

Range-wide cytokine diversity reveals candidate immune variants associated with chlamydial disease in koala

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Abstract

Disease outcomes are strongly influenced by host immune responses, yet the genetic basis of immune variation in koalas remains poorly understood. Chlamydial disease is a major threat to koala populations, and marked differences in infection and disease prevalence suggest an important role for host genetics. Here, we characterised coding and promoter-region variation in nine cytokine genes using whole-genome sequence data from 457 koalas sampled across Australia and investigated whether cytokine diversity corresponded with population-level differences in chlamydial infection and disease prevalence. Across seven polymorphic cytokine genes, we identified 18 coding SNPs, 32 alleles, and 37 promoter SNPs, including 17 located within predicted transcription factor binding motifs. Nine of eleven non-synonymous substitutions altered amino acid physicochemical properties, suggesting potential functional consequences for protein structure or signalling. IL12B and IL17F were particularly notable because all observed coding variation was non-synonymous, meaning that each allele encoded a distinct protein variant. IL12B also exhibited the greatest promoter diversity, with all variants located within predicted regulatory motifs. Cytokine diversity was highest in northern populations and declined southwards, mirroring broader patterns of genomic diversity. IL12B and IL17F allele frequencies differed among populations with contrasting chlamydial disease profiles, with some alleles more common where infection prevalence was high but disease prevalence was low. These patterns suggest IL12B and IL17F as promising candidate genes for future studies of disease susceptibility and resilience, and highlight the importance of immune gene diversity for the adaptive potential of wild populations.

Introduction

Disease is a major driver of wildlife population decline and can push even abundant species towards extinction by reducing survival, reproductive success, and long-term population viability (Langwig et al. 2015). Factors such as habitat loss, climate variability, pathogen pressures, and host genetic diversity can further interact to influence disease susceptibility and transmission across wildlife species (Pimm and Askins 1995; Wilcove et al. 1998). A number of infectious diseases are currently threatening wildlife populations (Daszak et al. 2000), including chytridiomycosis in frogs (Skerratt et al. 2007), morbillivirus in marine mammal species (Gulland and Hall 2007), and the rapid spread of Devil Facial Tumour Disease (DFTD) in Tasmanian devils (*Sarcophilus harrisii*) (Hawkins et al. 2006). Koalas (*Phascolarctos cinereus*), an iconic and widely distributed Australian marsupial, face multiple threats across their range, including habitat loss, chlamydial disease, and infection with koala retrovirus (KoRV). These threats can act synergistically, with infectious diseases contributing to reduced survival, reproductive success, and population viability. Given the substantial impacts of disease on koala populations, understanding how genetic variation in immune-related genes influences susceptibility, disease progression, and resilience is critical for effective conservation management.

Cytokines are key regulators of immune responses, balancing pathogen clearance with immunopathology during infection (Parkin and Cohen 2001). In humans, genetic variation in cytokine genes has been associated with chlamydial infection, with variants in both coding and non-coding regions influencing cytokine structure, expression, and regulation. These polymorphisms can alter immune responses, contributing to differences in infection susceptibility, disease progression, and host responses among individuals (Cantor et al. 2005; Ollier 2004). In humans, variation in cytokine genes have been reported at IL1B, IL2, IL12B, IL10 (Wang et al. 2005b), IL6 (Osiri et al. 1999), TNF and IFNG (Delaney et al. 2004). These differences may reflect adaptation to local environmental conditions or pathogen pressures, potentially influencing population-specific disease susceptibility (Turner et al. 2011).

Chlamydial infection is a leading cause of infertility in koalas, significantly reducing reproductive success and threatening the species' long-term viability. Two main *Chlamydia* species infect koalas, *C. pneumoniae* and *C. pecorum*, with *C. pecorum* being more prevalent and more commonly associated with disease (Polkinghorne et al. 2013). Transmission occurs mainly through sexual contact among adult koalas, although vertical transmission can also occur, with joeys becoming infected during suckling (Polkinghorne et al. 2013). The clinical signs include cystitis, sterility (Johnston et al. 2015), pneumonia, and blindness, ultimately leading to reduced reproductive output (McColl et al. 1984) and increased mortality (Blanshard 1994; Wan et al. 2011). Importantly, infection does not always result in clinical disease. Many infected koalas remain asymptomatic carriers, harbouring the bacterium without showing obvious symptoms (Polkinghorne et al. 2013).

Differences in disease susceptibility and clinical outcomes have been observed among koala populations, with some populations showing high infection rates but relatively low levels of clinical disease, while others experience more severe disease outcomes or remain asymptomatic (Nyari et al. 2017). Prevalence of *C. pecorum* infection ranges from 21% to 88% across koala populations (Quigley and Timms 2020), with higher levels of chlamydial disease reported in northern regions of Australia, particularly in Queensland (QLD) and northern New South Wales (NSW), where it has contributed to localised population declines and local extinctions (McCallum et al. 2018; Patterson et al. 2015; Polkinghorne et al. 2013). In contrast, Victorian koalas generally exhibit lower clinical severity associated with infection (Patterson et al. 2015). Several factors have been proposed to explain these regional differences, including environmental stressors, population density, and variation among pathogen strains (Robbins et al. 2020). However, host factors may also play an important role. Chlamydial infection does not always result in clinical disease, suggesting that differences in host immune responses may influence disease progression. Additionally, koalas are impacted by Koala Retrovirus (KoRV) infections (Melzer et al. 2000). KoRV has been linked to immunosuppression and increased susceptibility to secondary infections, potentially exacerbating the impacts of chlamydiosis (Denner and Young 2013). This immunosuppression may also contribute to the higher incidence of diseases and the overall health decline in infected populations.

The marked variation in chlamydial infection and disease prevalence observed among koala populations suggests that host immune factors may influence disease outcomes. Cytokines are key regulators of immune responses and inflammation, and genetic variation in cytokine genes has been linked to susceptibility, disease severity, and treatment outcomes in a range of infectious diseases. Consequently, cytokine variation may contribute to differences in chlamydial prevalence and disease progression among koala populations. Support for this hypothesis comes from human studies of *Chlamydia trachomatis*, where genetic variation in several cytokine genes, including CXCL8, IFNG, IL1B, IL6, IL10, IL12B, IL17A, IL17F and TNF, has been associated with differences in infection susceptibility and disease outcomes (Öhman et al. 2011; Poston et al. 2019; Wang et al. 2005a). Evidence from previous studies suggests that cytokine expression is influenced by disease status, viral co-infection, and environmental conditions. Koalas with chlamydial disease exhibit significantly higher expression of IL17, TNF α and IL10 than asymptotically infected individuals (Mathew et al. 2013b; Mathew et al. 2014). Similarly, koalas infected with KoRV-B show elevated expression of IL17A and IL10, together with altered expression of IFNG, IL4, IL6 and TNF α , with some responses varying seasonally (Maher and Higgins 2016). Environmental conditions may further shape cytokine responses, with koalas from the Liverpool Plains exhibiting greater and more variable expression of IL1B and IL6 than populations from the Southern Highlands and south-west Sydney (Fernandez et al. 2024). Collectively, these studies indicate that cytokine responses vary among individuals and populations and may contribute to differences in disease susceptibility and outcomes.

To date, cytokine genetic diversity has not been investigated in koalas. While studies of cytokine expression provide insights into immune activity, analyses of genetic variation can reveal the evolutionary and molecular mechanisms underpinning immune responses and disease susceptibility. Previous studies of MHC, TLR and AMP genes have revealed marked geographic structuring of immune diversity across the koala range and identified potentially functional variants that may influence pathogen recognition and immune responsiveness (Cui et al. 2025; Petrohilos et al. 2025; Silver et al. 2024). These findings suggest that other components of the immune system, including cytokines, may also contribute to population-level differences in disease susceptibility and resilience.

Here we examine genetic diversity within the exonic and promoter regions of nine key cytokine genes using data from 457 wild koalas across their entire range (Gates et al. 2025; Hogg et al. 2023). We investigate whether differences in chlamydial infection and disease prevalence among koala populations are associated with variation in cytokine genes by identifying immune-related genetic markers linked to disease susceptibility. This study provides insights into host-pathogen interactions and improves our understanding of the impacts of infectious disease on koala populations by using bioinformatic tools.

Methods

Study populations

The 457 whole-genome sequences (WGS) were sampled from 49 locations across their geographical range in Australia, including 413 samples across the east coast of Australia from Queensland, New South Wales and Victoria from the publicly available Koala Genome Survey dataset (NCBI BioProject PRJNA940526; metadata: DOI: 10.5281/zenodo.13777157) (Hogg et al. 2023; McLennan et al. 2025), an additional four from South West Victoria, and 40 additional samples from Kangaroo Island (Gates et al. 2025) and the Mount Lofty Ranges (South Australia). The methods for sample collection, genomic DNA extraction and sequencing have been described in detail in previous publications (McLennan et al. 2025). Following the methods of McLennan et al 2025, joint genotyping using DRGAEN Germline Pipeline (v3.8.4; Illumina) of samples aligned to the koala genome assembly (Koala phaCin_unsw_v4.1.fa) (Johnson et al. 2018) to produce a single, multi-sampling variant calling file (VCF) used for all downstream analyses. Based on the inferred population structure (McLennan et al. 2025), 457 koalas were divided into seven genomic clusters, including northern QLD (N_QLD) $n = 86$, southeast QLD and far northern NSW (SEQLD_FNNSW) $n = 101$, mid-coast NSW (M_NSW) $n = 64$, southern NSW (S_NSW) $n = 73$ and Victoria (VIC) $n = 76$, and South Australia (SA) $n = 40$ and Narrandera $n = 17$. The Narrandera population was considered a separate genetic cluster as it is an admixed population of koalas from French Island and a unknown population from the southeast QLD and far northern NSW genetic cluster (McLennan et al. 2025).

Cytokine characterisation

We selected nine cytokines for investigation that have been previously shown to be associated with chlamydial infection in humans, these include CXCL8, IFNG, IL1B, IL6, IL10, IL12B, IL17A, IL17F, and TNFa. To identify candidate single nucleotide polymorphisms (SNPs) within koala cytokine genes, “blastn” searches were performed in BLAST v2.2.30 (Altschul et al. 1990) using manually annotated koala cytokine reference sequences as queries (Peel et al. 2022) against the koala reference genome, with default parameters (phaCin_unsw_v4.1) (Johnson et al. 2018). Top blast hits for each gene were checked for open reading frames and start/stop codons

in IGV v2.17.0 (Robinson et al. 2011). Once the exonic regions of cytokine genes were identified within the genome, variants located within these regions were extracted and filtered using VCFtools v0.1.14 and BCFtools v1.3.1 (Danecek et al. 2011). Variants were filtered based on quality and frequency thresholds: minor allele frequency ≥ 0.05 , Phred-score > 20 , max-missingness = 0.9, minimum depth > 5 , minGQ > 10 , and maximum depth < 200 . Additionally, BCFtools v1.3.1 (Danecek et al. 2011) was used to remove sites with a strand-bias Phred score < 60 . SNPs were then annotated using SnpEff v5.2 (Cingolani et al. 2012) against the koala genome (Johnson et al. 2018), classifying exonic variants as synonymous or non-synonymous.

Individual genotype fasta files for each koala cytokine genes were generated from the filtered vcf file above using VCFtools v0.1.14 and FastaAlternateReferenceMaker within GATK v4.2.0.0 (McKenna 2010). Alleles were determined by first using GATK v4.2.1.0 FastaAlternateReferenceMaker to obtain the nucleotide sequence for seven cytokines in each individual (note, IL10 and TNF α contained no variation so were not included in further analyses). SeqPHASE (Flot 2010) was used to convert sequences into PHASE format. PHASE v2.1.1 (Stephens and Scheet 2005) was then used to determine alleles and estimate allele frequencies for each gene. The resulting files were imported into DNAsp v6.12.03 (Rozas et al. 2017) and analysed to calculate allele diversity, nucleotide diversity (π), and Tajima's D. Alleles that occurred in less than two individuals were excluded as potential errors. Allele frequencies of each cytokine gene for each genomic cluster were compared and visualised using ggplot2 v3.4.2 (Villanueva and Chen 2019) in RStudio v4.2.1 (Team 2020). BioEdit v7.2.5 (Hall et al. 2011) was used for SNP verification, protein translation of alleles, and comparison of amino acid sequences among alleles. Multiple sequence alignments were performed using ClustalW (Thompson et al. 2003) implemented in BioEdit to align koala cytokine protein sequences with human (*Homo sapiens*), cattle (*Bos taurus*), mouse (*Mus musculus*), and opossum (*Monodelphis domestica*) orthologs. The alignments were used to verify amino acid identity at non-synonymous SNP positions and assess the evolutionary conservation of residues. AlphaFold 3 (Jumper et al. 2021) was used for protein structure prediction. PROVEAN was used to predict the functional effects of non-synonymous mutations and to assess whether amino acid substitutions were likely to have functional impacts or be structurally conservative.

Cytokine promoter characterisation

Promoter regions were defined as genomic intervals upstream of the transcription start site (TSS), which typically span 1,000–2,000 bp and encompass core promoter elements and transcription factor binding sites (TFBSs) (Zhang 1998). In this study, promoters were defined as the 1,000 bp immediately upstream of the TSS (Tabach et al. 2007). This window was selected because most of the core promoter elements and TFBSs are located within the ~ 1000 bp surrounding the TSS. The ~ 1000 bp definition represents a balance between sensitivity for detecting regulatory variation and specificity for functionally relevant promoter elements. TSS for cytokine genes were obtained from genome annotation files, and gene strand orientation was used to ensure promoter coordinates were calculated and extracted in the correct transcriptional direction for each gene. Promoter sequences were extracted based on these coordinates, and SNP variants within promoter regions were identified and filtered using the same methods as described above.

Sequence motif discovery was performed using MEME (Multiple EM for Motif Elicitation) (<https://meme-suite.org/meme/>) to identify overrepresented sequence motifs within promoter regions (Bailey et al. 2015). Putative TFBSs were identified by scanning promoter sequences with FIMO (Find Individual Motif Occurrences) (Grant et al. 2011), using position frequency matrices from the JASPAR 2024 CORE vertebrate's non-redundant

database (Rauluseviciute et al. 2024). Genetic variants overlapping predicted binding sites were mapped to motif positions. The potential functional impact of variants was assessed by evaluating their position within the motif relative to conserved nucleotides, as defined by the JASPAR motif model. Each motif position has information content (IC) or base probability. Positions with high IC are critical for TF binding. Variants occurring at positions with high information content or altering highly preferred nucleotides were considered more likely to affect transcription factor binding and motif function and therefore expression profiles.

Cytokine variation and Chlamydia prevalence in populations

Chlamydia infection and disease prevalence data were obtained from a previous review study (Quigley and Timms 2020). Data included the prevalence of *C. pecorum* infection and disease reported in studies conducted from 2012 to 2019 across Australia. The study presented *Chlamydia* infection data from 20 distinct regions across Australia, with seven of these regions also providing data on Chlamydial disease prevalence. We retained infection and disease prevalence data only for locations where our genomic data overlapped with this disease data, these include Moreton Bay (N = 16), Port Macquarie (N = 13), French Island (N = 2), Kangaroo Island (N = 20) and Mount Lofty (N = 20). We investigated whether allele frequencies for each of the seven cytokines containing variation differed between locations with lower or higher *Chlamydia* prevalence and disease rate.

Results

We analysed SNP variation in nine cytokine genes across 457 wild koalas to assess genetic diversity and potential associations with *Chlamydia* infection and disease. After quality filtering, we identified 18 high-confidence biallelic SNPs across seven cytokine genes for downstream analyses (Table 1). Of the nine genes investigated, IL10 and TNF α were monomorphic and not analysed further. The number of SNPs observed in the seven polymorphic cytokines ranged from one (CXCL8) to four (IL1B and IL12B). We identified 32 alleles across these seven genes, ranging from two (CXCL8) to six (IL1B) (Table 1). Tajima's D-test was used to assess natural selection across different koala cytokines. Most genes showed a non-significant result, with significant positive Tajima's D values observed only for CXCL8 ($D = 2.19831$, $p < 0.05$) and IL6 ($D = 2.38611$, $p < 0.05$), suggesting deviations from neutral evolution that may reflect balancing selection or population demographic structure.

Table 1

Summary of cytokine genetic diversity in 457 koalas across nine cytokine genes. Eighteen SNPs and 32 alleles were identified across the cytokine genes examined. IL12B and IL17F were notable in containing exclusively non-synonymous variation, while IL10 and TNF were monomorphic across all sampled individuals. Measures of allelic diversity, nucleotide diversity (π), Tajima's D, and promoter region variation are shown for each gene. Positive (+) and negative (-) strands indicate transcriptional orientation. SNP counts are partitioned into synonymous (dS) and non-synonymous (dN) substitutions.

Gene	Scaffold	Strand	SNPs (dS:dN)	No. alleles	Allelic diversity	π	Tajima's D	promoter region
CXCL8	MSTS01000032.1	-	1(1:0)	2	0.4947	0.00127	2.19831*	15898653–15899652
IFNG	MSTS01000038.1	-	2(1:1)	3	0.3954	0.0008	0.54419	14484292–14485291
IL1B	MSTS01000147.1	+	4(1:3)	6	0.5268	0.00071	0.13253	907812–908811
IL6	MSTS01000051.1	-	3(3:0)	4	0.6907	0.00176	2.38611*	13471621–13472620
IL10	MSTS01000100.1	-	0	1	0	0	0	9803386–9804385
IL12B	MSTS01000045.1	+	4(0:4)	5	0.3752	0.00044	-0.28461	15027259–15028258
IL17A	MSTS01000055.1	-	2(1:1)	3	0.1339	0.00029	-0.56014	3839778–3840777
IL17F	MSTS01000055.1	+	2(0:2)	3	0.5333	0.00124	1.40377	3752646–3753645
TNF	MSTS01000255.1	-	0	1	0	0	0	2398541–2399540
Total			18	32				

Out of the 18 SNPs identified, seven were synonymous, and 11 were non-synonymous with nine of these resulting in a physicochemical change due to amino acid changes (Table 2). Two non-synonymous SNPs identified in IFNG and IL17A resulted in amino acid substitutions without changes in physicochemical properties of the residues. These suggest that the variants are unlikely to cause major changes in protein structures (Table 2). IL12B showed the highest diversity with four non-synonymous, followed by IL1B with 3 non-synonymous. All non-synonymous SNPs were predicted to be neutral by PROVEAN, suggesting that these amino acid substitutions may not be expected to have a significant impact on protein function. However, these variants may still contribute to phenotypic differences through effects on gene regulation, expression levels, mRNA stability or interactions with pathogens such as *Chlamydia*.

Table 2

Functional characteristics of non-synonymous cytokine variants identified across koala populations. Eleven non-synonymous SNPs were detected across five cytokine genes, with nine resulting in changes to amino acid physicochemical properties that may influence protein structure, stability or function. Variants occurred in cytokines involved in inflammation (IL1B), intracellular pathogen clearance (IFNG and IL12B), and Th17-mediated immune responses (IL17A and IL17F). Several substitutions were geographically restricted, particularly within IL1B, IL12B and IL17F, highlighting potential population-specific immune diversity.

Cytokine	Function	Position	AA site	AA change	Physicochemical change	Population
IFNG	Key for clearing the infected cells	Signal	45G > C	Met15Ile	None	S_NSW
IL1B	Trigger inflammation and recruit immune cells	N-terminal pro-domain	139T > C	Phe47Leu	Aromatic(hydrophobic)	N_QLD, SEQLD_FNNSW, S_NSW
		N-terminal pro-domain	355G > A	Ala119Thr	None/Polar	N_QLD, SEQLD_FNNSW, M_NSW
		IL1 family β -trefoil domain	787G > A	Asp263Asn	Negatively charged/Polar	S_NSW
IL12B	Key for clearing the infected cells	Disulfide bond	166A > G	Lys56Glu	Positively charged/Negatively charged	N_QLD, SEQLD_FNNSW, M_NSW, S_NSW, VIC, SA, Narrandera
		Disulfide bond	72G > T	Ala58Ser	None/Polar	N_QLD, SEQLD_FNNSW, M_NSW
		Mature cytokine domain	502G > A	Gly168Arg	None/Positively charged	N_QLD, SEQLD_FNNSW
		Disulfide bond	520A > G	Thr174Ala	Polar/None	N_QLD
IL17A	Promote inflammation to control bacterial growth	Cystine-knot domain	137G > T	Ser46Ile	Polar/None	S_NSW
IL17F	Promote inflammation to control bacterial growth	Signal	T > C	Val2Ala	None	N_QLD, SEQLD_FNNSW, M_NSW, S_NSW, VIC, SA, Narrandera
		Disulfide bond	88C > G	Gln130Glu	Polar/Negatively charged	N_QLD, SEQLD_FNNSW, M_NSW, S_NSW

There was higher genetic variation within the northern part of the koalas' range compared to the southern regions. Individuals from Queensland and northern NSW (SEQLD_FNNSW and N_QLD) had the highest number of cytokine alleles with 23 across the seven polymorphic genes, followed by the mid and southern NSW (M_NSW and S_NSW) with 20 alleles, and Victoria (VIC) and Narrandera having 14 alleles, and SA had the fewest with 11 alleles (Table 3). S_NSW had the highest number of unique cytokine alleles with three alleles at IFNG, IL17A and IL1B not found in other locations, followed by northern QLD with two unique cytokine alleles at IL1B and IL12B (Table 3). VIC and SA koalas typically showed a higher frequency of the most common allele and were typically dominated by a single allele (e.g. CXCL8_A2, IL17F_A3 and IL17A_A2) (Fig. 1). We saw differences in the most common alleles at CXCL8, IL17F and IL6, with CXCL8_A2 being highly prevalent in VIC and SA populations but CXCL8_A1 being more common in northern populations (Fig. 1).

Table 3

Cytokine allele diversity varies markedly across the koala range. Northern Queensland and southeast Queensland/far northern New South Wales populations harboured the highest number of cytokine alleles, while southern populations contained fewer alleles and were often dominated by a single variant. Several unique alleles were identified in northern Queensland and southern New South Wales, highlighting population-specific immune diversity. Values indicate the number of alleles detected within each genomic cluster, with unique alleles shown in parentheses.

Gene	No. Alleles in each genetic cluster						
	N_QLD	SEQLD_FNNSW	M_NSW	S_NSW	VIC	SA	Narrandera
CXCL8	2	2	2	2	2	2	2
IFNG	2	2	2	3(A3)	2	2	2
IL1B	5(A6)	4	3	3(A4)	2	2	2
IL6	3	4	3	3	3	2	3
IL12B	5(A5)	4	3	2	3	2	2
IL17A	2	2	2	3(A3)	1	1	1
IL17F	3	3	3	3	1	1	2
Total	23	23	20	20	14	11	14
Samples	86	101	64	73	72	40	17

We identified 37 SNPs within cytokine promoter regions. IFNG showed no variation within its promoter region, while other cytokines ranged between three (IL6 and TNF) and eight (CXCL8) SNPs (Table 4). We found southeast QLD and far-north NSW has the highest number of promoter SNPs with 35 SNPs, followed by north QLD with 32 SNPs. Narrandera and VIC have similar number of SNPs, with 18 and 17, respectively (Table 4). SA has the lowest number of SNP within promoters, with nine (Table 4). By aligning promoter sequences with human cytokine promoters, we predicted 17 SNPs within motifs and potentially influences expression. IL6 showed no variation within motifs, while other cytokines ranged between one (IL17A, IL17F and TNF) and six (IL12B) SNPs. All six SNPs found in the promotor region of IL12B are located within motifs.

Table 4

Promoter-region variation reveals extensive regulatory diversity across koala cytokine genes. Thirty-seven SNPs were identified within the promoter regions of eight cytokine genes, with 17 variants occurring within predicted transcription factor binding motifs and therefore having the potential to influence gene expression. IL12B was particularly notable, containing six promoter SNPs, all of which occurred within predicted motifs. Promoter diversity was highest in northern populations and lowest in southern populations, mirroring patterns observed for coding variation. Values in parentheses indicate the number of promoter SNPs located within predicted transcription factor binding motifs.

Gene	No. SNP at promoters	No. SNPs at motifs	No. SNP variants at promoter regions (motifs) in each population						
			N_QLD	SEQLD_FNNSW	M_NSW	S_NSW	VIC	SA	Narrandera
CXCL8	8	2	8 (2)	8 (2)	7 (1)	4 (0)	3 (0)	0	5 (0)
IL1B	5	4	4 (4)	4 (4)	0	4 (4)	0	0	0
IL6	3	0	3 (0)	3 (0)	3 (0)	2 (0)	2 (0)	2 (0)	2 (0)
IL10	4	2	3 (1)	3 (2)	4 (2)	4 (2)	4 (2)	3 (2)	3 (2)
IL12B	6	6	5 (5)	5 (5)	4 (4)	3 (3)	3 (3)	0	3 (3)
IL17A	4	1	2 (0)	4 (1)	4 (1)	2 (0)	1 (0)	1 (0)	1 (0)
IL17F	4	1	3 (0)	4 (1)	4 (1)	2 (1)	2 (1)	1 (1)	2 (1)
TNF	3	1	3 (1)	3 (1)	3 (1)	3 (1)	2 (1)	2 (1)	2 (1)
Total	37	17	32	35	29	25	17	9	18

Chlamydial infection prevalence and disease rates varied markedly amongst the subset of populations that overlapped with our study (Fig. 2). Mainland populations exhibited comparable and high chlamydial infection rates at Moreton Bay (47%), Port Macquarie (63%) and Mount Lofty (31%), but differed substantially in clinical disease rate, with higher disease prevalence at Moreton Bay (28%) and Port Macquarie (20%) compared to Mount Lofty (4%). French Island and Kangaroo Island were both reported as free of *C. pecorum* (Fig. 2). Several alleles exhibited marked frequency differences between high infection prevalence and high disease rates populations (HIHD), including Moreton Bay and Port Macquarie, and high infection prevalence and low disease rates (HILD) populations (Mount Lofty). For example, CXCL8_A1 and IL17F_A2 occurred at high allele frequency in HIHD populations (0.69–0.96 and 0.58–0.91) but was absent in HILD population (0%), respectively. Predominant alleles of CXCL8_A2, IL12B_A1, IL17F_A3 and IL17A_A2 were more frequent in low disease rate populations.

Discussion

Here, we provide the first range-wide assessment of cytokine genetic diversity in koalas and identify both coding and regulatory variants that may influence immune responsiveness and susceptibility to chlamydial disease. We detected 18 SNPs and 32 alleles across seven polymorphic cytokine genes, together with 37 promoter variants, many of which occur within predicted transcription factor binding motifs. Cytokine diversity was highest in

northern populations and lowest in southern populations, mirroring patterns previously observed for genome-wide diversity, MHC, TLR and AMP genes. Notably, variation in IL12B and IL17F differentiated populations with contrasting chlamydial disease profiles, highlighting these genes as promising candidates for future studies of disease susceptibility and resilience.

Previous studies have shown that marsupial cytokine repertoires are broadly comparable to those of eutherian mammals in the opossum (*Monodelphis domestica*), tammar wallaby (*Notamacropus eugenii*) and Tasmanian devil (*Sarcophilus harrisii*) (Belov et al. 2013; Morris et al. 2015; Wong et al. 2011; Wong et al. 2006). However, there are few studies that discuss marsupial cytokine variation. Koala cytokine diversity is similar to what is reported in wild field voles (*Microtus agrestis*), a common eutherian species, in which 18 SNPs across eight genes were identified in a sample of 665 individuals (Turner et al. 2011). The comparable cytokine diversity between koalas and field voles suggests that cytokine genes remain relatively diverse in koala populations.

We identified 18 SNPs across seven polymorphic cytokine genes involved in key immune pathways that regulate the balance between pathogen clearance and immunopathology during chlamydial infection. Because *Chlamydia* is an intracellular bacterium, effective host defence relies on coordinated innate, Th1 and Th17 immune responses (Scurlock et al. 2011). The cytokines characterised here include pro-inflammatory mediators (IL1B, IL6, TNF α and CXCL8) that drive early inflammatory responses, Th1-associated cytokines (IFNG and IL12B) that promote intracellular pathogen clearance, and Th17-associated cytokines (IL17A and IL17F) that regulate mucosal inflammation and neutrophil recruitment (Cohen and Cohen 1996; Li et al. 2018; Perry et al. 1997). Variation within these genes therefore has the potential to influence both protection against infection and the risk of inflammation-associated tissue damage, a hallmark of chlamydial disease (Rasmussen et al. 1997).

Nine of the 11 non-synonymous substitutions identified in cytokine exons resulted in changes to amino acid physicochemical properties, suggesting the potential to alter protein structure, stability or function. Although the functional consequences of these variants remain unknown, substitutions affecting charge, polarity or hydrophobicity can influence protein folding, receptor interactions and signalling efficiency, ultimately shaping immune responsiveness and disease susceptibility (Tsai 2017; Ullrich et al. 2020; Zhang et al. 2011). Several variants occurred in cytokines with central roles in regulating inflammatory responses to chlamydial infection, including IL1B, IL12B, IL17A and IL17F. Structural comparisons with human cytokines suggest that some substitutions occur within regions important for protein stability and function. Notably, several non-synonymous variants showed strong geographic structuring. The IL17F Gln130Glu substitution was detected only in northern populations, while all non-synonymous substitutions identified in IL1B and three of the four substitutions in IL12B were absent from southern populations. These patterns suggest the possibility of population-specific immune adaptation or divergent evolutionary histories.

Particularly noteworthy was the observation that IL12B and IL17F contained exclusively non-synonymous variation, meaning that all observed alleles encoded distinct protein variants. This finding suggests that genetic diversity within these cytokines may have functional consequences for immune responses to chlamydial infection. IL12B and IL17F form part of a linked immune signalling pathway that helps balance pathogen clearance and inflammation (Moschen et al. 2019). IL12B contributes to the production and maintenance of IL23 and promotes Th1 and Th17 immune responses, both of which are important for controlling intracellular pathogens such as *Chlamydia* (Scurlock et al. 2011). Downstream, IL17F regulates inflammatory responses at mucosal surfaces and contributes to neutrophil recruitment and bacterial control (Curtis and Way 2009; Onishi and Gaffen 2010). While activation of this pathway is critical for effective host defence, excessive or dysregulated signalling can promote

chronic inflammation, tissue damage and other pathological outcomes associated with persistent chlamydial disease (Holland et al. 1996). Consequently, variation within IL12B and IL17F may influence the balance between protective immunity and immunopathology, making these genes strong candidates for future investigations of disease susceptibility and resilience in koalas.

Variation in IL12B and IL17F emerged as one of the strongest signals associated with population differences in chlamydial infection and disease prevalence. Although individual-level infection and disease data were not available for the sequenced koalas, comparisons among populations with published chlamydial prevalence data revealed intriguing patterns. Allele frequencies at both IL12B and IL17F differed among population with contrasting disease profiles. In particular, populations with higher frequencies of IL17F_A3 tended to exhibit lower rates of clinical disease. These observations are consistent with the hypothesis that variation in cytokine genes may influence progression from infection to disease by altering the balance between pathogen clearance and inflammatory pathology (Wang et al. 2005b).

This interpretation is supported by previous studies demonstrating that cytokine expression varies with *Chlamydia* infection status in koalas (Mathew et al. 2014). Differences have been reported in the expression of IFNG, IL10 and IL17A between infected and uninfected individuals (Mathew et al. 2013a; Mathew et al. 2013b; Mathew et al. 2014), while KoRV-B-positive koalas show altered expression of several cytokines, including IL17A (Maher and Higgins 2016). Together, these findings suggest that the coding and regulatory variants identified in this study may contribute to differences in immune responsiveness among individuals and populations, although functional studies will be required to determine their effects on cytokine expression, signalling and disease outcomes.

In addition to coding variants that alter protein sequence, variation within cytokine promoter regions may influence immune responses by modifying transcription factor binding sites and, consequently, patterns of gene expression (Öhman et al. 2006). We identified 37 SNPs across the promoter regions of eight of the nine cytokines examined, 17 of which occurred within predicted transcription factor binding motifs. Particularly notable was IL12B, which contained the greatest promoter diversity, and for which all identified promoter variants occurred within predicted transcription factor binding motifs. Given the central role of IL12B in regulating Th1 and Th17 immune responses (Coffre et al. 2013), these variants may influence transcriptional regulation and contribute to differences in immune responsiveness among individuals and populations.

Together, the coding and regulatory patterns identified here suggest that cytokine genes form part of a broader landscape of geographically structured immune diversity across the koala range. Cytokine diversity was greatest in northern populations and declined towards the south, mirroring patterns previously reported for MHC, TLR and AMP genes, as well as genome-wide diversity. These results suggest that cytokine genes form part of a broader pattern of geographically structured immune variation that may contribute to differences in adaptive potential and disease resilience among populations.

The marked differences in cytokine allele frequencies among koala populations are consistent with the combined effects of pathogen-mediated selection and demographic history. Southern populations, particularly those in Victoria and South Australia, were often dominated by a single cytokine allele, reflecting the legacy of severe historical bottlenecks. Despite this reduced diversity, these populations generally experience lower levels of chlamydial disease, raising the intriguing possibility that some retained cytokine variants contribute to disease resilience. However, disentangling the effects of selection from historical population processes will require individual-level genotype and disease data. Regardless, these findings reinforce the importance of maintaining

immune gene diversity, as genetic variation provides the raw material for adaptation to future pathogen and environmental challenges.

Conclusion

This study provides the first range-wide assessment of cytokine genetic diversity in koalas and identifies both coding and regulatory variants that may contribute to differences in immune responsiveness and chlamydial disease outcomes. We identified substantial geographic structuring of cytokine diversity, with northern populations harbouring greater variation than southern populations, mirroring patterns previously observed across other immune gene families and the broader genome. In particular, IL12B and IL17F emerged as strong candidate genes associated with population differences in chlamydial infection and disease prevalence, while promoter variation, especially within IL12B, highlights the potential importance of regulatory mechanisms in shaping immune responses. Together, these findings expand our understanding of koala immunogenetics and provide a foundation for future studies linking cytokine variation to gene expression, disease susceptibility and resilience. Maintaining immune gene diversity will be critical for preserving the adaptive potential of koala populations facing ongoing disease and environmental change.

Declarations

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Funding Declaration

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Data availability

All raw sequence data will be deposited onto Dryad following acceptance of this manuscript.

Author Contribution

C.J.H. and K.B. supervised and conceived the project. J.C. undertook the characterization and the analysis, compiled the figures and tables and wrote the draft manuscript. L.W.S. assisted with the analysis and provided scripts. All authors reviewed and contributed to the final manuscript.

References

1. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)

2. Bailey TL, Johnson J, Grant CE, Noble WS (2015) The MEME suite. *Nucleic Acids Res* 43:W39–W49. <https://doi.org/10.1093/nar/gkv416>
3. Belov K, Miller RD, Old JM, Young LJ (2013) Marsupial immunology bounding ahead. *Aust J Zool* 61:24–40. <https://doi.org/10.1071/ZO12111>
4. Blanshard W (1994) Medicine and husbandry of koalas. *Wildlife, Proceedings*
5. Cantor MJ, Nickerson P, Bernstein CN (2005) The role of cytokine gene polymorphisms in determining disease susceptibility and phenotype in inflammatory bowel disease. *ACG* 100:1134–1142. <https://doi.org/10.1111/j.1572-0241.2005.40979.x>
6. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM (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* 6:80–92. <https://doi.org/10.4161/fly.19695>
7. Coffre M, Roumier M, Rybczynska M, Sechet E, Law HK, Gossec L, Dougados M, Bianchi E, Rogge L (2013) Combinatorial control of Th17 and Th1 cell functions by genetic variations in genes associated with the interleukin-23 signaling pathway in spondyloarthritis. *Arthritis Rheum* 65:1510–1521. <https://doi.org/10.1002/art.37936>
8. Cohen MC, Cohen S (1996) Cytokine function: a study in biologic diversity. *Am J Clin Pathol* 105:589–598. <https://doi.org/10.1093/ajcp/105.5.589>
9. Cui J, Batley KC, Silver LW, McLennan EA, Hogg CJ, Belov K (2025) Spatial variation in toll-like receptor diversity in koala populations across their geographic distribution. *Immunogenetics* 77:5. <https://doi.org/10.1007/s00251-024-01365-5>
10. Curtis MM, Way SS (2009) Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. *Immunology* 126:177–185. <https://doi.org/10.1111/j.1365-2567.2008.03017.x>
11. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST (2011) The variant call format and VCFtools. *Bioinformatics* 27:2156–2158. <https://doi.org/10.1093/bioinformatics/btr330>
12. Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science* 287:443–449. <https://doi.org/10.1126/science.287.5452.443>
13. Delaney NL, Esquenazi V, Lucas DP, Zachary AA, Leffell MS (2004) TNF- α , TGF- β , IL-10, IL-6, and INF- γ alleles among African Americans and Cuban Americans. Report of the ASHI minority workshops: part IV. *Hum Immunol* 65:1413–1419. <https://doi.org/10.1016/j.humimm.2004.07.240>
14. Denner J, Young PR (2013) Koala retroviruses: characterization and impact on the life of koalas. *Retrovirology* 10:108
15. Fernandez CM, Krockenberger MB, Valentina, Mella S, Wright BR, Crowther MS, Higgins DP (2024) A novel multi-variate immunological approach, reveals immune variation associated with environmental conditions, and co-infection in the koala (*Phascolarctos cinereus*). *Sci Rep-UK* 14:7260. <https://doi.org/10.1038/s41598-024-57792-7>
16. Flot JF (2010) SeqPHASE: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. *Mol Ecol Resour* 10:162–166. <https://doi.org/10.1111/j.1755-0998.2009.02732.x>
17. Gates K, Sandoval-Castillo J, Beaman JE, Da Silva KB, Saltr   F, Belov K, Hogg CJ, Bradshaw CJ, Beheregaray LB (2025) Conservation arks: genomic erosion and inbreeding in an Abundant Island population of koalas. *Mol Ecol* 34:e70097. <https://doi.org/10.1111/mec.70097>

18. Grant CE, Bailey TL, Noble WS (2011) FIMO: scanning for occurrences of a given motif. *Bioinformatics* 27:1017–1018. <https://doi.org/10.1093/bioinformatics/btr064>
19. Gulland FM, Hall AJ (2007) Is marine mammal health deteriorating? Trends in the global reporting of marine mammal disease. *EcoHealth* 4:135–150. <https://doi.org/10.1007/s10393-007-0097-1>
20. Hall T, Biosciences I, Carlsbad C (2011) BioEdit: an important software for molecular biology. *GERF bull biosci* 2:60–61
21. Hawkins CE, Baars C, Hesterman H, Hocking G, Jones ME, Lazenby B, Mann D, Mooney N, Pemberton D, Pyecroft S (2006) Emerging disease and population decline of an island endemic, the Tasmanian devil *Sarcophilus harrisii*. *Biol Conserv* 131:307–324. <https://doi.org/10.1016/j.biocon.2006.04.010>
22. Hogg CJ, Silver L, McLennan EA, Belov K (2023) Koala genome survey: an open data resource to improve conservation planning. *Genes* 14:546. <https://doi.org/10.3390/genes14030546>
23. Holland M, Bailey R, Conway D, Culley F, Miranpuri G, Byrne G, Whittle H, Mabey D (1996) T helper type-1 (Th1)/Th2 profiles of peripheral blood mononuclear cells (PBMC); responses to antigens of *Chlamydia trachomatis* in subjects with severe trachomatous scarring. *Clin Exp Immunol* 105:429–435. <https://doi.org/10.1046/j.1365-2249.1996.d01-792.x>
24. Johnson RN, O'Meally D, Chen Z, Etherington GJ, Ho SY, Nash WJ, Grueber CE, Cheng Y, Whittington CM, Dennison S (2018) Adaptation and conservation insights from the koala genome. *Nat Genet* 50:1102–1111. <https://doi.org/10.1038/s41588-018-0153-5>
25. Johnston S, Deif H, McKinnon A, Theilemann P, Griffith J, Higgins D (2015) Orchitis and epididymitis in koalas (*Phascolarctos cinereus*) infected with *Chlamydia pecorum*. *Vet Pathol* 52:1254–1257. <https://doi.org/10.1177/0300985815570069>
26. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A (2021) Highly accurate protein structure prediction with AlphaFold. *Nature* 596:583–589. <https://doi.org/10.1038/s41586-021-03819-2>
27. Langwig KE, Voyles J, Wilber MQ, Frick WF, Murray KA, Bolker BM, Collins JP, Cheng TL, Fisher MC, Hoyt JR (2015) Context-dependent conservation responses to emerging wildlife diseases. *Front Ecol Environ* 13:195–202. <https://doi.org/10.1890/140241>
28. Li Y, Wei C, Xu H, Jia J, Wei Z, Guo R, Jia Y, Wu Y, Li Y, Qi X (2018) The immunoregulation of Th17 in host against intracellular bacterial infection. *Mediat Inflamm* 2018:6587296. <https://doi.org/10.1155/2018/6587296>
29. Maher IE, Higgins DP (2016) Altered immune cytokine expression associated with KoRV B infection and season in captive koalas. *PLoS ONE* 11:e0163780. <https://doi.org/10.1371/journal.pone.0163780>
30. Mathew M, Beagley KW, Timms P, Polkinghorne A (2013a) Preliminary characterisation of tumor necrosis factor alpha and interleukin-10 responses to *Chlamydia pecorum* infection in the koala (*Phascolarctos cinereus*). *PLoS ONE* 8:e59958. <https://doi.org/10.1371/journal.pone.0059958>
31. Mathew M, Pavasovic A, Prentis PJ, Beagley KW, Timms P, Polkinghorne A (2013b) Molecular characterisation and expression analysis of interferon gamma in response to natural *Chlamydia* infection in the koala, *Phascolarctos cinereus*. *Gene* 527:570–577. <https://doi.org/10.1016/j.gene.2013.06.019>
32. Mathew M, Waugh C, Beagley KW, Timms P, Polkinghorne A (2014) Interleukin 17A is an immune marker for chlamydial disease severity and pathogenesis in the koala (*Phascolarctos cinereus*). *Dev Comp Immunol* 46:423–429. <https://doi.org/10.1016/j.dci.2014.05.015>

33. McCallum H, Kerlin DH, Ellis W, Carrick F (2018) Assessing the significance of endemic disease in conservation—koalas, chlamydia, and koala retrovirus as a case study. *Conserv Lett* 11:e12425
34. McColl K, Martin R, Gleeson L, Handasyde K, Lee A (1984) *Chlamydia* infection and infertility in the female koala (*Phascolarctos cinereus*). *Vet Rec.* <https://doi.org/10.1136/vr.115.25-26.655>
35. McLennan EA, Kovacs TG, Silver LW, Chen Z, Jaya FR, Ho SY, Belov K, Hogg CJ (2025) Genomics identifies koala populations at risk across eastern Australia. *Ecol Appl* 35:e3062. <https://doi.org/10.1002/eap.3062>
36. Melzer A, Carrick F, Menkhorst P, Lunney D, John BS (2000) Overview, critical assessment, and conservation implications of koala distribution and abundance. *Conserv Biol* 14:619–628
37. Morris KM, Cheng Y, Warren W, Papenfuss AT, Belov K (2015) Identification and analysis of divergent immune gene families within the Tasmanian devil genome. *BMC Genomics* 16:1017. <https://doi.org/10.1186/s12864-015-2206-9>
38. Moschen AR, Tilg H, Raine T (2019) IL-12, IL-23 and IL-17 in IBD: immunobiology and therapeutic targeting. *Nat Rev Gastroentero* 16:185–196. <https://doi.org/10.1038/s41575-018-0084-8>
39. Nyari S, Waugh CA, Dong J, Quigley BL, Hanger J, Loader J, Polkinghorne A, Timms P (2017) Epidemiology of chlamydial infection and disease in a free-ranging koala (*Phascolarctos cinereus*) population. *PLoS ONE* 12:e0190114. <https://doi.org/10.1371/journal.pone.0190114>
40. Öhman H, Tiitinen A, Halttunen M, Birkelund S, Christiansen G, Koskela P, Lehtinen M, Paavonen J, Surcel H (2006) IL-10 polymorphism and cell-mediated immune response to *Chlamydia trachomatis*. *Genes Immun* 7:243–249. <https://doi.org/10.1038/sj.gene.6364293>
41. Öhman H, Tiitinen A, Halttunen M, Paavonen J, Surcel H-M (2011) Cytokine gene polymorphism and *Chlamydia trachomatis*-specific immune responses. *Hum Immunol* 72:278–282. <https://doi.org/DOI:10.1016/j.humimm.2010.12.012>
42. Ollier WE (2004) Cytokine genes and disease susceptibility. *Cytokine* 28:174–178. <https://doi.org/10.1016/j.cyto.2004.07.014>
43. Onishi RM, Gaffen SL (2010) Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. *Immunology* 129:311–321. <https://doi.org/10.1111/j.1365-2567.2009.03240.x>
44. Osiri M, McNicholl J, Moreland L, Bridges SL (1999) A novel single nucleotide polymorphism and five probable haplotypes in the 5' flanking region of the IL-6 gene in African-Americans. *Genes Immun* 1:166–167. <https://doi.org/10.1038/sj.gene.6363652>
45. Parkin J, Cohen B (2001) An overview of the immune system. *Lancet* 357:1777–1789. [https://doi.org/10.1016/s0140-6736\(00\)04904-7](https://doi.org/10.1016/s0140-6736(00)04904-7)
46. Patterson JL, Lynch M, Anderson GA, Noormohammadi AH, Legione A, Gilkerson JR, Devlin JM (2015) The prevalence and clinical significance of *Chlamydia* infection in island and mainland populations of Victorian koalas (*Phascolarctos cinereus*). *J Wildl Dis* 51:309–317. <https://doi.org/10.7589/2014-07-176>
47. Peel E, Silver L, Brandies P, Zhu Y, Cheng Y, Hogg CJ, Belov K (2022) Best genome sequencing strategies for annotation of complex immune gene families in wildlife. *GigaScience* 11:giac100. <https://doi.org/10.1093/gigascience/giac100>
48. Perry LL, Feilzer K, Caldwell HD (1997) Immunity to *Chlamydia trachomatis* is mediated by T helper 1 cells through IFN-gamma-dependent and-independent pathways. *J Immunol* 158:3344–3352
49. Petrohilos C, Peel E, Silver LW, Belov K, Hogg CJ (2025) AMPed up immunity: 418 whole genomes reveal intraspecific diversity of koala antimicrobial peptides. *Immunogenetics* 77:11.

<https://doi.org/10.1007/s00251-024-01368-2>

50. Pimm SL, Askins RA (1995) Forest losses predict bird extinctions in eastern North America. *Proc Natl Acad Sci* 92:9343–9347. <https://doi.org/10.1073/pnas.92.20.9343>
51. Polkinghorne A, Hanger J, Timms P (2013) Recent advances in understanding the biology, epidemiology and control of chlamydial infections in koalas. *Vet Microbiol* 165:214–223. <https://doi.org/10.1016/j.vetmic.2013.02.026>
52. Poston TB, Lee DAE, Darville T, Zhong W, Dong L, O'Connell CM, Wiesenfeld HC, Hillier SL, Sempowski GD, Zheng X (2019) Cervical cytokines associated with *Chlamydia trachomatis* susceptibility and protection. *J Infect Dis* 220:330–339. <https://doi.org/10.1093/infdis/jiz087>
53. Quigley BL, Timms P (2020) Helping koalas battle disease—Recent advances in *Chlamydia* and koala retrovirus (KoRV) disease understanding and treatment in koalas. *FEMS Microbiol Rev* 44:583–605. <https://doi.org/10.1093/femsre/fuaa024>
54. Rasmussen SJ, Eckmann L, Quayle AJ, Shen L, Zhang Y-X, Anderson DJ, Fierer J, Stephens RS, Kagnoff MF (1997) Secretion of proinflammatory cytokines by epithelial cells in response to *Chlamydia* infection suggests a central role for epithelial cells in chlamydial pathogenesis. *J Clin Invest* 99:77–87. <https://doi.org/10.1172/jci119136>
55. Rauluseviciute I, Riudavets-Puig R, Blanc-Mathieu R, Castro-Mondragon JA, Ferenc K, Kumar V, Lemma RB, Lucas J, Chèneby J, Baranasic D (2024) JASPAR 2024: 20th anniversary of the open-access database of transcription factor binding profiles. *Nucleic Acids Res* 52:D174–D182. <https://doi.org/10.1093/nar/gkad1059>
56. Robbins A, Hanger J, Jelocnik M, Quigley BL, Timms P (2020) Koala immunogenetics and chlamydial strain type are more directly involved in chlamydial disease progression in koalas from two south east Queensland koala populations than koala retrovirus subtypes. *Sci Rep* 10:15013
57. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP (2011) Integrative genomics viewer. *Nat Biotechnol* 29:24–26. <https://doi.org/10.1038/nbt.1754>
58. Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol Biol Evol* 34:3299–3302. <https://doi.org/10.1093/molbev/msx248>
59. Scurlock AM, Frazer LC, Andrews CW Jr, O'Connell CM, Foote IP, Bailey SL, Chandra-Kuntal K, Kolls JK, Darville T (2011) Interleukin-17 contributes to generation of Th1 immunity and neutrophil recruitment during *Chlamydia muridarum* genital tract infection but is not required for macrophage influx or normal resolution of infection. *Infect Immun* 79:1349–1362. <https://doi.org/10.1128/iai.00984-10>
60. Silver LW, McLennan EA, Beaman J, da Silva KB, Timms P, Hogg CJ, Belov K (2024) Using bioinformatics to investigate functional diversity: a case study of MHC diversity in koalas. *Immunogenetics* 1–15. <https://doi.org/10.1007/s00251-024-01356-6>
61. Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N (2007) Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* 4:125–134. <https://doi.org/10.1007/S10393-007-0093-5>
62. Stephens M, Scheet P (2005) Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. *Am J Hum Genet* 76:449–462. <https://doi.org/10.1086/428594>

63. Tabach Y, Brosh R, Buganim Y, Reiner A, Zuk O, Yitzhaky A, Koudritsky M, Rotter V, Domany E (2007) Wide-scale analysis of human functional transcription factor binding reveals a strong bias towards the transcription start site. *PLoS ONE* 2:e807. <https://doi.org/10.1371/journal.pone.0000807>
64. Team RC (2020) RA language and environment for statistical computing. R Foundation for Statistical, Computing
65. Thompson JD, Gibson TJ, Higgins DG (2003) Multiple sequence alignment using ClustalW and ClustalX. *Curr Protoc Bioinform*:2.3. 1-2.3. 22. <https://doi.org/10.1002/0471250953.bi0203s00>
66. Tsai S-J (2017) Effects of interleukin-1beta polymorphisms on brain function and behavior in healthy and psychiatric disease conditions. *Cytokine Growth F R* 37:89–97. <https://doi.org/10.1016/j.cytogfr.2017.06.001>
67. Turner AK, Begon M, Jackson JA, Bradley JE, Paterson S (2011) Genetic diversity in cytokines associated with immune variation and resistance to multiple pathogens in a natural rodent population. *PLoS Genet* 7:e1002343. <https://doi.org/10.1371/journal.pgen.1002343>
68. Ullrich KA, Schulze LL, Paap E-M, Müller TM, Neurath MF, Zundler S (2020) Immunology of IL-12: An update on functional activities and implications for disease. *EXCLI J* 19:1563. <https://doi.org/10.17179/excli2020-3104>
69. Villanueva RAM, Chen ZJ (2019) ggplot2: elegant graphics for data analysis. *Meas-Interdiscip Res* 17:160–167. <https://doi.org/10.1080/15366367.2019.1565254>
70. Wan C, Loader J, Hanger J, Beagley K, Timms P, Polkinghorne A (2011) Using quantitative polymerase chain reaction to correlate *Chlamydia pecorum* infectious load with ocular, urinary and reproductive tract disease in the koala (*Phascolarctos cinereus*). *Aust Vet J* 89:409–412. <https://doi.org/10.1111/j.1751-0813.2011.00827.x>
71. Wang C, Tang J, Crowley-Nowick P, Wilson C, Kaslow R, Geisler W (2005a) Interleukin (IL)-2 and IL-12 responses to *Chlamydia trachomatis* infection in adolescents. *Clin Exp Immunol* 142:548–554. <https://doi.org/10.1111/j.1365-2249.2005.02946.x>
72. Wang C, Tang J, Geisler WM, Crowley-Nowick PA, Wilson CM, Kaslow RA (2005b) Human leukocyte antigen and cytokine gene variants as predictors of recurrent *Chlamydia trachomatis* infection in high-risk adolescents. *J Infect Dis* 191:1084–1092. <https://doi.org/10.1086/428592>
73. Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1998) Quantifying threats to imperiled species in the United States. *Bioscience* 48:607–615. <https://www.jstor.org/stable/1313420>
74. Wong ES, Papenfuss AT, Belov K (2011) Genomic identification of chemokines and cytokines in opossum. *J Interferon Cytokine Res* 31:317–330. <https://doi.org/10.1089/jir.2010.0045>
75. Wong ES, Young LJ, Papenfuss AT, Belov K (2006) In silico identification of opossum cytokine genes suggests the complexity of the marsupial immune system rivals that of eutherian mammals. *Immunome Res* 2:4. <https://doi.org/10.1186/1745-7580-2-4>
76. Zhang MQ (1998) Identification of human gene core promoters in silico. *Genome Res* 8:319–326. <https://doi.org/10.1101/gr.8.3.319>
77. Zhang X, Angkasekwinai P, Dong C, Tang H (2011) Structure and function of interleukin-17 family cytokines. *Protein Cell* 2:26–40. <https://doi.org/10.1007/s13238-011-1006-5>