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RESEARCH ARTICLE

Population connectivity patterns of genetic diversity, immune responses and exposure to infectious pneumonia in a metapopulation of desert bighorn sheep

Brian S. Dugovich[1] | Brianna R. Beechler[2] | Brian P. Dolan[2] | Rachel S. Crowhurst[3] | Ben J. Gonzales[4] | Jenny G. Powers[5] | Debra L. Hughson[6] | Regina K. Vu[4] | Clinton W. Epps[3] | Anna E. Jolles[1,2]

[1]Department of Integrative Biology, Oregon State University, Corvallis, Oregon, USA; [2]Carlson College of Veterinary Medicine, Oregon State University, Corvallis, Oregon, USA; [3]Department of Fisheries, Wildlife, and Conservation Sciences, Oregon State University, Corvallis, Oregon, USA; [4]California Department of Fish and Wildlife, Rancho Cordova, California, USA; [5]National Park Service, Biological Resources Division, Fort Collins, Colorado, USA and [6]National Park Service, Mojave National Preserve, Barstow, California, USA

Correspondence
Brian S. Dugovich
Email: bdugovich@gmail.com

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Abstract

1. Habitat fragmentation is an important driver of biodiversity loss and can be remediated through management actions aimed at maintenance of natural connectivity in metapopulations. Connectivity may protect populations from infectious diseases by preserving immunogenetic diversity and disease resistance. However, connectivity could exacerbate the risk of infectious disease spread across vulnerable populations.
2. We tracked the spread of a novel strain of *Mycoplasma ovipneumoniae* in a metapopulation of desert bighorn sheep *Ovis canadensis nelsoni* in the Mojave Desert to investigate how variation in connectivity among populations influenced disease outcomes.
3. *M. ovipneumoniae* was detected throughout the metapopulation, indicating that the relative isolation of many of these populations did not protect them from pathogen invasion. However, we show that connectivity among bighorn sheep populations was correlated with higher immunogenetic diversity, a protective immune response and lower disease prevalence. Variation in protective immunity predicted infection risk in individual bighorn sheep and was associated with heterozygosity at genetic loci linked to adaptive and innate immune signalling.
4. Together, these findings may indicate that population connectivity maintains immunogenetic diversity in bighorn sheep populations in this system and has direct effects on immune responses in individual bighorn sheep and their susceptibility to infection by a deadly pathogen.

Clinton W. Epps and Anna E. Jolles contributed equally to this work

5. Our study suggests that the genetic benefits of population connectivity could outweigh the risk of infectious disease spread and supports conservation management that maintains natural connectivity in metapopulations.

KEYWORDS
bighorn, connectivity, disease ecology, ecoimmunology, genetic diversity, mycoplasma

1 | INTRODUCTION

Habitat fragmentation and the accelerating emergence of infectious diseases are important and often synergistic drivers of anthropogenic biodiversity loss (Daszak et al., 2001). Fragmentation contributes to population declines and extinctions by eroding the genetic basis for adaptation to changing environments (Keyghobadi, 2007), whereas infectious diseases can reduce population resilience through their effects on individual fitness and population growth rates (McCallum, 2008). Habitat fragmentation can, in principle, be partly remediated through management interventions that maintain connectivity among populations. However, management for connectivity is sometimes met with scepticism, because connectivity acts as a double-edged sword (Hess, 1994): connectivity increases the risk of infectious disease transmission among populations but is essential to maintaining genetic diversity in fragmented populations (Hess, 1996); genetic diversity, in turn, may modulate the susceptibility or resistance to infectious diseases (McKnight et al., 2017). As such, the relative magnitude of fitness costs on disease transmission versus disease susceptibility determine the net impact of population connectivity on infectious disease risk (Figure 1a). The role of population connectivity in tempering infectious disease risk remains controversial as these costs and benefits have only begun to be quantified in natural wildlife host–parasite systems (e.g. Gompper et al., 2011). We evaluated effects of host population connectivity on outcomes of pathogen invasion in a metapopulation of wild desert bighorn sheep.

Desert bighorn sheep *Ovis canadensis nelsoni*, hereafter bighorn sheep, are uniquely adapted to the extreme environments they inhabit and are the only large herbivore in much of the Mojave Desert in southeastern California (Wehausen, 2005). In this region, bighorn sheep inhabit small island-like mountain ranges separated by desert flats that are largely avoided; each occupied range supports a relatively independent population. These populations are linked by occasional movements, and thus, function as a metapopulation in which population extinctions and recolonizations have been observed (Bleich et al., 1990; Epps et al., 2004, 2010). Movement between ranges, and thus gene flow, is predicted by distance, anthropogenic barriers (e.g. highways) and topography (Epps et al., 2005, 2007). Current bighorn sheep populations are small (Abella et al., 2011), and genetic drift is high (Epps et al., 2005), which has worrisome conservation implications if their fitness and resilience are compromised when challenged by infectious disease.

Bighorn sheep are vulnerable to infectious pneumonia transmitted from domestic sheep (Foreyt & Jessup, 1982). *Mycoplasma ovipneumoniae* is a key bacterial pathogen that causes respiratory disease in bighorn sheep (Besser et al., 2013), which often results in high mortality in adults and lambs at initial infection and sustained lamb mortality but few adult deaths in subsequent years (Cassirer & Sinclair, 2007). However, disease outcomes of infectious pneumonia are variable in bighorn sheep, depending on the pathogen strains (Kamath et al., 2019), host populations (Cassirer et al., 2017) and environmental context (Dekelaita et al., 2020). In May 2013, an epizootic of infectious pneumonia was detected in bighorn sheep in Mojave National Preserve at Old Dad Peak, resulting in extensive mortality of adults in that population, followed by reduced survival of affected adults across the metapopulation over three subsequent years of study (Dekelaita et al., 2020). This novel strain of *M. ovipneumoniae* was subsequently found throughout the metapopulation, missing only in a single, very isolated population—but further die-offs were not observed (Shirkey et al., 2021).

We investigated relationships between population connectivity, infection and immunity in the southern and central Mojave metapopulations of bighorn sheep (Abella et al., 2011) during this deadly and fast-moving outbreak of bighorn sheep pneumonia in 2013–14 (Figure 1b). At the population level, we expected connectivity to drive infection by facilitating *M. ovipneumoniae* spread among populations (Figure 1a, arrow 1). On the other hand, connectivity should also facilitate gene flow, preventing the loss of genetic diversity to drift (Figure 1a, arrow 2). We expected populations with greater genetic diversity to mount stronger protective immune responses (Figure 1a, arrow 3) that would reduce disease transmission within a population (Figure 1a, arrow 4). Given these associations, we expected indirect negative effects of connectivity and genetic diversity on disease occurrence (Figure 1a, arrows I-1 and I-3) and an indirect positive effect of connectivity on immunity (Figure 1a, arrow I-2). Thus, by forging a conduit of gene flow, connectivity might indirectly protect bighorn sheep from exposure by giving rise to stronger protective immunity that would limit transmission between conspecifics in a population. Finally, since hosts respond to current infection by mounting immune responses, we predicted current infection to be associated with stronger immune responses (Figure 1a, arrow 5). We expected the same patterns at the individual level. We combined data on population connectivity, genetic diversity and disease prevalence with individual-level data on immunogenetic heterozygosity, immune responses and infection susceptibility to understand the

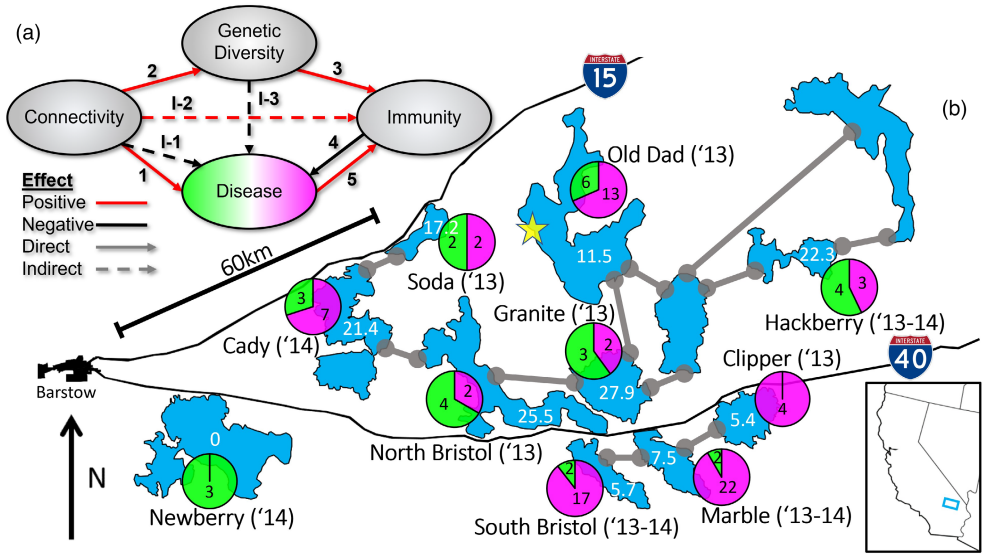

FIGURE 1 (a) Project conceptual diagram; (b) *Mycoplasma ovipneumoniae* exposure and connectivity varied throughout a desert bighorn sheep metapopulation in the Mojave Desert. Each blue area represents a mountain range in southeastern California, USA, and its associated desert bighorn sheep population (sampling years 2013–14). The grey bars show high gene flow links (F_{ST} <0.05, measured with 16 microsatellite loci, Epps et al., 2018) between populations, and the white value is the measure of overall connectivity for that population (demographic mean weighted closeness, Creech et al., 2014). The yellow star marks where the *M. ovipneumoniae* outbreak was first detected (May 2013). The pie charts represent *M. ovipneumoniae* seroprevalence in each population with green/magenta indicating seronegative/seropositive animals.

balance of risks and benefits of population connectivity in the face of invasion by a novel pathogen strain.

2 | MATERIALS AND METHODS

2.1 | Capture and sample collection

Bighorn sheep were captured in November of 2013 and October through November of 2014 for disease testing and global position satellite collaring of 10 populations (Cady, Clipper, Granite, Hackberry, Marble, Newberry, North Bristol, Old Dad, Soda and South Bristol; Figure 1b). These animals were caught by helicopter net-gunning and released after sample collection; blood samples were drawn from the jugular vein into sodium heparin, EDTA and non-coated blood tubes. Blood samples were either processed immediately for immunoassays or stored in liquid nitrogen for later use. Animals were captured and processed under the National Park Service Institutional Animal and Use Committee approved protocols (ACUP #PWR_MOJA_Epps.Powers_DesertBighorn_2013.A3, 2013–2015).

2.2 | Disease testing

We included PCR-based and serological diagnostic tests to track current infections and past exposure to *M. ovipneumoniae* (Table S1). Blood and nasal swabs were collected for *Mycoplasma ovipneumoniae* testing, and samples were stored at −20°C (swabs stored dry). Samples were sent to the Washington Animal Disease Diagnostic Laboratory (WADDL; Pullman, WA). At WADDL, DNA was extracted from nasal swab samples (MagMAX, ThermoFisher Scientific, Waltham MA) and tested using PCR for *M. ovipneumoniae* (Ziegler et al., 2014). For the results of the real-time PCR according to WADDL standard procedures, a cycle threshold score (Ct) cut-off range of 36–40 classified as indeterminate, and the test has a reported sensitivity and specificity of 99% and 91%, respectively (Johnson et al., 2022). To avoid complications with three classes of results with our small sample size, we interpreted as 'detected' if the Ct score was 36 or lower, and 'not detected' for a Ct above 36 which may slightly change the sensitivity and specificity but is unlikely to affect any conclusion in our manuscript. Serum was used for detection of antibodies against *M. ovipneumoniae* by monoclonal antibody-based competitive ELISA, and the results were interpreted as 'detected' if the test sample produced >50% inhibition, and 'not detected' if the test sample produced <50% inhibition. These cut-offs deliver 88% sensitivity and 99% specificity (Washington Animal Disease Diagnostic Laboratory, 2019). We chose cut-offs that optimized test specificity since we were tracking a current/recent disease outbreak due to a novel pathogen strain. Nevertheless, our results are broadly robust to excluding "indeterminate" animals with Ct scores/inhibition levels that are close to our thresholds (Table S2).

2.3 | Connectivity

Connectivity of populations was estimated using demographic mean weighted closeness (MWC), a metric developed in a previous study

that employed network theory to estimate pointwise indices of connectedness for each bighorn sheep population (Creech et al., 2014). That study developed network models based on bighorn sheep populations across the central and southern Mojave Desert of California, where connections between populations were established and weighted according to least cost paths generated by a landscape genetic model that included effects of slope and barriers on bighorn sheep movement (Epps et al., 2007). The landscape genetic model was derived from analysis of DNA samples from 392 bighorn sheep from 26 populations, including populations in this study, sampled 2000–2003. That model also allowed estimation of how gene flow—and thus dispersal—declined with effective distance (i.e. incorporating distance and landscape resistance to movement) along least cost paths, as well as a maximum effective distance beyond which gene flow or dispersal was unlikely. Therefore, Creech et al. (2014) weighted edges (links) among populations by the predicted strength of gene flow among each population pair based on landscape and pruned the population networks by removing edges that reflected effective distances larger than the maximum. The demographic (female-based, intended to capture dispersal by both sexes) model assumed that female movements also declined with effective distance at a similar rate as reflected by nuclear gene flow and was modified to reflect more philopatric behaviour by female bighorn sheep based on sharing of mitochondrial DNA haplotypes from the same 392 bighorn sheep and reported intermountain movements by radio-collared females (Creech et al., 2014), resulting in a lower maximum effective distance. Both models assume symmetrical gene flow and do not consider population size. For both models, Creech et al. (2014) chose population-level network metrics to reflect both short-term (direct) and long-term (stepwise) levels of connectivity, including demographic MWC, which reflects all stepwise connections of less than the maximum effective distance, weighted by the predicted strength of those connections. Although gene flow is both male and female-mediated in this system, demographic MWC was found to most strongly predict genetic diversity of populations at nuclear markers, likely because most gene flow occurs at the distances predicted by this model (Creech et al., 2014; Nickerson, 2014). This metric also incorporates multiple stepwise connections to other populations in the metapopulation, and thus is expected to better reflect long-term, metapopulation-level patterns of connectivity (Creech et al., 2014).

2.4 | Genetic diversity

We estimated genetic diversity from 15–25 bighorn sheep per population (198 total samples, Table S3) including those sampled for disease diagnostics and additional samples collected non-invasively from faecal pellets during 2013–2015. Eight microsatellite loci near known immune function genes (immune-linked [Nickerson, 2014]) and thirteen putatively neutral microsatellite loci (neutral-linked [Epps et al., 2018]) were genotyped (Table S4). Two of these immune-linked genes, BL4 and MHC1, were of particular interest due to relationships found in previous work (Epps et al., 2018; Plowright et al., 2017). BL4 is an immune-linked microsatellite near the locus for interferon gamma, which upregulates the adaptive immune response (Coltman et al., 2001). Major histocompatibility complex one (MHC1) is an immune-linked microsatellite for which increased diversity has been associated with better parasitic infection resistance and survival (Paterson et al., 1998). Sample collection, DNA extraction, genotyping, exclusion of duplicate samples and error rates are reported in Epps et al. (2018). We used several measures of genetic diversity. At the population level, we estimated allelic richness (corrected by subsampling based on the smallest sample size) and expected heterozygosity, averaged across loci for each population, using FSTAT (Goudet, 1995). At the individual level, we estimated what proportion of immune-linked and neutral loci were heterozygous (i.e. observed heterozygosity) using GENHET v3.1 (Coulon, 2010).

2.5 | Immunoassays

For each captured bighorn sheep, we measured the neutrophil, lymphocyte, monocyte, eosinophil, total white blood cell counts, bacterial killing ability of plasma and the ability of lymphocytes to proliferate in response to stimulation with pathogen-associated antigens. White blood cell counts described constitutive cellular immunity (Schneeberger et al., 2013) and an innate (neutrophils, monocytes and eosinophils) to adaptive (lymphocytes) axis, but there is considerable functional overlap (Tizard, 2017). Bacterial killing by mammalian plasma involves a range of effector proteins, such as complement proteins (Liebl & Martin, 2009) and natural antibodies (French & Neuman-Lee, 2012), reflecting innate killing mechanisms (Beechler et al., 2012; Dugovich et al., 2017). Lymphocyte proliferation is an adaptive immune response of B (antibody producing) and/or T (cytotoxic and helper) cells when stimulated with antigens produced by different groups of pathogens (Vance et al., 2004). Finally, the bacterial killing and lymphocyte proliferation assays (BKA and LPA) are considered functional immune assays compared to the morphometric counts of white blood cells (Downs & Stewart, 2014).

2.5.1 | Haematology

Total white blood cells were counted with a Leuko-TIC test kit (Medix #BLT-4013) according to the manufacturer's protocol. Differential blood cell counts were performed on blood smears. Absolute numbers of neutrophils, lymphocytes, monocytes and eosinophils were quantified.

2.5.2 | Bacterial killing assay

For the BKA, a previously established spectrophotometer method was employed (French & Neuman-Lee, 2012). Frozen plasma was thawed in an ice bath and was diluted 1:10 with phosphate-buffered saline to

a final volume of 150 μl, and 40 μl of each diluted sample was placed in duplicate on 96-well plates. An *E. coli* solution was created from freeze-dried commercial pellets (ATCC 8739) and diluted to 10^5 colony forming units/ml; 10 μl was added to the wells with plasma and four positive control wells of 40 μl of PBS. A pair of blank negative control wells at the end of the plate contained only 50 μl of PBS. The plate was incubated on a shaker at 37°C at 200 RPM for 30 min. Around 125 μl of tryptic soy agar broth (Sigma-Aldrich #22092) was added to all the wells and absorbance was measured at 300 nm. The plate was incubated on a shaker for 12 h, and the absorbance was checked again at 12 h. To calculate absorbance, the optical density at 0 h was subtracted from the optical density at 12 h. The bacterial killing proportion was calculated as

$$\frac{(\text{positive control absorbance} - \text{average experimental absorbance})}{\text{positive control absorbance}}.$$

2.5.3 | Lymphocyte proliferation assay

The LPA assessed the lymphocyte activation response to a mitogenic challenge, requiring fresh lymphocytes. The assay was therefore run in a mobile lab in the field. Lymphocytes were separated from the heparin blood tubes using UNI-SEP lymphoseparation tubes (Novamed #U-05). Then, 10 μl of this cell suspension was added to 90 μl of Trypan blue (Sigma-Aldrich #T8154), and the viable lymphocytes were counted on a haemocytometer. After the concentration of lymphocytes per μl was determined, the solution was diluted using AIM-V complete media (Life Technologies #12055091) to add one million lymphocytes per well. Lipopolysaccharide (LPS, Sigma-Aldrich #L3012) was used as a mitogen (Lewis et al., 2013). The samples were plated in duplicate on a 96-well plate with 1 μg LPS mitogen and the appropriate controls. The plates were incubated at 37°C in an incubator with increased carbon dioxide. This was accomplished in the field by placing a burning candle inside an incubator sealed with duct tape; the flame extinguished itself after converting a portion of oxygen in the incubator to carbon dioxide. A modified version of a colorimetric LPA was used (Cory et al., 1991): At 72 h, 20 μl of Alamar blue was added to the wells. At 96 h, the LPS well absorbance was measured. To calculate absorbance, the optical density at 600 nm was subtracted from the optical density at 570 nm from both sample wells and averaged. Negative values were set to zero. Cell proliferation after mitogen stimulation was calculated as

$$\frac{(\text{control absorbance} - \text{average experimental absorbance})}{\text{control absorbance}}.$$

Samples that did not meet acceptable endpoint criteria (i.e. control failure) were excluded from analysis.

2.6 | Data analysis

Of the 10 populations sampled, nine showed evidence of *M. ovipneumoniae* exposure (Table S1), but the Newberry population (totally isolated from the rest of the populations during the study period, Creech et al., 2014) did not. Therefore, to examine variation of disease exposure in a metapopulation, we excluded that population from the following analyses. Linear regression implemented in R version 3.6.1 (R Core Team, 2021) was used to examine the relationships between connectivity, genetic diversity and *M. ovipneumoniae* infection (Supporting Information: Population-level models). Despite uneven sampling, a Breusch-Pagan test revealed that the models were homoscedastic, and weighting was not required. To extricate a protective immune effect from an active immune response, the LPA and BKA were tested against PCR-prevalence and seroprevalence. Correlations between a protective immune response and genetic diversity/connectivity were then evaluated. For these univariate linear regression models, α was set at $p < 0.05$ and unadjusted R^2 was reported.

To summarize the disparate and overlapping effectors of an individual bighorn sheep's immune response, an individual-level immunophenotype was generated by using principal component analysis (PCA, R Core Team, 2021) on the white blood cell counts (total white blood cells, neutrophils, lymphocytes, monocytes and eosinophils), plasma bacterial killing and lymphocyte proliferation to generate different immune response axes. Covariates were scaled preceding the PCA to create a correlation matrix. We expected the active immune response to account for most of the variance. To identify immunoprotective eigenvectors, we utilized the sequential eigenvectors until the axes could no longer be interpreted.

To assess the relationship of disease status and the immunophenotype in individual bighorn sheep, logistic regression using a Bayesian hierarchical framework (Goodrich et al., 2020) and a logit link was used. Immunoprotective eigenvectors and observed heterozygosity were rescaled (Gelman, 2008). The binomial response variable was PCR status (PCR+/PCR−) or recovered status (seropositive/seronegative and excluding PCR+ individuals), and fixed effects were the individual($_i$) scores for the four eigenvectors, immune and neutral-linked observed heterozygosity, year and sex (Equation 1). To control for differences between populations, population($_j$) was added as a random effect

$$\begin{aligned}\text{logit}(\text{disease status}_{ij}) \sim\ & \beta_0 + \beta_1 PC1_i + \beta_2 PC2_i + \beta_3 PC3_i + \beta_4 PC4_i \\ & + \beta_5\, immune_i + \beta_6 neutral_i + \beta_7 sex_i + \beta_8 year_i + b_j,\end{aligned} \quad (1)$$

$$b_j \sim N(0, sigma_{pop}).$$

To evaluate immune-linked gene heterozygosity against an immunoprotective eigenvector while accounting for active infection, a Bayesian hierarchical model was used (Equation 2) to create a protective immunity model:

$$\begin{aligned}PC_{ij} \sim\ & \beta_0 + \beta_1 PCR_i + \beta_2 ADCYAP1_i + \beta_3 BL4_i + \beta_4 KP6_i + \beta_5 MHC1_i \\ & + \beta_6 MMP9_i + \beta_7 RAO1_i + \beta_8 TCRVB62_i + \beta_9 TGLA387_i \\ & + \beta_{10} sex_i + \beta_{11} year_i + b_j.\end{aligned} \quad (2)$$

Diagnostic procedures were performed according to Muth et al. (2018). Weakly informative priors (Supporting Information:

Individual-level model priors) and four Markov chains with 5000 discarded warmup iterations and 5000 test iterations were employed. Adapt delta was increased to 0.99 for all models. For numerical checks, there were no divergent transitions, effective sample sizes were greater than 4500 and $\hat{R}$ was less than 1.1 for all parameters. The modelled parameters fit the posterior predictive distributions well (Figure S1). 50% and 90% equal-tailed Bayesian credible intervals (BCI) and probability of direction (P_D; Makowski et al., 2019) were calculated to qualitatively describe eigenvector effect on disease infection status due to the somewhat subjective designation of eigenvector axis meaning.

3 | RESULTS

3.1 | Population-level associations

103 unique bighorn sheep in 10 populations were captured in November of 2013 and October through November of 2014 ($N = 72$, $N = 31$, respectively; Table S1). We found evidence of *M. ovipneumoniae* infection in all the populations we sampled except for the completely isolated Newberry population (Figure 1b). Seroprevalence was variable among the remaining populations, ranging from 33% (North Bristol) to 100% (Clipper) of bighorn sheep we sampled (mean population prevalence: 65%), but similar for both years ($\chi^2 = 0.66$, $df = 1$, $p = 0.416$), suggesting that antibody responses to prior *M. ovipneumoniae* infection remain detectable for at least a year. The proportion of animals harbouring current *M. ovipneumoniae* infections (i.e. PCR+) in the metapopulation declined over time from 60% in 2013 ($n = 70$, PCR+ = 42) to 16% in 2014 ($n = 31$, PCR+ = 5), indicating that the epizootic was transitioning towards a chronic disease-driven dynamic towards the end of our study period ($\chi^2 = 16.62$, $df = 1$, $p < 0.001$).

Connectivity negatively correlated with seroprevalence and positively correlated with immunogenetic diversity (Tables S5 and S6) as hypothesized in our conceptual model of the system (Figure 1a, arrows I-1, 2). We found a strong negative association between connectivity and *M. ovipneumoniae* seroprevalence; that is, we found less *M. ovipneumoniae* exposure in well-connected populations ($\beta = -0.026$, SE = 0.004, $p < 0.001$, $R^2 = 0.84$; Figure 2a), consistent with the hypothesized indirect effect of connectivity on infection (Figure 1a, arrow I-1) but not with the hypothesized direct effect (Figure 1a, arrow 1). This effect was robust to considering sampling year 2013 alone (Table S6). Allelic richness was strongly and positively correlated with connectivity for both neutral ($\beta = 0.034$, SE = 0.008, $p = 0.003$, $R^2 = 0.74$) and immune-linked ($\beta = 0.029$, SE = 0.006, $p = 0.001$, $R^2 = 0.79$, Figure 2b) microsatellite loci (Table S4), as hypothesized (Figure 1a, arrow 2). Both neutral ($\beta = -0.596$, SE = 0.153, $p = 0.006$, $R^2 = 0.68$) and immune-linked allelic richness ($\beta = -0.677$, SE = 0.198, $p = 0.011$, $R^2 = 0.62$; Figure 2c) appeared to have a protective effect against *M. ovipneumoniae* exposure in the populations we studied, in accordance with the hypothesized indirect relationship between population genetic diversity and disease (Figure 1a, arrow I-3). Immune-linked expected heterozygosity had comparable results (Tables S5 and S6).

When assessing population-level variation in adaptive (LPA) and innate (BKA) immune responsiveness (Table S7), we found the LPA to be negatively associated with *M. ovipneumoniae* seropositivity ($\beta = -1.679$, SE = 0.452, $p = 0.008$, $R^2 = 0.66$), whereas the BKA was positively correlated with being PCR+ ($\beta = 2.805$, SE = 0.534, $p = 0.001$, $R^2 = 0.80$; Figure 2d). This suggests that the ability to rapidly activate lymphocyte populations in response to a pathogen challenge may protect from *M. ovipneumoniae* infection (Figure 1a, arrow 4), and that hosts may upregulate their bacterial killing activity in response to *M. ovipneumoniae* infection (Figure 1a, arrow 5). Based on this, we investigated associations of the protective immune response (LPA) with genetic diversity and population connectivity. Allelic richness positively correlated with the population mean LPA for neutral ($\beta = 0.275$, SE = 0.082, $p = 0.012$, $R^2 = 0.62$) and more strongly for immune-linked ($\beta = 0.341$, SE = 0.089, $p = 0.007$, $R^2 = 0.68$, Figure 2e) microsatellite loci (Figure 1a, arrow 3). Finally, connectivity was positively associated with population adaptive immune responsiveness as assessed via the LPA ($\beta = 0.011$, SE = 0.003, $p = 0.007$, $R^2 = 0.67$; Figure 2f), consistent with the hypothesized link between connectivity and immunity (Figure 1a, arrow I-2).

3.2 | Individual-level associations

We summarized our measures of bighorn sheep immune responses using PCA, reducing the dimensionality of the observed immunophenotype to four interpretable axes (Figure S2a, Table S8). After removing individuals with incomplete immunoassay results, the immunophenotype PCA encompassed 81 individual bighorn sheep. The first axis (PC1, Figure 3a–c) describes 42.2% of the variance and reports the level of circulating white blood cells, a general measure of available immune effectors. PC2 (15.2% variance, Figure 3a) is defined by opposing effects of bacterial killing and lymphocyte proliferation; as such, it contrasts functional innate versus adaptive immunity. PC3 (13.8% variance, Figure 3b) represents an axis of functional immune responses (BKA and LPA) versus available adaptive immune effectors (lymphocyte count), and PC4 (12.0% variance, Figure 3c) charts variation in available monocytes. The fifth eigenvector had 10.1% of the variance but a difficult to interpret axis (Figure S2b) and was not used.

Bayesian logistic regression (Figure 4a,b, Table S9) identified elements of the animals' immune response that were protective against *M. ovipneumoniae* and others that were positively associated with infection. After animals with missing disease tests and genetic data were removed, the PCR and serology models had sample sizes of 79 and 41 animals, respectively. Current infection with *M. ovipneumoniae* (PCR+) was associated with increased cell counts (negative PC1, odds ratio [OR] = 0.092, 90% Bayesian credible interval [BCI] = 0.023, 0.344, probability of direction [P_D] = 99.9%), consistent with leukocytosis and upregulation of antibacterial innate immunity in response to active *M. ovipneumoniae* infection (Figure 4a).

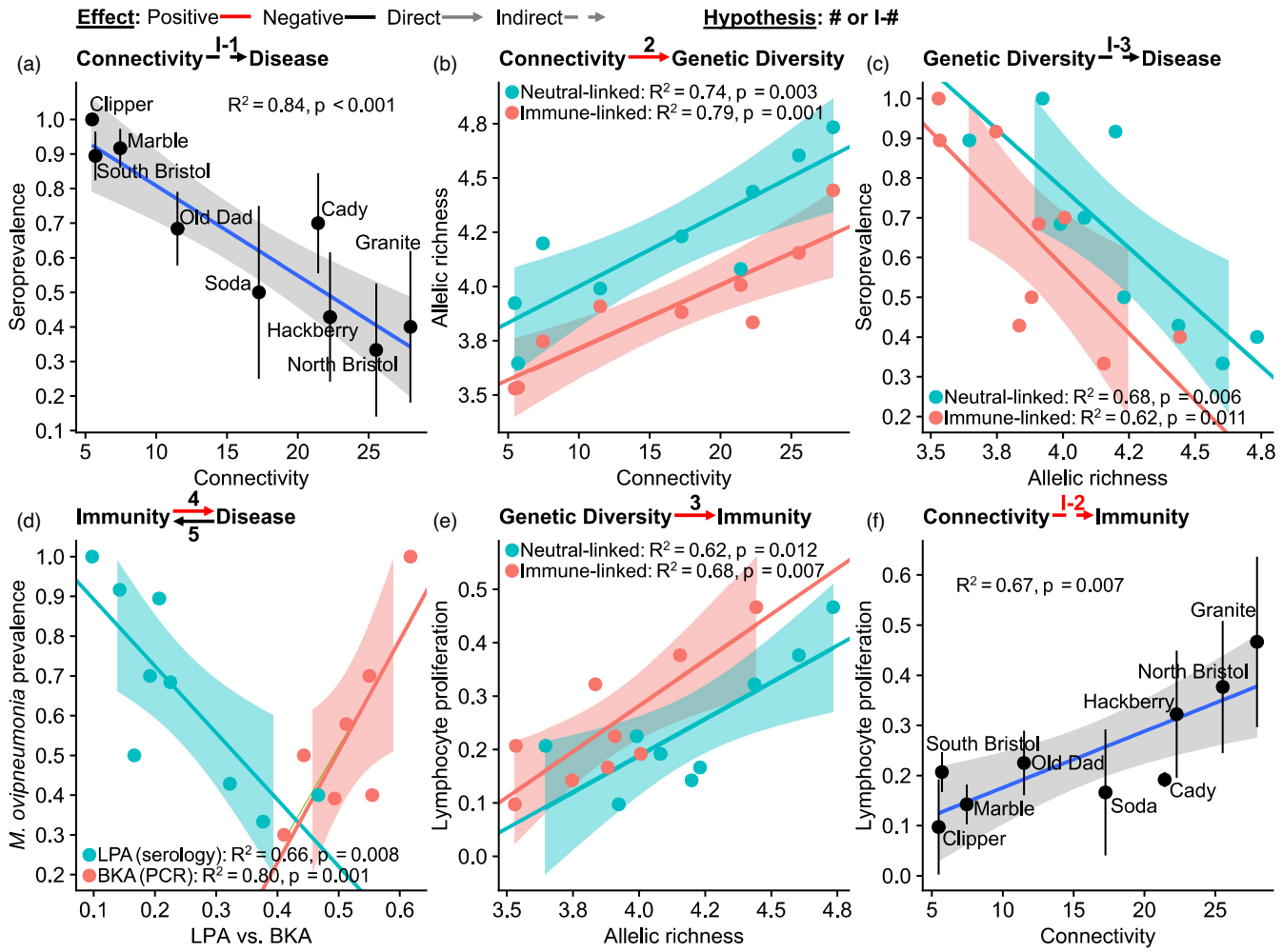

FIGURE 2 Increased connectivity was associated with lower *Mycoplasma ovipneumoniae* seroprevalence, higher immunogenetic diversity and stronger lymphocyte activation in desert bighorn sheep populations in the Mojave Desert. Potentially, connectivity indirectly decreased seroprevalence (a) by directly improving gene flow (b). This genetic diversity was also indirectly associated with decreased disease exposure (c). A plausible direct mechanism for this decreased pathogen invasion was found with improved lymphocyte proliferation (LPA) providing protective immunity against infection (higher in seronegative animals, (d), which was directly strengthened by genetic diversity (e). Finally, connectivity indirectly improved lymphocyte proliferation (f). The *x*-axis (d) is lymphocyte proliferation for the LPA and the proportion bacteria killed for the bacterial killing assay (BKA) which was upregulated in PCR+ animals. Standard error bars and 95% linear regression confidence intervals are shown.

On the other hand, a bias to adaptive over innate responses (PC2, OR = 0.255, 90% BCI = 0.075, 0.820, P_D = 97.3%) was associated with a lower likelihood of PCR+ status and may thus be protective against *M. ovipneumoniae* infection. PCR+ status was also negatively associated with year (OR = 0.089, 90% BCI = 0.008, 0.619, P_D = 98.5%), reflecting the observed decline in active *M. ovipneumoniae* infection from 2013 to 2014, consistent with a waning epizootic. These results document the reciprocal effects of immunity and infection shown in our conceptual model (Figure 1a, arrows 4–5).

Unlike current infection, past *M. ovipneumoniae* infection (seropositive, PCR- status) was not associated with increased cell counts (PC1), bolstering the interpretation that increased cell counts occurred as a response to active infection. By contrast, the protective association of an immune bias to adaptive over innate responses, while somewhat weaker, was also detectable in recovered hosts (PC2, OR = 0.25, 90% BCI = 0.036, 1.547, P_D = 89.3%). Compared to the current infection (PCR+) model, seropositive status was also associated with increased lymphocytes (PC3; OR = 2.531, 90% BCI = 0.452, 15.023, P_D = 81.1%; Figure 4c), which could indicate an upregulated memory lymphocyte response to past infection.

Interestingly, individual immune-linked heterozygosity showed opposing associations with respect to current infection and recovery from *M. ovipneumoniae* infection. Individual bighorn sheep with high immune-linked heterozygosity were more likely to be infected (PCR+; OR = 2.165, 90% BCI = 0.676, 7.326, P_D = 86.0%; Figure 4a) but were less likely to be found in the recovered class (seropositive; OR = 0.311, 90% BCI = 0.066, 1.301, P_D = 90.8%; Figure 4b). Based on the observed negative association between genetic diversity and *M. ovipneumoniae* at the population level, the pattern of higher odds of infection in individuals with high immunogenetic heterozygosity was surprising. Digging deeper into this relationship, we explored how immunophenotypic variation aligned with heterozygosity of the immune-linked

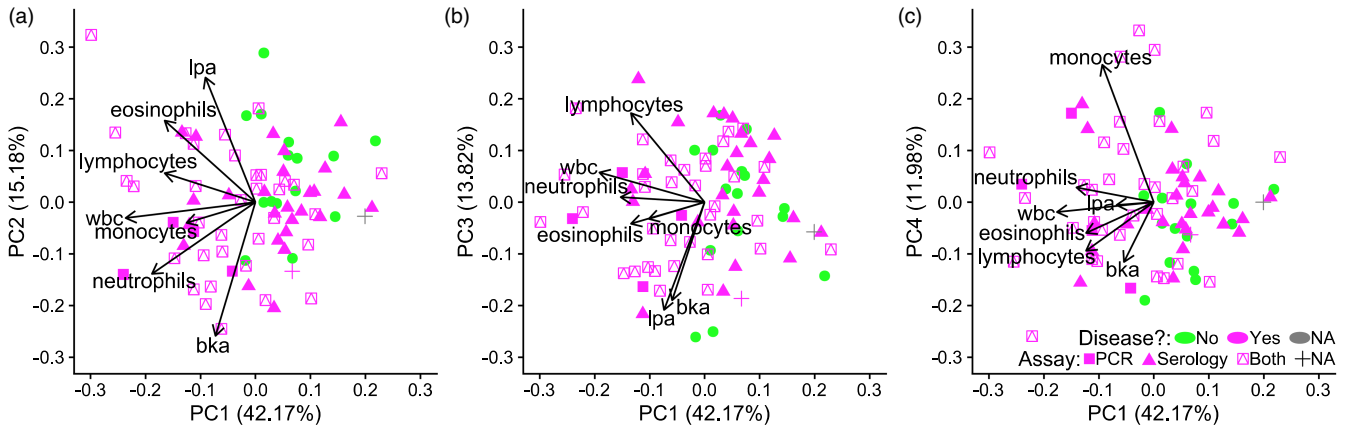

FIGURE 3 Principal component analysis of individual immunophenotype identified protective and active immunity against *Mycoplasma ovipneumoniae* in individual desert bighorn sheep in the Mojave Desert. Individual and total white blood cell counts (WBC), plasma bacterial killing assay (BKA) and lymphocyte proliferation assay (LPA) were used to measure individual immunocompetence, and sampling occurred 2013–2014. Negative PC1 (a–c) represented an active immune response. PC2 (a) represented an axis of innate to adaptive immunity. PC3 (b) represented an axis of functional immunity to a lymphocyte response. PC4 represented a monocyte response (c). Percentage of accounted variation is in the parentheses on the axes. One animal was PCR- and missing serology (grey cross), and another was seropositive but missing a PCR test (magenta cross).

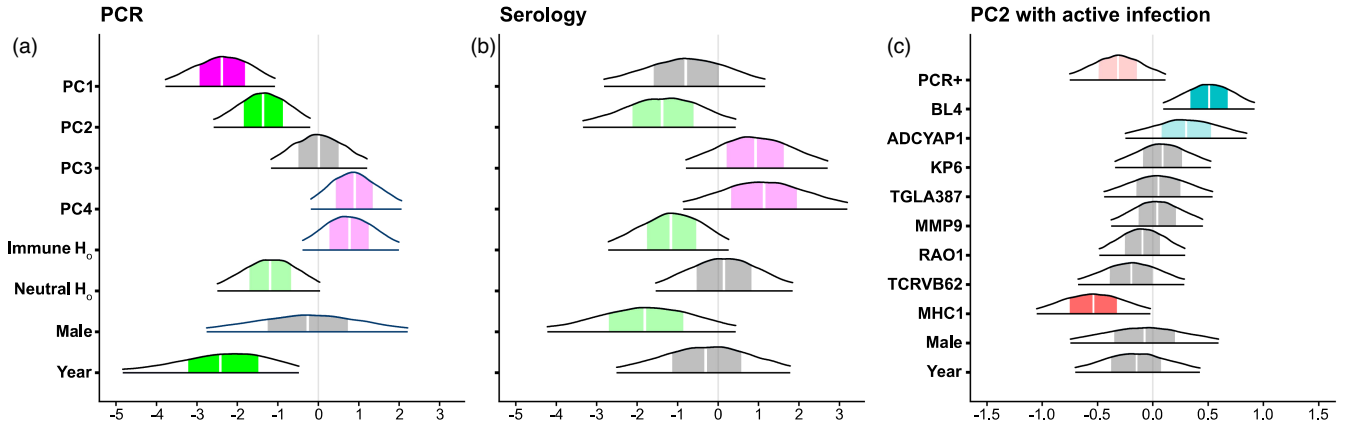

FIGURE 4 A protective immune response (PC2) was negatively associated with *Mycoplasma ovipneumoniae* infection, and specific immune-linked genes influenced this protective response in individual desert bighorn sheep in the Mojave Desert. Bayesian logistic regression identified increased adaptive immunity (PC2) as protective against *M. ovipneumoniae* while increased cell counts (negative PC1) and monocytes (PC4) were responding to active infection (a), and lymphocytes (PC3) and monocytes were higher in animals with past infections (b). Bayesian regression of PC2 correlated with BL4 and ADCYAP1 while MHC1 heterozygosity was negatively correlated (c). Immune and neutral-linked loci diversity is reported as observed heterozygosity (H_o). The serology model excluded PCR+ animals. The white line is the parameter mean, the coloured bar is the 50% Bayesian credible interval (BCI), and the posterior distribution is the 90% BCI. Magenta indicates increased disease risk (PC1's direction is negative).

microsatellite loci included in our study. Since PC2 showed the only protective immunity in the analyses above, we focused our investigation on dissecting how the immune-linked loci in our sample set affected the balance between functional innate and adaptive immune responses as characterized by PC2 (Table S10). Given that active *M. ovipneumoniae* infection affects the host's immune responses, our analyses controlled for PCR-status of the animals. Bayesian regression revealed associations between increased adaptive responsiveness (positive PC2) and heterozygosity at BL4 (mean = 0.510, 90% BCI =0.094, 0.921, P_D =97.8%) and ADCYAP1 (mean = 0.302, 90% BCI = -0.248, 0.848, P_D =81.7%). By contrast, MHC1 heterozygosity was associated with innate responsiveness (negative PC2; mean = −0.536, 90% BCI = −1.049, −0.021, P_D =95.7%; Figure 4c). As such, immune response patterns that may provide protection from *M. ovipneumoniae* infection (positive PC2, bias to adaptive over innate immune responses) align differentially with heterozygosity across immune-linked loci included in this study, providing a nuanced view of the association between individual level immunogenetic heterozygosity and *M. ovipneumoniae* infection status.

4 | DISCUSSION

An outbreak of infectious pneumonia, caused by a novel strain of the pathogen *M. ovipneumoniae*, was detected in 2013 in the Old Dad population of bighorn sheep (Shirkey et al., 2021), triggering

intensive disease testing throughout the Mojave metapopulation by the California Department of Fish and Wildlife during 2013–14. In concert with this effort, we evaluated immune responses and genetic diversity across nine populations of bighorn sheep to investigate how connectivity among populations affected infectious disease risk during the epizootic that ensued. Specifically, we were interested in evaluating the balance of risk (due to increased disease exposure) versus resistance (due to maintenance of genetic diversity and its effect on protective immune responses) conferred by population connectivity. Our results document that population connectivity was associated with genetic diversity, protective immunity and decreased *M. ovipneumoniae* exposure, as hypothesized in our conceptual framework (Figure 1a).

The association of connectivity with reduced *M. ovipneumoniae* seroprevalence was strong, explaining 84% of the variation in seroprevalence among populations. This result was robust and emerged no matter how we looked at the data—sampling year 2013-only, excluding PCR+ animals and even marginally with PCR status (Table S6). We hypothesized that the observed association between population connectivity and reduced infection might result from higher genetic diversity, including at immune-linked loci, in more well-connected populations, which might in turn facilitate more effective immune responses to *M. ovipneumoniae*, limiting pathogen transmission within infected populations. As such, we examined correlations between population connectivity, allelic richness and immune responsiveness. Our data confirmed that indeed, better-connected populations had higher allelic richness at neutral and immune-linked loci, and this was correlated to a stronger adaptive immune response. Closing the loop, we also found that stronger adaptive immune responses were associated with reduced *M. ovipneumoniae* seroprevalence in our study populations.

It is important to note that this does not necessarily mean that particular bighorn sheep were able to resist or be fully protected from infection, directly limiting prevalence in these populations. More plausibly, bighorn sheep with more efficient immune responses against *M. ovipneumoniae* might clear infection more rapidly and/or limit pathogen population growth more effectively, cutting down on infectiousness. Both mechanisms reduce the number of secondary infections each host is expected to pass on and will tend to limit the pathogen's transmission rate within a population. Second, it is clear that population connectivity, genetic diversity and immune response patterns are likely not the only factors affecting variation in outbreak size in these populations. Populations vary in forage conditions (Dekelaita et al., 2020), which could affect host condition and susceptibility to disease. Pathogen communities are likely to vary among populations (Sanchez et al., 2022), which could lead to distinct patterns of co-infections that might shape immunity and host outcomes in this polymicrobial disease. Little is known about the contact patterns of bighorn sheep within populations—but there is sure to be variation, reflecting resource distribution (e.g. water sources) and population densities. Future work should assess contact patterns within bighorn sheep populations and describe the parasite community context more fully. These investigations might explain some of the remaining variation in disease exposure after considering connectivity and/or uncover additional mechanisms underlying the observed connection between connectivity and disease exposure.

Finally, retrospective testing of previously collected sera revealed that the Mojave metapopulation had experienced *M. ovipneumoniae* infection in the past (Shirkey et al., 2021), albeit probably by different variants than the 2013 outbreak strain. As such, it is possible that some of our study populations may not have been naïve to *M. ovipneumoniae*, which could affect infection patterns across the metapopulation: If previously circulating strains of *M. ovipneumoniae* provided hosts with strongly cross-reactive antibodies to the 2013 strain, then variation in prior exposure among populations might contribute to the observed negative association between connectivity and *M. ovipneumoniae* prevalence. Indeed, at longer time scales, differences among populations in the diversity and frequency of infectious exposures might lead to local adaptation in immune response patterns. Future work could explore to what extent observed differences in immunophenotypes among bighorn sheep populations have a heritable component, and how they relate to variation in exposure risk profiles that arise from each population's position in the metapopulation network. Another alternate explanation for the observed negative correlation between population connectivity and disease exposure is that more connected populations suffered excess mortality, and the proportion of individuals sampled in these populations were the immunocompetent survivors. However, this scenario seems unlikely, because the only observed adult die-off (e.g. >50% mortality of ewes, J. Wehausen, unpubl. data) was at the initial outbreak at Old Dad Peak (Dekelaita et al., 2020; Shirkey et al., 2021).

Our study suggests that—at least in the case of the *M. ovipneumoniae* epizootic we tracked in bighorn sheep of the Mojave Desert—the benefits of maintaining genetic diversity and immune function through population connectivity could outweigh its risks in terms of accelerated spread of infection: *M. ovipneumoniae* spread successfully among most bighorn sheep populations during the outbreak we observed, regardless of isolation. Yet, its transmission within populations was reduced in better-connected, more genetically diverse populations. Notably, the only hypothesized relationship not observed in our study metapopulation was an increase in disease exposure in well-connected populations with one exception: the Newberry population, totally isolated from the rest of the study system (Figure 1b). While the Newberry population did not acquire *M. ovipneumoniae* infection during the study period, its low genetic diversity and protective immunity (Figure S3) may render it vulnerable to pathogen invasion. Indeed, *M. ovipneumoniae* was detected in 2018 (Shirkey et al., 2021), and follow-up work is underway to assess the impacts of the disease in this isolated population.

At the individual level, *M. ovipneumoniae* infection was associated with leukocytosis and upregulation of antibacterial innate immunity, as might be expected in response to an active bacterial infection. On the other hand, an immune bias to adaptive over innate responses decreased the odds of *M. ovipneumoniae* infection. Taken together, these findings are consistent with the hypothesized relationships laid out in our conceptual framework, where we expected

variation in immunity to reflect typical responses to current infection, as well as differences in susceptibility among individual hosts (Figure 1a, arrows 4 & 5).

In examining potential links between individual heterozygosity at immune-linked loci and *M. ovipneumoniae* infection, we expected, based on our findings at the population scale, to see a negative association. Instead, we found that individual bighorn sheep with high immunogenetic heterozygosity had higher odds of *M. ovipneumoniae* infection but were less likely to be found in the recovered (seropositive) class. As such, bighorn sheep with high immunogenetic heterozygosity may be more likely to acquire *M. ovipneumoniae* infection, and/or they linger in the infected class for longer—due to sluggish clearance of infection and perhaps low disease-induced mortality. These results point to a potential role for immunogenetic heterozygosity in shaping the outcomes of *M. ovipneumoniae* infection in bighorn sheep—especially disease progression to clearance and recovery versus chronic carriage of the pathogen. Previous studies investigating a genetic basis for the carrier status in *M. ovipneumoniae* infection have yielded mixed results: a study in a bighorn sheep herd in northeastern Oregon suggested a negative association between heterozygosity at a locus in the MHC1 gene complex and carrier status (Plowright et al., 2017); whereas a whole-genome association study based in the same bighorn sheep population found no difference in immunogenetic heterozygosity between carriers and non-carriers (Martin et al., 2021) but did identify two loci associated with carriage status.

Our study only included a small number of immune-linked loci, and we took an indirect approach, asking how heterozygosity at these loci mediates immune response patterns that seemed to play a role in the odds of *M. ovipneumoniae* infection and its outcomes. Heterozygosity at two loci, BL4 and ADCYAP1, which are both involved in the regulation of T-helper cell 1 responses, was associated with immune response patterns that may be protective against *M. ovipneumoniae* infection, whereas heterozygosity at an MHC1-linked locus was negatively associated with this protective immune pattern. More work is needed to understand the genetic mechanisms that contribute to disease progression in infectious pneumonia of bighorn sheep and clarify to what extent these mechanisms are consistent across bighorn sheep populations and environmental context. Our approach of connecting immunogenetic variation to immune response patterns relevant to *M. ovipneumoniae* infection may help provide mechanistic insights that can complement studies investigating immunogenomic and transcriptomic (Bowen et al., 2022) differences between hosts based on disease outcomes.

A central challenge in ecoimmunology is disentangling immune responses due to current infections from protective immunity that can prevent them: a "stronger" immune response may mark sickness or disease resistance. A variety of immunoassays is often required to distinguish these patterns (Downs & Stewart, 2014). An increase in white blood cells is expected during an active immune response (Tizard, 2017), and in this study, high white blood cell counts (negative PC1), and specifically, high monocytes (PC4), were associated with current *M. ovipneumoniae* infection. Strikingly, we detected a protective effect against *M. ovipneumoniae* of highly responsive lymphocyte activation both at the population and individual host levels: bighorn sheep populations with high mean lymphocyte proliferation capacity had lower seroprevalence of *M. ovipneumoniae*, and individual bighorn sheep with immune profiles emphasizing functional adaptive (LPA) over innate (BKA) responses were less likely to be currently (PCR+) or previously (seropositive) infected—even when controlling for their population of origin in our analyses. To explain the population-level linkage between functional adaptive immunity and reduced seroprevalence, one might expect that increased lymphocyte activation could lead to a more effective shutdown of pathogen proliferation in individual hosts resulting in absent or short-lived seroconversion (Gilbert et al., 2013), reduced disease transmission (Hawley & Altizer, 2011) and decreased pathogen persistence (Cassirer et al., 2018), but more work is needed. In vivo experimental work (more feasible in domestic than bighorn sheep) could evaluate how variation in lymphocyte proliferation and the rate of antibody production affect *M. ovipneumoniae* dynamics within individual hosts, linking cellular mechanisms to measures of infectiousness and population-level disease outcomes.

In describing host immune profiles, we discovered that one of the strongest axes in our PCA contrasted functional adaptive immunity (LPA) versus the functional innate immune response (*E. coli* plasma BKA), perhaps indicative of a trade-off in allocation of host resources to innate versus adaptive immune responses. As such, our finding that elevated levels of bacterial killing in host plasma were associated with active *M. ovipneumoniae* infection has at least two plausible (and non-exclusive) explanations. First, infected hosts activate the complement system (Dugovich et al., 2017), which contributes to bacterial killing, while other non-specific markers of inflammation may linger for days to weeks even after infection has been cleared (Glidden et al., 2017). Second, if there is an adaptive versus innate immune trade-off within individual bighorn sheep, then protection from infection in animals with strong adaptive responses would also be (spuriously) associated with relatively weak innate immunity. Trade-offs among immune responses in individual hosts may manifest only under resource restriction (Beechler et al., 2012; Jolles et al., 2008); as such, these patterns may well be context dependent and could vary with seasonal and interannual changes in nutritional availability. Bighorn sheep from this epizootic had less survival in areas with likely lower nutritional availability (higher population density and poorer forage quality, Dekelaita et al., 2020). Further work could also validate a *Mycoplasma ovipneumoniae* bacterial killing assay to better answer this question.

Loss of genetic diversity in small and isolated populations is an inevitable consequence of genetic drift (Fischer & Lindenmayer, 2007) and has been demonstrated in bighorn sheep (Poirier et al., 2019). At the population scale, this study revealed a protective effect of immunogenetic diversity against *M. ovipneumoniae* exposure; while effects of individual-level heterozygosity at immune-linked loci were nuanced, pointing to different processes driving disease patterns across biological scales. Our data allowed us to link host immune profiles to specific immunogenetic loci—even though our study only included a small, somewhat arbitrary assortment of immune-linked

loci. This constraint was driven by our use of field-collected faecal samples as a source of host DNA, which limits the depth of genetic information that can be obtained (Epps et al., 2019). Nonetheless, we were able to detect effects of heterozygosity in three immune-linked microsatellites, BL4, ADCYAP1 and MHC1 with the immune profile axis contrasting functional innate and adaptive responses (PC2). In bovids, including domestic and Soay sheep, BL4 is located near the gene for interferon gamma (Coltman et al., 2001), a cytokine that is central to upregulating adaptive immune responses including infections by *M. ovipneumoniae* (Jiang et al., 2016). Correspondingly, this locus appears to be under positive selection in Mojave bighorn sheep (Epps et al., 2018) and African buffalo (Lane-degraaf & Amish, 2015), suggesting fitness consequences of variation at this locus in our study metapopulation. ADCYAP1 regulates cytokine production including interleukin-6 and was associated with lower nematode burdens in bighorn sheep (Luikart et al., 2008). In mice inoculated with recombinant *M. ovipneumoniae* proteins, interleukin-6 was an early and late immune responder (Jiang et al., 2016).

Curiously, MHC1-linked heterozygosity correlated with higher innate immune function rather than the adaptive immune response, but MHC1 can be associated with both innate and adaptive immunity (Lukasch et al., 2017). Previous work in Rocky Mountain bighorn sheep has shown that individuals with higher MHC1 heterozygosity were less likely to be infected or be a persistent carrier with *M. ovipneumoniae* (Plowright et al., 2017), while work in desert bighorn sheep has demonstrated divergence among populations in MHC1 allelic expression (Dolan et al., 2020). Clearly, there is massive scope for future work to examine an expanded repertoire of immune system genes (including MHC1 genes) to understand variation in host responses to *M. ovipneumoniae* and other pathogens, track immunogenetic changes in the metapopulation and build a foundation for understanding these populations' capacity to respond to future infectious threats.

5 | CONCLUSIONS

This study examined the initial spread of a novel pathogen strain in a fragmented metapopulation and demonstrated reduced pathogen incursion in well-connected populations. In interpreting these findings for biological conservation, it is crucial to recognize the difference between maintaining connectivity among wildlife populations versus adopting approaches that create connectivity de novo. At evolutionary time scales, connectivity maintains genetic diversity which may be essential to protective immunity at the population scale (McKnight et al., 2017). However, most pathogens spread more quickly than host genes among populations. At ecological time scales, creating population connectivity is thus liable to exacerbate the risk of pathogen exposure before any protective effects of increased host genetic diversity can be realized. As such, our study lends strong support to management approaches that maintain natural connectivity or restore connectivity among recently fragmented wildlife populations, but we would caution against management actions that connect previously separate host populations. This bighorn sheep study system combines natural mountain range habitat fragmentation and added human barriers. It is not informative for evaluating the impacts of new connectivity on infectious disease risk, because connectivity in the system has been eroded, not enhanced.

In evaluating the role of population connectivity in mediating infectious disease risk, its role in pathogen persistence is a critical future area for investigation. In Rocky Mountain bighorn sheep, *M. ovipneumoniae* tends to persist endemically in its host populations once it has invaded (Cassirer et al., 2017), but it is yet unclear what role population connectivity may play in mediating pathogen persistence. It is possible that connectivity may facilitate long-term persistence of *M. ovipneumoniae*, especially in smaller populations of bighorn sheep: this could shift the balance of risks due to managing for maintenance of population connectivity. In addition, caution must be applied in extrapolating from our study system to other metapopulations, which may be dissimilar in network structure (Creech et al., 2017), genetic history (e.g. translocations [Jahner et al., 2019]), pathogen spillover risk from livestock (Cahn et al., 2011), pathogen variants (Johnson et al., 2022; Kamath et al., 2019) and population responses to infection (Manlove et al., 2016). Moreover, this study focused on one particular pathogen while wild animals experience exposure to a whole community of interacting micro and macroparasites (Jolles et al., 2008). It remains to be seen how connectivity affects the full gamut of infectious disease risks, and how immunogenetic variation functions in terms of responses to the pathogen community. These caveats notwithstanding, our study highlights the potential benefits of managing wildlife metapopulations for connectivity even in the face of deadly infectious disease threats.

Our study provides empirical evidence linking population connectivity, genetic diversity, immune function and a reduced infection burden at individual and population scales in a wild mammalian metapopulation. Threats to wildlife populations from increasing habitat fragmentation and accelerating infectious disease emergence can push wildlife management in opposing directions without clear options for ensuring long-term population health. Here, we show the value of maintaining population connectivity to preserve genetic diversity and the capacity for protective immunity as an insurance against the ravages of novel pathogen outbreaks. Connectivity may be a double-edged sword, but the benefits can cut sharper than the risks.

AUTHOR CONTRIBUTIONS

Brianna R. Beechler, Brian P. Dolan, Clinton W. Epps and Anna E. Jolles conceived the ideas, Clinton W. Epps acquired funding; Clinton W. Epps administered and Anna E. Jolles supervised the project; Brian S. Dugovich, Brianna R. Beechler, Brian P. Dolan, Rachel S. Crowhurst, Clinton W. Epps and Anna E. Jolles designed methodology; Ben J. Gonzales, Jenny G. Powers, Debra L. Hughson and Regina K. Vu provided resources; Brian S. Dugovich, Brianna R. Beechler, Brian P. Dolan, Rachel S. Crowhurst, Ben J. Gonzales, Regina K. Vu, Clinton W. Epps and Anna E. Jolles collected the data; Brian S. Dugovich analysed the data; Brian S. Dugovich, Brianna R. Beechler, Brian P. Dolan, Clinton W. Epps and Anna E. Jolles led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.5x69p8d2c (Dugovich et al., 2023)

ORCID

Brian S. Dugovich https://orcid.org/0000-0001-6729-745X
Brianna R. Beechler https://orcid.org/0000-0002-9711-4340
Brian P. Dolan https://orcid.org/0000-0001-5985-1887
Rachel S. Crowhurst https://orcid.org/0000-0003-2281-9693
Clinton W. Epps https://orcid.org/0000-0001-6577-1840
Anna E. Jolles https://orcid.org/0000-0003-3074-9912

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Summary of *M. ovipneumoniae* detection in desert bighorn sheep in the Mojave Desert. These animals were sampled in 2013 and/or 2014, and the populations are based on the mountain ranges they inhabit. PCR prevalence and seroprevalence are for animals with *Mycoplasma ovipneumoniae* antibodies (serology) or DNA (PCR) detected, respectively. The number in the parentheses is the total number of animals tested in that population.

Table S2. Model summary of censoring indeterminate *Mycoplasma ovipneumoniae* results in desert bighorn sheep in the Mojave Desert. Four PCR and 12 serology indeterminate results were censored. Due to loss of sample size in most populations, results were slightly weaker than the manuscript results, but the significant patterns remained. AR is allelic richness. LPA is lymphocyte proliferation assay, and BKA is bacterial killing assay. Significant results are bolded.

Table S3. Blood and faecal samples used to estimate genetic diversity. These samples were used to calculate allelic richness (AR) and expected heterozygosity (H_e) for neutral and immune-linked genes.

Table S4. Microsatellite loci used to calculate genetic diversity.

Table S5. Connectivity and genetic diversity models in desert bighorn sheep populations in the Mojave Desert. Neutral and immune-linked microsatellite loci were used to calculate allelic richness (AR) and expected heterozygosity (H_e). Significant results are bolded.

Table S6. M*ycoplasma ovipneumoniae* prevalence in desert bighorn sheep populations in the Mojave Desert by testing modality. Neutral and immune-linked microsatellite loci were used to calculate allelic richness (AR) and expected heterozygosity (H_e). Significant results are bolded. Sample sizes were too small to examine the 2014 results alone.

Table S7. Population-level immunity models in desert bighorn sheep. The bacterial killing assay (BKA) and lymphocyte proliferation assay (LPA) were tested against disease states. The protective immune effect of the LPA was then evaluated against genetic diversity and connectivity. Neutral and immune-linked microsatellite loci were used to calculate allelic richness (AR) and expected heterozygosity (H_e). Significant results are bolded.

Table S8. Principal component analysis table of loadings.

Table S9. Bayesian disease and immunity models in individual desert bighorn sheep in the Mojave Desert. Serology excluded PCR-positive animals. Principal components (PC) were derived from a principal component analysis of white blood cell counts and bacterial killing and lymphocyte proliferation assays. Immune and neutral-linked loci diversity is reported as observed heterozygosity (H_o). Bayesian credible intervals (BCI) are reported for the odds ratio (OR). Direction of effect is noted with probability of direction (P_D).

Table S10. Bayesian protective immunity (PC2) and immune-linked heterozygosity model in individual desert bighorn sheep in the Mojave Desert. Bayesian credible intervals (BCI) are reported. Direction of effect is noted with probability of direction (P_D).

Figure S1. Hierarchical logistic disease models' (a–b) and a hierarchical protective immunity model's (c) fit of posterior predictive distributions. The model fits (y) were compared to 100 generated datasets from the posterior predictive distributions (y_{rep}).

Figure S2. Principal component analysis diagnostics. Individual and total white blood cell counts (WBC), plasma bacterial killing assay (BKA) and lymphocyte proliferation assay (LPA) were used to measure individual immunocompetence, and desert bighorn sheep sampling occurred 2013 – 2014 in the Mojave Desert. The first four eigenvectors were used for data analysis and accounted for 83.2% of the total variance (a). PC5 was difficult to interpret and was not used for data analysis (b). One animal was PCR- and missing serology (gray cross), and another was seropositive but missing a PCR test (magenta cross).

Figure S3. The Newberry population of desert bighorn sheep in the Mojave Desert strengthened the metapopulation's relationship between connectivity and genetic diversity and had less than average mean lymphocyte proliferation. Connectivity formed strong linear relationships with both neutral-linked (a; $\beta = 0.041$, SE = 0.007) and immune-linked allelic richness at the population level ($\beta = 0.040$, SE = 0.008) with Newberry having the lowest connectivity and genetic diversity. The relationship between connectivity and LPA (lymphocyte proliferation assay) was significant (c; $\beta = 0.009$, SE = 0.003).

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