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Microbial surveillance versus cytokine responsiveness in native and non-native house sparrows

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The success of introduced species often relies on flexible traits, including immune system traits. While theories predict non-natives will have weak defences due to decreased parasite pressure, effective parasite surveillance remains crucial, as infection risk is rarely zero and the evolutionary novelty of infection is elevated in non-native areas. This study examines the relationship between parasite surveillance and cytokine responsiveness in native and non-native house sparrows, hypothesizing that non-natives maintain high pathogen surveillance while avoiding costly inflammation. We made this specific prediction, as this pattern could enable invaders to effectively mitigate pathogen risk in a manner commensurate with the life-history priorities of a colonizing organism (i.e. rapid maturation and high reproductive effort). To test this hypothesis, we measured *TLR*-2 and

TLR-4 expression, markers of pathogen surveillance and cytokine responses (changes in *IL-1β* and *IL-10*), regulators of inflammation, to a simulated bacterial infection. In non-native sparrows, we found that as *TLR*-4 expression increased, *IL-1β* and *IL-10* responses decreased, a relationship not observed in native sparrows. Additionally, higher body condition predicted larger *IL-1β* and *IL-10* responses in all birds. These findings suggest that high *TLR-4* surveillance may mitigate strong inflammatory responses in non-native sparrows, with pathological and resource-based costs driving immune variation among and within populations.

1. Introduction

Why do some organisms successfully colonize novel environments whereas others do not? A key factor is the flexibility of traits that enhance survival and/or reproduction [1], with immune defences being especially crucial [2,3]. Successful invaders tend to be those that balance reproductive needs against other priorities, including infection risk. The enemy release hypothesis (ERH) and the evolution of increased competitive ability hypothesis (EICA) exemplify the idea that invasion success is linked to immunity, in particular. According to the ERH, introduced organisms (termed non-native here) experience weaker selection on their immune systems due to the relative decrease in exposure to natural enemies [4]. This relative rarity allows them to allocate resources towards growth and reproduction rather than immune defences [5], thus allowing them to become more competitive (EICA). Consequently, successful colonizers are expected to exhibit weaker immune responses compared to native individuals, particularly at the forefront of an invasion where encounters with parasites should be few [6]. In support of this idea, one study found that a successful invader, the house sparrow (*Passer domesticus*), had a weaker inflammatory response compared to a less successful invader, the tree sparrow [7]. However, hypotheses like ERH and ECIA overlook the fact that while there is an overall reduction in exposure to parasites, non-native species are more likely to encounter more evolutionarily novel parasites [8], lineages or perhaps even species never encountered historically by their ancestors. Therefore, total immune suppression represents a large risk. Likely, the immune strategy of non-natives involves differential investment in components of defences that are effective at controlling mostly novel pathogens [6,9].

Not only do house sparrow populations vary immunologically, but given their wide distribution and success as an invader [10], they are ideal for questions regarding trait differences along invasion gradients. For example, during an active range expansion in Kenya, birds farther from their original site of introduction of the species (i.e. Mombasa) exhibited higher constitutive expression of Toll-like receptors (*TLRs*) than birds caught near the core [11]. Initially, this pattern may seem counter to EICA, especially given the function of *TLRs*, which recognize pathogen-associated molecular patterns and instigate various immune responses (e.g. inflammation) [12]. However, heightened TLR expression has been linked to quicker microbial detection across various species [13]. This upregulation may serve as a strategy to swiftly eliminate pathogens before they cause harm, especially in birds at the range's edge, where exposure to novel infections is more likely. Upon pathogen detection by *TLRs* on macrophages and heterophils, these leukocytes initiate the transcription and release of various pro-inflammatory (e.g. interleukin 1β (IL-1β)) and anti-inflammatory cytokines (e.g. interleukin 10 (IL-10)) [14], which work together to effectively control infections [15]. However, mounting an inflammatory response can be costly because of both the energetic/metabolic requirements of immune cells [16] and the indirect effects of the response, such as decreased activity and reproductive output [17]. Accordingly, theory predicts that the optimal immune response would balance the risk of infection (i.e. direct pathology from a pathogen) with the risk of immunopathology (i.e. collateral damage from the immune response) [17,18].

Cost–benefit frameworks like these can be used to predict immune response variation, but the fitness risks posed by infection and immunopathology are not always symmetrical. The optimal immune response arises from a combination of the life history [19] and demographic characteristics [20,21] of populations as well as the epidemiological setting [22–24]. For invading organisms, immunopathologies pose a particular risk as they can decrease reproductive fitness and survival [17,25,26]. This raises the question: why do we observe high parasite surveillance at the Kenyan house sparrow range edge, seemingly contradicting EICA, especially when house sparrows exhibit a subdued inflammatory response, at least relative to a congener? We hypothesize that increased surveillance does not always obligate a stronger inflammatory reaction and, more specifically, that non-native house sparrows would mount a weak inflammatory response even if microbial surveillance were high. In contrast, native house sparrows would exhibit a more robust inflammatory response commensurate with their level of microbial surveillance, reflecting a more traditional immune strategy.

In this study, we compared the relationship between constitutive *TLR* expression and cytokine responsiveness among five native (Spain, Norway, The Netherlands, Vietnam and Israel) and four non-native house sparrow populations (Australia, New Zealand, Senegal and Canada). We predicted that *TLR* expression would be related to cytokine expression within individual birds, but we expected a shallower relationship in non-natives compared to native sparrows. To test this prediction, we measured the expression of effectors (*IL-1β* and *IL-10*) and *TLRs* (*TLR-2* and *TLR-4*) in whole blood before and after administering lipopolysaccharide (LPS) from *Escherichia coli*, which mimics a bacterial infection [27] and has been extensively used in songbirds to induce inflammation [13]. Also, as immune responses are often condition-dependent [28], we asked whether the body condition index (BCI) of individual birds affected cytokine responsiveness. BCI was calculated as the residuals of a regression of body mass on a structural trait, hypothesizing that birds with a higher BCI, which indicates more mobilizable resources, would have a more robust cytokine response. The measure is based on the premise that better body condition equates to more resources available for defence [29], resulting in higher cytokine production [20,30,31].

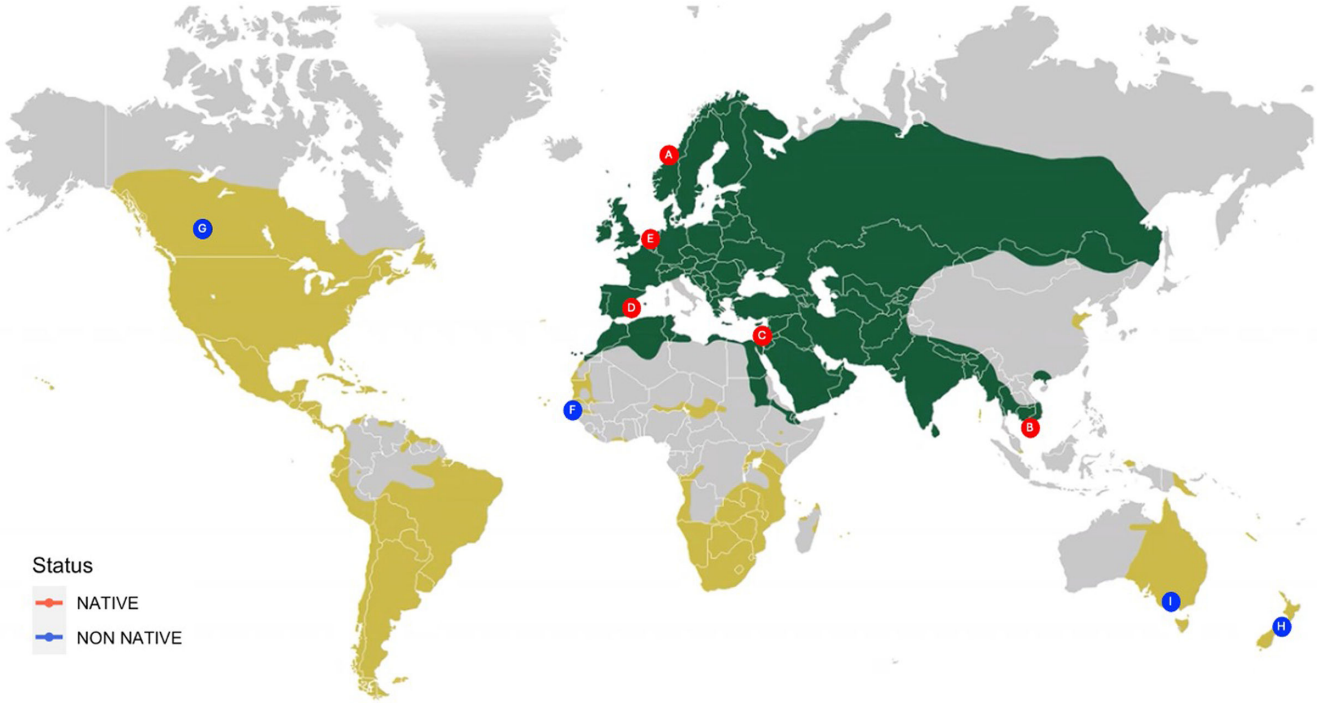

Figure 1. House sparrow (*Passer domesticus*) distribution as of 2019, with the green shading representing their native range and the yellow shading representing their introduced or non-native range. The locations of the sparrow populations sampled for this project are highlighted: native populations (locations A–E) are marked in red, while non-native populations (locations F–I) are marked in blue. (Figure adapted from [10].)

2. Methods

(a) Bird capture and care

During the non-breeding seasons of 2020–2023, we captured adult house sparrows ($n = 89$) via mist netting from sunrise to 11.00 in nine locations across the globe (figure 1) with exact location, dates and sample size available in the electronic supplementary material. Prioritizing the non-breeding season minimized potential confounding effects of reproductive activities, which could otherwise influence immune function [32], which allowed for more consistent and comparable measurements across individuals and locations. Upon capture, we measured wing chord (to 1 mm), tarsus length (to 0.1 mm) and body mass (to 0.1 g) and collected an approx. 50 µl blood sample from the brachial vein of each bird (stored in 300 µl of DNA/RNA Shield (Zymo R1100-50)). Immediately thereafter, we injected each bird with 100 µl of 1 mg ml^{-1} LPS (from *E. coli* 055:B5; Fisher L4005) in sterile saline subcutaneously over the breast muscle [33]. Post injection, we housed birds individually (but in visual and vocal contact with one another) in wire songbird cages (approximately 35.6 × 40.6 × 44.5) with food and water ad libitum. Eight hours post-injection, we took an additional approx. 10 µl of blood from the brachial vein. Forty-eight hours post-injection, we euthanized birds via isoflurane overdose and rapid decapitation, and approx. 50 µl of blood was again taken. Liver, spleen and gut samples were also taken for future studies and stored in 500 µl of DNA/RNA Shield. All samples were stored at −80°C until further processing. All animal research procedures adhered to local animal research guidelines and were approved in advance by both the USF IACUC (IS00011653) and the relevant authorities in the country of capture. Export and import of animal tissue were also compliant with all local US regulations according to USDA-APHIS and other appropriate permits.

(b) Molecular assays

(i) Target gene sequences and primer and probe design

Sequences for our target genes (i.e. *TLR-2*, *TLR-4*, *IL-10* and *IL-1β*) were identified using either *Passer domesticus* or *Passer montanus* genomes. To address potential concerns regarding DNA polymorphisms, we designed primers for conserved regions of exons by utilizing the BLAST alignment tool, ensuring a sequence similarity of at least 99% across multiple species to minimize the risk of SNP variation. Primers and probes for all four genes (detailed in the electronic supplementary material) were designed using Integrated DNA Technologies' PrimerQuest tool, using qPCR parameters (two primers and one probe). ZEN double-quenched probes were chosen, with either a FAM or HEX fluorescent dye used for different genes. For assays, all primers and probes were diluted to 10 µM concentration.

(ii) RNA extraction and cDNA synthesis

We extracted RNA from 50 µl of whole blood/shield mixture using a standard phenol : chloroform protocol [34]. For the full RNA extraction protocol, see the electronic supplementary material. Following extraction, RNA concentration quality was

assessed using a NanoDrop spectrophotometer, with special attention to the 260/280 ratio to ensure sample purity. Reverse transcription was performed using the iScript cDNA Synthesis kit (Bio-Rad 1708891), following the manufacturer's instructions.

(iii) Droplet digital polymerase chain reaction

We performed a droplet digital PCR (ddPCR) to quantify absolute copy numbers of the PCR targets. ddPCR reactions contained 5 µl ddPCR Multiplex Supermix (ddPCR Multiplex Supermix, 12005909, Bio-Rad); 2.25 µl forward primers (10 µM), 2.25 µl reverse primers (10 µM), 0.63 µl probe FAM, 0.63 µl probe HEX, 0.63 µl probe FAM+HEX (e.g. when 50% FAM + HEX, add 0.31 µl of each) and 3.5 µl sample (cDNA 1000 ng μl^{-1}). The ddPCR analysis was performed using the C1000 Touch™ Thermal Cycler with the 96-Deep Well Reaction Module (1851197, Bio-Rad). 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) using the droplet reader (QXDx Droplet Reader, 12008020, Bio-Rad). At the end of all runs, expression data were obtained using QuantaSoft™ Analysis Pro software (v. 1.05).

(c) Data analysis

We initially transformed all gene expression data using $\log_{10}$ to normalize distributions and reduce heteroscedasticity. Next, we tested for any baseline significant differences in gene expression between the native and non-native. Subsequently, we computed the change in gene expression following LPS injection of *IL-1β* and *IL-10*, denoted as delta (**Δ**). We directly assessed an individual's response to the LPS, as we had no predictions about pre-LPS values in relation to TLRs and for simplicity of interpretation.

We then examined whether population status (native or non-native), baseline TLR expression, or their interaction could predict cytokine deltas (**Δ**) using linear mixed models, with country of capture treated as a random effect. We then determined the body condition index (BCI) for each bird by regressing body mass against wing chord and saving standardized residuals from the models. In exploratory analyses, we found that wing chord regressed against body mass provided a better fit than tarsus against body mass, and wing chord was found to be a more repeatable measurement [35]. Due to sexual dimorphism in sparrows [10], BCI was calculated separately for males and females. Subsequently, we investigated whether BCI, along with its interaction with population status, could predict cytokine responsiveness and/or the ratio of **Δ***IL-1β* to **Δ***IL-10*. All linear mixed effects models were fitted using the 'lmer' function from the 'lme4' package (v. 4.3.2) [36]. Regression summaries were computed using the 'jtools' package (v. 4.3.2) [37]. All analyses were conducted using R v. 4.3.2.

3. Results

(a) Immune surveillance and cytokine responsiveness

Baseline gene expression levels of *IL-1β*, *IL-10*, *TLR-2* and *TLR-4* were comparable between native and non-native populations (electronic supplementary material, table S2). Also, neither **Δ***IL-1β* nor **Δ***IL-10* differed significantly by population status (**Δ***IL-1β*: estimate = 0.692, $p = 0.195$; **Δ***IL-10*: estimate = 0.476, $p = 0.380$). However, the interaction between *TLR* surveillance and population status was a significant predictor of cytokine responsiveness. Specifically, in non-native but not native birds, *TLR-4* expression was inversely related to **Δ***IL-10* (estimate = −0.623, $p = 0.037$) (figure 2, table 1) and **Δ***IL-1β* expression (estimate = −0.735, $p = 0.012$) (figure 2B, table 1). However, this pattern did not hold for *TLR-2*; neither *TLR-2* alone nor its interaction with population status predicted **Δ***IL-1β* (estimate = 0.11, $p = 0.631$) or **Δ***IL-10* (estimate = 0.227, $p = 0.358$; table 2).

(b) Body condition index and cytokine responsiveness

BCI did not differ by population status (estimate = 0.188, $p = 0.67$; figure 3A). However, **Δ***IL-1β* (estimate = 0.463, $p = 0.006$; figure 3B) and **Δ***IL-10* (estimate = 0.482, $p = 0.004$; figure 3C) were both positively related to BCI. Population status did not affect the manner in which **Δ***IL-1β* (estimate = −0.220, $p = 0.367$) or **Δ***IL-10* (estimate = −0.346, $p = 0.148$) related to BCI, however.

4. Discussion

We found that non-native house sparrows with high *TLR-4* expression showed reduced *IL-1β* and *IL-10* responsiveness, an effect not seen in natives. To our surprise, we also found that non-native sparrows with low *TLR-4* expression showed a heightened *IL-1β* and *IL-10* response. We also hypothesized that body condition would partly underlie the patterns of cytokine responsiveness and found it positively related to both *IL-1β* and *IL-10* responsiveness. However, these effects were consistent between population status, condition positively affected cytokine expression, but similarly in both groups. We offer several explanations for these findings and discuss their implications as follows.

(a) Immune surveillance and responsiveness

We found that native and non-native sparrows exhibit different relationships between *TLR-4* expression and cytokine responses. This result challenges the assumption that heightened microbial surveillance always leads to a strong cytokine response. The most parsimonious explanation for the negative relationship between cytokine responsiveness and surveillance involves the

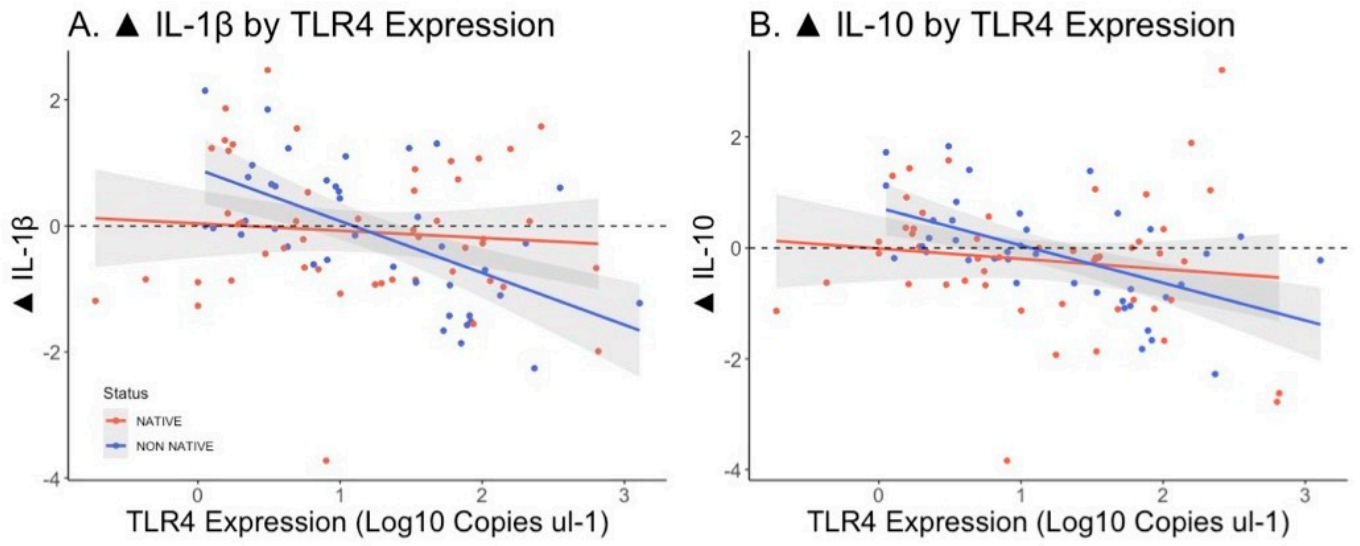

Figure 2. ΔIL-1β and ΔIL-10 are inversely related to TLR4 expression in non-native but not native house sparrows (*Passer domesticus*). Each symbol depicts the expression of a single bird, and trend lines depict linear relationships between variables with slopes differing between population status. The grey shaded area represents 95% confidence intervals.

Table 1. Effects of baseline TLR expression and invasion status on cytokine responsiveness (ΔIL-1β and ΔIL-10) in house sparrows.

dependent variable: ▲ IL-1β					
fixed effects	**estimate ± SE**	***t*-value**	***p*-value**	**variance**	**s.d.**
intercept	0.165 ± 0.343	0.481	0.631		
TLR2	−0.091 ± 0.170	−0.531	0.595		
status (non-native)	0.692 ± 0.534	1.296	0.195		
TLR4	−0.085 ± 0.200	−0.424	0.671		
status × TLR2	0.117 ± 0.243	0.481	0.631		
status × TLR4	**-0.735 ± 0.293**	**-2.504**	**0.012**		
country				0.079	0.282
marginal $R^2 = 0.21$; conditional $R^2 = 0.15$					

dependent variable: ▲ IL-10					
fixed effects	**estimate ± SE**	***t*-value**	***p*-value**	**variance**	**s.d.**
intercept	0.324 ± 0.348	0.929	0.353		
TLR2	−0.297 ± 0.173	−1.712	0.087		
status (non-native)	0.476 ± 0.542	0.878	0.380		
TLR4	−0.033 ± 0.203	−0.161	0.872		
status × TLR2	0.227 ± 0.247	0.920	0.358		
status × TLR4	**−0.623 ± 0.298**	**−2.089**	**0.037**		
country				0.081	0.285
marginal $R^2 = 0.19$; conditional $R^2 = 0.13$					

Linear mixed model results exploring the impact of TLR-2 and TLR-4 (surveillance molecules) on cytokine responsiveness (ΔIL-1β and ΔIL-10), along with the interaction between these molecules and invasion status (native versus non-native). Notably, the model included country as a random fixed effect and variables with p values less than 0.05 are bolded in the table.

speed and resolution of the inflammatory response. Non-native birds with high initial *TLR-4* expression may quickly detect and eliminate pathogens [13]; therefore, the smaller cytokine delta could be signalling the resolution of the inflammatory response. Conversely, non-native birds with lower initial *TLR-4* expression might take longer to respond, resulting in a larger cytokine response at 8 h and a more prolonged inflammatory response.

Alternatively, these patterns also reinforce that the inflammatory response is quite complex. In line with the EICA-refined hypothesis [6], variation in immune defences involves strategic trade-offs among *components* of the response, rather than simple upregulation or downregulation. Lee & Klasing [6] proposed that successful invaders should dampen the costly and/or least effective immune defences in favour of more efficient ones. Here, non-native birds might maintain effective pathogen surveillance and a more modest inflammatory response.

Table 2. Effects of body condition index (BCI) on cytokine responsiveness (ΔIL-1β and ΔIL-10) in native and non-native house sparrows.

dependent variable: ▲ IL-1β					
fixed effects	**estimate ± s.e.**	***t*-value**	***p*-value**	**variance**	**s.d.**
intercept	−0.041 ± 0.244	−0.168	0.866		
BCI	**0.463 ± 0.168**	**2.749**	**0.006**		
status (non-native)	−0.099 ± 0.366	−0.272	0.786		
BCI × status	−0.220 ± 0.243	−0.902	0.367		
country				0.194	0.441
marginal $R^2 = 0.27$; conditional $R^2 = 0.12$					

dependent variable: ▲ IL−10					
fixed effects	**estimate ± SE**	***t*-value**	***p*-value**	**variance**	**s.d.**
intercept	−0.173 ± 0.196	−0.881	0.378		
BCI	**0.482 ± 0.166**	**2.898**	**0.004**		
status (non-native)	0.048 ± 0.294	0.162	0.871		
BCI × status	−0.346 ± 0.239	−1.446	0.148		
country				0.084	0.290
marginal $R^2 = 0.17$; conditional $R^2 = 0.11$					

Linear mixed model results exploring the impact of the body condition index (BCI) on cytokine responsiveness (ΔIL-1β and ΔIL-10), along with the interaction with invasion status (native versus non-native). Notably, the model included country as a random fixed effect and variables with $p < 0.05$ are bolded in the table.

Indeed, we were surprised to see a negative relationship between *TLR-4* expression and cytokine responsiveness in non-native populations; our hypothesis was that relationships would be positive in all birds but more modest in non-natives than in natives. One phenomenon that could explain this unexpected result is endotoxin tolerance. Endotoxin tolerance, defined as a reduced responsiveness to Gram-negative pathogens due to repeated exposure to endotoxins (e.g. LPS) [27,38,39], has been observed across many taxa, from mammals [40] to songbirds [41]. Endotoxin tolerance arises through various mechanisms, primarily involving defects in *TLR-4* signalling pathways [38], and these defects can occur at the level of the receptor itself, its adaptor molecules, downstream signalling molecules or even transcription factors [38]. As mounting a robust inflammatory response can be costly and increase the risk of tissue damage; endotoxin tolerance can be understood as an adaptation that results in the reduced capacity of a cell to respond to LPS [38], mitigating the energy use and damage from constant activation [42,43]. House sparrows from Tampa, FL, where they are also non-native, have a notable degree of endotoxin tolerance [33]. Perhaps our results reflect a general disposition towards endotoxin tolerance in house sparrows as a species, but one that is particularly pronounced in non-native populations.

As previously explained, non-native organisms face an environment with reduced pressure from familiar pathogens but increased exposure to novel ones [8]. This scenario may lead to a strategy that involves enhanced surveillance for quick parasite detection combined with a controlled inflammatory response using endotoxin tolerance to mitigate the costs of inflammation [39]. Endotoxin tolerance might also explain certain behavioural phenomena. For example, female house sparrows expressing more *TLR-4* take greater foraging risks [44]. This heightened risk-taking behaviour could be attributed to their increased tolerance to endotoxins, enabling them to exploit a wider range of food sources, including those potentially contaminated with bacteria, without suffering the adverse effects of inflammation. This ability to utilize a broader variety of food resources could enhance survival of infection and confer a competitive advantage in certain environments [45]. Furthermore, endotoxin tolerance could be viewed as a regulatory mechanism, with parasite tolerance [26] representing the broader outcome. However, it should be noted that a refractory immune response due to endotoxin tolerance may also render the host vulnerable to secondary infections [46,47].

Another explanation for the negative relationship between surveillance and cytokine responsiveness involves the leukocyte composition of the blood, with heterophils followed by lymphocytes being the most abundant cell type in circulation [48,49]. Lymphocytes, both B and T, are central to adaptive immunity, with T cells recognizing specific antigens presented on cells and B cells producing immunoglobulins that bind specific antigens [50]. Heterophils, the most numerous leukocytes, are key components of innate immunity, rapidly migrating to sites of infection or injury to phagocytize pathogens and produce cytokines [51]. As the 'first responders', heterophils initiate acute inflammation and injury repair by releasing antimicrobial mediators and activating signalling pathways for chemokine and cytokine expression. Notably, given their abundance, heterophils predominantly drive TLRs and cytokine expression [51,52].

The heterophil to lymphocyte (H/L) ratio is highly sensitive to external stressors [48,49], as the body redistributes circulating lymphocytes from the blood to tissues [53], and all while stimulating the bone marrow to prioritize heterophil production during stressful states [48,54]. Notably, the H/L ratio often differs significantly between native and non-native populations [49], with invaders often exhibiting a higher proportion of heterophils. With heterophils containing pattern recognition receptors (e.g. TLRs), this increase could translate to a heightened expression of *TLR-4* [52]. However, this same stress-induced increase

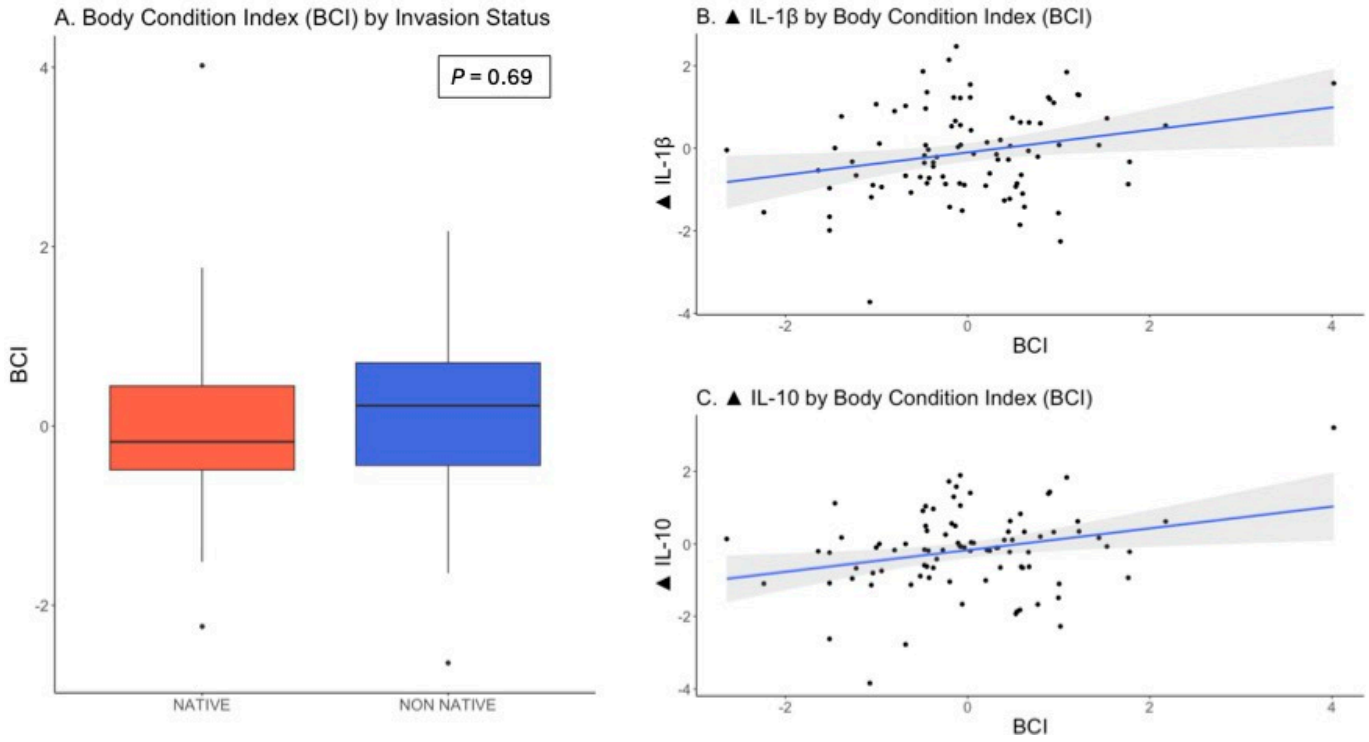

Figure 3. Cytokine responsiveness is positively related to body condition in both native and non-native house sparrows (*Passer domesticus*). (A). Body condition index (i.e. BCI, residuals from a regression of body mass and wing chord length) did not vary between house sparrow population types, with error bars depicting 95% confidence intervals. Panels (B) and (C) show that BCI was positively related to cytokine responsiveness, although population status did not affect these relationships. In (B) and (C), individual symbols derive from each bird with lines depicting regression relationships between variables, with the grey shaded area representing 95% confidence intervals.

in heterophils could explain a decrease in cytokine responsiveness. Glucocorticoids, known for their immunosuppressive effects [55,56], might promote the development of heterophils while concomitantly dampening the downstream signalling of TLRs by repressing the NF-κB pathway [57]. This could result in surveillance increasing while reducing cytokine responsiveness. Altogether, the mismatch between surveillance and responsiveness suggests immunological rewiring in non-native sparrows, absent in native populations.

(b) Body condition index and cytokine responsiveness

Although BCI did not differ between native and non-native birds, BCI was positively related to **Δ***IL-1β* and **Δ***IL-10*. Traditionally, a high BCI in passerines signifies that an animal has more fat content per unit physical size [58], providing a larger energy reserve for physiological functions [59,60]. Quantifying the metabolic costs of immune defence remains a challenge; however, evidence suggests a clear link between increased energy expenditure and proinflammatory cytokine upregulation. *In vivo* studies have demonstrated a 30% rise in respiration rates in animals stimulated with cytokines like TNF-α and IL-1β [61]. Furthermore, individuals mounting a pro-inflammatory response also exhibit a heightened demand for glucose and glutamine, which ultimately leads to the catabolism of the body's reserves of protein, carbohydrates and lipids [61–63].

Therefore, when considering the costs of immunity, individuals in poorer physiological states (i.e. low BCI) might face a trade-off [64]: either prioritize immediate immune defences or invest resources in other crucial traits for fitness [21]. This trade-off means that birds with a lower BCI may not have the necessary reserves to mount an effective immune response without compromising other vital functions [29]. In contrast, birds with a higher BCI have a surplus of resources for mounting robust inflammatory responses [65]. The relationship between BCI and *IL-10* was particularly surprising, though, as there is a lack of literature exploring the costs of anti-inflammatory cytokine expression. While the metabolic costs of pro-inflammatory responses have been studied extensively, less is known about the energy requirements and potential trade-offs associated with producing anti-inflammatory cytokines like IL-10. Altogether, birds with higher BCI are better equipped to both defend against parasites (e.g. through *IL-1β*) and protect against immunopathology (e.g. through *IL-10*).

5. Conclusion

Overall, our results challenge the notion that high parasite surveillance necessitates a strong immune response. Instead, we demonstrate that non-native house sparrows have a more nuanced response, one that allows some to have heightened microbial surveillance without triggering an overly sensitive 'smoke-detector'. This strategy could involve mechanisms such as endotoxin tolerance, which allows non-native sparrows to maintain vigilance against pathogens without the costs of

excessive inflammation [38,39]. Such a trait could provide a competitive edge by enabling these non-native birds to cope with a wider array of environmental challenges, including novel pathogens, while minimizing the trade-offs associated with immune activation.

Furthermore, understanding how non-native species respond to pathogens (i.e. resistance versus tolerance) offers valuable insight into disease dynamics [26]. This perspective is further emphasized when considering the well-established reservoir capabilities of successful invaders like the house sparrow [10,66,67]. Future research should not only investigate the mechanisms behind the regulation of inflammation and physiological trade-offs but also explore pressures that make immune flexibility important for facilitating range expansions and the ecological consequences of such variation.

Ethics. All animal research procedures adhered to local animal research guidelines and were approved in advance by both the USF IACUC (IS00011653) and the relevant authorities in the country of capture. Export and import of animal tissue were also compliant with all local US regulations according to USDA-APHIS and other appropriate permits.

Data accessibility. Data and analysis code are available from the Dryad Digital Repository [37].

Supplementary material is available online [68].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. K.M.M.: conceptualization, data curation, formal analysis, investigation, methodology, writing—original draft; G.M.: data curation, methodology, writing—review and editing; E.L.S.: data curation, methodology, writing—review and editing; C.Z.: methodology, resources, writing—review and editing; A.W.S.: conceptualization, data curation, formal analysis, funding acquisition, methodology, supervision, writing—review and editing; M.R.: methodology, resources, writing—review and editing; R.D.: methodology, resources, writing — review and editing; K.D.K.: conceptualization, data curation, formal analysis, funding acquisition, methodology, supervision, writing—review and editing; J.S.S.: investigation, methodology, writing—review and editing; H.J.: methodology, resources, writing—review and editing; K.J.M.: methodology, resources, writing—review and editing; T.V.: methodology, resources, writing—review and editing; H.T.P.: methodology, resources, writing—review and editing; B.J.: methodology, resources, writing—review and editing; K.L.B.: methodology, resources, writing—review and editing; M.T.: methodology, resources, writing—review and editing; J.B.: methodology, resources, writing—review and editing; L.B.M.: funding acquisition, investigation, methodology, project administration, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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