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The genetics of immune and infection phenotypes in wild mice, *Mus musculus domesticus*

Louise Cheynel[1] | Luke Lazarou[2] | Eleanor M. Riley[3] | Mark Viney[1]

[1]Department of Evolution, Ecology and Behaviour, University of Liverpool, Liverpool, UK

[2]School of Biological Sciences, University of Bristol, Bristol, UK

[3]Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh, UK

Correspondence
Louise Cheynel, Department of Evolution, Ecology and Behaviour, University of Liverpool, Liverpool, UK.
Email: cheynel.louise@gmail.com

Abstract

Wild animals are under constant threat from a wide range of micro- and macroparasites in their environment. Animals make immune responses against parasites, and these are important in affecting the dynamics of parasite populations. Individual animals vary in their anti-parasite immune responses. Genetic polymorphism of immune-related loci contributes to inter-individual differences in immune responses, but most of what we know in this regard comes from studies of humans or laboratory animals; there are very few such studies of wild animals naturally infected with parasites. Here we have investigated the effect of single nucleotide polymorphisms (SNPs) in immune-related loci (the major histocompatibility complex [MHC], and loci coding for cytokines and Toll-like receptors) on a wide range of immune and infection phenotypes in UK wild house mice, *Mus musculus domesticus*. We found strong associations between SNPs in various MHC and cytokine-coding loci on both immune measures (antibody concentration and cytokine production) and on infection phenotypes (infection with mites, worms and viruses). Our study provides a comprehensive view of how polymorphism of immune-related loci affects immune and infection phenotypes in naturally infected wild rodent populations.

KEYWORDS
cytokine, ecoimmunology, major histocompatibility, mouse, parasite, wild

1 | INTRODUCTION

Wild animals are under constant threat from a wide range of micro- and macroparasites present in their environment. Animals use a variety of behavioural and physiological responses to avoid and/or resist infection and the harm that it can cause. A key aspect of this are immune responses that actively defend individuals against infection. However, parasites also evolve to avoid these immune responses. The ubiquity of parasites and their continual evolution represents a strong selective force that hones the immune responses that animals make (Moran, 2002; Pilosof et al., 2014). This ongoing co-evolution between hosts and parasites shapes both parasites' genetic diversity and the genetic diversity of host loci whose products mediate the immune response, henceforth immune-related loci (Anderson & May, 1982; Frank, 2000).

In vertebrates, some immune-related loci, such as those in the major histocompatibility complex (MHC), are highly polymorphic (as reviewed in Radwan et al., 2020) and this genetic diversity is thought to be the result of the parasite-driven selection (Spurgin & Richardson, 2010). The MHC encodes proteins that present antigens to T lymphocytes, which can then result in an immune response (Klein, 1986). MHC heterozygosity is thought to be advantageous by facilitating the presentation of a wide variety of antigens, so widening the range of potential immune responses that can be generated; however, experimental work to test this hypothesis has provided mixed results (Doherty & Zinkernagel, 1975; Ilmonen et al., 2007;

Penn et al., 2002). Moreover, there is a much wider repertoire of immune-related loci that may also affect individuals' immune responses (Acevedo-Whitehouse & Cunningham, 2006). Animals use Toll-like receptors (TLRs) to detect bacterial and viral pathogen-associated molecular patterns (PAMPs). These TLRs then initiate signalling events, including via cytokines and chemokines, that ultimately activate innate and adaptive immune responses contributing to parasite resistance (Kawai & Akira, 2006). Most mammals have 10–12 TLR coding loci that detect different PAMPs (Roach et al., 2005). In humans, numerous studies have described associations between polymorphisms in TLR and cytokine-coding loci, and susceptibility or resistance to a range of infections (Mukherjee et al., 2019; Smith & Humphries, 2009). In humans, the cumulative contribution of non-MHC loci, such as those coding for cytokines or PAMP receptors, to the immune phenotype likely exceeds that of the MHC (Jepson et al., 1997; Roederer et al., 2015).

Most of our understanding of the effect of polymorphism in immune-related loci on immune and/or infection phenotypes comes from studies of humans or laboratory animals; few empirical studies have been conducted with wild animals. Major differences exist between the infection and immune states of wild animals and their laboratory-reared counterparts. This has been extensively studied for mice, showing the significantly different immunological effect of wild vs. laboratory gut microbiomes (Abolins et al., 2017; Beura et al., 2016; Rosshart et al., 2017). Outbred lines of laboratory mice are available, though these do not reflect the full genetic diversity of wild populations (Turner & Paterson, 2013). Given the disconnect between the immunological state, infection state and genetic diversity of wild and laboratory animals, the paucity of our understanding of how host genetics affects immune responses and infection phenotypes in wild animals is a notable knowledge gap, and points to the critical need to study these phenomena directly (Turner & Paterson, 2013).

To date, there have been about 30 immunogenetic studies on 16 different genera of wild mammals that sought specific associations between polymorphisms in immune-related loci and measures of immune or infection phenotypes (Table 1). Among these the effect of the MHC has been investigated most frequently and these studies commonly, but not universally, find effects of MHC diversity on micro- and macroparasite infection phenotypes. Studies of polymorphisms in TLR and cytokine-coding loci have also found associations with infection phenotypes. Most of these studies have studied the effects of polymorphism in immune-related loci on infection phenotypes (especially of macroparasite burden), and so there remains major gaps in our understanding of effects on immune responses themselves (but see Charbonnel et al., 2010; Cutrera et al., 2011; Turner et al., 2011; Huang et al., 2022). Overall, polymorphism in rather few immune-related loci has been studied in wild mammals, again in notable contrast to the very large number of loci studied in laboratory animals. It is not yet known whether these laboratory observed effects also occur in the wild. An important remaining question is therefore whether—and if so, how—naturally occurring polymorphism in immune-related loci impacts both immune and infection phenotypes in wild mammals. Wild mice have the potential

to be powerful study systems to improve our understanding of these relationships, because they will reveal effects occurring in natural environments, but with the advantage of exceptionally good genetic, physiological and immunological understanding of the study species, which has accumulated from decades of experimental laboratory work (Graham, 2021; Turner & Paterson, 2013).

Here we have investigated the effect of diversity in immune-related loci on a wide range of immune and infection phenotypes in wild house mice, *Mus musculus domesticus*. We first assessed the extent of genetic variation in 23 immune-related loci (coding for cytokines, TLRs or the MHC) in our study populations, finding variation in 14 of these loci among 435 mice. We then tested the effect of each genotype found in these 14 loci on 24 discrete immune phenotypes, encompassing both cellular and humoral components of the innate and the adaptive immune response. We also tested the effect of the genotype on infection phenotypes of nine different micro- and macroparasites (mites, worms, viruses and a bacterium). To look for effects of the genotype on the phenotype, we used a two-step model selection approach, firstly testing the effect of all non-genetic factors (site, sex, age and body mass), then adding each genetic factor to the model in turn to assess its effect on the relevant phenotype. Our study thus provides a uniquely complete picture of the association between genotype and immune phenotype, linking allele in 14 immune-related loci to 24 immune phenotypes and 9 infection phenotypes in wild rodents.

2 | METHODS

2.1 | Mice

The sampling, processing, determination of infection state and immune phenotyping of the mice have previously been reported (Abolins et al., 2017, 2018). In brief, 460 mice (*Mus musculus domesticus*) were live trapped from 12 sites in the southern United Kingdom (UK) between March 2012 and April 2014. Population genetic analyses show strong genetic structuring of mice among the different sample sites (Abolins et al., 2018). After being humanely killed, mice were sexed, weighed and measured from the tip of the snout to the base of the tail. Female mice that were later found to be pregnant had the mass of the foetuses subtracted from their total mass, and these values were used in all subsequent analyses. To estimate body condition, the Scaled Mass Index (SMI) was calculated as previously described (Peig & Green, 2009). Age was determined using eye lens mass as previously described (Rowe et al., 1985). The distribution of individuals by study site, sex and age class is given in Table S1.

2.2 | Immune measures

The immune state of the mice was assessed by measuring (i) 12 immune cell populations, (ii) the concentration of 3 immunoglobulins, and (iii) the concentration of 9 cytokines produced after in vitro

TABLE 1 Studies in wild mammals, testing for associations between genetic variation in specific immune-related loci (i.e. MHC, TLR and/or cytokines) and measures of immune or infection phenotypes. These articles were searched for from the Web of Science using combinations of key words 'immuno-genetic', 'genetic variation', 'immunity', 'infection' and 'wild' on 1st January 2022 This table aims to summarize results from previous work that are directly comparable with those reported in this paper.

Species	Loci	Infection phenotype	Immune trait	Conclusions	Study
Alpine ibex (*Capra ibex*)	MHC, TLR	*Mycoplasma conjunctivae, Brucella*	—	MHC heterozygosity positively correlated with *M. conjunctivae* infection; polymorphism in TLR has effects on brucellosis	Brambilla et al. (2018), Quéméré et al. (2020)
Badger (*Meles meles*)	MHC	*Trypanosoma pestanai*, MHV, enteric bacteria (*Salmonella, Yersinia, Campylobacter*), *Coccidia*, helminth, ectoparasites	—	Association of MHC alleles and infection phenotype but not with co-infection status; heterozygote advantage	Sin et al. (2014)
Bank vole (*Myodes glareolus*)	MHC, TLR	Helminths, *Borrelia afzelii*; *Cryptosporidium, Bartonella, Babesia, Haemobartonella, Trypanosoma, Hepatozoon*	—	Intermediate number of MHC alleles associated with low helminth infection; association between TLR2 polymorphism and *Borrelia* infection; effect of TLR1 and TLR5 alleles on *Bartonella* infection, not on nematodes; associations between MHC DQB locus haplotypes and *Borrelia* infection status: one haplotype was associated with lower risk of infection, another with higher risk of infection	Kloch et al. (2010), Kloch et al. (2018), Scherman et al. (2021), Tschirren et al. (2013)
Voles (*Microtus agrestis, M. oeconomus*)	MHC, TLR, cytokines	Ectoparasites, nematodes, cestodes, *Babesia, Bartonella,* helminth FEC, *Mycoplasma, Trypanosoma, Bartonella*	Cytokines, immunity related transcription factors	Genetic diversity at cytokine loci is an important source of individual variation in immunity and pathogen resistance; MHC alleles predict infection status	Kloch et al. (2013), Turner et al. (2011)
Hairy footed gerbil (*Gerbillurus paeba*)	MHC	Helminth morphotypes, FEC	—	Association of alleles of DRN exon 2 and FEC, but not of infection status or intensity of infection	Harf and Sommer (2005)
Long-tailed giant rat (*Leopoldamys sabanus*)	MHC	Helminth morphotypes, FEC	—	Association of diversity of MHC alleles and resistance to helminth infection	Lenz et al. (2009)
Plains zebra (*Equus quagga*)	MHC	Nematode FEC, ticks	—	Association of rare alleles and greater FEC and of common alleles with greater tick burdens	Kamath et al. (2014)
Roe deer (*Capreolus capreolus*)	TLR	*Toxoplasma gondii, Chlamydia abortus*	—	Antagonistic selection on TLR2 polymorphisms; limited support for heterozygote advantage	Quéméré et al. (2021)
Soay sheep (*Ovis aries*)	MHC, cytokines, others	Nematode FEC	Antibodies	Association of MHC and immunoglobulin heavy constant loci (IGH) complex with [Ig] and nematode resistance; little support for candidate genes explaining parasitological and immunological traits; IFN-γ allele associated with reduced FEC and increased [IgA].	Brown et al. (2013), Coltman et al. (2001), Huang et al. (2021), Paterson et al. (1998), Sparks et al. (2019)

TABLE 1 (Continued)

Species	Loci	Infection phenotype	Immune trait	Conclusions	Study
Spotted suslik (*Spermophillus suslicus*)	MHC	Helminth FEC, *Coccidia*, blood parasites (*Anaplasma, Babesia, Mycoplasma, Trypanosoma*)	–	Increase in parasites results in a development of novel associations between MHC alleles and parasite resistance	Biedrzycka and Kloch (2016)
Striped mouse (*Rhabdomys pumilio*)	MHC	Helminth FEC	–	Divergent-allele advantage; no heterozygote advantage	Froeschke and Sommer (2005, 2012)
Talas tuco-tuco (*Ctenomys talarum*)	MHC	Helminth FEC, ectoparasites	Antibody production, leukocytes profiles	Associations between MHC class II DRB locus, parasite intensity and humoral immune response	Cutrera et al. (2011, 2014)
Tome's spiny rat (*Proechimys semispinosus*)	TLR	Helminth FEC, *Hepacivirus*	–	Association of TLR4 haplotypes, nematode intensity and virus prevalence	Heni et al. (2020)
Water voles (*Arvicola scherman, A. terrestris*)	MHC	Helminths, *Coccidia*, viruses (puumala, cowpox, LCMV), ectoparasites, *Bartonella*	Cell-mediated immunocompetence	Associations between MHC alleles, parasite intensity and parasite species were found; MHC heterozygosity and lowered parasitism; negative association of MHC heterozygosity and immune response	Charbonnel et al. (2010), Oliver et al. (2009), Tollenaere et al. (2008)
California sea lion (*Zalophus californianus*)	MHC	–	Phytohaemagglutinin (PHA)-induced inflammation	Two-month-old pups with a specific MHC-DRB locus tended to have less effective inflammatory response	Montano-Frías et al. (2016)
Reddish-grey mouse lemur (*Microcebus griseorufus*)	MHC	Helminths, adenovirus	–	No evidence of an association between MHC diversity and adenovirus or helminth infection status	Montero et al. (2021)

Abbreviations: FEC, faecal egg counts; GI, gastrointestinal; LCMV, Lymphocytic choriomeningitis virus; MHV, murine hepatitis virus; TLR, Toll Like Receptor; MHC, Major Histocompatibility Complex.

stimulation of spleen cells with 4 different stimuli (anti-CD3/anti-CD28, CpG, lipopolysaccharide and peptidoglycan). Together, these measures therefore encompass cellular and humoral components of the innate and adaptive immune responses, so providing a broad assessment of humoral and cellular immune responses (as reported in Abolins et al. (2017, 2018)).

The 12 cell populations measured were flow cytometric counts of splenic NKp46+ Natural Killer (NK) cells; CD19+ B cells; CD11c+ Dendritic cells (DCs); CD8+ T cells; CD4+ T cells; CD25+ FoxP3+ Treg cells; F4/80+ Ly6G- macrophages; F4/80+ Ly6G-low monocytes; F4/80+ Ly6G-intermediate hyper-granulocytic myeloid cells; F4/80 variable Ly6G-High polymorphonuclear (PMN) cells; FSC-low PMNs neutrophils; and FSC-High PMNs myeloid-derived suppressor cells (MDSC) (as Abolins et al., 2017). Each of the 12 cell counts were then scaled to the mouse body mass, using the same formula as the one used for the calculation of the SMI (see above), but replacing mouse mass with the count for each cell type. The relevant scaling component was calculated separately for each of the 12 cell types. This results in the count of each immune cell for each mouse scaled to the length of the average mouse. Scaling the immune cells in this manner, rather than using a proportion of each cell type, allowed us to use count data in our models while still controlling for effects of mouse body size.

The immunoglobulins measured were the serum concentrations of total IgG, total IgE and the faecal concentration of total IgA, all of which were assayed by ELISA (Abolins et al., 2017). The cytokines measured were IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-12p40, IL-12p70, IL-13, MIP-2α, which were assayed by in vitro stimulation of splenocytes (as above) followed by multiplex bead array assay of cytokine concentration (Abolins et al., 2017).

2.3 | Infection phenotype

The number of fur mites, *Myocoptes musculinus*, was determined as previously described and classified into the categories: 0–10, 11–20, 21–30, 31–40, 41–50, 51–100, 101–200, 201–300, 301–400, 401–500, 501–1000, 1001–1500 and 1501–2000 (Weldon et al., 2015). The number of intestinal nematodes was determined by gut examination as previously described (Weldon et al., 2015). Current or prior infection with Corona, Mouse Hepatitis, Sendai, Minute, Noro and Parvo viruses and to *Mycoplasma pulmonis* was inferred from serological data, as previously described (Abolins et al., 2017).

2.4 | Single nucleotide polymorphism identification and genotyping

Our approach to identifying single nucleotide polymorphisms (SNPs) was (i) to identify loci of immunological interest, (ii) within these loci to search for sites where there were variants known to differ between strains of laboratory mice, and (iii) search for regions

encompassing these sites that were likely to be suitable for PCR amplification. Using this approach, we identified 23 loci coding for products of immunological interest, specifically 15 coding for cytokines (IL-1a, IL-1b, IL-2, IL-4, IL-6, IL-10, IL-2a, IL-2b, IL-13, IL-17a, IL-17F, IFN-γ, TNF, IL2-rg, CD40lg), 3 coding for TLRs (TLR4, 5 and 9), 5 for products of the murine MHC H2 (H2-Aa, H2-Ab1, H2-Eb1, H2-K1, H2-D1 which are classical Class 1 and Class 2 MHC genes, Kumánovics et al., 2003). We also selected, as a control, a locus encoding a non-immunological product (Myo1a). We then PCR-amplified fragments of these loci in a randomly selected sub-set of 25 wild mice from the different sites, and sequenced those amplicons to identify putative SNPs. We then selected 23 of these SNPs (in 21 immune-related loci: IL-1a, IL-1b [2 SNPs, IL-1b_U, IL-1b_N], IL-2, IL-4, IL-6, IL-10, IL-2a, IL-2b, IL-13, IL-17a [2 SNPs, IL-17a_U, IL-17a_N], IL-17F, IFN-γ, TNF, IL2-rg, CD40lg, TLR4, TLR5, TLR 9, H2-Aa, H2-Ab1, H2-Eb1) that we then Kompetitive Allele Specific PCR (KASP) genotyped in our full set of wild mice, which was done by LGC Genomics, UK. We found that 14 (IL-1a, IL-1b_U, IL-6, IL-10, IL-3, IL-17a_U, IL-17a_N, IL-17F, TNF, TLR5, TLR 9, H2-Aa, H2-Ab1 and H2-Eb1) of these 23 SNPs were polymorphic in this full set of mice (Lazarou, 2019) and these are the data that are analysed here. The control locus Myo1a was also successfully amplified, sequenced and two SNPs (Myo1a_1, Myo1a_2) were found to be polymorphic in these mice. A summary table of the SNPs identified and variants found is provided in Table S2.

The PCR amplification of these fragments used the primer sequences shown in Table S3. The reactions consisted of 1 μL of genomic DNA, 4 μL (10 μM) of each primer, 5 μL (20 units) of Platinum Taq High fidelity Buffer (Life Technologies), 1 μL of dNTPs (10 mM each) (ThermoFisher Scientific), 2 μL of the manufacturer's supplied MgSO4 (Life Technologies) and 32 μL of water (Sigma Aldrich), which were then cycled through the following conditions: denaturation at 94°C for 30 s, annealing temperature 15 s, elongation 68°C with time depending on product, calculated at a rate of 30 s per kb and a final elongation at 68°C for 10 min. Annealing temperatures were optimized using gradient PCR with C57/BL6 target DNA. The 25 wild mice used for SNP discovery were those defined in Abolins et al. (2017, 2018) (SNPs identified are shown in Table S4 and variants detected are shown in Table S5). Successful PCR products were purified using the GeneJet PCR clean-up kit (Thermo Fisher Scientific), following the manufacturer's instructions and single-read sequencing of the purified products was carried out by Eurofins Genomics, UK.

2.5 | Statistical analyses

We tested all possible associations between individual SNPs and immune or infection phenotypes. We used generalized linear models for continuous variables (immune measures and worm number) and ordinal logistic regression for categorial data (i.e. intensity of infection with mites and microparasites). Each immune or infection phenotype was used as the response variable in the model. Raw data

were used when the immune parameter had a Gaussian distribution, while traits with a non-Gaussian distribution were $\log(X+1)$ transformed before analysis to improve the normality of the data.

As our dataset comprised concentrations of nine different cytokines produced in vitro after spleen cell stimulation with four different stimuli (i.e. 36 cytokine measures for each mouse), we performed a principal component analysis (PCA) on all mice for which all 36 cytokine measures were available ($N = 172$) to identify the main axes of variation, for which we used the R package 'ade4' (Dray & Dufour, 2007). We removed data for three mice that contributed abnormally to the PCs (i.e. contributing 8% of the first principal component) possibly because of a severe inflammatory state. PC1 and PC2 represent 34% and 19% (total 53%), respectively, of the covariation among the cytokine measures. PC1 was mostly influenced by IL-1B, IL-10, IL-12p40, IL-12p70, IL-13, IL-4; PC2 was mostly influenced by IL-6, IFN-γ and MIP-2α (Table S6). PC1 thus appears to represent overall cytokine concentration, weighted more to specific cytokines and PC2 to Th1/Th2-related cytokines. For cytokine measures, we therefore used PC1 and PC2 as the response variables in the modelling approach below.

Our modelling approach had two steps. Firstly, for each immune or infection phenotype, we built a base model to investigate the effect of potential non-genetic confounders, specifically site effects (nine sites, Figure 1), age (linear function and three classes, Table S1), sex (two classes: male and female), and body condition (SMI, continuous). We merged data from some closely located sites with small numbers of mice, giving us nine sites for analysis across the whole population (Figure 1; Table S1). The three age classes were chosen to differentiate individuals ecologically according to their behavioural and life-history state, linked to their likelihood of encountering a range of environmental antigens as: 0–6 weeks (immature animals unlikely to be venturing far from their nest site); 7–12 weeks (young animals unlikely to have reproduced); >12 weeks (sexually mature adults). We

tested the two-way interactions between site, age and sex. Among female mice, 15% were pregnant. We tested the effect of pregnancy on immune traits, finding that there was only an effect on the cytokine measures, and so we included pregnancy as a random factor in models analysing the cytokine PCAs. Secondly, we tested the effect of the SNPs in the immune-related loci, with the different allele combinations tested as fixed effects (three classes: homozygous 1, homozygous 2, heterozygous). Depending on the variables retained in the base model, two-way interactions between SNP, site, sex and age were also included. Using a model selection procedure based on the Akaike information criterion (AIC, Burnham & Anderson, 2002), we retained the model with the lowest AIC. When the difference in AIC between competing models was less than 2, we retained the model with the fewest parameters to satisfy parsimony rules (Burnham & Anderson, 2002). Residuals plots were checked to ensure the fit of the selected regression models. Finally, to be more selective about the associations identified in our study, the significance of the genetic effects selected with the model testing was also tested using deletion testing and the log-likelihood ratio test (McCullagh & Nelder, 1989). The *p*-values obtained from this additional deletion testing step are reported in Table 2 and provided in Table S12. All statistical modelling was performed using R version 4.0.5 (R Core Team, 2021).

3 | RESULTS

We genotyped 435 mice for 23 SNPs in 21 immune-related loci; 14 of these 23 SNPs were found to be polymorphic. We successfully genotyped 378 (87%) mice for all 14 SNPs; the remaining 57 (13%) mice were successfully genotyped for at least 11 SNPs. The minor allele frequencies and the observed and expected heterozygosity are shown in Tables S7 and S8, respectively. The distribution of each SNP among sample sites is shown in Table S9.
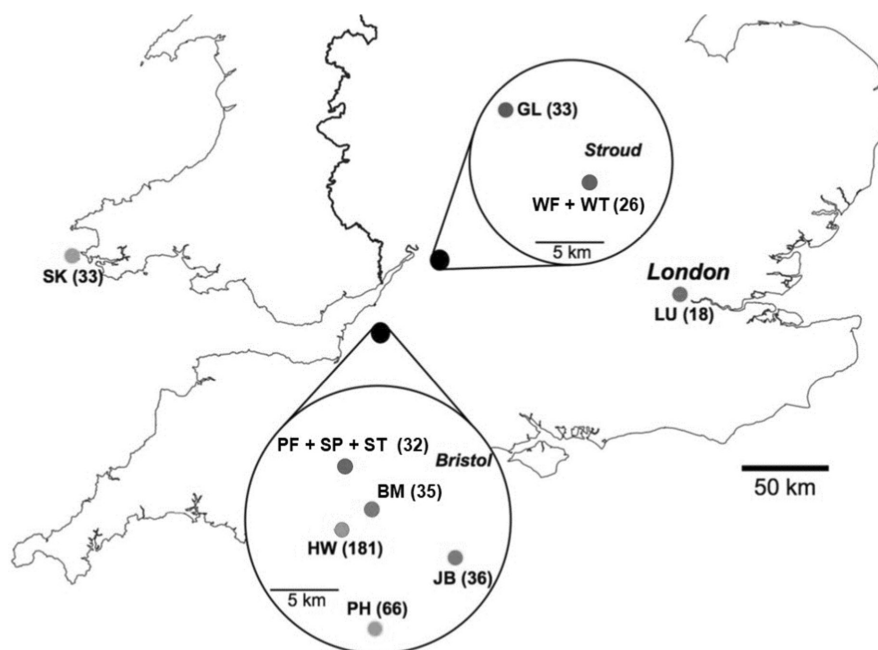

FIGURE 1 Wild mice sampled from across the southern UK. The 9 sampling sites are shown by letter designations, with the number of animals obtained at each site shown in parentheses. Figure adapted from Abolins et al. (2018).

3.1 | Genetic variation in immune-related loci

There was a high level of variation in the MHC loci within our wild mouse population. H2-Ab was the most diverse H-2 locus, with three different genotypes present at similar frequencies and with a heterozygosity of 0.33. For H2-Aa and H2-Eb, one genotype dominated (found in 85% and 70% of mice, respectively) and with lower heterozygosity (0.09 and 0.21, respectively).

For four of the cytokine-coding loci (IL-1a, IL-1b_U, IL-17a_U and IL-17F), there was relatively high level of genetic diversity, with overall heterozygosity between 0.12 and 0.34. In contrast, the other cytokines were each dominated by one very common genotype (89%–95%), and had low heterozygosity (0.01–0.06). A similar situation pertains for the two TLR loci where one genotype dominated (95% and 96% for TLR5 and TLR9, respectively) with a heterozygosity of 0.04.

The degree of variation at different loci differs among sampling sites (Figure 1, Table S9). For example, mice from the island site SK ($N = 27$) are invariant at all MHC SNPs, mice from sites BM, HW, JB, LU and WF + WT were polymorphic at 1 or 2 MHC SNPs, and mice from sites GL, PH and ST + SP + PH were polymorphic at all three MHC loci. For SNPs in the cytokine-coding loci, IL-1b_U and IL-17a_U polymorphism was limited to only some sample sites. The IL-6, IL-10, TNF and TLR loci generally had low levels of polymorphism that was often restricted to a few sample sites (including just one site for TLR-9).

3.2 | Genetic effects on immune phenotypes

We tested the effect of both non-genetic factors and of the three different classes of allele combinations within MHC, cytokine and TLR coding loci on the immune phenotype of wild mice. We first tested the effect of non-genetic factors, finding strong effects of both site and age on all immune parameters, and of body mass (SMI) on cellular components of the immune phenotype, as in Abolins et al. (2018) (Table 2). We then tested the effect of the three different combination of alleles of each SNP on the immune phenotype (Tables 2 and 3). We found no relationships between the control locus and the immune and infection phenotypes. Full details of the two-step model selection are reported in Table S10, effect estimates of all the models selected are shown in Table S11, and the *p*-values of the log-likelihood ratio test after deletion testing are reported in Table S12.

3.2.1 | Antibody concentration

We found strong associations between the genotype of the MHC and cytokine-coding loci and the concentration of antibodies (Table 2; Figure 2b). IgE concentration was strongly associated with IL-1a and IL-1b_U polymorphisms ($p < 0.001$ for both). Specifically, mice with the homozygous TT genotype for both IL-1a and IL-1b_U

had the highest concentrations of IgE, while those with the other two homozygous genotypes (CC for IL-1a and GG IL-1b_U) had the lowest IgE concentrations; heterozygous individuals had intermediate IgE concentrations. This pattern is suggestive of an allele dosage effect. The concentration of IgE was also associated with the IL-17a_U genotype (Table 2), where heterozygous mice had lower concentrations of IgE than either homozygous genotype (Figure 2b). Finally, the MHC H2-Ab genotype showed an association with the concentration of IgG (Table 2), where heterozygous mice had lower concentrations than either of the two homozygous genotypes, that is GG and AA genotype (Figure 2b). While these patterns are evident in the whole data set, effects of IL-1a or IL-17a_U genotype on IgE concentration and of H2-Aa polymorphism on IgG concentration also varied among sites and among mouse age classes (Table 3, Table S11).

3.2.2 | Cytokine production

We also found associations between the genotype of cytokine-coding loci and the PCA of cytokine responses (Table 2, Figure 2c). Polymorphism in IL1-b_U was associated with PC1 ($p < 0.05$), which mainly reflects cytokines IL-1B, IL-10, IL-12p40, IL12p70, IL-3 and IL-4. Specifically, heterozygous GT or homozygous GG mice had higher PC1 values than the most common genotype TT (Table 3). The genotype of the MHC locus H2-Eb was associated with PC1 ($p < 0.001$), with heterozygous AG mice having lower PC1 values than the rare AA genotype or the most common GG genotype (Table 3). The genotype of IL-17a_U was associated with cytokine PC2 ($p < 0.05$), which mainly reflects cytokines IL-6, IFN-γ and MIP-2α; heterozygous mice had higher PC2 values than the homozygous genotypes (Table 3). Concerning the PCA analyses, we generally note that PC1 has negative loadings for all measures and thus indicates variation in the levels of cytokine production, while PC2 presents a more qualitative separation between IFN-γ and IL-6 against IL-13 and IL-4, consistent with representing the Th1/Th2 phenotype.

3.2.3 | Immune cells

We found some associations between the MHC and cytokine loci genotypes and immune cell numbers (Table 2, all $p > 0.05$; Figure 2a). The H2-Aa genotype was associated with the number of DCs, CD4⁺ and CD8⁺ T cells, and MDSC: mice with the rare AA genotype had more cells than either of the other genotypes (Figure 2a). In contrast, the rare AA H2-Eb genotype was associated with fewer CD4⁺ T cells compared to the other genotypes (Table 3). Finally, there were some associations between the genotype of IL-17F and IL-17a_U and the number of PMNs (Table 2). Mice heterozygous for IL-17F had more PMNs than either homozygous genotype (Table 3), whereas mice with the most common GG IL-17a_U genotype had more PMNs than the other rarer genotypes (Table 3). As above, some of these associations varied among sample sites (Table S11).

TABLE 2 Effect of polymorphism in immune-related loci on immune phenotypes. Results of linear models where first the effect of different age functions (linear, classes), sex (female vs. male), site (9 sites), and scaled mass index were tested to select the base model of non-genetic factors. Secondarily, the effect of each polymorphism was tested and shaded boxes indicate where genetic effects were selected in the model (Table S10).

Immune phenotype	Non-genetic factors	Effect of genetic variation					
		MHC			Cytokines		
		H2-Aa	H2-Ab	H2-Eb	IL-1a	IL-1b_U	IL-6
Cells							
NKp46+ NK cells	Site × age (linear)+SMI						** (CT<CC<TT)
CD19+ B cells	Site + age (linear) × sex + SMI						
CD11c+DC	Site × age (linear)+SMI	** (AG<GG<AA)					** (CT<CC<TT)
CD8+ T cells	Age (linear)+SMI	* (AG,GG<AA)					* (CT<CC<TT)
CD4+ T cells	Site + age (linear)+SMI	** (AA<GG<AG)		** (AA<AG,GG)			*** (CC<CT<TT)
CD25+ FoxP3+ Tregs	Site × age (linear)+SMI						* (CT<CC<TT)
F4/80+ macrophages	Age (linear)+SMI						
F4/80+ monocytes	Site × age (linear)+SMI						
F4/80+ HGM	Site × age (linear)+sex + SMI						
Ly6G+ PMN	Site + age (classes)+SMI						
Ly6G+ neutrophils	Site × age (linear)+SMI						* (CT<TT<CC)
MDSC	site × age (linear)+SMI	* (AG<AA,GG)					** (CT<TT<CC)
Antibodies							
IgG	Site + age (classes)		* (GG<AG<AA)				
IgA	Site × sex + age (classes)						* (TT<CT<CC)
IgE	Site + sex + age (classes)				*** (TC<TT<CC)	*** (GT,GG<TT)	* (TT<CT,CC)
Cytokine PCA							
PC1 (34% variation)	Site × sex			*** (AG<AA,GG)		* (TT<GT<GG)	* (CC<CT<<TT)
PC2 (19% variation)	Site × sex						

Note: The stars shows the significance of the selected genetic effects, determined by deletion testing and log-likelihood ratio test, as: *** for $p < 0.001$; ** for $p < 0.01$; * for $p < 0.05$ and for $p > 0.05$ (Table S12). Loci where the minor allele frequency (MAF) is >0.1 are shown in bold (Table S7); loci with a MFA <0.1 are not bold and these results should be interpreted with more caution. DC is Dendritic Cells; F4/80+ HGM is F4/80+ hyper-granulocytic myeloid cells; Ly6G+ PMN is Ly6G+ polymorphonuclear cells; MDSC is myeloid-derived suppressor cells.

For some loci, there was too little genetic variation to allow us to draw strong conclusions about their association with immune phenotypes. However, we found a recurring tendency for associations between alleles of IL-6, IL-10, IL-13 and TNF coding loci and many different components of the immune phenotype (Table 2). This interesting trend would benefit from further study. We did not, however, find any striking associations between the two TLR loci, nor the IL-17a_N cytokine locus, and the immune phenotype.

3.3 | Genetic effects on infection phenotypes

As above, we first tested the effect of non-genetic factors on infection phenotypes (Table 4). Again, we found strong site and age effects on virus seropositivity, mite and worm burden, and of SMI on mite and worm burden, as in Abolins et al. (2018) (Table 4). We then tested the effect of the three different allele combinations in each SNP on infection phenotypes (Tables 4 and 5). Full details of

IL-10	IL-13	IL-17a_U	IL-17a_N	IL-17F	TNF	TLR	
						TLR-5	TLR-9
** (CT<CC<TT)	* (CC<TC<TT)				** (TT<TG<GG)		
*** (CT<CC<TT)	* (CC<TC<TT)				** (TG,TT<GG)		
*** (CT<CC<TT)	* (CC<TC<TT)						
* (CC,CT<TT)							
*** (CT<CC,TT)	** (CC<TC<TT)				** (TT<TG<GG)		
	** (CC<TC<TT)						
* (CT<CC,TT)	* (CC<TC,TT)						
	* (CC<TC<TT)						
		* (GA<AA<GG)		*** (CC<TT<<CT)			
*** (CT<CC<TT)							
*** (CT<CC<TT)			* (AA<GG<AG)				
							** (CC<TC)
		*** (GG<GA<AA)			* (TG,TT<GG)		
*** (CT<CC)					*** (TT<<GG)		
* (CT<CC)		* (GG<AA<GA)					

the two-step model selection are reported in Table S10, effect estimates of all the models selected are shown in Table S11, and the *p*-values of the log-likelihood ratio test after deletion testing are reported in Table S12.

We found a strong association between MHC H2-Aa genotype and mite burden: mice with the rare homozygous AA genotype had fewer mites than those with the other, more common genotypes (Tables 4 and 5, $p < 0.05$). MHC H2-Ab genotype was associated with worm burden (Table 4, $p < 0.01$), with a general tendency for mice with the AA genotype to have higher worm burdens than the two other genotypes. However, this general pattern obscures some local variation between sites (see all effect estimates of the H2-Ab x site interaction in Table S11); for instance, H2-Ab heterozygous mice from site WF + WT have higher worm burdens than the other genotypes.

TABLE 3 The effect of different genotypes on immune phenotypes. The parameter estimates for genetic effects for loci where the minor allele frequency is >0.1 are shown. Parameter estimates for other factors (age, site, SMI, etc.), interactions and other loci are in Table S11. In Variable the genotype in brackets is the genotype to which the comparison is made.

Locus	Immune trait	Model selected	Df	Variable	Parameter estimate ± SE	t value	p
H2-Aa	DCs	H2-Aa + site × age (linear) + SMI	22	Intercept	4.75±0.33	14.53	***
				AG (AA)	−0.47±0.16	−2.85	**
				GG (AA)	−0.27±0.18	−1.51	–
	CD4+ T cells	H2-Aa × site + age (linear) + SMI	19	Intercept	5.57±0.19	29.02	***
				AG (AA)	0.27±0.34	0.79	–
				GG (AA)	0.16±0.16	1.00	–
	CD8+ T cells	H2-Aa + age (linear) + SMI	6	Intercept	5.72±0.11	50.27	***
				AG (AA)	−0.14±0.09	−1.55	–
				GG (AA)	−0.17±0.07	−2.44	*
	MDSC	H2-Aa + site × age (linear) + SMI	22	Intercept	3.48±0.34	10.23	***
				AG (AA)	−0.39±0.19	−2.10	*
				GG (AA)	−0.07±0.21	−0.34	–
H2-Ab	IgG	H2-Ab × age (classes) + site	18	Intercept	4.20±0.09	47.41	***
				AG (AA)	−0.25±0.11	−2.30	*
				GG (AA)	−0.35±0.11	−3.31	**
H2-Eb	CD4+ T cells	H2Eb × site + age (linear) + SMI	18	Intercept	5.32±0.17	30.43	***
				AG (AA)	0.37±0.20	1.85	–
				GG (AA)	0.44±0.13	3.44	***
	Cytokines PC1	H2Eb + site × sex	20	Intercept	−6.76±1.65	−4.11	***
				AG (AA)	−1.75±0.99	−1.75	–
				GG (AA)	−0.04±0.96	−0.05	–
IL-1a	IgE	IL1a × site + age (classes) + sex	25	Intercept	3.23±0.28	11.74	***
				TC (CC)	−1.08±0.29	−3.69	***
				TT (CC)	−0.67±0.25	−2.66	**
IL-1b_U	Cytokines PC1	IL1b_U + site × sex	20	Intercept	−5.45±1.58	−3.45	***
				GT (GG)	0.33±0.79	0.42	–
				TT (GG)	−1.78±0.94	−1.88	–
	IgE	IL1b_U + site + sex + age (classes)	15	Intercept	2.05±0.12	17.53	***
				GT (GG)	0.08±0.07	1.15	–
				TT (GG)	0.31±0.09	3.47	***
IL-17a_U	Ly6G+ PMN	IL-17a_U × site + age (classes) + SMI	21	Intercept	3.44±0.31	11.25	***
				GA (AA)	−0.27±0.27	−1.00	–
				GG (AA)	0.20±0.37	0.56	–
	IgE	IL-17a_U × site + age (classes) + sex	23	Intercept	2.38±0.09	27.72	***
				GA (AA)	−0.39±0.44	−0.90	–
				GG (AA)	−0.96±0.20	−4.79	***
	Cytokines PC2	IL-17a_U + site × sex	20	Intercept	−3.36±0.99	−3.37	***
				GA (AA)	1.27±0.87	1.46	–
				GG (AA)	−0.98±0.85	−1.15	–
IL-17F	Ly6G+ PMN	IL-17F × site + age (classes) + SMI	28	Intercept	2.55±0.73	3.50	***
				CT (CC)	1.15±0.75	1.52	–
				TT (CC)	0.53±0.76	0.70	–

Note: Statistical significance is shown by: ⁻ for $p < 0.1$, * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$. Degrees of freedom is df. DC is dendritic cells; MDSC is myeloid-derived suppressor cells; Ly6G+ PMN is Ly6G+ polymorphonuclear cells.
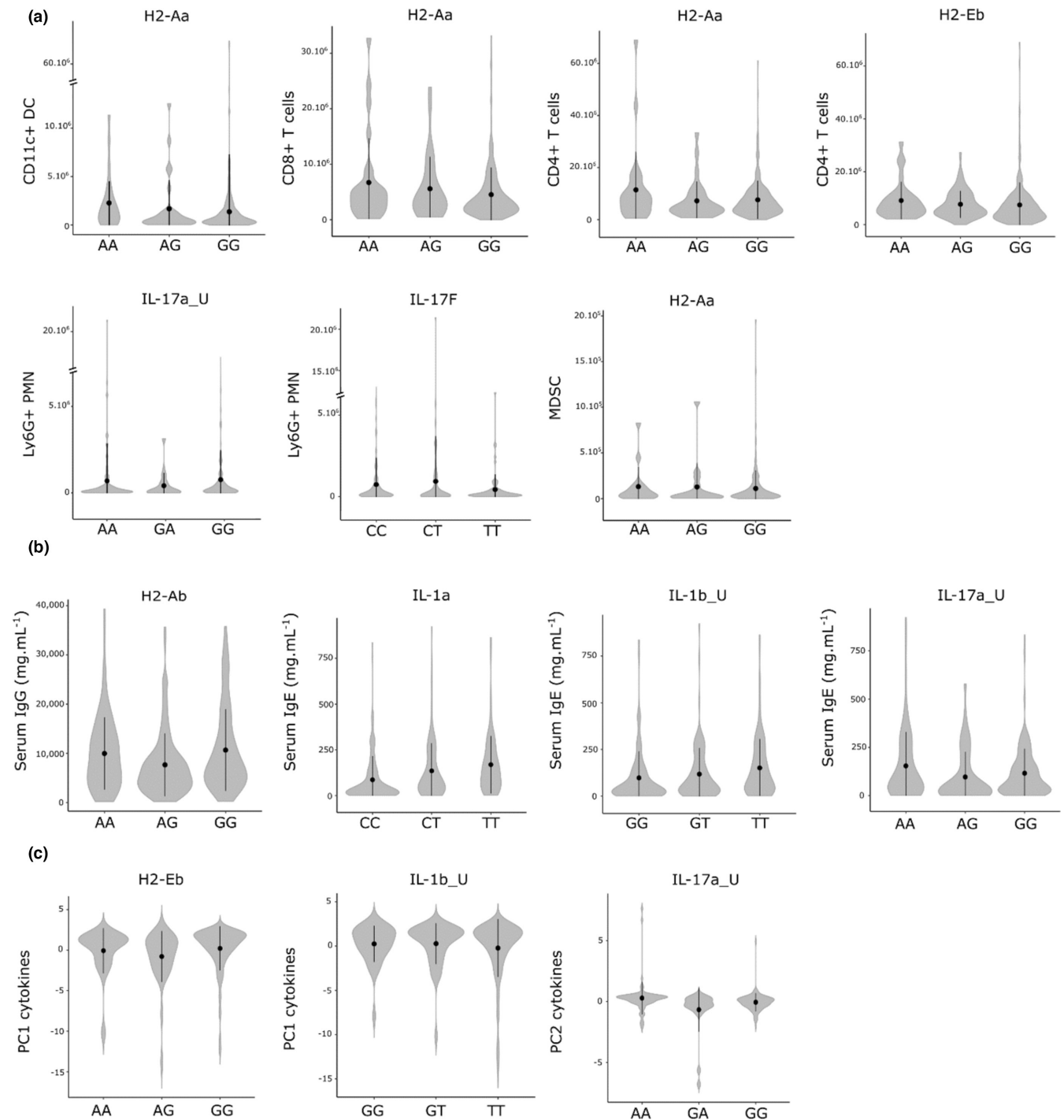
FIGURE 2 Immune phenotypes in mice of different genotypes at loci where there was a model-identified association of the polymorphism and the phenotype, for (a) scaled cell numbers, (b) antibody concentration and (c) cytokine PCA axes. The violin plots show the raw immune data distribution with the mean shown by the black dot and SD shown by the black line. Only associations of loci where the minor allele frequency (MAF) is >0.1 are shown.

We also found associations between the genotype of MHC loci and seropositivity for virus infections (Table 4). For example, mice with AA or AG H2-Ab genotypes were less likely to be seropositive for Minute virus compared with mice with the most common GG genotype (Table 5, $p < 0.05$); mice that were heterozygous at the H2-Aa locus were less likely to be seropositive for Mouse Hepatitis virus (MHV) infection than either of the two homozygous genotypes

(Table 5, $p < 0.01$). Finally, mice heterozygous at the H2-Eb locus were less likely to be seropositive for norovirus—but more likely to be seropositive for parvovirus—compared with the two other homozygous genotypes (Table 5, $p < 0.05$ for both).

We found only limited evidence of associations between genotypes of cytokine-coding loci and infection phenotypes (Tables 4 and 5), though mice that were heterozygous at the IL-1a locus were

TABLE 4 Effect of polymorphism in immune-related loci on infection phenotypes. Results of linear models where first the effect of different age functions (linear, classes), sex (female vs. male), site (9 sites) and scaled mass index were tested to select the base model of non-genetic factors. Secondarily, the effect of each polymorphism was tested and shaded boxes indicate where genetic effects were selected in the model (Table S10).

Infection phenotype	Non-genetic factors	Effect of genetic variation				
		MHC			Cytokines	
		H2-Aa	H2-Ab	H2-Eb	IL-1a	IL-1b_U
Macroparasites						
Mites	Site + age (classes)+SMI	* (AA < AG,GG)				
Worms	Site + age (linear)+SMI		** (AG,GG < AA)			
Microparasites						
Norovirus	Site + age (classes)			* (AG < AA < GG)		
Parvovirus	Site + age (classes)			* (AA < GG < AG)		
Minute virus	Site + age (classes)		* (AG < AA < GG)		* (CC < TT < TC)	
Mouse hepatitis virus	Site + age (classes)	** (AG < AA,GG)				
Sendai virus	Site + age (classes)					
Coronavirus	Site + age (linear)					
Mycoplasma pulmonis	Site + age (classes)					

Note: The stars shows the significance of the selected genetic effect, determined by deletion testing and the log-likelihood ratio test, as: *** for $p < 0.001$; ** for $p < 0.01$; * for $p < 0.05$ and for $p > 0.05$ (Tables S12). Loci where the minor allele frequency (MAF) is >0.1 are shown in bold (Table S7); loci with a MFA < 0.1 are not bold and these results should be interpreted with more caution.

more likely to be seropositive for Minute virus genotypes ($p < 0.05$). Finally, we found a recurring association of IL-6 alleles and both macroparasite (worm number, $p < 0.01$) and microparasite infection markers (MHV, sendai virus and *M. pulmonis*; Table 4, $p < 0.05$ for all). We also found an association of the TLR-9 locus and the mite infection phenotype (Table 4, $p < 0.01$). However, for these loci, the relative abundance of the different genotypes means that these results should be considered with caution and would benefit from further study.

4 | DISCUSSION

We tested the effect of the genotype of 14 immune-related loci on a wide range of immune and infection phenotypes in wild UK house mice, *Mus musculus domesticus*. These results bring new insights to the field of immunogenetics in three ways. First, this is one of the first studies of which we are aware where the effect of a large number of immune-related loci (MHC, cytokines and TLR) on multiple immune and infection phenotypes have been studied in a wild mammal. Second, these effects have been characterized in a large sample of mice from nine different sample sites, which is rare in a wild species where field constraints often limit sample sizes. Third, these results are of particular interest because they also consider infection phenotypes, which are the result of natural processes of infection, co-infection, and host immune responses occurring in these populations.

Most of the immune-related loci that we investigated were polymorphic, though there were differences in the representation of genotypes at loci among mice from different sites. Overall the observed genetic variation was most notable for the MHC and cytokine-coding loci, but was much less extensive for the TLR coding loci (known to be highly conserved in vertebrates), which may explain why we found comparatively fewer clear effects of polymorphism in TLR coding loci. We did, however, find strong associations between the MHC and cytokine-coding loci on different components of the immune phenotype—principally on antibody concentration and on cytokine production—but also on infection phenotypes. The effects of the MHC loci on immune and infection state are consistent with previous results in natural populations (Table 1). Interestingly, we found that the genotype of the MHC H2-Ab locus was associated with both IgG concentration and intestinal worm burden and there is the possibility that this is causal. Associations between MHC loci and antibody response in wild populations have been described in other mammals such as Soay sheep (Huang et al., 2022; but also see in other species reported in Gaigher et al., 2019). We also found associations between the MHC H2-Aa locus and various populations of DCs, CD4+ and CD8+ T cells, and MDSCs, as well as on ectoparasite burden.

We found major associations between the genotype of cytokine-coding loci and antibody concentration. Specifically, the serum concentration of IgE was associated with genotypes of IL-1a, IL1b_U, IL-6 IL-17a_U and TNF, with different IL-1a, IL1b_U, IL-17a_U genotypes having quite strikingly different concentrations. Genotypes of

							TLR	
IL-6	IL-10	IL-13	IL-17a_U	IL-17a_N	IL-17F	TNF	TLR-5	TLR-9
							– (AA<GG<AG)	** (TC<CC)
** (CT<CC<TT)								
	** (CC<TT<<CT)							
* (CT<TT<CC)						– (TG<TT<GG)		
* (CC<CT,TT)						** (TT<<GG<TG)		* (TC<CC)
				* (AA<<AG<<GG)				
* (CC<CT<TT)								

IL-6 were also associated with the concentration of faecal IgA. The potential mechanism of these effects on antibody concentration is that these polymorphisms affect (i) animals' antibody production per se, (ii) animals' resistance or susceptibility to a wide range of infections, which is manifest as different antibody concentrations or (iii) a combination of these.

Among the cytokine-coding loci that we investigated, we found a range of associations with cell populations, the concentration of antibodies and cytokine PCs. Many cytokines are multifunctional and widely connected in the generation on an immune response (Fonseca dos Reis et al., 2021), such that we should not be surprised that diversity at cytokine-coding loci may have pleiotropic phenotypic effects. Five cytokine-coding loci (IL-1a, IL-1b, IL-6, IL-17a and TNF) were associated with the concentration of IgE, and an overlapping set of 5 (IL-1b, IL-6, IL-10, IL-17a and TNF) were associated with cytokine PCs. Moreover, a further combination of 5 of these (IL-1a, IL-6, IL-10, IL-17a and TNF) was also associated with measures of infection. The associations between these genotypes and both immune and infection phenotypes might be the result of causal immunological relationships, although further evidence would be need to substantiate this. Causal relationships remain very difficult to establish in natural populations subject to diverse backgrounds, compared with animal models in the laboratory raised in common, tightly controlled environments. Polymorphism in IL-10 may be having pleiotropic effects on immune and infection phenotypes, which is consistent with IL-10 being known to play a central role in regulating immune responses to a wide range of micro- and macroparasites

(Couper et al., 2008). Similarly, polymorphism in IL-6 also appears to be pleiotropic, which is also consist with IL-6's role in integrating different aspects of an immune response, and where in humans suppression of IL-6 function increases the risk of serious infection (Rose-John et al., 2017). However, we need to be cautious in interpreting these data since the measures of microparasite infection are serological, and so may be confounded with individuals' antibody production per se, which we also found to be affected by polymorphism in these and other loci.

While we focus on SNPs within these loci of immunological interest the associations we report are to the SNP and to regions that are linked to it, and so it would be inappropriate to seek causal relationships between the focal SNP and altered protein structure as underlying any direct or indirect immunological effect. While it would be of interest to understand how such genetic variation alters protein structure and function, our study was not designed to study such mechanisms.

Our measures of viral infection were serological, and while this is an easy-to-use approach it is potentially problematic because it is itself an immunological measure. An advantage of serological diagnosis is that it shows evidence of historical infection, though in the mice studied here their median age is 6–7 weeks (maximum 20–39, for male and females, respectively; Abolins et al., 2017), meaning that in many cases these serological diagnoses are often likely of relatively recent viral infection. Future work would benefit by using more specific viral diagnosis, as well as considering a wider range of parasites, but also host pathology.

TABLE 5 The effect of different genotypes on infection phenotypes. The parameter estimates for genetic effects for loci where the minor allele frequency >0.1 are shown. Parameter estimates for other factors (age, site, SMI, etc.), interactions, and other loci are in Table S11. In Variable the genotype in brackets is the genotype to which the comparison is made. Statistical significance is represented by: for $p < 0.1$, * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$. Degrees of freedom is df.

Locus	Parasite	Model	df	Variable	Parameter estimate ± SE	t-value	p
H2-Aa	Mites	H2-Aa + site + age (classes)+SMI	21	Intercept (0|50)	0.33±0.98	0.33	–
				Intercept (50|100)	0.53±0.98	0.54	–
				Intercept (100|200)	1.11±0.98	1.14	–
				Intercept (200|300)	2.24±0.98	2.28	*
				Intercept (300|400)	2.62±0.99	2.66	**
				Intercept (400|500)	2.75±0.99	2.79	**
				Intercept (500|1000)	3.63±0.99	3.66	***
				Intercept (1000|1500)	4.78±1.01	4.75	***
				AG (AA)	1.91±0.76	2.50	*
				GG (AA)	1.80±0.84	2.15	*
	Mouse hepatitis virus	H2-Aa + site + age (classes)	15	Intercept (0|1)	−1.90±1.88	−1.01	–
				Intercept (1|2)	−0.02±1.87	−0.01	–
				Intercept (2|3)	1.61±1.88	0.86	–
				AG (AA)	−2.64±1.97	−1.34	–
				GG (AA)	−0.04±1.84	−0.02	–
H2-Ab	Worms	H2-Ab × site + age (linear)+SMI	25	Intercept	0.69±0.22	3.15	**
				AG (AA)	−0.10±0.24	−0.42	–
				GG (AA)	−0.12±0.41	−0.29	–
	Minute virus	H2-Ab + site + age (classes)	15	Intercept (0|1)	1.59±0.52	3.03	**
				Intercept (1|2)	2.99±0.55	5.40	***
				Intercept (2|3)	3.82±0.59	6.53	***
				AG (AA)	−0.25±0.45	−0.55	–
				GG (AA)	0.76±0.45	1.69	–
H2-Eb	Norovirus	H2-Eb x age (classes)+site	19	Intercept (0|1)	1.90±0.64	2.95	**
				Intercept (1|2)	4.41±0.69	0.69	***
				Intercept (2|3)	6.17±0.77	0.77	***
				AG (AA)	−0.64±0.57	−1.13	–
				GG (AA)	0.42±0.46	0.92	–
	Parvovirus	H2-Eb+site + age (classes)	16	Intercept (0|1)	−1.25±0.51	−2.43	*
				Intercept (1|2)	0.18±0.51	0.36	–
				Intercept (2|3)	0.97±0.51	1.92	-
				Intercept (3|4)	3.13±0.54	5.85	***
				AG (AA)	1.02±0.40	2.51	**
				GG (AA)	0.56±0.38	1.48	–
IL-1a	Minute virus	IL1a+site + age (classes)	15	Intercept (0|1)	2.56±0.66	3.86	***
				Intercept (1|2)	3.94±0.69	5.74	***
				Intercept (2|3)	4.73±0.71	6.62	***
				TC (CC)	1.10±0.46	2.37	*
				TT (CC)	0.75±0.52	1.43	–

In our results, it is notable that we found a large number of significant associations that occurred at loci with low minor allele frequencies. There are two points to consider in respect of this. First, that for loci with low minor allele frequencies there is more limited statistical power to detect significant effects. Secondly, that if immune phenotypes are being affected by loci with low minor allele

frequencies, then this may be suggestive of frequency-dependant selection occurring in these populations.

The associations that we have found do not provide strong support for the existence of heterozygote advantage; this is in line with the inconclusive evidence for this phenomenon in other wild mammals. Rather, we found that some rare homozygous genotypes are less parasitized than more common homozygous or heterozygous genotypes; for example, mice with the rare AA MHC H2-Aa genotype had fewer mites than the other two genotypes.

More generally, our results show that there is genetic diversity affecting immune response and infection phenotypes in wild mice and that, presumably, this diversity is being maintained in these populations by balancing selection. While parasites, generally, are strong selective pressures on their hosts and their immune responses, different parasites will have different selection pressures, especially where these different parasites stimulate different immune responses. Consequently, the different and changing infections and co-infections that individual animals have likely represent the continually shifting selection pressure acting on these populations. Moreover, immune responses do not act in isolation with respect to an animal's fitness. Immune responses are energetically expensive and can themselves cause harm to the host, such that an individual's fitness may not be maximized by generating a maximally effective anti-parasite response.

While the present study focusses on genetic effects on measures of the immune response, previous analysis of this same cohort (Abolins et al., 2018) has revealed how non-genetic factors including body condition, age, infection status and season also affect immune state; this was confirmed in our analysis here. Taken together, these studies reveal the complexity of genetic and non-genetic factors affecting immune status and infection phenotypes in wild animals. Although it is difficult to compare the relative magnitude of the genetic effects between the diverse immune and infection traits measured with different methods and scales, the strong associations that we have observed between genetic effects and antibody concentrations or cytokine production appear to be especially significant.

For some of the immune-related loci (including IL-6, IL-10, IL-13, TNF, TLR), there was a substantial imbalance in the frequency of different genotypes that limited our analytical power. Previous studies have noted that effective sample sizes can limit what can be studied in wild populations and have suggested minimum sample sizes of at least 200 individuals (Gaigher et al., 2019). It is notable that even with our large sample size of more than 400 mice, the relative under-representation of some genotypes has constrained the power of our analyses. Previous genetic analyses of these same mice using putatively neutral loci showed genetic differentiation among mice from the different sites (Abolins et al., 2018), again consistent with differences in genotype frequencies in the immune-related loci that we have observed. Moreover, previous analyses of the immune distance among these mice found that mice within one sample site were on average more immunologically similar to each other, compared with mice from other sample sites (Abolins et al., 2018). In agreement with both of these analyses, in the present study we also found significant effects of sample site on immune and infection phenotypes. Genetic and immunological differences among mice at different sites are therefore a notable feature of the mouse populations that we have studied. This host genetic and immunological diversity therefore presents a heterogeneous environment in which parasites are selected and are evolving, and where hosts continue to evolve to respond to that threat.

5 | CONCLUSION

In conclusion, this work has conducted a large-scale study of the genetics of immune and infection phenotypes in wild mice, which adds to the rather limited set of studies in wild mammals. We have detected a range of genetic associations between multiple components of the immune response and on infection phenotypes. Of particular note, we have found significant associations of polymorphism with cytokine-coding loci on the serum concentration of IgE antibodies, and of MHC with the serum concentration of IgG antibodies. There is considerable genetic diversity in the loci that we have studied, suggestive of balancing selection acting, likely due to shifting patterns of infection and co-infection that occur in wild animal populations. Results such as those we present here could be investigated further, for example by undertaking detailed structure–function studies in laboratory animal systems, to seek to understand how the genetic effects we describe functionally affect immune responses. However, such an approach needs to be cognisant of the context in which these immune responses are produced. Thus, while parasites do cause harm to their hosts, which immune responses can ameliorate, the costliness and risk of immunopathology means that maximal anti-parasite immune response may not always maximize fitness.

ORCID

Louise Cheynel https://orcid.org/0000-0003-2292-4143

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SUPPORTING INFORMATION

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