Beyond neutral loci: Examining immune gene variation in tigers *(Panthera tigris)*

B.V. Aditi[1], Uma Ramakrishnan[1, 2]

[1] National Centre for Biological Sciences-TIFR

[2] Senior Fellow, Wellcome Trust DBT India Alliance

Abstract:

Genetic vulnerability of wild populations is generally assessed using neutral loci, which may not accurately reflect a population's adaptive potential. In contrast, diversity at loci putatively involved in fitness and survival, such as immune genes, could be a better proxy for future survival. Research on immune genes in wild species has traditionally focused on the MHC loci. We investigate five families of non-MHC immune genes -Tumor Necrosis Factor, Interleukin, Toll-like Receptor, Leukocyte Immunoglobulin-Like Receptors, and Chemokine - involved in adaptive and innate immunity in the tiger *(Panthera tigris)*, an endangered carnivore. We compare immune gene diversity to neutral diversity using publicly available resequenced whole genomes from 107 tigers representing all extant subspecies and subpopulations (within India) with different demographic histories.

Immune receptor genes show high nucleotide diversity (mean: 0.0019) compared to neutral loci (0.0008) and immune signalling genes (0.0004), suggesting positive selection. However, heterozygosity of immune genes does not differ significantly from neutral loci, suggesting strong genetic drift. Populations with histories of bottleneck and inbreeding show reduced genetic diversity and high mutation load but some immune loci retain polymorphisms even in inbred populations. Genetic differentiation and site frequency spectra of immune loci correspond to patterns from neutral loci but Toll-like receptor genes show signatures associated with balancing selection. Our findings indicate that genetic drift is likely the predominant evolutionary force shaping genetic variation in bottlenecked endangered species such as tigers, even at immune loci that are typically subject to selection. However, remnants of past balancing selection might preserve important variation, potentially influencing population survival.

1. Introduction:

Human activities have caused habitat loss and fragmentation, resulting in the recent decline in biodiversity across the globe (Barnosky et al. 2011; Scanes 2018; Bodo, Gimah, and Seomoni 2021). Despite significant conservation action, many species continue to be at risk. Carnivores, particularly Felidae, have continued to decline in numbers and rank higher in vulnerability in the IUCN Red List Index (Ripple et al. 2014; Fernández-Sepúlveda and Martín 2022). These species tend to be large-bodied, apex predators that require large habitats and naturally occur at low densities (Cardillo et al. 2008). Habitat loss and fragmentation sequesters such species into small and often isolated populations (Ripple et al. 2014). Population genetic and island biogeography theories predict genetic erosion in small, isolated populations due to random genetic drift and inbreeding (Frankham 1996; Johnson, Adler, and Cherry 2000; Keller and Waller 2002). The chance effects of genetic drift can outweigh selection, cause disadvantageous alleles to increase in frequency, while reducing advantageous alleles (Allendorf 1986; Masel 2011). Restricted mating opportunities could result in inbreeding, which rapidly increases homozygosity of deleterious mutations (Alho et al. 2009; Robinson et al. 2016; Khan et al. 2021) and manifest in reduced fitness or inbreeding depression (Slate et al. 2000; Bolund et al. 2010; Bataillon and Kirkpatrick, 2020; Kardos et al. 2023). This decline in individual fitness can lead to a reduction in effective population size and population growth

rate, ultimately resulting in extinction (Gilpin 1986). Since the 1980s, numerous case studies from captive and wild populations have provided empirical evidence for this process, commonly referred to as the extinction vortex (Beilharz 1982; Ralls, Ballou, and Templeton 1988; Keller et al. 1994; Saccheri et al. 1998; Slate et al. 2000; Young and Clarke 2000; Liberg et al. 2005; Fagan and Holmes 2006; Höglund 2009; Blomqvist et al. 2010; Godwin et al. 2020).

Understanding the mechanisms behind population extinction is important to design and prioritise conservation management. While the generality of genetic effects are well understood (Wilder et al. 2023), the genomic regions responsible for these processes and the fitness effects remain unclear and are challenging to identify in wild populations. Population geneticists have typically relied on genetic correlates of extinction or endangerment to inform and prioritize conservation efforts. Broadly, these correlates could be negative (higher mutation load and/or lower genetic diversity) or positive (high genetic diversity and/or lower mutation load compared to similar taxa or populations). Several studies find correlation between genetic diversity and fitness; such studies typically focus on neutral loci such as microsatellites or genome-wide markers (Hansson and Westerberg 2002; Johnson and Dunn 2006; Chapman et al. 2009; Taylor et al. 2010; Annavi et al. 2014; Velando, Barros, and Moran 2015). Recent advancements in gene and variant prediction algorithms have allowed quantification of mutation load (Robinson et al. 2023; Grossen and Ramakrishnan 2024). Several studies have revealed elevated homozygous mutation load (inbreeding load) in small, inbred populations when compared to large populations (Xue et al. 2015; Robinson et al. 2018; Bataillon and Kirkpatrick, 2020).

However, several endangered populations with very low genome-wide diversity persist, seemingly evading the extinction vortex (Jones et al. 2008; Robinson et al. 2016; Mathur and DeWoody 2021; Morin et al. 2021). The demographic and fitness impacts of deleterious mutations remain poorly understood (Grossen and Ramakrishnan 2024). Hence, it is debated whether genetic diversity and load are good correlates for population survival. Reported correlations between genetic diversity and fitness might exist because neutral loci might include fitness-related loci. Publication biases might underrepresent studies showing lack of correlation. Moreover, most new mutations are mildly deleterious and beneficial mutations are rare (Eyre-Walker and Keightley 2007), challenging the assumption that high genome-wide variation might indicate adaptive potential.

Our understanding of genetic effects on population persistence/extinction might be clearer if we distinguish between adaptive and neutral loci. Evolutionary processes differentially shape genetic variation at different loci (Slatkin 1987; Charlesworth and Charlesworth 2017). Neutral loci are under weak or no selection and are shaped by mutation, recombination, genetic drift and gene flow (Kimura 1983). Adaptive variation (with a direct effect on fitness) is consequently influenced by different types of selection together with neutral evolutionary processes (Fisher et al. 1930; Endler, 1986; Whitlock and Barton 1997). Hence studying the same genetic correlates at adaptive variation might be a better approach to better understand extinction risk or adaptive potential (Frankham 2005; Hedrick 2001; Flanagan et al. 2018; Teixeira and Huber 2021). Because adaptive loci are affected by selection, patterns of variation at these loci are also expected to be different from that at neutral loci. However, evolution in small populations tends to be dominated by drift, and selection is weak (Keller and Waller 2002; Pardo et al. 2005; Masel 2011). In other words, demographic history and small population size may result in the depletion of both neutral and adaptive genetic diversity (Sutton et al. 2011; Ochoa et al. 2020; Minias et al. 2023).

Studies on adaptive variation have attempted to understand levels of variation and detect selective pressures at putatively fitness-related loci including genes related to reproduction (Carneiro et al. 2015) and immunity (Brandt et al. 2018; Davies et al. 2021). Immune genes are known to have a

direct effect on survival and reproduction, and are an ideal candidate for studying adaptive variation, (Janeway et al. 2001; Netea, Wijmenga, and O'Neill 2012; Hedrick, Lee, and Buchanan 2013). Loss of genetic diversity at immune genes is associated with increased disease susceptibility (Reid et al. 2007; Brock et al. 2015; Duffield et al. 2019). Most studies on population genetics of immune genes focus on a single family- the Major Histocompatibility Complex (MHC) which is involved in adaptive immunity (Aguilar et al. 2004; Alcaide 2010; Castro-Prieto, Wachter, and Sommer 2010; Edwards et al. 2000; Edwards, Wakeland, and Potts 1995; Radwan, Biedrzycka, and Babik 2010; Rivero-de Aguilar et al. 2016; Sutton et al. 2011; Wang et al. 2014). MHC genes represent a very small proportion of the immune gene repertoire (Jepson et al. 1997; Acevedo-Whitehouse and Cunningham 2006). Hence, studies are now expanding to other immune gene families to understand immunogenetic variation better. Genes for Toll-Like Receptor (TLR) and Chemokine are characterized in several wild species including those that exist in small populations (Turner et al. 2011; Grueber et al. 2012; Ellis, Turner, and Knight 2012; Grueber, Wallis, and Jamieson 2014; Morris et al. 2015; Gilroy et al. 2017; Vlček et al. 2023).

Evolutionary and population genetic studies have identified contrasting roles of drift and selection in shaping immunogenetic variation (Alcaide 2010; Bollmer et al. 2011; Luo et al. 2012; Cortázar-Chinarro et al. 2017). One common observation is that genes that code for receptors, which directly interact with pathogen molecules (e.g. MHC and TLR genes) are under positive selection (balancing or diversifying selection), whereas genes that code for signalling molecules such as Interleukin and Chemokine are under negative selection (Mukherjee et al. 2009). Several studies find that immune genetic diversity in bottlenecked populations reflects that at neutral loci (Bollmer et al. 2011; Morris et al. 2015; Bateson et al. 2016), but other studies observe higher polymorphisms in genes under balancing selection, preventing erosion of genetic diversity at these loci even in such vulnerable populations (Edwards et al. 2000; Oliver and Piertney 2012; Gilroy et al. 2017).

In this study, we attempt to understand genetic correlates of endangerment in non-MHC immune gene of an endangered carnivore- tiger. Tigers have experienced population decline and habitat fragmentation (Mondol, Karanth, and Ramakrishnan 2009; Natesh et al. 2017; Zhang et al. 2023), have low neutral genetic diversity but are now exhibiting demographic recovery(Armstrong et al. 2021; Jhala et al. 2021). Currently, six extant tiger subspecies/populations are recognized based on genetic data: Bengal tiger Panthera *tigris tigris* (Linnaeus, 1758), Sumatran tiger *P.t.sumatrae* (Pocock, 1929), Indochinese tiger *P.t. corbetti* (Mazak 1968), Malayan tiger *P.t.jacksoni*, Amur tiger *P.t. altaica* (Temminck, 1844) and South China tiger *P.t. amoyensis* (Hilzheimer, 1905). Malayan and Indochinese tigers are closest in geography to South China tigers and retain considerable genome-wide genetic diversity in spite of population decline. Based on demographic models (Armstrong et al., 2021), Bengal/Indian tigers were the first to diverge and expanded into the Indian subcontinent. Currently, this subspecies constitutes 75% of the global population of wild tigers (Jhala et al., 2025). Indian tigers show strong genetic differentiation and exist in at least three subpopulations: The North West (NW) Indian subpopulation is small and isolated while Central Indian (CI) and South Indian (SI) subpopulations are large and connected (Natesh et al. 2017; Armstrong et al. 2021). The Northwest Indian subpopulation shows genomic signatures of inbreeding (Khan et al. 2021). Genetic data from both Amur and Sumatran tigers reveal signatures of past bottlenecks and founding events, as well as natural selection at certain loci (Liu et al. 2018; Armstrong et al. 2021). In this paper, we only analyse subpopulation structure in Bengal/Indian tigers, as understanding of population structure in other subspecies remains poor. Limited genomic analyses suggest lack of population structuring in Amur tigers, but some structuring in Malayan tigers (Armstrong et al. 2021). Additionally, the different subspecies of tigers exhibit

different evolutionary histories with varying population bottleneck sizes in the last century (Armstrong et al., 2021). However, processes that tend to decrease diversity: habitat loss, habitat fragmentation and poaching resulting in smaller population sizes are common to all.

Studies on immune genes in tigers and other felids have revealed signatures of positive, balancing, and purifying selection in MHC genes, and also reveal low allelic diversity compared to humans or domestic cats (Castro-Prieto, Wachter, and Sommer 2010; Pokorny, Sharma, and Tiedemann 2010; Wei et al. 2010; Shukla et al. 2023). While these findings highlight past selection and recent genetic diversity loss, they are often limited to a few immune gene loci and populations. Currently available whole-genome resequencing data and assemblies for various tiger subspecies, offer a unique opportunity to investigate immunogenetic variation at a broader scale, including multiple subspecies and their subpopulations, particularly those with known histories of disease exposure (Amur tigers (Gilbert, Dvornicky-Raymond, and Bodgener 2023; Northwest Indian tigers (Sidhu et al. 2019). With emerging infectious diseases known to affect tiger and other carnivore populations, studying immunogenetic variation could inform conservation efforts by contributing to a broader generalisation regarding adaptive genome-wide diversity and probabilities of extinction or resilience.

Owing to the complexity of MHC genes, it is difficult to assemble these loci even with long read genomic data. Hence, we exclude these genes from this study. We attempt to understand adaptive variation at five families of non-MHC immune genes in terms of nucleotide diversity and heterozygosity. We classify these genes into two categories based on their immune functions, reflecting the possible selective forces acting on them. Those under positive selection i.e. genes coding for pathogen recognition molecules that tend to be receptor proteins, and those under purifying selection (e.g. genes coding for mediator cytokine molecules), that tend to be involved in signalling. We compare genetic variation, genetic differentiation and mutation load in these two categories with putatively neutral loci present in intergenic regions. Specifically, we ask the following questions: i) Do immune genes exhibit patterns of variation distinct from neutral loci and do these patterns reflect population histories? ii) Is variation at immune gene loci genetically differentiated across subspecies and Indian subpopulations? iii) Does site frequency spectra of immune gene loci vary from neutral loci suggesting selective pressures? iv) Do immune genes carry mutation load, and does this correlate with known patterns of demographic history in terms of inbreeding, population size and connectivity? We attempt to identify populations of conservation concern based on accumulated mutation load and lower genetic diversity.

2. Methods:

2.1 Identification of immune genes

Immune gene sequences were extracted from the annotation file of a female wild Indian Tiger genome assembly (GenBank ID: GCA_021130815.1) (PanTigT.MC.v3, Shukla et al. 2023). The associated General Feature Format file was examined to extract rows containing terms related to the immune genes of interest. We searched for genes coding for Toll-Like Receptors (TLR), Leucocyte Immunoglobulin-Like receptors (LILR), Interleukin (IL), Chemokine (CC or CXC) and Tumor Necrosis Factor (TNF). These genes are well characterized and known to be involved in immunity. The search results were manually reviewed, and sequences with verified gene names and completely assembled Open Reading Frames were selected for further analysis. Genes were chosen for the study if the translated protein were immune receptors or signalling molecules and was excluded if present on sex chromosome or unplaced scaffolds. In the case of numerous entries of the same gene with similar start and end positions, the longest sequence was retained. The annotation also provided information on the start and end positions of exons and coding sequence. This allowed us to determine the start and end positions of the entire gene as well as the exons.

2.2 Whole genome resequencing data and initial processing

We downloaded resequenced genomes of six subspecies of tigers from European Nucleotide Archive and China National GenBank in the form of raw Fastq files (Liu et al. 2018; Khan et al. 2021; Armstrong et al. 2021; Du et al. 2022; Zhang et al. 2023). In total, 107 genomes were downloaded, trimmed using default parameters of Trimmomatic (Bolger, Lohse, and Usadel 2014) and adapter sequences as mentioned in the published studies and aligned to the Indian tiger reference PanTigT.MC.v3 published in Shukla et al. 2023 with bwa-mem. The dataset includes genomes of wild or wild caught individuals as well as captive individuals. The South China genomes are all from captive individuals whereas all Bengal tiger genomes are from wild individuals. The details of samples and accession numbers are listed in Supplementary Table 1. These samples were further classified into subspecies as per the Subspecies column and Indian subpopulations of South India, Central India and Northwest India as per the Indian Subpopulation column in Supplementary Table 1. A summary of the number of samples in each subspecies or subpopulation group and its demographic background can be found in Supplementary Table 2.

2.3 SNP calling and filtering for neutral and immune loci

Since intergenic loci are not expected to be subject to selection, they were chosen as surrogates for neutral loci. We defined intergenic loci as 50 kb upstream and downstream of genic regions throughout the genome (as in Armstrong et al. 2021). We extracted the start and end positions of intergenic regions in the autosomes from the Indian Tiger annotation mentioned above into a bed file and added a buffer of 50kb upstream and downstream the start and end of the genes. The Variant Call Format (VCF) files containing both variant and invariant sites were created using bcftools (Danecek et al. 2021). An mpileup was created followed by identification of variants which were annotated with the relevant depth tags using the --annotate tag. Using bcftools norm, redundant lines were eliminated and variants were left aligned. VCFtools (Danecek et al. 2011) was used to filter the resulting two VCF files for standard quality filters of minimum base quality of 30 and minimum genotype quality of 30 and to remove alleles occurring three times or less (minor allele count filter of three). We also exclude insertions and deletions since the identification of structural variants is not reliable with the

SNP calling method we employ. Further, variant and invariant sites were filtered separately. We excluded SNPs with a Hardy Weinberg p-value less than 0.0001 (to account for genotyping errors) and retained only biallelic sites (to reduce errors). We retained SNPs that were present in at least 70 percentage of the individuals and finally filtered out SNPs in the top and bottom 0.25 quantile of mean depth (as in Khan et al. 2021). Finally, the filtered variant and invariant site VCFs were merged using bcftools concat command (Danecek et al. 2021).

2.4 Nucleotide diversity, Heterozygosity and F_{ST} estimation

Nucleotide diversity and heterozygosity were estimated in two ways for immune receptor, immune signalling and intergenic loci: a) with all samples pooled as a single population, b) samples subset for each subspecies and each Indian subpopulation. The first approach aims to understand the distribution of diversity values for the immune receptor, immune signalling and neutral regions for the tiger species whereas the second approach allows us to make this same comparison at subspecies and subpopulation level.

Heterozygosity was calculated as the proportion of observed heterozygotes for every exonic SNP in immune genes and all SNPs in intergenic loci. Specifically, the –hardy option in VCFtools (Danecek et al. 2011) was used to obtain observed genotypes and proportion of observed heterozygotes was calculated by summing up number of observed heterozygotes and dividing by total observed genotypes.

Pairwise Hudson's F_{ST} (Hudson, Slatkin, and Maddison 1992) was estimated for both immune and intergenic datasets to account for unequal sample size across different groupings. These distributions were visualized using boxplots. The subsets of pairwise F_{ST} were further visualized as follows: i) F_{ST} of Amur with all other subspecies, since Amur tigers were exposed to Canine Distemper Virus; ii) F_{ST} of Sumatran tigers with all other subspecies, since they underwent a founding event and exist on an island and iii) Rest of the subspecies and Indian subpopulation level comparisons

2.5 Comparison of site frequency spectrum to infer selection

In order to test for selection at immune genes, we estimated the unfolded site frequency spectrum (SFS) across all 107 genomes for synonymous and nonsynonymous SNPs, and compared this with the unfolded SFS at neutral loci. In order to account for the difference in number of neutral and immune sites examined, we divided each bin with the total number of SNPs for that category. SFS was estimated using angsd -doSaf 1 and realSFS programs, and was polarized using a genomic alignment of closely related species -domestic cat, lion and cheetah- as described in Khan et al, 2021. With this approach, type of selection can be inferred as follows: i) Purifying selection if the SFS of non-synonymous loci was skewed more towards the right when compared to neutral and synonymous SFS, ii) Positive selection if the SFS of non-synonymous loci was skewed more towards the left compared to neutral and synonymous SFS and iii) Balancing selection if the SFS of nonsynonymous loci peaked at the centre of the SFS when compared to neutral and synonymous SFS.

To detect synonymous and nonsynonymous variants, Ensembl Variant Effect Prediction (McLaren et al. 2016) was used to predict effect of the variants in immune genes with the custom annotation files, the tag –everything and –pick-allele. Variants predicted to be synonymous and missense were used to create SFS for synonymous and nonsynonymous SFS respectively.

2.6 Statistical tests

Differences in nucleotide diversity of immune receptor, signalling and neutral loci between populations was tested with a Zero Inflated Gamma Generalized Linear Model implemented through glmmTMB R package (Brooks et al. 2017) (available on github as well as CRAN) and interpreted using p-value and estimate. Differences between heterozygosity of immune receptor, signalling and intergenic loci and differences in site frequency spectra of neutral, synonymous and nonsynonymous SNPs was tested with a Binomial Generalized Linear Model, and interpreted using p-value as well as pseudo-R squared. Differences in pairwise Hudson's F_{ST} were estimated with a Gaussian model implemented through glm() function in RStudio and model fit was assessed using p-value and pseudo R squared. High values of these estimates indicated a good model fit; the estimate in zero-inflated GLM was required to assess deviation from the null hypothesis, which was the diversity at neutral loci in this study. The differences in heterozygosity between populations were tested with Kruskal Wallis p-value test in RStudio since these were three or more distributions. To identify genes with high nucleotide diversity, we identified windows of signalling immune genes with nucleotide diversity in the top two quantiles and receptor immune genes in top quantile of this distribution. Genes with high heterozygosity were identified based on boxplot outliers in using the boxplot() function in R.

2.7 Identification of deleterious mutation and estimation of mutation load

Results of Variant Effect prediction from Ensembl Variant Effect Prediction (McLaren et al. 2016) was used to identify deleterious mutations. Missense variants could potentially change the structure of the protein product but this change could be benign or deleterious. Hence, the potential for pathogenicity of identified missense variants was predicted using PolyPhen2 (Adzhubei, Jordan, and Sunyaev 2013) using the protocol HumVar2 which compares known pathogenic amino acid changes in humans to classify query mutations into benign, possibly damaging and probably damaging. Possibly and probably damaging variants were considered putatively deleterious variants (pDeleterious). Variants that were classified as putatively Loss of Function (pLOF were transcript_ablation, splice_donor_variant, splice_acceptor_variant, stop_gained, frameshift_variant, inframe_insertion, inframe_deletion, splice_region_variant. Putatively deleterious variants were assumed to be mildly deleterious while pLOF variants were considered to be strongly deleterious.

The ancestral state for each pDeleterious and pLOF allele was inferred from the majority allele present in the genomic alignment of domestic cat, lion and cheetah as described in Khan et al, 2021. Sites at which ancestral alleles were missing were excluded from the estimation of mutation load while derived alleles were retained. We estimated mutation load for Indian subpopulations to contrast mutation load between the small, isolated and inbred Northwest Indian population with the two large connected Indian populations (South Indian and Central Indian). We also compared load in inbred South China tigers with Malayan tigers that are geographically close to South China tigers, but are not inbred. Mutation load was estimated in terms of number of alleles using VCFtools –hwe tag to find the number of loci that were homozygous and heterozygous for pDeleterious and pLOF allele. Homozygous load helped us to understand realized or inbreeding load whereas heterozygous load estimated the masked load in a population.

3. Results

3.1 Summary of Immune genes identified

We were able to identify 91 Immune Genes spanning 11,57,251 bp across five gene families related to immunity with complete and contiguous gene sequences. Of these, two gene families code for receptor molecules that are involved in adaptive and innate immunity. Toll-Like Receptor (TLR) code for receptor molecules that recognize pathogens as an innate immune mechanism. Leukocyte-like Immunoglobulin Like Receptor (LILR) molecules interact with MHC receptors and regulate the activity of NK Cells and are part of the adaptive immune response. The other three gene families are involved in innate immunity. TNF, Interleukin and Chemokines gene families code for small, secreted molecules called cytokines and their ligands that reach sites of immune reactions and aid in the process by inducing inflammation and signalling.

Supplementary Table 3 lists the names of the genes in each family and Supplementary Table 4 includes the number of genes, sizes of genes and number of SNPs in these gene families.

3.2 Patterns of genetic diversity at immune genes in tigers

To assess patterns of genetic diversity at immune genes and neutral loci, we estimated nucleotide diversity over 500 bp windows and heterozygosity for each SNP as detailed in section 2.4 approach a). Our results revealed that mean nucleotide diversity at receptor loci (0.0019, 95 % CI: 0.0005-0.0041) is significantly higher than neutral loci (0.0008, 95 % CI: 0.0008-0.0008) (Figure 1a) whereas signalling loci show lowest mean nucleotide diversity (0.0003, 95 % CI: 0.0003-0.0004). Mean heterozygosity at receptor loci (0.1645, 95 % CI: 0.1370- 0.1948) and signalling loci (0.1437, 95 % CI: 0.1299-0.1576) is lower than neutral loci (0.1973, 95 % CI: 0.1972 - 0.1975) (Figure 1 b) but this difference is not significant (zero inflated and binomial GLM results, Supplementary Table 5 and Supplementary Table 6, respectively).

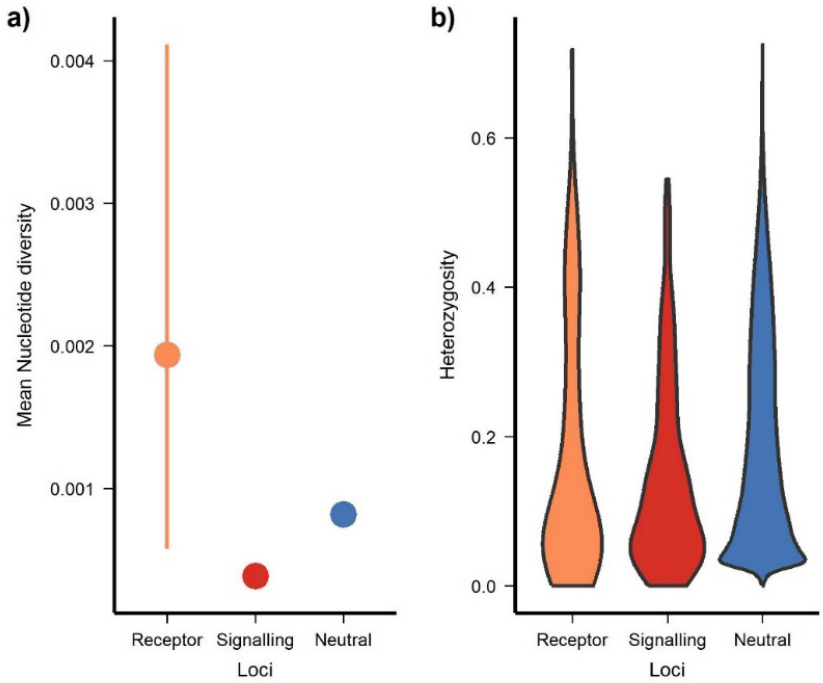

Figure 1: Distribution of a) nucleotide diversity (with 95% confidence intervals around the mean (Significant difference from neutral in: Receptor *, Signalling n.s) and b) heterozygosity at receptor and signalling immune genes and neutral loci (No significant differences between groups) (Significance levels: *** < 0.000, ** < 0.001, * < 0.01, ns < 0.05)**

We also examined the distribution of nucleotide diversity and heterozygosity values at each family of immune genes. We found significant differences in nucleotide diversity of TLR and LILR genes when compared to neutral loci but no significant differences in heterozygosity was found (Figure 2). We observe a higher mean nucleotide diversity and higher heterozygosity at the receptor genes (LILR and TLR) (Figure 2a, Figure 2b, Supplementary Table 7) (zero-inflated and binomial GLM results, Supplementary Table 8 and Supplementary Table 9, respectively).

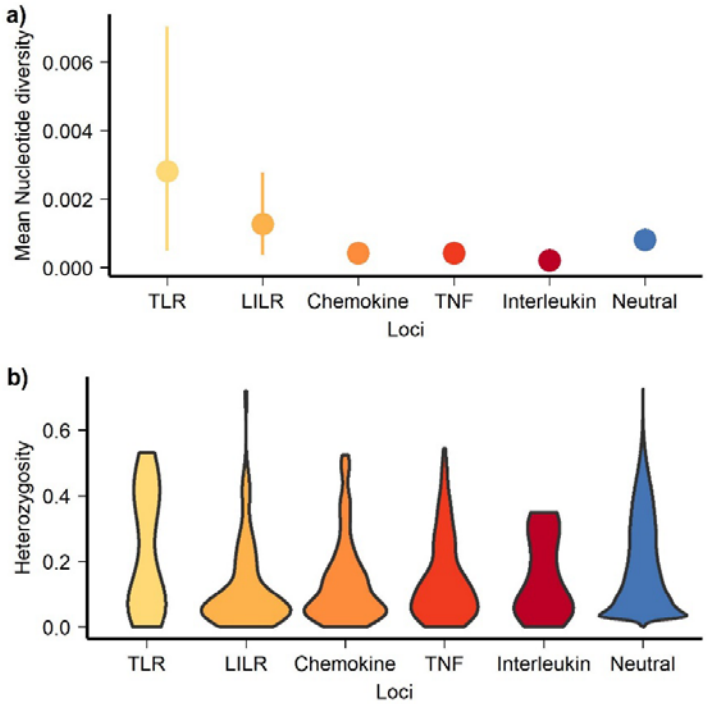

Figure 2: Distribution of a) nucleotide diversity (with 95% confidence intervals) (Significant difference from neutral in: TLR *, LILR ***, Chemokine, n.s, TNF n.s, Interleukin n.s) and b) heterozygosity at different families of immune genes (No significant differences between groups) (Significance levels: *** < 0.000, ** < 0.001, * < 0.01, ns < 0.05)**

3.3 Differences in immune gene diversity across subspecies and Indian subpopulations of tigers

We investigated the genetic diversity patterns for neutral and immune loci across range-wide subspecies as detailed in section 2.4 approach b). Since we found no significant differences in heterozygosity of different gene families, we combine them to contrast immune genes with neutral loci in this analysis. Nucleotide diversity at receptor loci is significantly different from neutral loci so we compare receptor and signalling loci separately with neutral loci.

Our results show that both nucleotide diversity (Figure 3, Supplementary Table 10) and heterozygosity (Figure 4, Supplementary Table 11) vary similarly across subspecies at both immune and neutral loci. While these differences were significant in most comparisons of nucleotide diversity, none of the differences were significant in comparisons of heterozygosity (zero-inflated and binomial, GLM results Supplementary Tables 12, 13, 14 and 15). Sumatran (SUM) tigers have the least nucleotide diversity at immune and neutral loci (Figure 3a) and South China (SOC) tigers have least heterozygosity at immune and neutral loci (Figure 4a). Malayan (MAL) tigers have the highest mean nucleotide diversity and heterozygosity at immune and neutral loci (Figure 3a and 4a). In populations within India, the nucleotide diversity and heterozygosity at immune loci follow a pattern similar to neutral loci (Figure 3b and 4b). The large, connected populations (CI and SI) have higher nucleotide diversity and heterozygosity when compared to the small, isolated population (NW). The differences in distributions across populations as tested by Kruskal-Wallis's test were found to be significant except for nucleotide diversity of receptor loci in Indian subpopulations (Supplementary Table 16 and Supplementary Table 17).

Receptor loci have consistently and significantly high nucleotide diversity for subspecies, as well as Indian subpopulations (Supplementary Table 12). The only exception are Indochinese (INC) tigers, where nucleotide diversity for immune loci fell within inferred nucleotide diversity values (Figure 3a). Signalling loci showed lower mean nucleotide diversity when compared to neutral diversity in all populations examined but this difference is not significant in all populations (Figure 3, Supplementary Table 12).

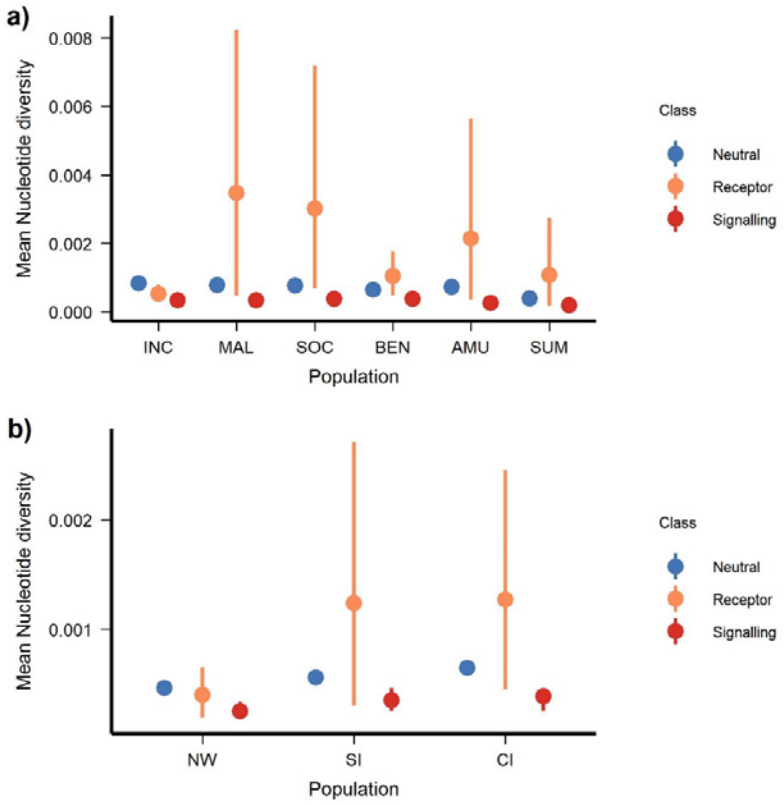

Figure 3: a) Mean and confidence intervals for nucleotide diversity at immune receptor, immune signalling and neutral loci in extant sub-species of tigers including INC (Indochinese), MAL (Malayan), SOC (South China), BEN (Bengal), AMU (Amur) and SUM (Sumatran) (Significant difference from neutral at receptor loci in: INC n.s , MAL*, SOC***, BEN***, AMU***, SUM ***; Significant difference from neutral at signalling loci in: INC *, MAL*, SOC***, BEN***, AMU n.s., SUM, n.s; Significant difference across subspecies: Receptor loci *, Signalling loci *** and neutral loci ***) b) Mean and confidence intervals for nucleotide diversity at immune receptor, immune signalling and neutral loci in Indian tiger populations NW (Northwest), CI (Central India) and SI (South India). (Significant difference from neutral at receptor loci in: NW*, SI***, CI***; Significant difference from neutral at signalling loci in: NW***, SI***, CI***; Significant difference across subpopulations: Receptor loci n.s., Signalling loci *** and neutral loci ***) (Significance levels: *** < 0.000, ** < 0.001, * < 0.01, ns < 0.05)**

Although the mean and median for both diversity indices were much lower in the Northwest Indian (inbred) population, there are several outliers in this distribution as well as for Amur (bottlenecks and disease epidemic), Sumatran (island population) and South China (inbred) tigers. These outliers are from sites across receptor and signalling genes and are not restricted to any one gene family. Interestingly, we found that the outliers in different populations were in the same few genes (listed in Supplementary Table 18).

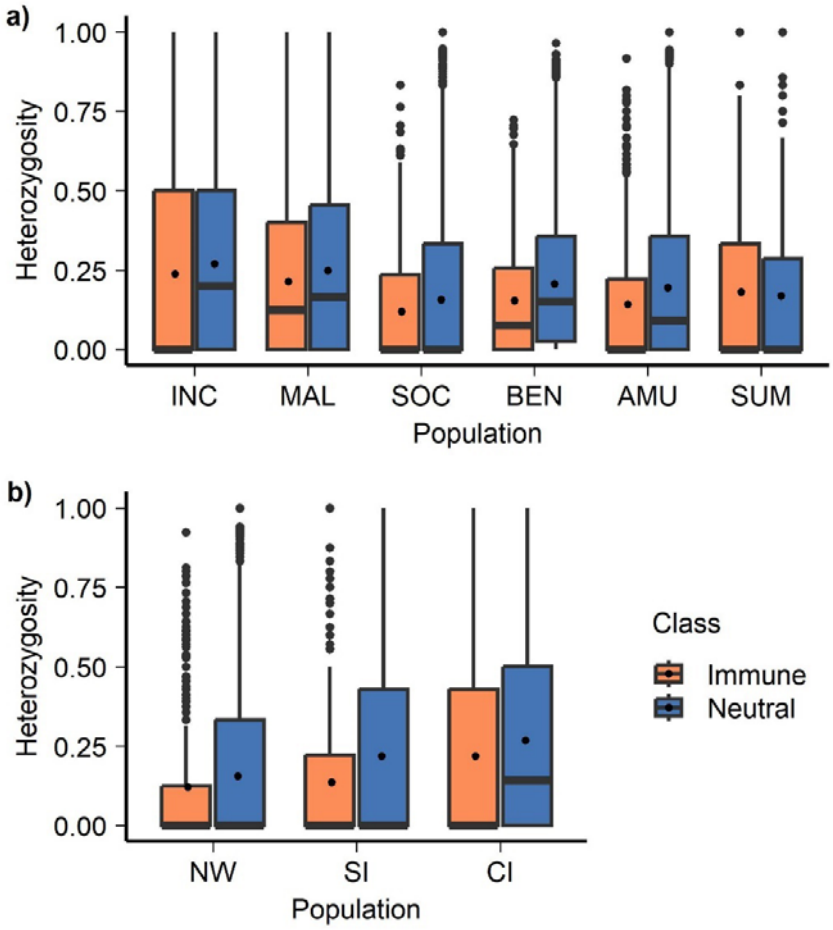

Figure 4: a) Box and whisker plots for heterozygosity at Immune and Neutral loci for extant subspecies of tigers including INC (Indochinese), MAL (Malayan), SOC (South China), BEN (Bengal), AMU (Amur) and SUM (Sumatran) (No significant differences found) (Significant difference across subspecies: Immune loci * and neutral loci ***) b) Box and whisker plots for heterozygosity at Immune and Neutral loci for different populations of Indian tigers NW(Northwest), CI (Central India) and SI (South India) (No significant differences found) (No significant differences found) (Significant difference across subspecies: Immune loci *** and neutral loci ***) (Significance levels: *** < 0.000, ** < 0.001, * < 0.01, ns < 0.05)**

3.4 Genetic differentiation at immune gene and neutral loci between populations of tigers

Given the differences in habitats (and associated pathogens) of the different subspecies and subpopulations of tigers, it is possible that differential pathogen pressure leads to genetic differentiation at immune loci. We compared genetic differentiation at immune loci to that at neutral loci using the Hudson's F_{ST} calculation for tiger subspecies (Figure 5a) and Indian subpopulations (Figure 5b). We found no significant difference in F_{ST} distributions between immune receptor, signalling and neutral loci (Gaussian GLM, Supplementary Table 19).

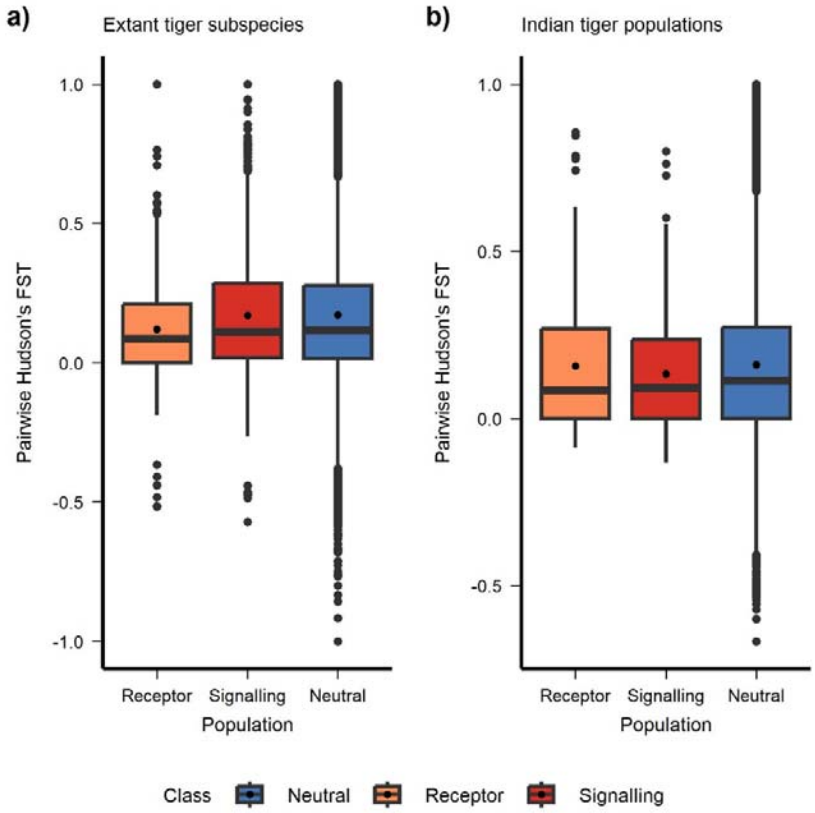

Figure 5: a) Box and whisker plots for Fixation index at immune receptor, immune signalling and Neutral loci for different subspecies of tigers b) Box and whisker plots for Fixation index at immune receptor, immune signalling and neutral loci for different populations of Indian tigers (No significant differences found) (Significance levels: * < 0.000, ** < 0.001, * < 0.01, ns < 0.05)**

We also examined F_{ST} at immune and neutral loci for populations that have history of disease or drastic demographic change. Both scenarios could lead to elevated F_{ST} values. We compared F_{ST} for Amur, Sumatran and South China to all other tiger populations and the three Indian subpopulations to each other and found no significant differences. Sumatran tigers had the highest mean F_{ST} at both neutral and immune loci. Amur tigers had slightly higher mean F_{ST} at immune loci when compared to neutral loci. Northwestern Indian tigers were the most genetically differentiated amongst the three Indian tiger populations, and similar patterns were reflected in immune genes with no significant difference between neutral and immune loci (Supplementary Figure 1 and Supplementary Figure 2, Gaussian GLM Supplementary Table 20).

3.5: Site frequency spectrum of immune and neutral loci to infer selective pressure

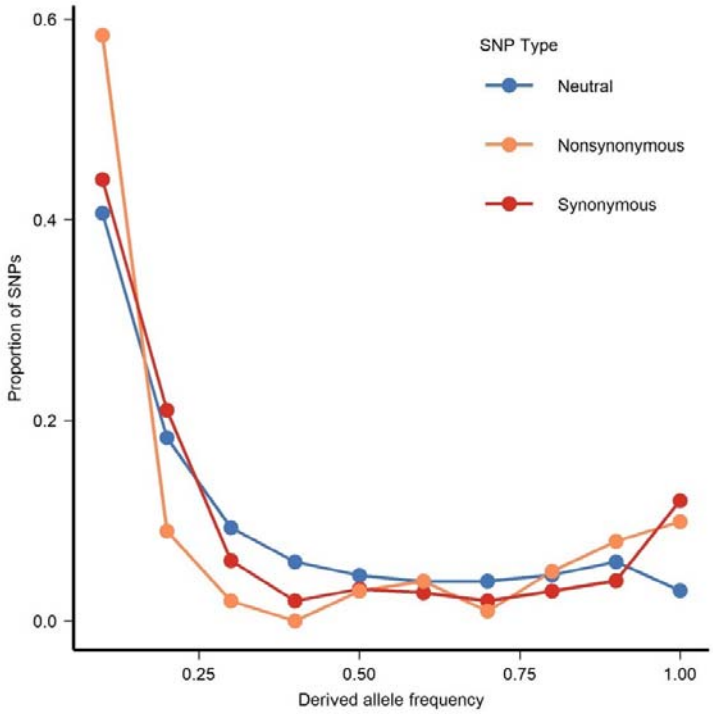

Figure 6: Comparison of SFS of neutral SNPs with that of immune synonymous and nonsynonymous SNPs (no significant differences found) (Significance levels: * < 0.000, ** < 0.001, * < 0.01, ns < 0.05)**

Unfolded SFS for synonymous and nonsynonymous variants were found to not differ significantly from neutral SFS (Supplementary Table 21). However, proportion of nonsynonymous SNPs at lower frequency was higher than neutral or synonymous SNPs which could indicate a weak purifying selection (Figure 6). Since, TLR and LILR genes have significantly higher nucleotide diversity than neutral loci, we assessed selective pressure at these loci through SFS of synonymous and non-synonymous SNPs (Figure 7). We found that the SFS of TLR genes differed from neutral SFS with more non-synonymous variants at intermediate frequencies but again, this difference was not significant (Supplementary Table 21). The SFS of LILR genes was not different from neutral SFS. The TLR genes that were found to harbor SNPs with high heterozygosity were TLR2, TLR5 and TLR9.

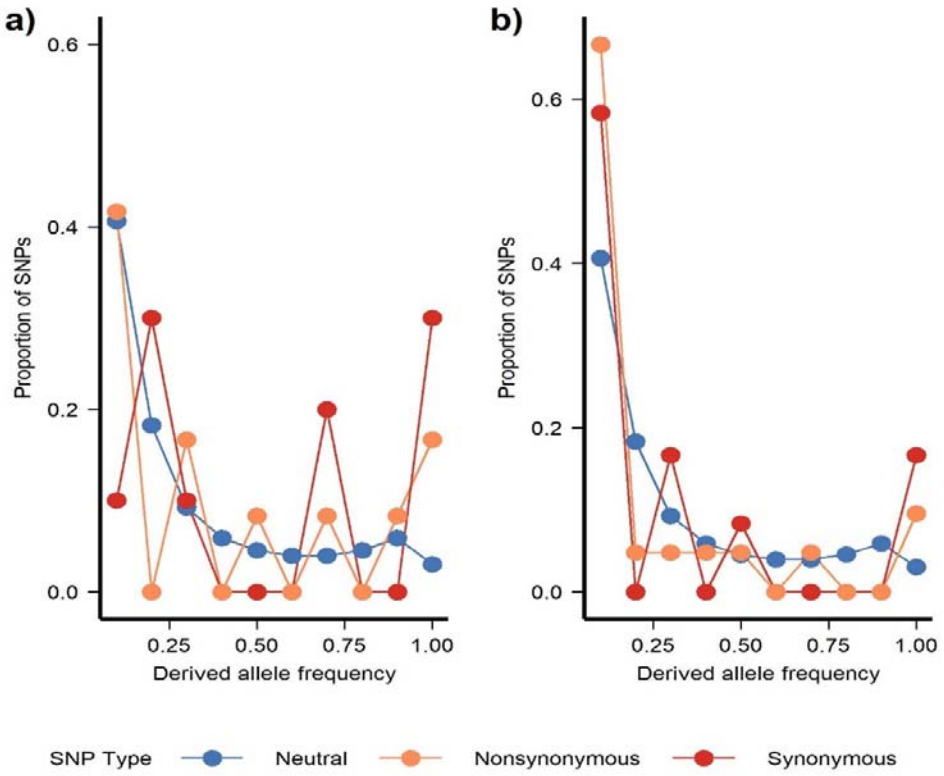

Figure 7: Comparison of SFS of neutral SNPs with that of synonymous and nonsynonymous SNPs at a) TLR genes and b) LILR genes (no significant differences found) (Significance levels: * < 0.000, ** < 0.001, * < 0.01, ns < 0.05)**

3.6: pDeleterious and pLOF mutation load in Indian populations

The pDeleterious and pLOF mutations identified from VEP and further confirmed through Polyphen (Supplementary Figure 3) were used to estimate mutation load in the Indian subpopulations. Most mutation load was found to be masked (Figure 8). We found that large connected populations examined (SI, CI and MAL) have higher masked pDeleterious load than the inbred populations (NW and SOC). Inbreeding load, estimated as homozygous damaging alleles, was lower than masked load in all populations for both pDeleterious and pLOF. The two inbred populations harbored pLOF load (both masked and inbreeding) but SOC tigers lacked any homozygous deleterious mutations (Figure 8).

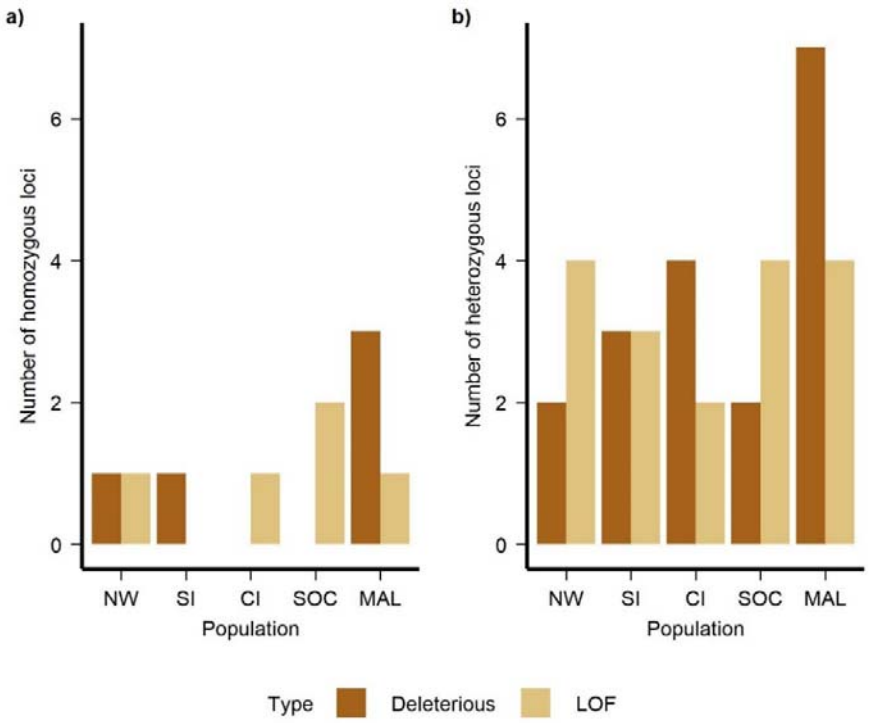

Figure 8: a) Homozygous and b) masked burden of pDeleterious and pLOF (Loss of Function) load in small, isolated inbred NW (Northwest) population and large, connected populations SI (South India) and CI (Central India); inbred South China (SOC) and population with high genetic diversity MAL (Malayan) tigers

We found homozygous pDeleterious mutations in CCR2 (MAL), CCL8 (MAL) and TLR5 (NW and SI). The homozygous pLoF mutations were in CCL5 (CI, MAL and SOC), CXCR2 (SOC) and TNFSF11 (NW).

4. Discussion

4.1 Heterozygosity and nucleotide diversity at different immune gene families revealed contrasting patterns

We characterized variation at several families of immune genes in tigers spanning adaptive and innate immune functions. Given the availability of gene annotations and genomic data, we were able to examine a total of 91 genes spanning 11,57,251 bp, as opposed to a few genes and shorter stretches studied through molecular methods such as PCR (Pokorny, Sharma, and Tiedemann 2010; Morris et al. 2015). Using genetic diversity indices of nucleotide diversity and heterozygosity at intergenic regions, we inferred neutral diversity, against which we compare the same diversity indices for immune genes. Immune genes that code for receptors had significantly higher nucleotide diversity, but heterozygosity did not differ significantly from neutral loci at both receptor and signalling immune loci. Given that nucleotide diversity does not erode as quickly as heterozygosity (Mathur et al. 2023; Sahoo et al. 2023), it is likely that TLR and LILR genes may have undergone positive selection in the past, potentially because they interact with pathogen and MHC molecules (Hirayasu and Arase 2015; Kloch et al. 2018). However, all immune gene families appear to be largely evolving under genetic drift, irrespective of the type of immune function they are involved in.

4.2 Patterns of genetic diversity of immune genes mirror that of neutral loci across populations with differing demographic history

To further ascertain whether drift is the major evolutionary force in tigers, we contrasted immune and neutral variation between the different subspecies of tigers and the different populations of Indian tigers which are known to have varying effective sizes and connectivity status. All tiger subspecies have undergone range contractions and population decline (Liu et al. 2018; Harihar et al. 2018; Armstrong et al. 2021). We find that the changes in neutral variation are reflected in immune gene variation as well, suggesting that most immune genes examined are evolving under genetic drift. Receptor genes have significantly higher nucleotide diversity but this is correlated with neutral diversity across populations. Our results substantiate inferences from earlier studies that most loci in coding regions of the tiger genome are influenced by drift, regardless of which subspecies or population, potentially due to demographic fluctuations and bottlenecks (Armstrong et al. 2021). Most studies examining adaptive variation examine one or few populations which have undergone bottlenecks or exist in both mainland and island populations. To our knowledge, this is the first study examining adaptive variation in range wide populations of a species, and one of the few studies with populations of diverse demographic histories.

The Malayan, Indochinese and Bengal (Figure 3a and Figure 4a) tigers were found to have the highest genetic diversity at both immune and neutral loci. Indochinese and Malayam tigers are geographically close to the ancestral tiger population (South China tiger) whereas Bengal tigers have the largest population size of extant tigers. Receptor immune genes have low nucleotide diversity and do not differ significantly from neutral loci in Indochinese tigers, suggesting these genes might not have been under positive selection in Indochinese tigers. Sumatran tigers (that live on the island of Sumatra) have the lowest nucleotide diversity at immune and neutral loci as expected from island populations (Clegg et al. 2002). The South China tigers that are extinct in the wild and are inbred as a captive population was found to have the lowest homozygosity at immune and neutral loci as expected in an inbred population. Amur tigers, having undergone strong recent bottlenecks similar to Sumatran tigers, have genetic diversity similar to Sumatran tigers at both immune and neutral loci.

Our results substantiate findings in several other endangered species, where decline in population size and isolation leads to loss of adaptive genetic diversity, along with neutral diversity. Such patterns have been found for immune gene diversity in tasmanian devils (Morris et al. 2015), black grouse (Strand et al. 2012), dunnocks (Lara et al. 2020), snub-nosed monkeys (Luo et al. 2012).

4.3 Several immune genes retain high polymorphisms even in bottlenecked populations

We observe several genes having high heterozygosity values in the Northwestern population of Indian tigers, which is small and isolated and has low genome wide heterozygosity. This is also true in Sumatran, South China and Amur tigers (Supplementary Table 18). These are genes performing diverse immune functions, and not just receptor genes. Some of these genes, like C-X-C chemokine receptor type 4 are known to have been under positive selection in tigers and several Interleukin genes are known to be under positive selection in the Panthera lineage (Shukla et al. 2023). Overall, this suggests that despite a bottleneck and drift, some loci might retain high polymorphisms, potentially maintained by past selection. Such variation has been described previously as “ghosts of selection past,” and might get eroded with time due to drift debt (Oliver and Piertney 2012; Gilroy et al. 2017; Davies et al. 2021). Interestingly, a previous study on MHC Class I and Class II genes in Asiatic lions

and Indian tigers which found 'hyper polymorphic' hotspots even when allelic diversity was low (Sachdev, 2005.; Pokorny, Sharma, and Tiedemann 2010).

4.4 Patterns of genetic differentiation at immune loci in tigers are similar to differentiation at neutral loci

We used fixation index or F_{ST} to contrast differentiation at subspecies level and amongst Indian populations while comparing with differentiation at neutral loci. Our findings re-iterate the predominant role of drift in shaping evolution of tigers, including immune loci. F_{ST} values for population pairs, where one (a) had a history of disease (e.g. Amur tigers (Gilbert et al. 2014)), (b) had undergone major bottlenecks (e.g. South China and North western Indian tigers), or (c) had undergone founding events (e.g. Sumatran tigers in the Sunda islands) were not significantly different for immune gene and neutral loci. Amur tigers had a higher mean F_{ST} at immune loci than neutral loci, which could indicate selection for certain alleles that protect from Canine distemper virus but this difference is not significant. Our results align with population genetic theory that suggests that small populations that have been through bottlenecks are expected to be affected more by drift than selection (Keller and Waller 2002; Whitlock 2002; Masel 2011). Additionally, tigers being a solitary species, with very little social contact with other tigers and felids, the possibility of infection through horizontal transmission is limited. Oral consumption and nasal exposure could be two possible modes of infection, of which the latter is less likely. This could have prevented a high prevalence of infections and weaker pathogen mediated selection, making it difficult to detect over the strong signatures of demographic history.

4.5 TLR genes show signatures of balancing selection

TLR and LILR genes have higher heterozygosity and nucleotide diversity than other gene families, and could be candidates for balancing selection. SFS for synonymous and nonsynonymous SNPs at these two loci found higher frequencies of nonsynonymous SNPs at intermediate frequencies in TLR genes but not in LILR genes. Such signal is associated with balancing selection in previous studies (Bernhardsson, 2012.; Fijarczyk and Babik 2015; Bitarello et al. 2018; Brandt et al. 2018). After MHC loci, it is TLR loci that are frequently found to be under balancing selection (Kloch et al. 2018). TLR genes are involved in recognition of structurally conserved molecules of microbes such as virus, bacteria and fungus and are an essential part of innate immunity(Netea, Wijmenga, and O'Neill 2012). These functions of TLR genes could have led to positive balancing selective pressures on them (Fornůsková et al. 2013; Khan et al. 2019). However, there are also several studies that find purifying selection acting on TLR genes (Stefanović et al. 2020; Mukherjee, Ganguli, and Majumder 2014; Netea, Wijmenga, and O'Neill 2012; Raven et al. 2017). The absence of pLOF mutations in TLR genes in tigers suggests the action of purifying selection. This was the only gene family to lack any pLOF variants. Given these contradicting results, it is possible that there is a relaxation of selection constraints on TLR genes, whereby deleterious variants are removed by purifying selection and selection might also increase diversity at certain parts of these genes (Babik et al. 2015; Podlaszczuk et al. 2020). Nevertheless, both evidences point out to potential mechanisms of genomic resilience-purging and retention of functional diversity-that could contribute to population persistence.

We have not used the traditional selection tests (such as Tajima's D, McDonald Kreitman's test, etc.) to distinguish between neutral and non-neutral evolution since these approaches cannot distinguish between selective and demographic events (Poh et al. 2014). All subspecies of tigers have undergone drastic bottlenecks throughout their evolutionary history (Armstrong et al. 2021), making the use of

such approaches redundant. A previous study with the same Indian tiger reference used here found some of the Interleukin and Chemokine genes to be under positive selection through a phylogenetic approach (Shukla et al. 2023). Interestingly, all of the genes detected to be under positive selection were signalling genes and none of the immune receptor genes were detected to be under positive selection. Our comparison of unfolded site frequency spectra for synonymous immune, nonsynonymous immune and neutral SNPs found higher frequency of nonsynonymous SNPs at lower derived allele frequencies suggesting purifying selection and drift to largely shape immune gene diversity (Bernhardsson, 2012; Pepperell et al. 2013; Fijarczyk and Babik 2015).

4.6 Mutation load in immune genes are similar to genome-wide mutation load in inbred tiger populations

Estimating mutation load may help characterize variation in immune gene loci. Overall, load at immune genes is quite low, as only 30 potentially damaging loci were identified. For this load, we attempted to identify pathogenicity of missense mutations (instead of assuming all missense mutations to be pDeleterious). PolyPhen (Adzhubei, Jordan, and Sunyaev 2013) helped classify the extent of potential impact of damaging mutations. These results indicate that most of the missense variants are benign (Supplementary Figure 3). We were able to discern potential damaging effect for twenty missense mutations. There were several missense mutations for which Polyphen assignment was not possible, maybe because we are working with a non-model organism (Kovalev et al. 2018). Polyphen assignments were largely successful in TLR, Interleukin and Chemokine missense mutations, probably because these genes are well characterized for several species (Areal, Abrantes, and Esteves 2011; Bollmer et al. 2011; Morris et al. 2015; Kloch et al. 2018).

We found that damaging mutation load varied across subpopulations of Indian tigers as observed from genome-wide studies (Khan et al. 2021). However, for immune gene loci, pLoF load was higher than pDeleterious load in the Northwest Indian tigers. We also observed higher pLoF than pDeleterious in South China tigers. Khan et al. 2021 found lower pLoF than pDeleterious in the Northwest Indian tiger populations and attributed it to purging. However, they did not investigate possible pathogenicity of these mutations, and this might explain differences in results. These results are important, as such load could be detrimental to the population when exposed to new pathogens (Asano et al. 2022).

4.7 Mutation load and diversity sheds light on populations of conservation importance

Loss of protective polymorphisms in immune genes could result in increased susceptibility to novel infectious diseases, resulting in further population decline (Sommer 2005). Tasmanian devils are particularly notorious for lowered immune function and increased susceptibility to facial tumours that have contributed to population decline (Morris et al. 2015; Strickland et al. 2024). There are few studies on specific mechanisms that result in lowered immune genetic diversity due to technical challenges. Galapagos sea lions with higher homozygosity are known to have lowered immunity when exposed to invasive species and pollution (Brock et al. 2015). Inbred song sparrows are known to have lowered immunity to parasitic infections varying with sex (Reid et al. 2007). Although many immune genes undergo somatic recombination to increase variety of pathogen recognition motifs, genetic diversity of immune genes is known to aid in production of many protein motifs that could help identify novel pathogens and impart disease resistance (Trowsdale and Parham 2004).

Host immunity appears to be an important facet for survival of tigers and other carnivore populations in the future. Several species of large carnivores have suffered disease epidemics in the recent past leading to population decline (Mccarthy, Shaw, and Goodman 2007; Becker et al. 2013). Canine

distemper virus epidemics have affected African wild dogs, African and Asiatic lions and Amur tigers (Mccarthy, Shaw, and Goodman 2007; Roelke-Parker et al. 2010; Gilbert et al. 2014; Mourya et al., 2019). Inbreeding in lions in South Africa is known to increase susceptibility to bovine tuberculosis whereas the Devil Facial Tumour disease epidemic in Tasmanian devil has led to a population crash (Trinkel et al. 2013; Morris et al. 2015).

In our study, we find that the Sumatran and South China subspecies have low nucleotide diversity and heterozygosity respectively and hence, we suggest are most likely to be susceptible to new infections. We caution that the inbred Northwestern subpopulation of Indian tigers also has low neutral and immune gene diversity. A previous study has confirmed the prevalence of Canine Distemper Virus in the region (Sidhu et al. 2019), and we suggest active disease surveillance in this population to avoid exposure. The reduced homozygous load and remnants of heterozygosity at certain immune genes in Northwest Indian and South China populations might yet help the populations persist even in the event of disease exposure. However, in the presence of genetic drift, this variation could be eventually lost (Gargiulo, Budde, and Heuertz 2024). Conservation measures such as genetic rescue must be cautioned of the possibility of introducing deleterious alleles that are purged out in these inbred populations but are present as masked load in larger populations.

4.8 Use and opportunities of genomics in studying adaptive variation of threatened species

Recent methodological developments have contributed to making predictions of load easier (PolyPhen2, SNPEff, CADD) and analysing large scale data quicker (eg. Pixy, which accounts for unequal sample sizes and missing data, angsd which is suitable for low coverage data) (Cingolani et al. 2012; Adzhubei, Jordan, and Sunyaev 2013; Korneliussen, Albrechtsen, and Nielsen 2014; Rentzsch et al. 2019; Korunes and Samuk 2021). Yet, the availability of large-scale whole genome resequencing data and genome assemblies is rare for many endangered species, particularly in the global South (Khan and Tyagi 2021). Our study demonstrates the potential of using genomic data beyond its initial research purpose, particularly for conservation, since we have not generated any new data and utilized publicly available genomes.

Apart from the genetic correlates currently used to study endangered species, in order to understand the adaptive potential of a population, it would be ideal to better understand beneficial mutations. Identifying populations that carry alleles for disease resistance could help find suitable source for genetic rescue of populations at risk. However, this information of fitness-in this case, proved resistance of a mutation to infections- is difficult to obtain. Even variants identified as pDeleterious and pLOF are bioinformatic predictions that might not necessarily be damaging (Kovalev et al. 2018). Only experiments establishing susceptibility to disease and increased disease mortality could truly identify deleterious and beneficial mutations (Hedrick, Lee, and Buchanan 2013). Unfortunately, it is very difficult to carry out such studies in wild species, particularly elusive carnivores. One way to prioritize alleles where functional relevance should be explored is through temporal genomic sampling. Sampling of historical DNA could help identify the loss of adaptive variation and even restore it through genetic rescue strategies (Reynes et al. 2024).

While our study highlights concordance between neutral and immunogenetic variation, generality of such patterns is important to investigate in other endangered species. As shown in Kardos et al. 2021, population genetic theory and empirical evidence in several studies show that neutral variation is indicative of extinction risk and genome wide variation is important for population viability. A comprehensive understanding of functional variation can be obtained only for species with genomic information and access to samples, often limited to few charismatic endangered species. Our results

add to the literature that suggests that when such information is not available, genome-wide neutral markers would be indicative of adaptive potential. In some cases, adaptive loci might better inform conservation priorities (Oliver and Piertney 2012) and situations where gene targeted approaches are more useful than neutral loci (such as locally adapted alleles) have been described in Flanagan et al. 2018 and Kardos and Shafer 2018.

5. Conclusion

Our study attempts to answer a longstanding question in conservation genomics on the correlation between neutral and adaptive variation in genetically depauperate populations with differing demographic histories. We show that immunogenetic variation, if measured as heterozygosity correlates with neutral variation. However, when measured as nucleotide diversity, immune genes coding for receptor molecules (that interact with pathogens and other MHC molecules) have higher genetic variation than neutral loci in tigers. Populations that have undergone bottlenecks and founding effects have lower immune gene (as well as neutral loci) diversity, and show slightly higher differentiation, implicating the dominant role of neutral processes in such populations. However, despite drift, some receptor coding immune genes and immune signalling loci retain high polymorphisms. Of these, TLR genes were found to show possible signature of balancing selection, although this was difficult to test given these strongly bottlenecked populations. We identified populations and subspecies with putatively disadvantageous immune gene variation. Finally, we conclude that in the absence of large-scale genomic data, such as in our study, neutral markers could adequately inform managers about the vulnerability of populations and species to extinction risks from pathogens and infectious diseases.

Acknowledgments:

We are thankful to Laura Bertola and Anubhab Khan for comments on the manuscript; Megan Aylward, Mayuresh Gangal, Sabarinathan Radhakrishnan, Vinay Sagar, Praveen Prakash, Marty Kardos, Harsh Shukla, Mihir Joshi and Kushal Suryamohan for discussion and inputs and Kritagnya Vadar for help with mining annotations. BVA was supported by the NCBS/Tata Institute of Fundamental Research (TIFR) graduate program. The NCBS data cluster used is supported under project 12-R&D-TFR-5.04-0900, Department of Atomic Energy, Government of India. This work was supported by the NCBS/TIFR internal plan fund awarded to UR (Project Identification RTI 4006, Department of Atomic Energy, Government of India) and DBT Wellcome Trust Senior Fellowship (IA/S/16/2/502714) awarded to UR.

References:

Acevedo-Whitehouse, Karina, and Andrew A. Cunningham. 2006. "Is MHC Enough for Understanding Wildlife Immunogenetics?" *Trends in Ecology and Evolution* 21 (8): 433–38. https://doi.org/10.1016/j.tree.2006.05.010.

Adzhubei, Ivan, Daniel M. Jordan, and Shamil R. Sunyaev. 2013. "Predicting Functional Effect of Human Missense Mutations Using PolyPhen-2." *Current Protocols in Human Genetics* 76 (1): 7.20.1-7.20.41. https://doi.org/10.1002/0471142905.hg0720s76.

Aguilar, Andres, Gary Roemer, Sally Debenham, Matthew Binns, David Garcelon, and Robert K. Wayne. 2004. "High MHC Diversity Maintained by Balancing Selection in an Otherwise Genetically Monomorphic Mammal." *Proceedings of the National Academy of Sciences of the United States of America* 101 (10): 3490–94. https://doi.org/10.1073/PNAS.0306582101.

Alcaide, Miguel. 2010. "On the Relative Roles of Selection and Genetic Drift in Shaping MHC Variation." *Molecular Ecology* 19 (18): 3842–44. https://doi.org/10.1111/J.1365-294X.2010.04772.X.

Alho, Jussi S., Bo Göran Lillandt, Sonja Jaari, and Juha Merilä. 2009. "Multilocus Heterozygosity and Inbreeding in the Siberian Jay." *Conservation Genetics* 10 (3): 605–9. https://doi.org/10.1007/S10592-008-9588-Z.

Allendorf, Fred W. 1986. "Genetic Drift and the Loss of Alleles versus Heterozygosity." *Zoo Biology* 5 (2): 181–90. https://doi.org/10.1002/zoo.1430050212.

Annavi, Geetha, Christopher Newman, Christina D. Buesching, David W. Macdonald, Terry Burke, and Hannah L. Dugdale. 2014. "Heterozygosity–Fitness Correlations in a Wild Mammal Population: Accounting for Parental and Environmental Effects." *Ecology and Evolution* 4 (12): 2594–2609. https://doi.org/10.1002/ECE3.1112.

Areal, Helena, Joana Abrantes, and Pedro J. Esteves. 2011. "Signatures of Positive Selection in Toll-like Receptor (TLR) Genes in Mammals." *BMC Evolutionary Biology* 11 (1): 368. https://doi.org/10.1186/1471-2148-11-368.

Armstrong, Ellie E, Anubhab Khan, Ryan W Taylor, Alexandre Gouy, Gili Greenbaum, Alexandre Thiéry, Jonathan T Kang, et al. 2021. "Recent Evolutionary History of Tigers Highlights Contrasting Roles of Genetic Drift and Selection." *Molecular Biology and Evolution* 38 (6): 2021. https://doi.org/10.1093/molbev/msab032.

Asano, Takaki, Takanori Utsumi, Reiko Kagawa, Shuhei Karakawa, and Satoshi Okada. 2022. "Inborn Errors of Immunity with Loss- and Gain-of-Function Germline Mutations in STAT1." *Clinical and Experimental Immunology* 212 (2): 96–106. https://doi.org/10.1093/cei/uxac106.

Babik, Wieslaw, Katarzyna Dudek, Anna Fijarczyk, Maclej Pabijan, Michal Stuglik, Rafal Szkotak, and Piotr Zielinski. 2015. "Constraint and Adaptation in Newt Toll-Like Receptor Genes." *Genome Biology and Evolution* 7 (1): 81–95. https://doi.org/10.1093/GBE/EVU266.

Barnosky, Anthony D., Nicholas Matzke, Susumu Tomiya, Guinevere O. U. Wogan, Brian Swartz, Tiago B. Quental, Charles Marshall, et al. 2011. "Has the Earth's Sixth Mass Extinction Already Arrived?" *Nature* 471 (7336): 51–57. https://doi.org/10.1038/nature09678.

Bataillon, Thomas, and Mark Kirkpatrick#. 2020. "Inbreeding Depression Due to Mildly Deleterious Mutations in Finite Populations: Size Does Matter." *Genet. Res., Camb* 75:75–81. https://doi.org/10.1017/S0016672399004048.

Bateson, Zachary W., Susan C. Hammerly, Jeff A. Johnson, Michael E. Morrow, Linda A. Whittingham, and Peter O. Dunn. 2016. "Specific Alleles at Immune Genes, Rather than Genome-Wide Heterozygosity, Are Related to Immunity and Survival in the Critically Endangered Attwater's Prairie-Chicken." *Molecular Ecology* 25 (19): 4730–44. https://doi.org/10.1111/mec.13793.

Becker, Matthew S., Nicholas M. Sakuya, Cathleen A. Hanlon, Jassiel M'soka, Mike R. Dunbar, Rachel McRobb, Egil Droge, Are R. Berentsen, and Wigganson Matandiko. 2013. "Rabies, Canine Distemper, and Canine Parvovirus Exposure in Large Carnivore Communities from Two Zambian Ecosystems." *Vector-Borne and Zoonotic Diseases* 13 (9): 643–49. https://doi.org/10.1089/vbz.2012.1233.

776 Beilharz, R. G. 1982. "The Effect of Inbreeding on Reproduction in Mice." *Animal Science* 34 (1): 49–
777 54. https://doi.org/10.1017/S0003356100000465.
778 Bernhardsson, Carolina. n.d. "Molecular Population Genetics of Inducible Defense Genes in Populus
779 Tremula."
780 Bitarello, Bárbara D, Cesare de Filippo, João C Teixeira, Joshua M Schmidt, Philip Kleinert, Diogo
781 Meyer, and Aida M Andrés. 2018. "Signatures of Long-Term Balancing Selection in Human
782 Genomes." *Genome Biology and Evolution* 10 (3): 939–55.
783 https://doi.org/10.1093/gbe/evy054.
784 Blomqvist, Donald, Angela Pauliny, Mikael Larsson, and Lars-Åke Flodin. 2010. "Trapped in the
785 Extinction Vortex? Strong Genetic Effects in a Declining Vertebrate Population." *BMC*
786 *Evolutionary Biology* 10 (1): 33. https://doi.org/10.1186/1471-2148-10-33.
787 Bodo, Tombari, Batombari Gbidum Gimah, and Kemetonye Joy Seomoni. 2021. "Deforestation and
788 Habitat Loss: Human Causes, Consequences and Possible Solutions." *Journal of Geographical*
789 *Research* 4 (2): 22–30. https://doi.org/10.30564/jgr.v4i2.3059.
790 Bolger, Anthony M., Marc Lohse, and Bjoern Usadel. 2014. "Trimmomatic: A Flexible Trimmer for
791 Illumina Sequence Data." *Bioinformatics* 30 (15): 2114.
792 https://doi.org/10.1093/BIOINFORMATICS/BTU170.
793 Bollmer, Jennifer L., Elizabeth A. Ruder, Jeff A. Johnson, John A. Eimes, and Peter O. Dunn. 2011.
794 "Drift and Selection Influence Geographic Variation at Immune Loci of Prairie-Chickens."
795 *Molecular Ecology* 20 (22): 4695–4706. https://doi.org/10.1111/J.1365-294X.2011.05319.X.
796 Bolund, Elisabeth, Katrin Martin, Bart Kempenaers, and Wolfgang Forstmeier. 2010. "Inbreeding
797 Depression of Sexually Selected Traits and Attractiveness in the Zebra Finch." *Animal*
798 *Behaviour* 79 (4): 947–55. https://doi.org/10.1016/J.ANBEHAV.2010.01.014.
799 Brandt, Débora Y.C., Jônatas César, Jérôme Goudet, and Diogo Meyer. 2018. "The Effect of Balancing
800 Selection on Population Differentiation: A Study with HLA Genes." *G3: Genes, Genomes,*
801 *Genetics* 8 (8): 2805–15. https://doi.org/10.1534/g3.118.200367.
802 Brock, Patrick M, Simon J Goodman, Ailsa J Hall, Marilyn Cruz, and Karina Acevedo-whitehouse.
803 2015. "Context-Dependent Associations between Heterozygosity and Immune Variation in a
804 Wild Carnivore." *BMC Evolutionary Biology*, 1–10. https://doi.org/10.1186/s12862-015-
805 0519-6.
806 Brooks, Mollie, E., Kasper Kristensen, Koen Benthem J.,van, Arni Magnusson, Casper Berg W., Anders
807 Nielsen, Hans Skaug J., Martin Mächler, and Benjamin Bolker M. 2017. "glmmTMB Balances
808 Speed and Flexibility Among Packages for Zero-Inflated Generalized Linear Mixed Modeling."
809 *The R Journal* 9 (2): 378. https://doi.org/10.32614/RJ-2017-066.
810 Cardillo, Marcel, Georgina M Mace, John L Gittleman, Kate E Jones, Jon Bielby, and Andy Purvis.
811 2008. "The Predictability of Extinction: Biological and External Correlates of Decline in
812 Mammals." *Proceedings of the Royal Society B: Biological Sciences* 275 (1641): 1441–48.
813 https://doi.org/10.1098/rspb.2008.0179.
814 Carneiro, Miguel, Vicente Piorno, Carl-Johan Rubin, Joel M. Alves, Nuno Ferrand, Paulo C. Alves, and
815 Leif Andersson. 2015. "Candidate Genes Underlying Heritable Differences in Reproductive
816 Seasonality between Wild and Domestic Rabbits." *Animal Genetics* 46 (4): 418–25.
817 https://doi.org/10.1111/age.12299.
818 Castro-Prieto, Aines, Bettina Wachter, and Simone Sommer. 2010. "Cheetah Paradigm Revisited:
819 MHC Diversity in the World's Largest Free-Ranging Population." *Molecular Biology and*
820 *Evolution* 28 (4): 1455–68. https://doi.org/10.1093/molbev/msq330.
821 Chapman, J. R., S. Nakagawa, D. W. Coltman, J. Slate, and B. C. Sheldon. 2009. "A Quantitative
822 Review of Heterozygosity-Fitness Correlations in Animal Populations." *Molecular Ecology* 18
823 (13): 2746–65. https://doi.org/10.1111/J.1365-294X.2009.04247.X.
824 Charlesworth, B., and D. Charlesworth. 2017. "Population Genetics from 1966 to 2016." *Heredity*.
825 Nature Publishing Group. https://doi.org/10.1038/hdy.2016.55.

Cingolani, Pablo, Adrian Platts, Le Lily Wang, Melissa Coon, Tung Nguyen, Luan Wang, Susan J. Land, Xiangyi Lu, and Douglas M. Ruden. 2012. "A Program for Annotating and Predicting the Effects of Single Nucleotide Polymorphisms, SnpEff: SNPs in the Genome of Drosophila Melanogaster Strain W1118; Iso-2; Iso-3." *Fly* 6 (2): 80–92. https://doi.org/10.4161/fly.19695.

Clegg, Sonya M., Sandie M. Degnan, Craig Moritz, Arnaud Estoup, Jiro Kikkawa, and Ian P. F. Owens. 2002. "MICROEVOLUTION IN ISLAND FORMS: THE ROLES OF DRIFT AND DIRECTIONAL SELECTION IN MORPHOLOGICAL DIVERGENCE OF A PASSERINE BIRD." *Evolution* 56 (10): 2090–99. https://doi.org/10.1111/j.0014-3820.2002.tb00134.x.

Cortázar-Chinarro, Maria, Ella Z. Lattenkamp, Yvonne Meyer-Lucht, Emilien Luquet, Anssi Laurila, and Jacob Höglund. 2017. "Drift, Selection, or Migration? Processes Affecting Genetic Differentiation and Variation along a Latitudinal Gradient in an Amphibian." *BMC Evolutionary Biology* 17 (1). https://doi.org/10.1186/s12862-017-1022-z.

Danecek, Petr, Adam Auton, Goncalo Abecasis, Cornelis A. Albers, Eric Banks, Mark A. DePristo, Robert E. Handsaker, et al. 2011. "The Variant Call Format and VCFtools." *Bioinformatics* 27 (15): 2156–58. https://doi.org/10.1093/bioinformatics/btr330.

Danecek, Petr, James K Bonfield, Jennifer Liddle, John Marshall, Valeriu Ohan, Martin O Pollard, Andrew Whitwham, et al. 2021. "Twelve Years of SAMtools and BCFtools." *GigaScience* 10 (2): giab008. https://doi.org/10.1093/gigascience/giab008.

Davies, Charli S., Martin I. Taylor, Martijn Hammers, Terry Burke, Jan Komdeur, Hannah L. Dugdale, and David S. Richardson. 2021. "Contemporary Evolution of the Innate Immune Receptor Gene TLR3 in an Isolated Vertebrate Population." *Molecular Ecology* 30 (11): 2528–42. https://doi.org/10.1111/mec.15914.

Du, Hairong, Jingjing Yu, Qian Li, and Minghai Zhang. 2022. "New Evidence of Tiger Subspecies Differentiation and Environmental Adaptation: Comparison of the Whole Genomes of the Amur Tiger and the South China Tiger." *Animals* 12 (July):1817. https://doi.org/10.3390/ani12141817.

Duffield, Kristin R., Kylie J. Hampton, Thomas M. Houslay, John Hunt, Ben M. Sadd, and Scott K. Sakaluk. 2019. "Inbreeding Alters Context-dependent Reproductive Effort and Immunity in Male Crickets." *Journal of Evolutionary Biology* 32 (7): 731–41. https://doi.org/10.1111/jeb.13478.

Edwards, Scott V., Joe Gasper, Daniel Garrigan, Duane Martindale, and Ben F. Koop. 2000. "A 39-Kb Sequence around a Blackbird Mhc Class II Gene: Ghost of Selection Past and Songbird Genome Architecture." *Molecular Biology and Evolution* 17 (9): 1384–95. https://doi.org/10.1093/oxfordjournals.molbev.a026421.

Edwards, Scott V., E. K. Wakeland, and W. K. Potts. 1995. "Contrasting Histories of Avian and Mammalian Mhc Genes Revealed by Class II B Sequences from Songbirds." *Proceedings of the National Academy of Sciences of the United States of America* 92 (26): 12200–204. https://doi.org/10.1073/pnas.92.26.12200.

Ellis, J. S., L. M. Turner, and M. E. Knight. 2012. "Patterns of Selection and Polymorphism of Innate Immunity Genes in Bumblebees (Hymenoptera: Apidae)." *Genetica* 140 (4–6): 205–17. https://doi.org/10.1007/s10709-012-9672-7.

Endler, J.A. (1986) Natural Selection in the Wild. Princeton University Press, Princeton.https://www.scirp.org/reference/referencespapers?referenceid=1320621.

Eyre-Walker, Adam, and Peter D. Keightley. 2007. "The Distribution of Fitness Effects of New Mutations." *Nature Reviews Genetics* 8 (8): 610–18. https://doi.org/10.1038/nrg2146.

Fagan, William F., and E. E. Holmes. 2006. "Quantifying the Extinction Vortex." *Ecology Letters* 9 (1): 51–60. https://doi.org/10.1111/j.1461-0248.2005.00845.x.

Fernández-Sepúlveda, Jaime, and Carlos A. Martín. 2022. "Conservation Status of the World's Carnivorous Mammals (Order Carnivora)." *Mammalian Biology* 102 (5–6): 1911–25. https://doi.org/10.1007/S42991-022-00305-8/FIGURES/5.

Fijarczyk, Anna, and Wiesław Babik. 2015. “Detecting Balancing Selection in Genomes: Limits and Prospects.” *Molecular Ecology* 24 (14): 3529–45. https://doi.org/10.1111/mec.13226.

Fisher, R. A., Henry Bennett, R. A. Fisher, and Henry Bennett, eds. 1999. *The Genetical Theory of Natural Selection: A Complete Variorum Edition*. Oxford, New York: Oxford University Press.

Flanagan, Sarah P., Brenna R. Forester, Emily K. Latch, Sally N. Aitken, and Sean Hoban. 2018. “Guidelines for Planning Genomic Assessment and Monitoring of Locally Adaptive Variation to Inform Species Conservation.” *Evolutionary Applications* 11 (7): 1035–52. https://doi.org/10.1111/eva.12569.

Fornůsková, Alena, Michal Vinkler, Marie Pagès, Maxime Galan, Emmanuelle Jousselin, Frederique Cerqueira, Serge Morand, Nathalie Charbonnel, Josef Bryja, and Jean-François Cosson. 2013. “Contrasted Evolutionary Histories of Two Toll-like Receptors (Tlr4 and Tlr7) in Wild Rodents (MURINAE).” *BMC Evolutionary Biology* 13 (1): 194. https://doi.org/10.1186/1471-2148-13-194.

Frankham, Richard. 1996. “Relationship of Genetic Variation to Population Size in Wildlife.” *Conservation Biology* 10 (6): 1500–1508. https://doi.org/10.1046/j.1523-1739.1996.10061500.x.

Frankham,Richard. 2005. “Genetics and Extinction.” *Biological Conservation* 126 (2): 131–40. https://doi.org/10.1016/j.biocon.2005.05.002.

Gargiulo, Roberta, Katharina B. Budde, and Myriam Heuertz. 2024. “Mind the Lag: Understanding Genetic Extinction Debt for Conservation.” *Trends in Ecology & Evolution*, November. https://doi.org/10.1016/j.tree.2024.10.008.

Gilbert, Martin, Zachary Dvornicky-Raymond, and Jessica Bodgener. 2023. “Disease Threats to Tigers and Their Prey.” *Frontiers in Ecology and Evolution* 11 (April):1135935. https://doi.org/10.3389/fevo.2023.1135935.

Gilbert, Martin, Dale G. Miquelle, John M. Goodrich, Richard Reeve, Sarah Cleaveland, Louise Matthews, and Damien O. Joly. 2014. “Estimating the Potential Impact of Canine Distemper Virus on the Amur Tiger Population (Panthera Tigris Altaica) in Russia.” *PLOS ONE* 9 (10): e110811. https://doi.org/10.1371/JOURNAL.PONE.0110811.

Gilpin, Michael E. 1986. “Minimum Viable Populations: Processes of Species Extinction.” *Conservation Biology: The Science of Scarcity and Diversity*, 19–34.

Gilroy, D. L., K. P. Phillips, D. S. Richardson, and C. van Oosterhout. 2017. “Toll-like Receptor Variation in the Bottlenecked Population of the Seychelles Warbler: Computer Simulations See the ‘ghost of Selection Past’ and Quantify the ‘drift Debt’.” *Journal of Evolutionary Biology* 30 (7): 1276–87. https://doi.org/10.1111/jeb.13077.

Godwin, Joanne L., Alyson J. Lumley, Łukasz Michalczyk, Oliver Y. Martin, and Matthew J. G. Gage. 2020. “Mating Patterns Influence Vulnerability to the Extinction Vortex.” *Global Change Biology* 26 (8): 4226–39. https://doi.org/10.1111/gcb.15186.

Grossen, Christine, and Uma Ramakrishnan. 2024. “Genetic Load.” *Current Biology* 34 (24): R1216–20. https://doi.org/10.1016/j.cub.2024.11.004.

Grueber, Catherine E., Graham P. Wallis, and Ian G. Jamieson. 2014. “Episodic Positive Selection in the Evolution of Avian Toll-like Receptor Innate Immunity Genes.” *PLoS ONE* 9 (3). https://doi.org/10.1371/journal.pone.0089632.

Grueber, Catherine E., Graham P. Wallis, Tania M. King, and Ian G. Jamieson. 2012. “Variation at Innate Immunity Toll-Like Receptor Genes in a Bottlenecked Population of a New Zealand Robin.” *PLOS ONE* 7 (9): e45011. https://doi.org/10.1371/JOURNAL.PONE.0045011.

Hansson, Bengt, and Lars Westerberg. 2002. “On the Correlation between Heterozygosity and Fitness in Natural Populations.” *Molecular Ecology* 11 (12): 2467–74. https://doi.org/10.1046/J.1365-294X.2002.01644.X.

Harihar, Abishek, Pranav Chanchani, Jimmy Borah, Rachel Jane Crouthers, Yury Darman, Thomas N E Gray, Shariff Mohamad, et al. 2018. “Recovery Planning towards Doubling Wild Tiger Panthera Tigris Numbers: Detailing 18 Recovery Sites from across the Range,” 1–16.

Hedrick, Philip W. 2001. "Conservation Genetics: Where Are We Now?" *Trends in Ecology & Evolution* 16 (11): 629–36. https://doi.org/10.1016/S0169-5347(01)02282-0.

Hedrick, Philip W., Rhonda N. Lee, and Colleen Buchanan. 2013. "Canine Parvovirus Enteritis, Canine Distemper, and Major Histocompatibility Complex Genetic Variation in Mexican Wolves." *Journal of Wildlife Diseases* 39 (4): 909–13. https://doi.org/10.7589/0090-3558-39.4.909.

Hirayasu, Kouyuki, and Hisashi Arase. 2015. "Functional and Genetic Diversity of Leukocyte Immunoglobulin-like Receptor and Implication for Disease Associations." *Journal of Human Genetics* 60 (11): 703–8. https://doi.org/10.1038/jhg.2015.64.

Höglund, Jacob. 2009. "The Extinction Vortex, Is Genetic Variation Related to Extinction?" In *Evolutionary Conservation Genetics*, edited by Jacob Höglund, 0. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780199214211.003.0001.

Hudson, R R, M Slatkin, and W P Maddison. 1992. "Estimation of Levels of Gene Flow from DNA Sequence Data." *Genetics* 132 (2): 583–89. https://doi.org/10.1093/genetics/132.2.583.

Janeway, Charles A., Paul Jr. Travers, Mark Walport, and Mark J. Shlomchik. 2001. "Immunobiology: The Immune System in Health and Disease," 910. https://doi.org/10.1017/CBO9781107415324.004.

Jepson, A, W Banya, F Sisay-Joof, M Hassan-King, C Nunes, S Bennett, and H Whittle. 1997. "Quantification of the Relative Contribution of Major Histocompatibility Complex (MHC) and Non-MHC Genes to Human Immune Responses to Foreign Antigens." *Infection and Immunity* 65 (3): 872.

Jhala, Yadvendradev, Rajesh Gopal, Vaibhav Mathur, Prodipto Ghosh, Himmat Singh Negi, Sunita Narain, Satya Prakash Yadav, Amit Malik, Rajendra Garawad, and Qamar Qureshi. 2021. "Recovery of Tigers in India: Critical Introspection and Potential Lessons." *People and Nature* 3 (2): 281–93. https://doi.org/10.1002/pan3.10177.

Jhala, Yadvendradev V., Ninad Avinash Mungi, Rajesh Gopal, and Qamar Qureshi. 2025. "Tiger Recovery amid People and Poverty." *Science* 387 (6733): 505–10. https://doi.org/10.1126/science.adk4827.

Johnson, Jeff A., and Peter O. Dunn. 2006. "Low Genetic Variation in the Heath Hen Prior to Extinction and Implications for the Conservation of Prairie-Chicken Populations." *Conservation Genetics* 7 (1): 37–48. https://doi.org/10.1007/s10592-005-7856-8.

Johnson, Kevin P., Frederick R. Adler, and Joshua L. Cherry. 2000. "Genetic and Phylogenetic Consequences of Island Biogeography." *Evolution* 54 (2): 387–96. https://doi.org/10.1111/j.0014-3820.2000.tb00041.x.

Jones, Carl, WILLARD HECK, Richard Lewis, YOUSOOF MUNGROO, Glenn Slade, and TOM CADE. 2008. "The Restoration of the Mauritius Kestrel Falco Punctatus Population." *Ibis* 137 (June):S173–80. https://doi.org/10.1111/j.1474-919X.1995.tb08439.x.

Kardos, Marty, Ellie E. Armstrong, Sarah W. Fitzpatrick, Samantha Hauser, Philip W. Hedrick, Joshua M. Miller, David A. Tallmon, and W. Chris Funk. 2021. "The Crucial Role of Genome-Wide Genetic Variation in Conservation." *Proceedings of the National Academy of Sciences of the United States of America* 118 (48): e2104642118. https://doi.org/10.1073/pnas.2104642118.

Kardos, Marty, and Aaron B. A. Shafer. 2018. "The Peril of Gene-Targeted Conservation." *Trends in Ecology & Evolution* 33 (11): 827–39. https://doi.org/10.1016/j.tree.2018.08.011.

Kardos, Marty, Yaolei Zhang, Kim M. Parsons, Yunga A, Hui Kang, Xun Xu, Xin Liu, et al. 2023. "Inbreeding Depression Explains Killer Whale Population Dynamics." *Nature Ecology & Evolution* 7 (5): 675–86. https://doi.org/10.1038/s41559-023-01995-0.

Keller, Lukas F., Peter Arcese, James N. M. Smith, Wesley M. Hochachka, and Stephen C. Stearns. 1994. "Selection against Inbred Song Sparrows during a Natural Population Bottleneck." *Nature* 372 (6504): 356–57. https://doi.org/10.1038/372356a0.

Keller, Lukas F, and Donald M Waller. 2002. "Inbreeding Effects in Wild Populations" 17 (5): 19–23.

Khan, Anubhab, Kaushalkumar Patel, Harsh Shukla, Ashwin Viswanathan, Tom van der Valk, Udayan Borthakur, Parag Nigam, et al. 2021. "Genomic Evidence for Inbreeding Depression and Purging of Deleterious Genetic Variation in Indian Tigers." *Proceedings of the National Academy of Sciences* 118 (49): e2023018118. https://doi.org/10.1073/PNAS.2023018118.

Khan, Anubhab, and Abhinav Tyagi. 2021. "Considerations for Initiating a Wildlife Genomics Research Project in South and South-East Asia." *Journal of the Indian Institute of Science* 101 (2): 243–56. https://doi.org/10.1007/s41745-021-00243-3.

Khan, Imran, Emanuel Maldonado, Liliana Silva, Daniela Almeida, Warren E. Johnson, Stephen J. O'Brien, Guojie Zhang, Erich D. Jarvis, M. Thomas, and Agostinho Antunes. 2019. "The Vertebrate TLR Supergene Family Evolved Dynamically by Gene Gain/Loss and Positive Selection Revealing a Host-Pathogen Arms Race in Birds." *Diversity* 11 (8): 131. https://doi.org/10.3390/d11080131.

Kimura, Motoo. 1983. *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9780511623486.

Kloch, Agnieszka, Marius A. Wenzel, Dominik R. Laetsch, Olek Michalski, Anna Bajer, Jerzy M. Behnke, Renata Welc-Falęciak, and Stuart B. Piertney. 2018. "Signatures of Balancing Selection in Toll-like Receptor (TLRs) Genes – Novel Insights from a Free-Living Rodent." *Scientific Reports* 8 (1): 8361. https://doi.org/10.1038/s41598-018-26672-2.

Korneliussen, Thorfinn Sand, Anders Albrechtsen, and Rasmus Nielsen. 2014. "ANGSD: Analysis of Next Generation Sequencing Data." *BMC Bioinformatics* 15 (1): 356. https://doi.org/10.1186/s12859-014-0356-4.

Korunes, Katharine L., and Kieran Samuk. 2021. "Pixy: Unbiased Estimation of Nucleotide Diversity and Divergence in the Presence of Missing Data." *Molecular Ecology Resources* 21 (4): 1359–68. https://doi.org/10.1111/1755-0998.13326.

Kovalev, Maxim S., Anna A. Igolkina, Maria G. Samsonova, and Sergey V. Nuzhdin. 2018. "A Pipeline for Classifying Deleterious Coding Mutations in Agricultural Plants." *Frontiers in Plant Science* 9 (November). https://doi.org/10.3389/fpls.2018.01734.

Lara, Carlos Esteban, Catherine E. Grueber, Benedikt Holtmann, Eduardo S.A. Santos, Sheri L. Johnson, Bruce C. Robertson, Gabriel J. Castaño-Villa, Malgorzata Lagisz, and Shinichi Nakagawa. 2020. "Assessment of the Dunnocks' Introduction to New Zealand Using Innate Immune-Gene Diversity." *Evolutionary Ecology* 34 (5): 803–20. https://doi.org/10.1007/s10682-020-10070-0.

Liberg, Olof, Henrik Andrén, Hans Christian Pedersen, Håan Sand, Douglas Sejberg, Retter Wabakken, Mikael Åkesson, and Staffan Bensch. 2005. "Severe Inbreeding Depression in a Wild Wolf (Canis Lupus) Population." *Biology Letters* 1 (1): 17–20. https://doi.org/10.1098/rsbl.2004.0266.

Liu, Yue Chen, Xin Sun, Carlos Driscoll, Dale G. Miquelle, Xiao Xu, Paolo Martelli, Olga Uphyrkina, James L.D. Smith, Stephen J. O'Brien, and Shu Jin Luo. 2018. "Genome-Wide Evolutionary Analysis of Natural History and Adaptation in the World's Tigers." *Current Biology* 28 (23): 3840-3849.e6. https://doi.org/10.1016/j.cub.2018.09.019.

Luo, Mao Fang, Hui Juan Pan, Zhi Jin Liu, and Ming Li. 2012. "Balancing Selection and Genetic Drift at Major Histocompatibility Complex Class II Genes in Isolated Populations of Golden Snub-Nosed Monkey (Rhinopithecus Roxellana)." *BMC Evolutionary Biology* 12 (1): 207. https://doi.org/10.1186/1471-2148-12-207.

Sachdev, M., Sankaranarayanan, R., Reddanna, P., Thangaraj, K. and Singh, L. (2005), Major histocompatibility complex class I polymorphism in Asiatic lions. Tissue Antigens, 66: 9-18. https://doi.org/10.1111/j.1399-0039.2005.00432.x

Masel, Joanna. 2011. "Genetic Drift." *Current Biology* 21 (20): R837–38. https://doi.org/10.1016/j.cub.2011.08.007.

1027 Mathur, Samarth, and J. Andrew DeWoody. 2021. "Genetic Load Has Potential in Large Populations
1028 but Is Realized in Small Inbred Populations." *Evolutionary Applications* 14 (6): 1540–57.
1029 https://doi.org/10.1111/eva.13216.
1030 Mathur, Samarth, Andrew J. Mason, Gideon S. Bradburd, and H. Lisle Gibbs. 2023. "Functional
1031 Genomic Diversity Is Correlated with Neutral Genomic Diversity in Populations of an
1032 Endangered Rattlesnake." *Proceedings of the National Academy of Sciences of the United*
1033 *States of America* 120 (43). https://doi.org/10.1073/pnas.2303043120.
1034 Mccarthy, Alex J, Marie-anne Shaw, and Simon J Goodman. 2007. "Pathogen Evolution and Disease
1035 Emergence in Carnivores," no. October, 3165–74. https://doi.org/10.1098/rspb.2007.0884.
1036 McLaren, William, Laurent Gil, Sarah E. Hunt, Harpreet Singh Riat, Graham R. S. Ritchie, Anja
1037 Thormann, Paul Flicek, and Fiona Cunningham. 2016. "The Ensembl Variant Effect
1038 Predictor." *Genome Biology* 17 (1): 122. https://doi.org/10.1186/s13059-016-0974-4.
1039 Minias, Piotr, Patrycja Podlaszczuk, Piotr Indykiewicz, Mateusz Ledwoń, Jacek Nowakowski, Amelia
1040 Chyb, and Tomasz Janiszewski. 2023. "Genetic Variation at Innate and Adaptive Immune
1041 Genes – Contrasting Patterns of Differentiation and Local Adaptation in a Wild Gull."
1042 *Heredity* 131 (4): 282–91. https://doi.org/10.1038/s41437-023-00645-2.
1043 Mondol, Samrat, K. Ullas Karanth, and Uma Ramakrishnan. 2009. "Why the Indian Subcontinent
1044 Holds the Key to Global Tiger Recovery." *PLoS Genetics* 5 (8).
1045 https://doi.org/10.1371/journal.pgen.1000585.
1046 Morin, Phillip A., Frederick I. Archer, Catherine D. Avila, Jennifer R. Balacco, Yury V. Bukhman,
1047 William Chow, Olivier Fedrigo, et al. 2021. "Reference Genome and Demographic History of
1048 the Most Endangered Marine Mammal, the Vaquita." *Molecular Ecology Resources* 21 (4):
1049 1008–20. https://doi.org/10.1111/1755-0998.13284.
1050 Morris, Katrina M., Belinda Wright, Catherine E. Grueber, Carolyn Hogg, and Katherine Belov. 2015.
1051 "Lack of Genetic Diversity across Diverse Immune Genes in an Endangered Mammal, the
1052 Tasmanian Devil (*Sarcophilus Harrisii*)." *Molecular Ecology* 24 (15): 3860–72.
1053 https://doi.org/10.1111/mec.13291.
1054 Mourya, Devendra T., Pragya D. Yadav, Sreelekshmy Mohandas, R. F. Kadiwar, M. K. Vala, Akshay K.
1055 Saxena, Anita Shete-Aich, et al. n.d. "Canine Distemper Virus in Asiatic Lions of Gujarat State,
1056 India - Volume 25, Number 11—November 2019 - Emerging Infectious Diseases Journal -
1057 CDC." Accessed February 10, 2025. https://doi.org/10.3201/eid2511.190120.
1058 Mukherjee, Souvik, Debdutta Ganguli, and Partha P. Majumder. 2014. "Global Footprints of Purifying
1059 Selection on Toll-Like Receptor Genes Primarily Associated with Response to Bacterial
1060 Infections in Humans." *Genome Biology and Evolution* 6 (3): 551–58.
1061 https://doi.org/10.1093/gbe/evu032.
1062 Mukherjee, Souvik, Neeta Sarkar-Roy, Diane K. Wagener, and Partha P. Majumder. 2009. "Signatures
1063 of Natural Selection Are Not Uniform across Genes of Innate Immune System, but Purifying
1064 Selection Is the Dominant Signature." *Proceedings of the National Academy of Sciences of*
1065 *the United States of America* 106 (17): 7073–78. https://doi.org/10.1073/pnas.0811357106.
1066 Natesh, Meghana, Goutham Atla, Parag Nigam, Yadvendradev V. Jhala, Arun Zachariah, Udayan
1067 Borthakur, and Uma Ramakrishnan. 2017. "Conservation Priorities for Endangered Indian
1068 Tigers through a Genomic Lens." *Scientific Reports* 7 (1): 1–11.
1069 https://doi.org/10.1038/s41598-017-09748-3.
1070 Netea, Mihai G., Cisca Wijmenga, and Luke A. J. O'Neill. 2012. "Genetic Variation in Toll-like
1071 Receptors and Disease Susceptibility." *Nature Immunology* 13 (6): 535–42.
1072 https://doi.org/10.1038/ni.2284.
1073 Ochoa, Alexander, Michael Broe, Emily Moriarty Lemmon, Alan R. Lemmon, Darin R. Rokyta, and H.
1074 Lisle Gibbs. 2020. "Drift, Selection and Adaptive Variation in Small Populations of a
1075 Threatened Rattlesnake." *Molecular Ecology* 29 (14): 2612–25.
1076 https://doi.org/10.1111/mec.15517.

1077 Oliver, Matthew K., and Stuart B. Piertney. 2012. "Selection Maintains MHC Diversity through a
1078 Natural Population Bottleneck." *Molecular Biology and Evolution* 29 (7): 1713–20.
1079 https://doi.org/10.1093/molbev/mss063.
1080 Pardo, Luba, Ian Mackay, Ben Oostra, Cornelia Duijn, and Yurii Aulchenko. 2005. "The Effect of
1081 Genetic Drift in a Young Genetically Isolated Population." *Annals of Human Genetics* 69
1082 (May):288–95. https://doi.org/10.1046/J.1469-1809.2005.00162.x.
1083 Pepperell, Caitlin S., Amanda M. Casto, Andrew Kitchen, Julie M. Granka, Omar E. Cornejo, Eddie C.
1084 Holmes, Bruce Birren, James Galagan, and Marcus W. Feldman. 2013. "The Role of Selection
1085 in Shaping Diversity of Natural M. Tuberculosis Populations." *PLOS Pathogens* 9 (8):
1086 e1003543. https://doi.org/10.1371/journal.ppat.1003543.
1087 Podlaszczuk, Patrycja, Piotr Indykiewicz, Janusz Markowski, and Piotr Minias. 2020. "Relaxation of
1088 Selective Constraints Shapes Variation of Toll-like Receptors in a Colonial Waterbird, the
1089 Black-Headed Gull." *Immunogenetics* 72 (4): 251. https://doi.org/10.1007/S00251-020-
1090 01156-8.
1091 Poh, Yu Ping, Vera S. Domingues, Hopi E. Hoekstra, and Jeffrey D. Jensen. 2014. "On the Prospect of
1092 Identifying Adaptive Loci in Recently Bottlenecked Populations." *PLoS ONE* 9 (11).
1093 https://doi.org/10.1371/journal.pone.0110579.
1094 Pokorny, Ina, Reeta Sharma, and Ralph Tiedemann. 2010. "MHC Class I and MHC Class II DRB Gene
1095 Variability in Wild and Captive Bengal Tigers (Panthera Tigris Tigris)," 667–79.
1096 https://doi.org/10.1007/s00251-010-0475-7.
1097 Radwan, Jacek, Aleksandra Biedrzycka, and Wiesław Babik. 2010. "Does Reduced MHC Diversity
1098 Decrease Viability of Vertebrate Populations?" *Biological Conservation* 143 (3): 537–44.
1099 https://doi.org/10.1016/j.biocon.2009.07.026.
1100 Ralls, Katherine, Jonathan D. Ballou, and Alan Templeton. 1988. "Estimates of Lethal Equivalents and
1101 the Cost of Inbreeding in Mammals." *Conservation Biology* 2 (2): 185–93.
1102 Raven, Nynke, Simeon Lisovski, Marcel Klaassen, Nathan Lo, Thomas Madsen, Simon Y. W. Ho, and
1103 Beata Ujvari. 2017. "Purifying Selection and Concerted Evolution of RNA-Sensing Toll-like
1104 Receptors in Migratory Waders." *Infection, Genetics and Evolution* 53 (September):135–45.
1105 https://doi.org/10.1016/j.meegid.2017.05.012.
1106 Reid, Jane M, Peter Arcese, Lukas F Keller, Kyle H Elliott, Laura Sampson, and Dennis Hasselquist.
1107 2007. "Inbreeding Effects on Immune Response in Free-Living Song Sparrows (Melospiza
1108 Melodia)," no. November 2006, 697–706. https://doi.org/10.1098/rspb.2006.0092.
1109 Rentzsch, Philipp, Daniela Witten, Gregory M Cooper, Jay Shendure, and Martin Kircher. 2019.
1110 "CADD: Predicting the Deleteriousness of Variants throughout the Human Genome." *Nucleic*
1111 *Acids Research* 47 (D1): D886–94. https://doi.org/10.1093/nar/gky1016.
1112 Reynes, Lauric, Louise Fouqueau, Didier Aurelle, Stéphane Mauger, Christophe Destombe, and
1113 Myriam Valero. 2024. "Temporal Genomics Help in Deciphering Neutral and Adaptive
1114 Patterns in the Contemporary Evolution of Kelp Populations." *Journal of Evolutionary Biology*
1115 37 (6): 677–92. https://doi.org/10.1093/jeb/voae048.
1116 Ripple, William J., James A. Estes, Robert L. Beschta, Christopher C. Wilmers, Euan G. Ritchie, Mark
1117 Hebblewhite, Joel Berger, et al. 2014. "Status and Ecological Effects of the World's Largest
1118 Carnivores." *Science* 343 (6167): 1241484. https://doi.org/10.1126/science.1241484.
1119 Rivero-de Aguilar, J., H. Westerdahl, J. Martínez-de la Puente, G. Tomás, J. Martínez, and S. Merino.
1120 2016. "MHC-I Provides Both Quantitative Resistance and Susceptibility to Blood Parasites in
1121 Blue Tits in the Wild." *Journal of Avian Biology* 47 (5): 669–77.
1122 https://doi.org/10.1111/JAV.00830.
1123 Robinson, Jacqueline A., Caitlin Brown, Bernard Y. Kim, Kirk E. Lohmueller, and Robert K. Wayne.
1124 2018. "Purging of Strongly Deleterious Mutations Explains Long-Term Persistence and
1125 Absence of Inbreeding Depression in Island Foxes." *Current Biology* 28 (21): 3487-3494.e4.
1126 https://doi.org/10.1016/j.cub.2018.08.066.

Robinson, Jacqueline A., Diego Ortega-Del Vecchyo, Zhenxin Fan, Bernard Y. Kim, Bridgett M. vonHoldt, Clare D. Marsden, Kirk E. Lohmueller, and Robert K. Wayne. 2016. "Genomic Flatlining in the Endangered Island Fox." *Current Biology* 26 (9): 1183–89. https://doi.org/10.1016/j.cub.2016.02.062.

Robinson, Jacqueline, Christopher C. Kyriazis, Stella C. Yuan, and Kirk E. Lohmueller. 2023. "Deleterious Variation in Natural Populations and Implications for Conservation Genetics." *Annual Review of Animal Biosciences* 11 (Volume 11, 2023): 93–114. https://doi.org/10.1146/annurev-animal-080522-093311.

Roelke-Parker, M E, L Munson, C Packer, R Kock, S Cleaveland, M Carpenter, S J O'Brien, et al. 2010. "A Canine Distemper Virus Epidemic in Serengeti Lions (Panthera Leo) (Vol 379, Pg 441, 1996)." *Nature* 464 (7290): 942. https://doi.org/Doi 10.1038/Nature08888.

S, Sommer. 2005. "The Importance of Immune Gene Variability (MHC) in Evolutionary Ecology and Conservation." *Frontiers in Zoology* 2 (October). https://doi.org/10.1186/1742-9994-2-16.

Saccheri, Ilik, Mikko Kuussaari, Maaria Kankare, Pia Vikman, Wilhelm Fortelius, and Ilkka Hanski. 1998. "Inbreeding and Extinction in a Butterfly Metapopulation." *Nature* 392 (6675): 491–94. https://doi.org/10.1038/33136.

Sahoo, Bismay, Gargee Das, Priyanka Nandanpawar, Nirjharini Priyadarshini, Lakshman Sahoo, Prem Kumar Meher, Uday Kumar Udit, Jitendra Kumar Sundaray, and Paramananda Das. 2023. "Genetic Diversity and Genome-Scale Population Structure of Wild Indian Major Carp, Labeo Catla (Hamilton, 1822), Revealed by Genotyping-by-Sequencing." *Frontiers in Genetics* 14 (May):1166385. https://doi.org/10.3389/fgene.2023.1166385.

Scanes, Colin G. 2018. "Chapter 19 - Human Activity and Habitat Loss: Destruction, Fragmentation, and Degradation." In *Animals and Human Society*, edited by Colin G. Scanes and Samia R. Toukhsati, 451–82. Academic Press. https://doi.org/10.1016/B978-0-12-805247-1.00026-5.

Shukla, Harsh, Kushal Suryamohan, Anubhab Khan, Krishna Mohan, Rajadurai C. Perumal, Oommen K. Mathew, Ramesh Menon, et al. 2023. "Near-Chromosomal de Novo Assembly of Bengal Tiger Genome Reveals Genetic Hallmarks of Apex Predation." *GigaScience* 12. https://doi.org/10.1093/gigascience/giac112.

Sidhu, Nadisha, Jimmy Borah, Sunny Shah, Nidhi Rajput, and Kajal Kumar Jadav. 2019. "Is Canine Distemper Virus (CDV) a Lurking Threat to Large Carnivores? A Case Study from Ranthambhore Landscape in Rajasthan, India." *Journal of Threatened Taxa* 11 (9): 14220–23. https://doi.org/10.11609/jott.4569.11.9.14220-14223.

Slate, J., L. E.B. Kruuk, T. C. Marshall, J. M. Pemberton, and T. H. Clutton-Brock. 2000. "Inbreeding Depression Influences Lifetime Breeding Success in a Wild Population of Red Deer (Cervus Elaphus)." *Proceedings of the Royal Society B: Biological Sciences* 267 (1453): 1657–62. https://doi.org/10.1098/rspb.2000.1192.

Slatkin, Montgomery. 1987. "Gene Flow and the Geographic Structure of Natural Populations." *Science* 236 (4803): 787–92. https://doi.org/10.1126/science.3576198.

Stefanović, Milomir, Mihajla Djan, Nevena Veličković, Yasin Demirbaş, Ladislav Paule, Csongor István Gedeon, Annika Posautz, Christoph Beiglböck, Anna Kübber-Heiss, and Franz Suchentrunk. 2020. "Purifying Selection Shaping the Evolution of the Toll-like Receptor 2 TIR Domain in Brown Hares (Lepus Europaeus) from Europe and the Middle East." *Molecular Biology Reports* 47 (4): 2975–84. https://doi.org/10.1007/s11033-020-05382-x.

Strand, Tanja M., Gernot Segelbacher, María Quintela, Lingyun Xiao, Tomas Axelsson, and Jacob Höglund. 2012. "Can Balancing Selection on MHC Loci Counteract Genetic Drift in Small Fragmented Populations of Black Grouse?" *Ecology and Evolution* 2 (2): 341–53. https://doi.org/10.1002/ece3.86.

Strickland, Kasha, Menna E. Jones, Andrew Storfer, Rodrigo K. Hamede, Paul A. Hohenlohe, Mark J. Margres, Hamish I. McCallum, Sebastien Comte, Shelly Lachish, and Loeske E. B. Kruuk. 2024. "Adaptive Potential in the Face of a Transmissible Cancer in Tasmanian Devils." *Molecular Ecology* 33 (21): e17531. https://doi.org/10.1111/mec.17531.

1178 Sutton, Jolene T., Shinichi Nakagawa, Bruce C. Robertson, and Ian G. Jamieson. 2011. "Disentangling
1179 the Roles of Natural Selection and Genetic Drift in Shaping Variation at MHC Immunity
1180 Genes." *Molecular Ecology* 20 (21): 4408–20. https://doi.org/10.1111/J.1365-
1181 294X.2011.05292.X.
1182 Taylor, Sabrina S., Rebecca J. Sardell, Jane M. Reid, Thomas Bucher, Nathan G. Taylor, Peter Arcese,
1183 and Lukas F. Keller. 2010. "Inbreeding Coefficient and Heterozygosity–Fitness Correlations in
1184 Unhatched and Hatched Song Sparrow Nestmates." *Molecular Ecology* 19 (20): 4454–61.
1185 https://doi.org/10.1111/J.1365-294X.2010.04824.X.
1186 Teixeira, João C., and Christian D. Huber. 2021. "The Inflated Significance of Neutral Genetic Diversity
1187 in Conservation Genetics," March. https://doi.org/10.1073/pnas.2015096118.
1188 Trinkel, Martina, Dave Cooper, Craig Packer, and Rob Slotow. 2013. "Inbreeding Depression
1189 Increases Susceptibility To Bovine Tuberculosis in Lions: An Experimental Test Using an
1190 Inbred–Outbred Contrast Through Translocation." *Journal of Wildlife Diseases* 47 (3): 494–
1191 500. https://doi.org/10.7589/0090-3558-47.3.494.
1192 Trowsdale, John, and Peter Parham. 2004. "Defense Strategies and Immunity-Related Genes."
1193 *European Journal of Immunology*. Eur J Immunol. https://doi.org/10.1002/eji.200324693.
1194 Turner, Andrew K., Mike Begon, Joseph A. Jackson, Janette E. Bradley, and Steve Paterson. 2011.
1195 "Genetic Diversity in Cytokines Associated with Immune Variation and Resistance to Multiple
1196 Pathogens in a Natural Rodent Population." Edited by Bret A. Payseur. *PLoS Genetics* 7 (10):
1197 e1002343. https://doi.org/10.1371/journal.pgen.1002343.
1198 Velando, Alberto, Álvaro Barros, and Paloma Moran. 2015. "Heterozygosity–Fitness Correlations in a
1199 Declining Seabird Population." *Molecular Ecology* 24 (5): 1007–18.
1200 https://doi.org/10.1111/MEC.13092.
1201 Vlček, Jakub, Matěj Miláček, Michal Vinkler, and Jan Štefka. 2023. "Effect of Population Size and
1202 Selection on Toll-like Receptor Diversity in Populations of Galápagos Mockingbirds." *Journal
1203 of Evolutionary Biology* 36 (1): 109–20. https://doi.org/10.1111/JEB.14121.
1204 Wang, Biao, Robert Ekblom, Ignas Bunikis, Heli Siitari, and Jacob Höglund. 2014. "Whole Genome
1205 Sequencing of the Black Grouse (Tetrao Tetrix): Reference Guided Assembly Suggests Faster-
1206 Z and MHC Evolution." *BMC Genomics* 15 (1): 180. https://doi.org/10.1186/1471-2164-15-
1207 180.
1208 Wei, Kun, Zhihe Zhang, Xiaofang Wang, Wenping Zhang, Xiao Xu, Fujun Shen, and Bisong Yue. 2010.
1209 "Lineage Pattern, Trans-Species Polymorphism, and Selection Pressure among the Major
1210 Lineages of Feline Mhc-DRB Peptide-Binding Region." *Immunogenetics* 62 (5): 307–17.
1211 https://doi.org/10.1007/s00251-010-0440-5.
1212 Whitlock, Michael C. 2002. "Selection, Load and Inbreeding Depression in a Large Metapopulation."
1213 *Genetics* 160 (3): 1191 LP – 1202.
1214 Whitlock, Michael C, and N H Barton. 1997. "The Effective Size of a Subdivided Population." *Genetics*
1215 146 (1): 427–41. https://doi.org/10.1093/genetics/146.1.427.
1216 Wilder, Aryn P., Megan A Supple, Ayshwarya Subramanian, Anish Mudide, Ross Swofford, Aitor
1217 Serres-Armero, Cynthia Steiner, et al. 2023. "The Contribution of Historical Processes to
1218 Contemporary Extinction Risk in Placental Mammals." *Science (New York, N.Y.)* 380 (6643):
1219 eabn5856. https://doi.org/10.1126/science.abn5856.
1220 Xue, Yali, Javier Prado-Martinez, Peter H. Sudmant, Vagheesh Narasimhan, Qasim Ayub, Michal
1221 Szpak, Peter Frandsen, et al. 2015. "Mountain Gorilla Genomes Reveal the Impact of Long-
1222 Term Population Decline and Inbreeding." *Science (New York, N.Y.)* 348 (6231): 242–45.
1223 https://doi.org/10.1126/science.aaa3952.
1224 Young, Andrew Graham, and Geoffrey Maurice Clarke. 2000. *Genetics, Demography and Viability of
1225 Fragmented Populations.*
1226 Zhang, Le, Tianming Lan, Chuyu Lin, Wenyuan Fu, Yaohua Yuan, Kaixiong Lin, Haimeng Li, et al. 2023.
1227 "Chromosome-Scale Genomes Reveal Genomic Consequences of Inbreeding in the South

China Tiger: A Comparative Study with the Amur Tiger." *Molecular Ecology Resources* 23 (2): 330–47. https://doi.org/10.1111/1755-0998.13669.

Data availability statement:

No new data was generated for this study. Accession information for the data used is available in the supplementary file.

Author contributions:

Conceived and designed the analysis: BVA and UR. Performed the analysis: BVA. Wrote the paper: BVA and UR.