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RESEARCH ARTICLE

Congeneric Rodents Differ in Immune Gene Expression: Implications for Host Competence for Tick-Borne Pathogens

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ABSTRACT

Mice in the genus *Peromyscus* are abundant and geographically widespread in North America, serving as reservoirs for zoonotic pathogens, including *Borrelia burgdorferi* (*B. burgdorferi*), the causative agent of Lyme disease, transmitted by *Ixodes scapularis* ticks. While the white-footed mouse (*Peromyscus leucopus* (*P. leucopus*)) is the primary reservoir in the United States, the deer mouse (*P. maniculatus*), an ecologically similar congener, rarely transmits the pathogen to biting ticks. Understanding the factors that allow these similar species to serve as a poor and competent reservoir is critical for understanding tick-borne disease ecology and epidemiology, especially as climate change expands the habitats where ticks can transmit pathogens. Our study investigated immunological differences between these rodent species. Specifically, we compared the expression of six immune genes (i.e., TLR-2, IFN-γ, IL-6, IL-10, GATA-3, TGF-β) broadly involved in bacterial recognition, elimination, and/or pathology mitigation in ear biopsies collected by the National Ecological Observatory Network (NEON) as part of their routine surveillance. A principal components analysis indicated that immune gene expression in both species varied in two dimensions: TLR2, IFN-γ, IL-6, and IL-10 (comprising PC1) and TGF-β and GATA3 (comprising PC2) expression tended to covary within individuals. However, when we analyzed expression differences of each gene singly between species, *P. maniculatus* expressed more TLR2, IL-6, and IL-10 but less IFN-γ and GATA3 than *P. leucopus*. This immune profile could partly explain why *P. leucopus* is a better reservoir for bacterial pathogens such as *B. burgdorferi*.

1 | Introduction

Mice in the genus *Peromyscus* are among the most abundant and widespread small mammals in North America, with several species occupying a wide range of terrestrial habitats (e.g., woodlands, brushlands, tropical and temperate rainforests, grasslands, savannas, swamps, deserts, and alpine habitats) (Bedford and Hoekstra 2015). Of particular interest for disease ecology and public health are the white-footed mouse, *Peromyscus leucopus* (*P. leucopus*), and the deer mouse, *Peromyscus maniculatus* (*P. maniculatus*), both reservoirs for the pathogens of various zoonoses, including Lyme disease, babesiosis, anaplasmosis, and hantavirus pulmonary syndrome (Cook and Barbour 2015; Milovic, Duong, and Barbour 2024). Lyme disease is the most common zoonosis in the United States (Mead 2015; Zinck et al. 2023) and is caused by the transmission of the spirochete bacterium, *Borrelia burgdorferi* (*B. burgdorferi*), through the bite of the black-legged tick, *Ixodes scapularis* (*I. scapularis*). In humans, infection with this bacterium can lead to long-term arthritic disease and other adverse outcomes.

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Summary

- *Peromyscusleucopus* and *P. maniculatus* are relevant to Lyme disease.
- Host competence variation between two *Peromyscusspecies* may be due to differences in immunity.
- P. maniculatus showed higher expression of genes associated withbacterial resistance than P. leucopus.

Small mammals may play an essential role in the Lyme disease cycle, as ticks can only become infected with *B. burgdorferi* after feeding on an infected host. *P. leucopus* are often implicated as important hosts because the infection has little or no effect on the health of this species, but uninfected larval ticks often feed on (and become infected by) *P. leucopus* (Ostfeld and Keesing 2000; Barbour 2017). Intriguingly, although *P. maniculatus* are morphologically and ecologically similar to *P. leucopus* (Rand et al. 1993), *P. maniculatus* is not regarded as a major host of *B. burgdorferi*. Moreover, a recent study showed that when both species were present in the same area, infection with *B. burgdorferi* was more common in *P. leucopus* (Larson, Lee, and Paskewitz 2018). As yet, it remains unclear how these ecologically similar species are so distinct in their propensity to serve as reservoirs for zoonotic pathogens.

Resolving how *P. leucopus* and *P. maniculatus* serve as zoonotic reservoirs is important because both species co-occur across a large portion of the United States (Long 1996; Myers et al. 2009; Eisen et al. 2017). In the upper Midwestern US, for example, which is considered a growing hotspot for tick-borne diseases (Eisen et al. 2017), both species are abundant. When the species overlap geographically, *P. leucopus* is more common in dry, brushy habitats, environments that favor the black-legged tick (*I. scapularis*), whereas *P. maniculatus* is commonly found in habitats with high canopies, which may reduce its encounter rates with ixodid ticks (Long 1996; Guerra et al. 2002). Additionally, *P. leucopus* and *P. maniculatus* use torpor differently, with *P. maniculatus* being more likely to enter torpor during the winter (Pierce and Vogt 1993). This metabolic difference could also lead to less interactions with black-legged ticks. In a recent study conducted in Maine, a region along the expansion front for *I. scapularis*, the authors examined the potential differences between the roles of *P. leucopus* and *P. maniculatus* in the maintenance of tick populations and in the tick-borne pathogen transmission cycle (Dill et al. 2024). They found the frequency and intensity of *I. scapularis* infestations were significantly higher for *P. leucopus* than for *P. maniculatus*. However, they did not detect any significant difference in pathogen prevalence between the two species (Dill et al. 2024).

Probably, host competence partly underpins the distinct ecological roles of each *Peromyscus* species with respect to zoonotic disease. Host competence can be defined as the ability of a host to transmit a pathogen to another host or vector (Martin et al. 2016, 2019; VanderWaal and Ezenwa 2016; Ashley and Demas 2017; White, Forester, and Craft 2018). The different propensities of each species to contribute to local disease dynamics thus derive from some combination of behaviors (such as those that expose hosts to ticks or grooming behaviors that reduce tick numbers) and aspects of the immune response (Gervasi et al. 2015; Martin et al. 2016; Adelman and Hawley 2017). To date, despite the important roles of these two *Peromyscus* species in multiple zoonoses, we still understand little about differences in immunological aspects of host competence. A few exceptions involve studies of lab-bred individuals of these and other *Peromyscus* species. In one study, *P. leucopus* and *P. maniculatus* exhibited strong fevers but weak or no reductions in activity and food intake when injected with lipopolysaccharide (LPS), an immunogenic element of Gram-negative bacteria (Martin, Weil, and Nelson 2008). In another, *P. maniculatus* had a stronger skin inflammatory response than *P. leucopus* after exposure to a T cell-specific antigen (Martin, Weil, and Nelson 2007). Whereas these studies suggest that the immune responses of these species could make them differently effective reservoirs for *B. burgdorferi*, both studies were not designed to address immunity to specific zoonotic pathogens and both involved captive-bred animals studied in the lab. As host competence is essential in predicting disease outbreaks and understanding the transmission dynamics of infectious diseases (VanderWaal and Ezenwa 2016; Ashley and Demas 2017; White, Forester, and Craft 2018; Martin et al. 2019), there is value in research directed at revealing the immunological basis of host competence between species, particularly involving free-living individual animals.

Recent conceptual frameworks illustrate how variation in host competence can be decomposed into resistance and tolerance (Martin et al. 2019), an approach that might be particularly useful for understanding the different roles of *P. leucopus* and *P. maniculatus* in disease ecology. In the broad sense, hosts facing an infection can either resist a pathogen or tolerate it (Råberg, Graham, and Read 2009). Resistance occurs when the host's immune system identifies the pathogen (Tschirren et al. 2013), triggering an immunological cascade (Strle et al. 2011) to reduce pathogen burden. In host tolerance, the immune response does not ameliorate disease by eliminating infection; instead, it mitigates the detrimental effects of pathogen on the host through various means (Jackson et al. 2014; Martin et al. 2019) including promotion of tissue repair, reduction of oxidative damage, or decreased inflammatory cytokine and cell activities (Råberg 2014). In this light, resistant hosts are probably often incompetent hosts as the burden never reaches transmissible levels for long enough to be ecologically consequential. Conversely, tolerant hosts could be quite transmissible of pathogens and hence competent for long periods, especially if their health is uncompromised by infection (Råberg 2014; Martin et al. 2019).

In this study, we compared the expression of several immune genes relevant to resistance and tolerance of *B. burgdorferi* infections. Vertebrates generally discriminate pathogens such as *B. burgdorferi* via Toll-like receptor 2 (TLR2) (Yamamoto and Gaynor 2001) and other membrane or cytosolic factors that unequivocally distinguish pathogen and host-derived molecules (Janeway and Medzhitov 2002). Once bound by bacterial lipoproteins, TLR2 triggers an immunological cascade, including the cytokines, interferon (IFN)-γ and interleukins (IL)-1 and 6 (Tau and Rothman 1999; Akdis et al. 2016; Kopitar-Jerala 2017; Bockenstedt, Wooten, and Baumgarth 2022). These cytokines, among other actions, stimulate local leukocytes to clear or at

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least reduce bacterial burden. Coincident with resistance responses, other cytokines initiate bacterial tolerance. This endpoint is partly accomplished by the anti-inflammatory cytokines, IL-10, tumor growth factor-β (TGF-β), and gata-binding protein 3 (GATA3) (Kondělková et al. 2010; Iyer and Cheng 2012; Jackson et al. 2014). Our hypothesis in this study was that the immune profile of *P. leucopus* would take a more tolerance-oriented form than *P. maniculatus*. To test this hypothesis, we obtained ear biopsies from mice captured at two sites where both species are common and Lyme disease is a comparatively high risk for humans (Centers for Disease Control and Prevention [CDC] 2025). These biopsies were collected by the staff of the National Ecological Observatory Network (NEON), a nationwide initiative of the US National Science Foundation (Dantzer et al. 2023). We predicted that *P. leucopus* from NEON sites where these congeners co-occur would exhibit lower expression of genes related to bacterial resistance and higher expression of genes related to bacterial tolerance, than *P. maniculatus*.

2 | Materials and Methods

2.1 | Animals and Study Site

Ear tissue (pinnae) biopsies from *P. leucopus* ($N = 55$) and *P. maniculatus* ($N = 38$) were collected by NEON staff in 2022 from UNDE—University of Notre Dame Environmental Research Center, Michigan (*P. leucopus:* $N = 28$, 12♂ 16♀; *P. maniculatus:* $N = 9$, 8♂ 1♀), and STEI—Steigerwalt-Chequamegon, Wisconsin (*P. leucopus:* $N = 27$, 13♂ 14♀; *P. maniculatus*: $N = 29$, 19♂ 10♀), two locations separated by 115 km. The trapping and processing of mice followed NEON protocols for small mammals (data product DP1.10072.001). Ear biopsies (~2 mm in diameter and weighing < 1 mg) were taken as part of routine sampling. Immediately upon collection, biopsies were placed in labeled 2 mL cryovials and kept on dry ice in the field to prevent RNA degradation. Subsequently, these samples were stored at −80°C at NEON facilities. In November 2022, all samples were shipped to the University of South Florida (USF) overnight on dry ice. The samples were maintained at −80°C upon arrival at USF until RNA extraction. We measured gene expression in ear biopsies partly because ticks are often found attached to pinnae (Ostfeld, Miller, and Schnurr 1993; Fellin and Schulte-Hostedde 2022), and previous studies found a positive relationship between pathogen load in ear tissue and transmission success from host to feeding ticks (Råberg 2012; Zinck et al. 2023). Animal collections were performed under authorization from IACUC (# IS00009477).

2.2 | RNA Extraction

Tissue biopsies (< 1 mg) were transferred to 2 mL reinforced screwcap tubes (Fisherbrand Bulk tubes, 15-340-162) with two ceramic beads (2.8 mm; Fisherbrand Bulk beads, 15-340-160) and 500 μL cold (4°C) Trizol reagent (Invitrogen, 15596018). Samples were then homogenized (Fisherbrand Bead Mill 24 Homogenizer, 15-340-163) at 4.5 m/s for 5 cycles of 30 s with 30-s intervals, and homogenates were transferred to sterile 1.5 mL microtubes and stored for 5 min at room temperature (23°C) to allow the dissociation of nucleoprotein complexes. Cold chloroform (100 μL) was then added to tubes (4°C), vortexing vigorously for 15 s. This mixture was stored for 5 min at room temperature and then centrifuged (12,000 x g, 15 min, 4°C). The clear layer containing RNA was transferred to a new 1.5 mL microtube. In these new microtubes, 250 μl cold (4°C) isopropyl alcohol was added to precipitate RNA with 1.25 μl of 20 mg/mL of glycogen (RNA grade, Thermo Scientific, R0551) to improve pellet visualization. Samples were then agitated (10 s) and incubated overnight in a −20°C freezer. The following day, samples were centrifuged (12,000g, 10 min, 4°C), supernatants were disposed, and 500 μL cold ethanol (75%, 4°C) was added to each microtube to wash samples. The samples were then centrifuged again (12,000g, 5 min, 4°C), the supernatants disposed, the samples centrifuged once more (12,000g, 3 min, 4°C), and the remaining ethanol evaporated. The dried pellets were then resuspended with 30 μL RNase-free water, and due to the small size of the ear samples and the low RNA yields, RNA concentration of all samples was measured via Qubit (Invitrogen, Qubit 4 Fluorometer, Thermo Scientific, USA) to optimize synthesis of cDNA and/or identify samples unviable for analysis (RNA yield < 18 ng/μL).

2.3 | Conversion of RNA Into Complementary DNA (cDNA)

Reverse transcription was performed for each extracted RNA sample using 500 ng RNA, 4 μL 5X reaction mix, and 1 μL reverse transcriptase (iScript cDNA Synthesis kit, 1708891, Bio-Rad) in RNase-free water to obtain a total volume of 20 μL, following the manufacturer's instructions. Samples were placed in a thermocycler, following the program: 25°C for 5 min, 46°C for 20 min, 96°C for 1 min, and holding at 4°C.

2.4 | Droplet Digital Polymerase Chain Reaction (ddPCR)

Droplet digital PCR (ddPCR) uses a water-in-oil micro-droplet approach to quantify absolute copy numbers of a polymerase chain reaction (PCR) target and hence does not require a housekeeping gene for normalization (Hindson et al. 2011; Whale, Huggett, and Tzonev 2016). This technique is optimal for our goals because it performs well when tissue is modestly bioactive (i.e., ear biopsies) and when targets of expression are expected to be rare (Hindson et al. 2011; Whale, Huggett, and Tzonev 2016). ddPCR reactions contained 5 μL ddPCR Multiplex Supermix (ddPCR Multiplex Supermix, 12005909, Bio-Rad); 4.5 μL target primer (10 μM; forward + reverse mix), 0.63 μL probe FAM, 0.63 μL probe HEX, and 0.63 μL probe FAM + HEX (e.g., when 50% FAM + HEX, add 0.31 μL of each; when 60% FAM + 40% HEX, add 0.38 FAM + 0.25 HEX), and 6 μL sample (cDNA 25 ng/μL). Next, 22 μL of each sample was gently transferred to the middle row of the cartridge (DG8 Cartridges and Gaskets, 1864007, Bio-Rad), and 71 μL of droplet generation oil (Droplet Generation Oil for Probes, 863005, Bio-Rad) was added into the bottom row of a cartridge. Cartridges were covered with gaskets and placed on a droplet generator (QX200 Droplet Generator, 1864002, Bio-Rad). Each sample was apportioned into 15,000–20,000 nanoliter-sized oil droplets, which then were transferred to a 96-well plate (ddPCR 96-Well Plates, 12001925, Bio-Rad).

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Plates were covered with foil (PCR Plate Heat Seal, foil, pierceable 1814040, Bio-Rad), sealed (PX1 PCR Plate Sealer, 1814000, Bio-Rad), and transferred to a thermal cycler (C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module, 1851197, Bio-Rad). Reaction conditions were: 95°C for 10 min, followed by 48 cycles of 94°C for 50 s and 58°C (Triplex A: IL-10, IFN-γ, and IL-6) or 62°C (Triplex B: GATA3, TLR, and TGF-β) for 2 min. These cycles were followed by a melting curve of 98°C for 10 min and holding at 4°C. After amplification, the droplets were separated and counted as either positive (i.e., having the target sequence of interest) or negative (i.e., not having the target sequence of interest) (Taylor, Laperriere, and Germain 2017) using the droplet reader (QXDx Droplet Reader, 12008020, Bio-Rad). To ensure the reliability of our results, we included both a positive control (a synthetic DNA strand of each target sequence, created using Integrated DNA Technologies (IDT) g-Block gene fragment product) and a negative control (water) in all assays. At the end of all runs, expression data were obtained using QuantaSoft Analysis Pro software (version 1.05).

2.5 | Species ID Confirmation

Because *P. leucopus* and *P. maniculatus* can be difficult to differentiate morphologically (Kamler et al. 1998), we conducted a PCR genotyping of all individuals in the study following the methods in Tessier, Noël, and Lapointe (2004). The primers specific to *P. leucopus* (F9263) and *P. maniculatus* (F9197) target the 159 and 225-bp fragments of COIII mitochondrial gene, respectively, in conjunction with the reverse primer H9375. DNA extracts for vouchers of both species were used as positive controls to ensure accurate *Peromyscus* spp. assignments (voucher specimens from NEON: NEON01I9X (D00000189338) *P. maniculatus* and NEON03ST1 (D00000189339) *P. leucopus*).

Amplifications were carried out in 10.75 μL reaction volumes including 5 μL of Bioline MyTaq Mix, 0.2 μL of each primer, 1 μL template RNA, and water to complete the total volume. PCR conditions were as follows: after initial denaturation at 95°C for 1 min, cycling was performed for 30 cycles of 15 s at 95°C, 15 s at 56°C, and 15 s at 72°C with a final extension of 1 min at 72°C. Amplification success was checked by running PCR products on 2% agarose gel stained with SYBR Safe DNA Gel Stain (Invitrogen—S33102) and by revealing bands under UV light. According to NEON, 52% of animals were identified as *P. leucopus* ($N = 48$) and 48% as *P. maniculatus* ($N = 45$). Our molecular identifications were similar, but for us, 59% of samples were *P. leucopus* ($N = 55$) and 41% were *P. maniculatus* ($N = 38$). There were 11 animals identified morphologically by NEON as *P. leucopus* that were genetically identified by us as *P. maniculatus*. Additionally, there were four animals that NEON identified morphologically as *P. leucopus* that were genetically confirmed by us as *P. maniculatus*.

2.6 | Statistical Analyses

Our first goal was to discern whether and how immune gene expression clustered in the two *Peromyscus* species. In a previous study with *P. leucopus*, a principal components analysis grouped the six genes we measured here into two principal components (Assis et al. 2024). To compare those results to the present data, our first step was to again use a Varimax-normalized principal component analysis (PCA) on $\log_{10}(N+1)$ transformed gene expression data for each species separately. Following the Kaiser criterion, we considered only principal components with eigenvalues > 1 as appropriate for further investigation. Once the PCA revealed immunological differences between species, we decided to compare each gene individually between species. For this comparison, we used multivariate analysis of variance (MANOVA) with transformed gene expression ($\log_{10}(N+1)$ of TLR2, IFN-γ, IL-6, IL-10, TGF-β, and GATA3) as the response variables. Fixed effects in this model included mouse species (*P. leucopus* or *P. maniculatus*), mouse sex (male or female), and the interaction between species and sex. We did not include the capture location in the analysis, as we had insufficient samples of both species and sexes at both capture sites. All analyses were run with IBM SPSS Statistics 29, and graphs were made using Excel (version 2409).

3 | Results

PCA revealed two components with eigenvalues > 1 in both species. In *P. leucopus*, the two PCs accounted for 85.87% of the total variation in gene expression (Table 1A). The first component (55.25% variation) was positively associated with TLR2, IFN-γ, IL-6, and IL-10 expression (Table 1B). The second component (30.62%) was positively related to TGF-β and GATA3 expression

TABLE 1A | Components retained by principal component analysis (PCA) of six immune gene targets among *Peromyscus leucopus*.

Total variance explained			
		Initial eigenvalues	
Component	Total	% of variance	Cumulative %
1	**3.315**	**55.25**	**55.25**
2	**1.837**	**30.62**	**85.87**
3	0.532	8.87	94.74
4	0.250	4.17	98.91
5	0.056	0.93	99.85
6	0.009	0.16	100.00

Note: Extraction method: Principal component analysis. Components with eigenvalues > 1 are highlighted in bold.

TABLE 1B | Individual immune gene expression loadings on each of the two PCA axis (after varimax rotation with Kaiser normalization) for *Peromyscus leucopus*.

	Component	
	1	2
TLR2	**0.783**	−0.060
IFN-γ	**0.844**	0.077
IL-6	**0.958**	−0.165
IL-10	**0.954**	−0.165
TGF-β	−0.066	**0.982**
GATA3	−0.086	**0.979**

Note: The variables showing a higher contribution for each component are highlighted in bold.

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(Table 1B). For *P. maniculatus*, the two PCs accounted for 84.60% of the total variation in gene expression (Table 2A). The first component (55.51% variation) was positively associated with TLR2, IFN- γ, IL-6, and IL-10 expression (Table 2B), and the second component (29.09%) was positively related to TGF-β and GATA3 expression (Table 2B). As IFN-γ was not as strongly correlated with PC1 for *P. maniculatus* as it was for *P. leucopus*, we compared the expression of all genes separately between species.

MANOVA revealed that gene expression was only dependent on species, not sex or the interaction of sex and species (Table 3). Compared to *P. maniculatus*, *P. leucopus* exhibited significantly lower expression of TLR2 (Partial $\eta^2 = 0.073$, $p = 0.010$), IL-6 (Partial $\eta^2 = 0.084$, $p = 0.005$), and IL-10 (Partial $\eta^2 = 0.074$, $p = 0.009$), and significantly greater gene expression of IFN-γ (Partial $\eta^2 = 0.342$, $p \leq 0.001$) and GATA3 (Partial $\eta^2 = 0.060$, $p = 0.019$) than *P. maniculatus* (Figure 1).

TABLE 2A | Components retained by principal component analysis (PCA) of six immune gene targets among *Peromyscus maniculatus*.

Total variance explained			
		Initial eigenvalues	
Component	Total	% of variance	Cumulative %
1	**3.330**	**55.51**	**55.51**
2	**1.746**	**29.09**	**84.60**
3	0.712	11.87	96.46
4	0.124	2.06	98.53
5	0.084	1.40	99.93
6	0.004	0.07	100.00

Note: Extraction method: Principal component analysis. Components with eigenvalues > 1 are highlighted in bold.

TABLE 2B | Individual immune gene expression loadings on each of the two PCA axis (after varimax rotation with Kaiser normalization) for *Peromyscus maniculatus*.

	Component	
	1	2
TLR2	**0.932**	0.171
IFN-γ	**0.575**	0.217
IL-6	**0.981**	0.001
IL-10	**0.985**	0.008
TGF-β	0.075	**0.969**
GATA3	1.367	**0.951**

Note: The variables showing a higher contribution for each component are highlighted in bold.

4 | Discussion

Understanding species-specific variation in host competence is essential to advancing our understanding of host–pathogen interactions and zoonoses. Our examination of two important rodent hosts of several tick-borne pathogens, including *B. burgdorferi*, the causative agent of Lyme disease, reveals stark differences in the expression of genes related to resistance. In particular, TLR2, IL-6, and IL-10 expression were greater in *P. maniculatus* than in *P. leucopus*; all three of these genes relate to recognizing and eliminating (i.e., resisting) bacterial pathogens. In contrast, GATA3 expression was higher in *P. leucopus* than *P. maniculatus*; and this transcription factor was already found to be a key determinant for host tolerance to extracellular pathogens in voles (Jackson et al. 2014). Finally, the lower expression of IFN-γ in *P. maniculatus* was unexpected and, hence, especially worthy of future investigation, especially given the role of *P. maniculatus* in hantavirus transmission. Below we discuss the relevance of these results to host competence differences between species.

4.1 | Patterns of Resistance and Tolerance

The duration, intensity, and hence transmissibility of infections result from a balance between tissue damage and pathogen control (Glass 2012). Defensive responses involving tissue damage control should provide pathogen resistance mechanisms that lead to negligible immunopathology and effective pathogen clearance (Martin et al. 2019). Bacterial resistance often involves innate immune responses, particularly early pathogen detection and rapid killing or clearance efforts. Relevant genes include pathogen recognition receptors, like TLRs (Schneider and Ayres 2008; Glass 2012; Martin et al. 2019), and proinflammatory cytokines that provide rapid and generic defense through various means. Tolerance-associated factors, on the other hand, help reduce collateral damage from innate immunity or amelioration of the effects of pathogen toxins. Genes involved in bacterial tolerance include sensors of pathogen growth, metabolic disruption, and damage, as well as

TABLE 3 | Multivariate general linear model analysis of gene expression in *Peromyscus* mice ear tissue biopsies.

Effect	Value	*F*	Hypothesis df	Error df	*p* value	Partial η^2
Intercept	0.971	471.141[a]	6	84	**< 0.001**	0.971
Species	0.611	21.973[a]	6	84	**< 0.001**	0.611
Sex	0.057	0.842[a]	6	84	0.541	0.057
Species * Sex	0.027	0.381[a]	6	84	0.889	0.027

Note: Model parameters: Species (*P. leucopus* or *P. maniculatus*), Sex (male or female), Genes (TLR2, IFN-γ, IL-6, IL-10, TGF-β, and GATA3). Since we could not assume that the covariance of dependent variables (in this case, the six gene expression levels) was equal across the groups defined by our factors (species and sex), we are reporting the results for the Pillai's Trace, a more robust multivariate test, which is less sensitive to violations of this assumption. Design: Intercept + Species + Sex + Species * Sex. Variables with $p \leq 0.05$ are highlighted in bold.
[a]Exact statistic.

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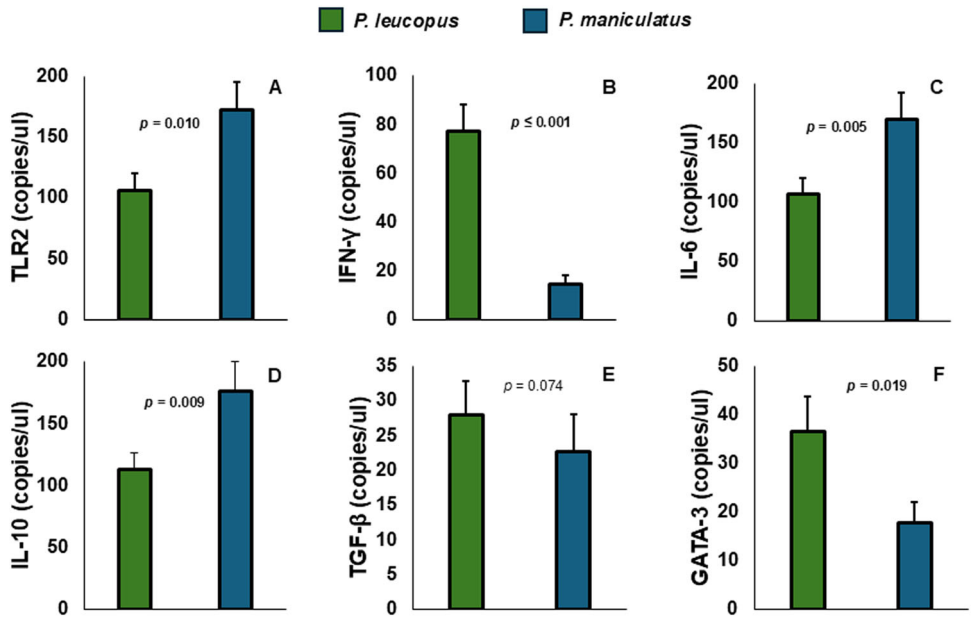

FIGURE 1 | Immune gene expression in *Peromyscus* mice. Gene expression in the ear biopsies of *P. leucopus* and *P. maniculatus*. (A) TLR2. (B) IFN-γ. (C) IL-6. (D) IL-10. (E) TGF-β. (F) GATA3. Bars represent mean gene expression and standard error. Significant statistical differences are in bold.

genes affecting host tissue repair and immune system regulation (Schneider and Ayres 2008; Glass 2012; Martins et al. 2019).

A common feature of vertebrate zoonotic reservoir hosts is their ability to be infected without experiencing significant harm. This capacity, which can also be viewed as tolerance, is often achieved through minimal inflammation to limit the collateral damage associated with infection (Martin et al. 2019). Here, we found that *P. leucopus* exhibited lower expression of genes related to resistance, including immune surveillance (TLR2), pro- (IL-6), and anti-inflammatory (IL-10) responses compared to *P. maniculatus*. It is intriguing that even though *P. leucopus* and *P. maniculatus* are often sympatric, ecologically similar, and of comparable body size, they exhibited quite distinct immunological profiles. Mainly when a previous study that compared five closely related *Peromyscus* species found that *P. maniculatus* and *P. leucopus* showed similar behavioral responses to a simulated bacterial infection with LPS, exhibiting strong fever responses and minimal sickness behaviors (e.g., reduced activity and food intake), distinguishing them from the other species in the study (Martin, Weil, and Nelson 2008). Even though another study found that *P. leucopus* exhibited a significantly lower ear cutaneous inflammatory response (driven by T cells) compared to five other *Peromyscus* species, including *P. maniculatus* (Martin, Weil, and Nelson 2007), the specific immunological genes responsible for this distinct inflammatory response in *P. leucopus* likely do not overlap extensively with those measured in the current study. Thus, even though *P. leucopus* and *P. maniculatus* share some similarities in behavioral responses to infection; there are reports of previous divergent immunological strategies, particularly regarding inflammation between these species. On the other hand, for TGF-β and GATA3, genes expected to foster tolerance, were expressed more in *P. leucopus* than *P. maniculatus*. High expression of GATA3 was found to improve tolerance to macroparasites in a wild population of voles (*Microtus agrestis*) (Jackson et al. 2014). In response to helminths, GATA3 upregulates IL-5, enhancing both the number and function of eosinophils, while for ectoparasites, such as ticks, GATA3 upregulates the function of basophils (Zon et al. 1993; Wen et al. 2021). Basophils are important for the manifestation of acquired tick resistance, which can diminish the chance of pathogen transmission from infected ticks to the host (Karasuyama et al. 2018). However, the specific mechanisms by which GATA3 may contribute to host tolerance of microparasites, such as bacteria, remains to be determined.

The differences in the immune profiles of these two congeners we found suggest that *P. leucopus* is less effective at resisting but more effective at tolerating *B. burgdorferi* than *P. maniculatus*. Even though we did not find a difference in TGF-β expression between species, the other gene expected to foster tolerance, there are many possible explanations for the absence of the expected difference. Most involve the complexity of a protracted immune response involving many cell types and defensive proteins over the course of the bacterial infection. Indeed, as we only had frozen biopsies to study, we could also not discern from which cells the gene expression differences we *did* find derive. Our prime directive here was to sacrifice experimental control, as would be possible with lab studies of captive rodents, in favor of obtaining immunological data from sympatric, free-living mice. This approach, though lack depth, enabled us to capture gene expression in its natural context. The conspicuous shortcoming of this approach, however, is the lack of detailed infection histories for each individual, such as whether or when they were bitten by ticks or infected with bacteria, and the inability to describe immune responses over time in pinnae and other tissues. In finding intriguing differences in gene expression at the sites where most tick bites occur among free-living mice, our study sets the stage for future studies (i.e., single-cell RNA expression) in controlled laboratory settings with experimentally infected and uninfected individuals after different

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points of bacterial exposures. This work, too, will be crucial for pinpointing specific aspects of immune responses affecting the outcomes of host–pathogen interactions and, hence, differences in competence between species.

4.2 | Possible IFN-γ Roles in Host Competence

Among the six genes we considered, IFN-γ expression was the most different between species. The difference is interesting and relevant to our original hypothesis, as IFN-γ can orchestrate several protective functions against a variety of infections (Kak, Raza, and Tiwari 2018). IFN-γ is primarily secreted by activated T cells and natural killer (NK) cells and can promote macrophage activation, mediate antiviral and antibacterial immunity, and control cellular proliferation and apoptosis (Tau and Rothman 1999; Kak, Raza, and Tiwari 2018; Torelli et al. 2018). Specifically for *B. burgdorferi* and related bacteria, IFN-γ plays an important role in host pathology (Miller et al. 2008; Strle et al. 2011). For example, in humans, an initial IFN-γ response, followed by upregulation of IL-4, is associated with nonchronic manifestations of Lyme borreliosis, including skin local infection (i.e., erythema migrans) and early neuroborreliosis (i.e., subacute meningitis, facial palsy, and radiculitis), arthritis, or carditis (Widhe et al. 2004). A persistent IFN-γ response may even lead to chronic Lyme borreliosis, which is associated with long-lasting symptoms (> 6 months), namely, chronic neuroborreliosis (i.e., radiculitis, facial palsy, chronic encephalomyelitis), paresthesia, headache, general pain, cognitive dysfunction, chronic arthritis, or fatigue (Widhe et al. 2004). In *Mus musculus* (*M. musculus*), Lyme disease-susceptible C3H/HeN mice produced significantly higher levels of IFN-γ than disease-resistant BALB/c mice, which produced little to none. IFN-γ may impede early control of spirochete growth in C3H/HeN susceptible mice, as treatment with neutralizing anti-IFN-γ monoclonal antibodies significantly reduced both joint swelling and spirochete burdens compared to control mice (Keane-Myers and Nickel 1995). In the context of our study, the above results suggest that elevated levels of IFN-γ might contribute to higher bacterial loads in individual mice, partly explaining why *P. leucopus* is a more competent host for *Borrelia* compared to *P. maniculatus*. While we were unable to measure *B. burgdorferi* burden or prior exposure in our study, our findings suggest that future research on *B. burgdorferi* and its relationship to immunity in wild *Peromyscus* mice would be highly valuable.

5 | Conclusion and Future Perspectives

Our findings suggested that differences in immune responses may partly underlie host competence differences or bacterial pathogens between these two *Peromyscus* species. The distinct expression of important immune genes underscores the need for further, more systematic, and experimental studies comparing the immune response of these congeners to different pathogens. Our approach was biased toward describing natural and, thus, ecologically important variation in wild animals, and even with low-resolution tools and opportunistic sampling, we revealed interesting patterns. However, conducting laboratory-based, time-series studies involving dose-appropriate and experimental *Borrelia* infections would be particularly informative. Such an approach would also allow for an accurate assessment of bacterial burden and transmissibility, particularly if paired with tick-feeding studies. According to Long et al. (2019), differences in response to infection between *P. leucopus* and *M. musculus* are likely due to the differences between the species' genes and transcriptional factors. We agree, but we also encourage further examination of the role of IFN-γ and GATA3 in *Peromyscus* spp., in particular. Efforts to understand resistance and tolerance and hence competence for *B. burgdorferi* and other zoonotic pathogens cannot be wholly understood via the study of model organisms, mainly because *M. musculus* and related species are not natural reservoirs of most zoonotic pathogens and because lab mice and rats (*Rattus rattus*) last shared an ancestor with *Peromyscus* > 25 mya (Ramsdell et al. 2008).

Author Contributions

Conceptualization: V.R.A., J.L.O., L.B.M. Methodology: V.R.A., G.C. Data collection: V.R.A., G.C., A.M.B. Data analyses: V.R.A., G.C., L.B.M. Funding acquisition: L.B.M., J.L.O. Writing–original draft: V.R.A., G.C., L.B.M. Writing–review and editing: V.R.A., G.C., A.M.B., J.L.O., L.B.M.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The original data used in this manuscript will be available at the Mendeley Data, through the 10.17632/d9b924c8w5.1.

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