = minimum number of alleles per individual; S = number of polymorphic sites; Syn/NonSyn = number of synonymous and nonsynonymous sites; h = number of haplotypes/alleles; pi = nucleotide diversity; K = average number of differences between sequences.

Geographic group	*n*	A	SD	max A	min A	Private alleles	S	Syn/ NonSyn	h	pi	K
MHC class I exon 2											
Central	23	5.70	1.10	8	5	0	48	55.10/ 136.90	12	0.08680	17.273
South	17	5.90	1.70	9	3	2	51	55.25/ 136.75	18	0.08661	17.235
Chiloé Island	23	5.96	1.50	9	3	0	48	58.96/ 145.04	12	0.08901	17.712
Extreme South	9	6.00	1.40	8	3	0	42	54.94/ 137.06	12	0.07827	15.576
Argentina	1	9.00	–	–	–	0	45	59.04/ 144.96	9	0.08836	17.583
TOTAL	73	5.90	1.40	9	3	2	51	55.25/ 136.75	18	0.08081	17.431
MHC class I exon 3											
Central	29	4.28	1.07	6	2	2	25	47.88/ 150.12	11	0.05635	11.382
South	18	4.10	1.20	6	2	1	29	48.03/ 149.97	10	0.06205	12.533
Chiloé Island	25	2.88	0.60	4	2	0	22	48.00/ 150.00	7	0.05658	11.429
Extreme South	7	3.70	1.70	6	1	0	22	48.00/ 150.00	7	0.05658	11.429
Argentina	2	3.50	0.71	4	3	0	20	47.75/ 150.25	4	0.05116	10.333
TOTAL	81	3.70	1.19	6	1	3	30	47.97/ 150.03	12	0.06098	10.804
MHC class II-DRB exon 2											
Central	40	5.10	1.85	10	2	4	69	56.26/ 180.74	17	0.11856	28.218
South	25	4.88	1.39	8	3	4	88	56.33/ 180.67	17	0.13109	31.199
Chiloé Island	40	5.20	1.49	9	3	1	69	56.54/ 180.46	14	0.11755	27.978
Extreme South	18	4.83	1.86	8	2	2	82	56.69/ 180.31	14	0.12661	30.133
Argentina	2	4.00	0.00	4	4	1	51	57.67/ 179.33	6	0.10588	25.200
TOTAL	125	5.03	1.63	10	2	12	109	56.24/ 180.76	26	0.14546	34.618

except for a significantly lower number of alleles per individual for Chiloé Island group at MHC class I exon 3 (C1-E3) (estimate = −1.40, *p*-value = <0.0001) (Table 3, Fig. 3-G,H). MHC diversity showed no statistically significant differences between males and females, and did not change over time (years) (Table S3).

3.2. Positive selection

We tested for evidence of selection across entire MHC exons, and found gene-wide positive selection in MHC class I exon 3 (Log (L) = −462.568, AIC = 1016.68, Mean = 2.095, CoV = 2.126, *p*-value = 0.0022) and MHC class II-DRB exon 2 (Log (L) = −1468.40, AIC = 3084.18, Mean = 0.8782, CoV = 1.037, *p*-value = 0.0429). No evidence for gene-wide positive selection was found in MHC Class I exon 2 (Log (L) = −701.904, AIC = 1523.23, Mean = 1.189, CoV = 0.3428, *p*-value = 0.4138) (Table 4).

In both exons under selection, we explored site-by-site tests for selection and identified nine codons putatively under positive selection (2 sites in class I exon 3, 7 sites in class II-DRB exon 2), with partial coincidence (5 of 28 sites) with putative antigen recognition sites in domestic cat MHC (Smith and Hoffman, 2001; Yuhki et al., 2008) (Fig. 4, Tables S4, S5, S6).

3.3. Landscape associations

We found a significantly higher number of MHC alleles per individual in guignas inhabiting landscapes with houses present (MHC Class I exon 3; C1-E3) (estimate = 0.720; *p*-value = 0.038) and with a lower percentage of vegetation cover (C1-E3) (estimate = −0.010; *p*-value = 0.026) (Table 3, Fig. 3-A,B). Associations among MHC diversity and other landscape and anthropogenic disturbance variables were non-significant (Table S7).

Since Chiloé Island had lower MHC diversity at class I exon 3, we compared the Chiloé Island group with the rest of the continental geographic groups regarding the predictors presence of houses and vegetation cover. We found no statistically significant differences (C1-E3 dataset; Table S9). We also explored linear mixed models including the categorical variable 'island versus continent' as a random factor, but some models failed to converge (Tables S10, S11, S12).

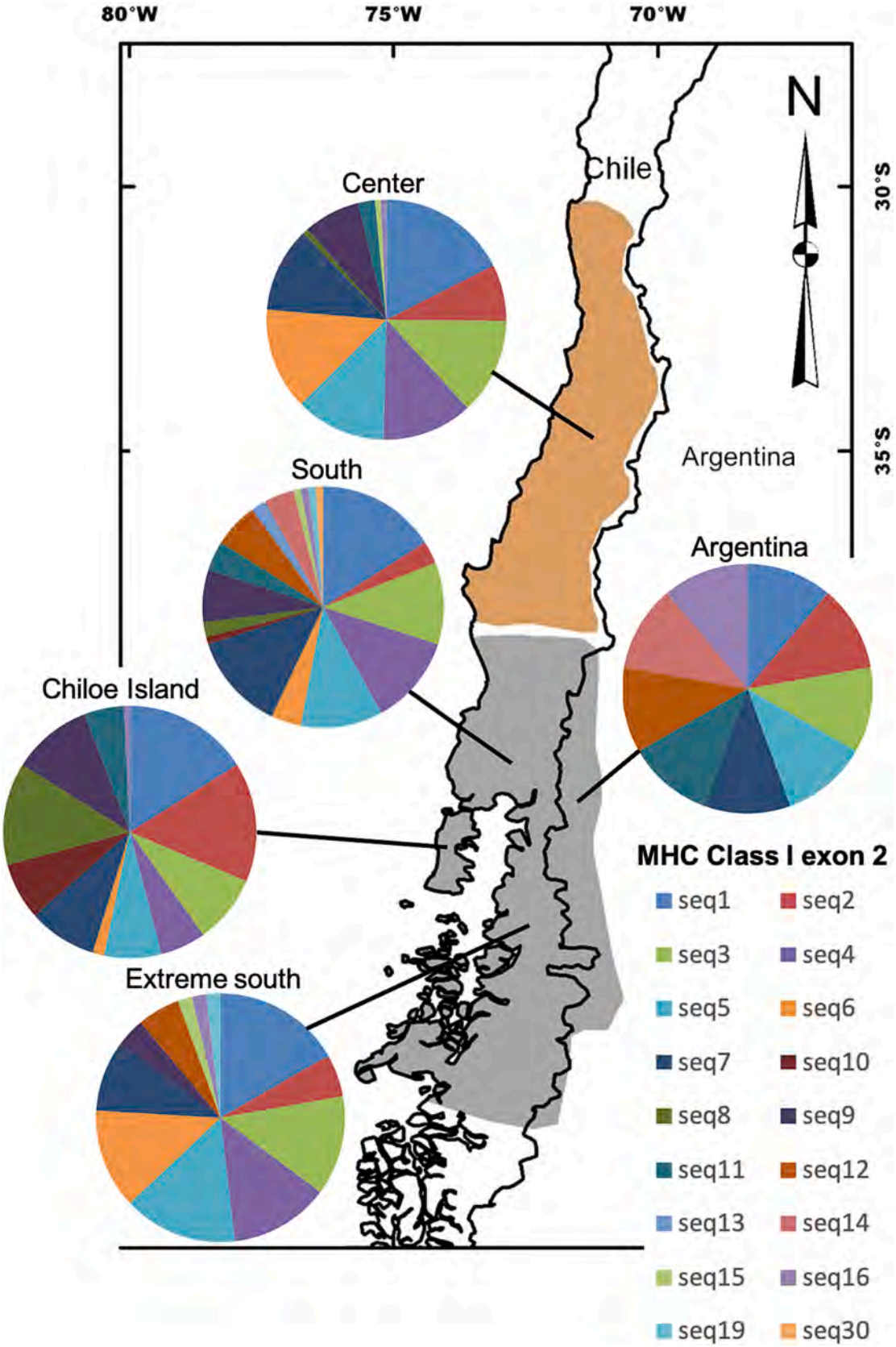

Fig. 2. Geographic distribution of genetic diversity at MHC Class I exon 2 across L. *guigna* geographic groups. The distribution and frequencies of 18 different MHC alleles are shown by the pie charts. Similar patterns were observed for the other two studied exons. Brown area = *L. g. tigrillo* subspecies distribution. Grey area = *L. g. guigna* subspecies distribution.

Table 3
Statistically significant predictor effects on guigna MHC diversity (response variable) based on linear modelling. All the remaining models are shown in Tables S3, S7, S8.

Predictor	Var Type Pred	Response	Var type Resp	Parameter	Estimate	Std error	*p*-value	*n*
Landscape and anthropogenic disturbance variables								
Percentage of vegetation cover	Continuous	A (C1-E3)	Count	Intercept	4.21	0.24	< 0.0001	81
				vegcov	−0.01	0.0044	**0.0261**	
Presence/ absence of houses	Cat, Binary	A (C1-E3)	Count	Intercept	3.14	0.31	< 0.0001	81
				Preshouse (n = 67)	0.72	0.34	**0.0382**	
Parasite infection variables								
Total presence / absence of CR helminths	Cat, Binary	A (C1-E2)	Count	Intercept	5.17	0.16	<0.0001	14
				PApresCR1 (n = 2)	1.83	0.42	**0.0010**	
Intensity of infection with CR helminths	Continuous	A (C1-E2)	Count	Intercept	5.16	0.11	<0.0001	14
				PAloadCR	0.94	0.13	**<0.0001**	
Helminth species richness	Continuous	A (C1-E2)	Count	Intercept	4.77	0.33	<0.0001	14
				PAsp.richness	0.26	0.11	**0.0299**	
Coinfection	Continuous	A (C1-E2)	Count	Intercept	4.40	0.38	<0.0001	13
				Coinfection	0.28	0.09	**0.0091**	
Geographic variables								
Geographic group	Categorical	A (C1-E3)	Count	Intercept	4.28	0.20	<0.0001	81
				pop2 (n = 18)	−0.16	0.32	0.6033	
				pop3 (n = 25)	−1.40	0.29	**<0.0001**	
				pop4 (n = 7)	−0.56	0.44	0.2089	
				pop5 (n = 2)	−0.78	0.77	0.3164	
Geographic groups: continent versus island	Cat, Binary	A (C1-E3)	Count	Intercept	4.13	0.14	<0.0001	81
				popCI1 (n = 25)	−1.25	0.25	**<0.0001**	

Var type pred = Variable type predictor; Var type resp. = variable type response; A = number of alleles per individual; n = sample size. Significant p-values (<0.05) shown in bold. Cat = Categorical; Std = Standard; vegcov = percentage of vegetation cover; preshouse = presence/absence of houses (0 = absence; 1 presence); PA = Macroparasite (helminth); CR = Cardiorespiratory; PApresCR = Total presence/absence of CR PA (0 = negative, 1 = positive); PAloadCR = Load of CR PA (intensity of infection); PAsp.richness = PA species richness; Coinfection = number of simultaneous infections with micro-macroparasites; pop = Geographic group (1 = Central, 2 = South, 3 = Chiloé Island, 4 = Extreme South, 5 = Argentina); popCI = Geographic groups continent (pop 1,2,4,5) versus island (pop 3) (0 = continent, 1 = island). Predictor n = number of entries/values for each predictor.

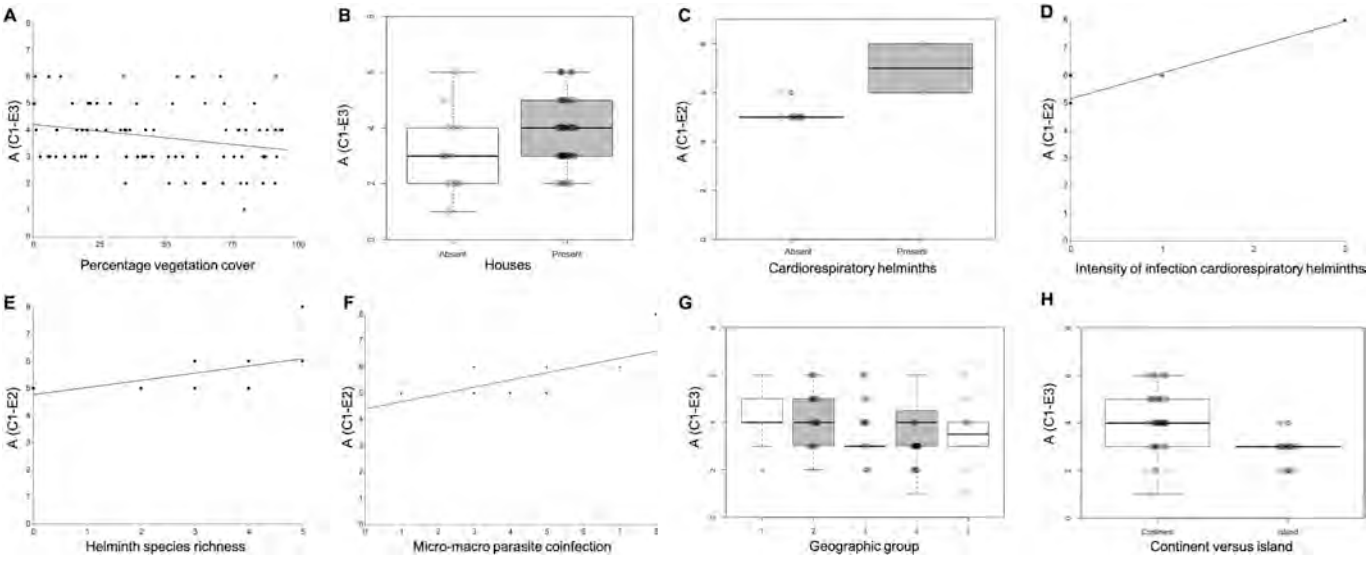

Fig. 3. Statistically significant relationships among predictor effects and MHC diversity responses (Linear models). Panels A to H: (A) Percentage of vegetation cover (C1-E3), (B) Presence/absence of houses (C1-E3), (C) Presence/absence of CR helminths (C1-E2), (D) Load (intensity of infection) of CR helminths (C1-E2) (n = 14), (E) Helminth species richness (C1-E2), (F) Micro-macro parasite co-infection (C1-E2), (G) Geographic group (C1-E3) (1: Central, 2: South, 3: Chiloé Island, 4: Extreme south, 5: Argentina), (H) Continent versus island (C1-E3). All Y axes: A = number of alleles per individual for specific exons (C1-E2 = class I exon 2; C1-E3 = class I exon 3); CR = cardiorespiratory. Lines were fitted using a Linear model (Parameters can be found in Table 4). Box plots (B,C,G,H): boxes represent 25 %–75 % values of data set, whiskers represent minimum - maximum values, black dots represent the mean. Full modelling results are presented in Tables S7 and S8.

3.4. Micro-macroparasite infection associations

For microparasites, specifically FeLV (n = 84), Carnivore protoparvovirus-1 (n = 85), hemoplasmas (n = 83) and feline paramyxovirus (n = 31), we found no statistically significant associations between infection status and MHC diversity (Table S8). For macroparasites, we found a significantly higher number of MHC alleles per individual in guignas infected with CR helminths (MHC Class I exon 2; C1-E2) (estimate = 1.83; p-value = 0.0010), with a higher load of CR helminths (intensity of infection) (C1-E2) (estimate = 0.94; p-value =

Table 4
Tests for gene-wide positive selection in guigna MHC exons studied. Rate distribution column shows the percentage frequency distribution (percentage of observations for each value), the mean and coefficient of variation for each estimate.

Model	Log (L)	AIC-c	Params.	Rate distribution
MHC class I exon 2				
Unconstrained	−701.904	1523.23	57	Tested ω 0.9570 (57.054 %), 1.000 (20.311 %), 1.941 (22.635 %) Mean = 1.189, CoV = 0.3428
Unconstrained				Synonymous rates 0.000 (39.082 %), 1.509 (56.056 %), 3.166 (4.8618 %) Mean = 1.000, CoV = 0.8743
Constrained	−702.093	1521.42	56	Tested ω 1.000 (11.438 %), 1.000 (41.804 %), 1.000 (46.758 %) Mean = 1.000, CoV = 0.000
Constrained				Synonymous rates 0.000 (39.472 %), 1.512 (55.958 %), 3.371 (4.5700 %) Mean = 1.000, CoV = 0.8934
MHC class I exon 3				
Unconstrained	−462.568	1016.68	43	Tested ω 0.000 (81.883 %), 0.1975 (0.000 %), 11.56 (18.117 %) Mean = 2.095, CoV = 2.126
Unconstrained				Synonymous rates 0.000 (65.805 %), 0.6048 (30.802 %), 23.98 (3.3930 %) Mean = 1.000, CoV = 4.316
Constrained	−467.994	1025.28	42	Tested ω 0.000 (62.171 %), 0.1883 (0.000 %), 1.000 (37.829 %) Mean = 0.3783, CoV = 1.282
Constrained				Synonymous rates 0.000 (67.220 %), 1.363 (29.224 %), 16.92 (3.5560 %)= Mean = 1.000, CoV = 3.118
MHC class II-DRB exon 2				
Unconstrained	−1468.40	3084.18	71	Tested ω 0.000 (51.823 %), 0.9994 (0.000 %), 1.823 (48.177 %) Mean = 0.8782, CoV = 1.037
Unconstrained				Synonymous rates 0.1717 (44.900 %), 0.9139 (35.004 %), 3.001 (20.096 %) Mean = 1.000, CoV = 1.056
Constrained	−1470.86	3086.94	70	Tested ω 0.000 (34.895 %), 0.9202 (0.000 %), 1.000 (65.105 %) Mean = 0.6511, CoV = 0.7321
Constrained				Synonymous rates 0.1874 (46.611 %), 0.9596 (33.902 %), 3.014 (19.488 %) Mean = 1.000, CoV = 1.048

<0.0001) and with higher helminth species richness (C1-E2) (estimate = 0.26; p-value = 0.0299) (n = 29) (Table 3, Fig. 3-C,D,E,F). For micro-macroparasite co-infection, we found a significantly higher number of MHC alleles per individual in guignas with higher number of simultaneous infections (MHC Class I exon 2; C1-E2) (estimate = 0.28; p-value = 0.0091) (n = 28) (Table 3, Fig. 3-F). Associations among MHC diversity and other micro-macroparasite infection variables were non-significant (Table S8).

4. Discussion

In this study, we integrated MHC sequence diversity with multiple measures of anthropogenic disturbance at the landscape scale and micro-macroparasite infection data. We tested the hypothesis that human-dominated landscapes, with greater densities of domestic and companion animals, presented increased novel pathogen selection pressures to adjacent wild guigna populations, compared to more natural habitats, which would be detected as higher MHC genetic diversity.

We found that MHC diversity of individuals varied significantly with a range of predictors associated with the presence of humans and their domestic animals in the landscape (proxy for pathogen pressure), and higher individual pathogen infection/loads. Specifically, higher MHC genetic diversity was detected in guignas inhabiting areas where houses were present and percentage of vegetation cover was lower. We also found higher MHC diversity in guignas that were infected with, and with a higher intensity of infection of, CR helminths, with higher helminth species richness, and with higher co-infection with micro and/or macroparasites. Guignas inhabiting landscapes with higher human, livestock, and (non-vaccinated; López-Jara et al., 2021) domestic cat and dog densities are more likely to have contact with and be exposed to domestic animal pathogens compared to those in natural forest landscapes where no (or very few) humans and their domestic animals dwell (Sacristán et al., 2019b). In previous studies, human-dominated landscapes have been associated with a higher prevalence with FeLV and Carnivore protoparvovirus-1 in guignas (Sacristán et al., 2021a, 2021b). Therefore, higher pathogen loads in human-disturbed landscapes may be acting as selective pressures shaping MHC diversity for guigna populations. These overall findings provide support for the hypothesis that MHC variation is (at least partially) maintained by balancing selection driven by pathogenic selective pressures. Our results support our prediction that MHC diversity in guignas would be highest in human-dominated landscapes (higher parasite exposure) and in infected individuals (higher parasite load).

This study supports the 'Evolutionary equilibrium' scenario, in which a population with low MHC diversity is under relaxed selection pressure when exposed to low parasite diversity, and vice versa (Meyer-Lucht et al., 2010). Other studies have recorded low MHC diversity associated with high gastrointestinal parasite load in marsupials supporting the 'Unbalanced situation' scenario (i.e. a recent loss in MHC diversity leading to a lack of resistance alleles and increased micro-macroparasite burden) (Meyer-Lucht et al., 2010), found no signatures of selection in MHC loci despite an increase in parasitic infections in a small rodent (Biedrzycka and Kloch, 2016), observed that nematode loads and virus prevalence were influenced by the landscape and specific Toll-like receptors (TLRs) haplotypes in a generalist rodent (Heni

Fig. 4. Site-by-site test for positive selection in MHC class I exon 3 and MHC class II-DRB exon 2. Codons identified as putatively under positive selection (shown in grey): Two in MHC class I exon 3, and seven in MHC class II-DRB exon 2, with partial coincidence with putative antigen recognition sites in domestic cat MHC (showed with asterisks) (as described in Smith and Hoffman, 2001, Yuhki et al., 2008).

et al., 2020), or found MHC heterozygosity and specific variants were associated to nematode infection resistance in pandas (Zhu et al., 2020) (but see examples of positive correlations between MHC diversity and pathogen load in Introduction). A meta-analysis across 112 mammal species found that MHC nucleotide diversity increased with parasite richness for bats and ungulates but decreased with parasite richness for carnivores (Winternitz et al., 2013). Contrasting evidence is therefore observed in the few studies that have directly addressed an association between MHC diversity and pathogen loads in vertebrates. Differences may be explained by the fact that stable populations at host–parasite equilibrium should show a positive association between MHC diversity and parasite load/diversity, whereas recently bottlenecked populations are predicted to show the opposite trend (Radwan et al., 2014).

A previous study with neutral markers showed that small, isolated guigna populations inhabiting fragmented landscapes harbored less genetic diversity compared to continuous forests for this forest-associated species (Napolitano et al., 2015b). If neutral demographic processes were the dominant drivers of MHC diversity in guignas, then in human-dominated landscapes we should expect to find a similar pattern of low genetic diversity as that found with neutral markers. However, in this study we found the opposite pattern: Guignas in anthropogenically disturbed landscapes had higher MHC diversity compared to those inhabiting more natural landscapes with higher vegetation cover and no human presence. We also found evidence for positive selection at MHC loci based on the ratio of nonsynonymous to synonymous substitution rates. Therefore, our results support the hypothesis that anthropogenic selective processes influence guigna MHC evolution and diversity.

Both pathogen-driven selective pressures and neutral genetic processes potentially influence MHC evolution and diversity (Kamath and Getz, 2012). It has been previously recorded in other studies that parasite-driven selection can counteract the loss of MHC alleles due to drift, and several species with extremely low neutral genetic variance have managed to retain polymorphism within the MHC (Aguilar et al., 2004; Biedrzycka and Kloch, 2016; Marmesat et al., 2017). This may be the case for guigna populations, as evidenced by their contrasting genetic diversity patterns for neutral (demographic) versus adaptive MHC genetic diversity (this study). The MHC diversity found in guignas suggests genetic drift has not eroded variability across populations and that selection is maintaining MHC diversity. Large population sizes, high gene flow, and/or similar selection pressures may maintain MHC variation across a species range (Sallaberry-Pincheira et al., 2016), and in the case of guignas high gene flow across mainland populations has been recorded at neutral markers (Napolitano et al., 2014). Different functional properties of MHC molecules are probably being maintained across populations (Kamath and Getz, 2012), further emphasizing the important role of human factors in driving immunogenetic selection in this wildlife species. In other species, it has been reported that neutral demographic processes could have a larger influence on immune-gene evolution and variation than selection (Miller et al., 2010; Grueber et al., 2013; Elbers et al., 2017), or, that despite balancing selection shaping MHC variation in the long-term, on a shorter timescale genetic drift can substantially affect MHC in bottlenecked and fragmented populations, leading to depletion of polymorphism (Miller and Lambert, 2004; Mainguy et al., 2007; Radwan et al., 2010).

Overall genetic diversity for MHC exons in guignas across populations was average to high, compared with the same exon diversity observed in other wild cat (Castro-Prieto et al., 2011a, 2011b; Wang et al., 2009; Hendrickson et al., 2000) and carnivore species (Hosotani et al., 2020; Wilbert et al., 2020; Pizarro et al., 2021; Bartocillo et al., 2021). This suggests that overall, guignas have a relatively high adaptive capacity and potentially high resilience to diseases transmitted from domestic and/or invasive animals (but see Sacristán et al., 2019a, 2021a, 2021b, Ortega et al., 2021), which may facilitate the long-term persistence of guigna populations. However, this scenario may change if current land-use trends and associated threats persist in the Chilean Winter Rainfall-Valdivian Forests biodiversity hotspot (Echeverría et al., 2006; Rodríguez-Echeverry et al., 2018; Noh et al., 2019), especially for a habitat specialist like the guigna, and if these changes are faster than the host's adaptation. Although guigna appears to have maintained a high degree of diversity relative to other wild cats, they risk losing functional MHC alleles if populations become smaller and/or more fragmented, as has been observed in other Chilean threatened carnivores (Pizarro et al., 2021).

Balancing selection would be expected to maintain or enhance genetic diversity in a population whereas directional selection would reduce variation by favoring specific alleles (Radwan et al., 2020; Serieys et al., 2015). Therefore, theory predicts that population divergence (i.e., between-population differences) should be less for loci under balancing selection and greater at loci under directional selection compared with neutral loci (Bernatchez and Landry, 2003; Piertney and Oliver, 2006). In our MHC study, overall high genetic diversity and alleles evenly distributed across geographic groups compared with a previous study in neutral loci (Napolitano et al., 2014), supports that the positive selection detected may correspond to balancing selection.

It is perhaps surprising that the associations between MHC diversity and macroparasite (helminth) infection, load and richness were with MHC class I, rather than class II. MHC class I genes have been described to code for molecules that display intracellular peptides (e.g., viruses), whereas MHC class II for molecules presenting extracellular peptides (e.

g., bacteria, helminths) (Neefjes et al., 2011), suggesting a stronger mechanistic hypothesis for an association with MHC class II. However, these mechanisms are not clearcut (Fleischer et al., 2022) as both MHC class I and II have been linked to the clearance of hepatitis C (McKiernan et al., 2004) and viral and helminth infections (Montero et al., 2021), while class II has been described to be involved in the clearance of influenza (Luckey et al., 2019). For microparasites studied here, FeLV, FIV, hemoplasmas, Carnivore protoparvovirus-1 and paramyxovirus, it is possible that our sample size was insufficient to detect the full scope of MHC interactions, as the prevalences of these infections were low (3–23 %). Also, parasites and pathogens are just one possible selection pressure that may shape MHC diversity, and in multiple ways: multiple infections, pathogen interactions, differences in pathogen diversity, and selective pressures changing over time may all play a role (Biedrzycka and Kloch, 2016). Several other pathogens not assessed in this study may also be infecting guignas and interacting among them and with MHC, such as viral infections that frequently cause immunodepression and thus may be related with higher infection intensities of co-occurring pathogens (Munson et al., 2008). Extreme climatic conditions may also disrupt historic stable dynamics between co-existing pathogens and their susceptible hosts and promote coinfections that may result in catastrophic population mortality (Munson et al., 2008).

We acknowledge that sample size for some of the tested variables is small, which could limit the inferences from our findings. With a larger sample size, we would also have the power to conduct multivariate analysis and explore more complex relationships potentially not revealed in our study. However, this study was designed to prioritise complete geographic distribution of the sampling, targeting a range of habitat types and levels of human interaction, in order to test our hypotheses. This study is also the first stage of a larger, long-term research, in which we plan to include additional data generated over time, to help us better understand the relationship between immune diversity, disease resilience and fitness in a changing environment.

Most research on genetic variation at immune genes have focused on MHC, which has an advantage for comparison purposes across studies. However, non-MHC immune genes have been associated to approximately half of the genetic diversity involved in pathogen resistance (Jepson et al., 1997). Therefore, integrating additional biomarkers such as antibodies, cytokines and other immunologically important loci (i.e., Toll-like receptors; TLR) with MHC genetic diversity data is needed to support the results obtained in this study and to broaden our current understanding of wildlife immunogenetics (Acevedo-Whitehouse and Cunningham, 2006; Serieys et al., 2015).

MHC diversity is key to investigate the evolutionary and adaptive potential of threatened wild populations in the face of emerging infectious disease threats (O'Brien and Yuhki, 1999; Bernatchez and Landry, 2003; Sommer, 2005; Acevedo-Whitehouse and Cunningham, 2006; Castro-Prieto et al., 2011a, 2011b, 2012), and to reveal how selection can promote and maintain genetic variation in natural populations (Radwan et al., 2020). Human activities can change the adaptive landscape that vertebrates experience, which in turn impacts their pathogen susceptibility and transmission; these complex processes can be understood by examining the MHC. However, the association between immunogenetic diversity and pathogens, and how it is shaped by habitat disturbance, is understudied in wildlife, despite the fact that it has been suggested as useful and necessary to understand the role of immunogenetic diversity in the long-term conservation of populations and species (Radwan et al., 2010). Considering both immunogenetic and ecological factors is necessary to achieve a comprehensive picture of the effects of anthropogenic changes on wildlife health (Heni et al., 2020). Therefore, continuous pathogen surveillance and monitoring of neutral and adaptive genetic diversity in populations across different geographic groups and time frames may inform complex processes and interactions in natural systems under varying environmental conditions.

Under the One Health concept, where the health of wildlife is interdependent with the health of humans and domestic animals, wildlife studies have important implications. They can dually serve for the monitoring of host health and immune genetic diversity, and the surveillance of potential reservoir hosts for the spillover of zoonotic pathogens between humans and wildlife (Altizer et al., 2018). Disease emergence and re-emergence is a growing problem in both human and wildlife populations, and the evolutionary potential of wild populations to cope with infectious diseases by means of their immune genetic variation is relevant in the currently changing world (Daszak et al., 2000).

Here we integrated multiple approaches to understand functional immunogenetic variation, pathogenic selective processes and drivers of MHC variability in natural environments that are impacted by human activities to varying degrees. This study is the first comprehensive, landscape-level assessment of immunogenetic variation in guignas from across their entire distribution, and the first integrating MHC diversity and multiple micro-macroparasite selective pressures across landscapes with differential human disturbance in Chile. The results presented here contribute to evidence-based decisions for future conservation and management programs of this threatened species, and will likely be applicable to other species particularly in the face of emerging infectious pathogens. MHC has also been highlighted as a potentially useful genetic marker to guide conservation management, reflecting adaptive instead of neutral genetic diversity, therefore complementary to neutral genetic assessments for conservation purposes (Manlik et al., 2019). Our results enhance our knowledge on the evolutionary and adaptive potential of vertebrates in the face of emerging infectious disease threats and increasing anthropogenic global change, and can be used to inform the effects of anthropogenic disturbance on wildlife, and the human-wildlife disease interface.

CRediT authorship contribution statement

Conceived and designed the study: CN. Performed field work, data collection and provided samples: CN, IS, FA, EA, SG, MJL-J, JC, EH-H. Performed lab work: IS. Analyzed the data: CN. Interpreted and curated the data: CN, EP, CEG, IS. Wrote the paper and prepared tables and figures: CN. Contributed in writing the paper: IS, EP, CEG. All authors discussed the results and contributed to editing the manuscript.

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Declaration of competing interest

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Data availability

Genetic sequences were submitted to the GenBank database under the accession numbers (OQ932874-OQ932903).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166289.

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