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RESEARCH ARTICLE

Costs of immunity and their role in the range expansion of the house sparrow in Kenya

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ABSTRACT

There are at least two reasons to study traits that mediate successful range expansions. First, dispersers will found new populations and thus impact the distribution and evolution of species. Second, organisms moving into new areas will influence the fate of resident communities, directly competing with or indirectly affecting residents by spreading non-native or spilling-back native parasites. The success of invaders in new areas is likely mediated by a counterbalancing of costly traits. In new areas where threats are comparatively rare, individuals that grow rapidly and breed prolifically should be at an advantage. High investment in defenses should thus be disfavored. In the present study, we compared the energetic, nutritional and collateral damage costs of an inflammatory response among Kenyan house sparrow (*Passer domesticus*) populations of different ages, asking whether costs were related to traits of individuals from three different capture sites. Kenya is among the world's most recent range expansions for this species, and we recently found that the expression of *Toll-like receptors* (*TLRs*), leukocyte receptors that instigate inflammatory responses when bound to microbial elements, was related to the range expansion across the country. Here, we found (contrary to our expectations) that energetic and nutritional costs of inflammation were higher, but damage costs were lower, in range-edge compared with core birds. Moreover, at the individual level, *TLR-4* expression was negatively related to commodity costs (energy and a critical amino acid) of inflammation. Our data thus suggest that costs of inflammation, perhaps mediated by *TLR* expression, might mitigate successful range expansions.

KEY WORDS: Invasion, Pest species, Enemy release, Trade-off, Energetics

INTRODUCTION

Many species are innocuous when introduced outside their native ranges, barely maintaining viable populations. However, some invaders become extremely damaging (Parker et al., 2013), with damage occurring directly through predation or competition or indirectly through the transmission of parasites (Raffel et al., 2008; Kelly et al., 2009). Surprisingly, we still know little about what traits comprise successful invaders, especially for vertebrates. Perhaps the best-studied introduced vertebrate species is the cane toad (*Rhinella marina*), which spread across Australia in ∼80 years (Phillips et al., 2006; Kolbe et al., 2009; Brown and Shine, 2014; Brown et al., 2015a,b; Rollins et al., 2015). Other non-native vertebrates, particularly rodents and songbirds (Losos et al., 1997; Kolbe et al., 2004; Lee et al., 2004, 2005; Fassbinder-Orth et al., 2013; Vilcinskas et al., 2013; White et al., 2013; Morand et al., 2015; Tian et al., 2015), have gained some recent attention, but the diversity of research approaches makes generalizations about facilitators of range expansions premature.

One pattern that is emerging involves immune system architecture. For instance, variation in the regulation of inflammation appears important to the invasion success of the house sparrow (*Passer domesticus*) (Liebl and Martin, 2012, 2013, 2014; Liebl et al., 2013; Coon et al., 2014; Coon and Martin, 2014; Martin et al., 2014a,b; Martin and Liebl, 2014; Schrey et al., 2014), one of the world's most common birds. Compared with a less successful congener, the tree sparrow (*Passer montanus*), the house sparrow invests little energy in inflammatory immune responses (Lee et al., 2005) at one site in its introduced range, biasing its defenses towards adaptive responses (Lee et al., 2006). In Kenya, where house sparrows were introduced to Mombasa in approximately 1950 (Lewis and Pomeroy, 1989), inflammation seems to have mitigated spread over the last 60 years. Range-edge birds have become quite distinct from birds at the core (Liebl and Martin, 2012, 2013, 2014; Coon et al., 2014; Coon and Martin, 2014; Martin and Liebl, 2014), in particular in terms of *Toll-like receptor* (*TLR*) expression. Indeed, twice previously (Martin et al., 2014a,b), we found that *TLR-4 and TLR-2* expression from leukocytes (blood samples) was higher in range-edge than in core Kenyan house sparrows. TLRs are pattern-recognition receptors (PRRs), meaning that they serve an important role in host surveillance for infectious disease threats (Medzhitov and Janeway, 1998). For instance, TLR-2 and TLR-4 are predominantly responsible for detecting Gram-positive and Gram-negative threats, respectively, whereas TLR-3 detects some viral threats (Alcaide and Edwards, 2011). TLRs perform these functions by binding constituents of pathogens, namely molecules such as lipopolysaccharide, peptidoglycan and others that do not exist in hosts but play an integral role in the physiology and structural integrity of the infecting organism.

Because of these particular functional roles for TLRs in immune defense, we proposed that population differences in *TLR* expression might represent adaptations useful at the range edge and/or disadvantageous traits selected against at the range core. In terms of adaptation, more *TLR* could help range-edge birds control their parasites better (Martin et al., 2014a,b). On the range edge, where enemies are probably rarer yet comparatively more novel than at the core, greater TLR expression might be an especially critical aspect

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of broadly effective, fast-acting inflammation, providing sufficient defense against vital threats. In terms of disadvantages at the core, inflammation is among the most expensive and self-damaging immune defenses available to vertebrates. So, in the absence of strong selection for its persistence, core individuals might express little *TLR* to minimize the costs of parasite exposures, which can be handled effectively through other mechanisms (e.g. B- or T-cell-mediated immune memory).

Here, we investigated aspects of the second possibility, asking whether *TLR-4* expression was associated with the costs of inflammation (Klasing, 2004) among sparrows living at three sites across Kenya differing in time since colonization. First, we compared *TLR-4* expression in circulating leukocytes to determine whether previously observed among-population patterns were reproducible. Then, we compared the relative impacts of *TLR-4* expression and site of capture on three costs of inflammation: energetic, nutritional and collateral damage. Each cost type has different ramifications for hosts and hence the ecology and evolution of hosts and parasites (Cressler et al., 2015). Energetic costs entail increased calorie turnover, which could impose trade-offs with other host life functions. Nutritional costs involve, among other things, the assimilation of critical amino acids, which could also impose trade-offs with other life functions. Unlike calories though, critical amino acid costs cannot be compensated by reductions in other activities; additional consumption is mandatory to avoid trade-offs. Collateral damage costs, particularly for inflammation (Raberg et al., 2009), include DNA and cellular damage, and are a common consequence of the activity of effectors (i.e. oxidative burst) of this broadly protective defense mechanism. Once inflammation costs were measured, we asked whether variation in costs was predicted by *TLR-4* expression at the individual level and site of capture at the population level. We expected that birds from the range edge would exhibit larger costs of inflammation than core birds and that these costs would be positively related to *TLR-4* expression at the individual level (Raberg et al., 2009).

MATERIALS AND METHODS

Sparrow capture and husbandry

House sparrows, *Passer domesticus* (Linnaeus 1758), were caught using mist nets between 06:00 h and 12:00 h from the cities of Mombasa (n=12), Nairobi (n=14) and Nakuru (n=14) in early July 2013. Mombasa is likely the point of introduction of house sparrows to Kenya (Martin et al., 2014a,b). Nairobi, the capital city located ∼500 km from Mombasa, was probably colonized around 1980 and is thus presumed to be of intermediate age. Nakuru (650 km from Mombasa) is one of the most recently colonized cities in Kenya (∼1990), yet also possesses a population dense enough to enable capture of sufficient individuals for study. To attempt to avoid confounds in site comparisons associated with timing of sampling, we conducted the project first in Nakuru followed by Mombasa and finally Nairobi. We well recognize that the inclusion of only three populations in our study limits inference (because factors such as climate, elevation, parasite communities and many other factors that differentiate sites could also impact immune costs and/or *TLR-4* expression). Nevertheless, we could not include additional populations and also ensure all measurements were made in under 3 weeks (to minimize any seasonal/climatic influences on results). The USF IACUC as well as the Kenya Wildlife Service approved all methods in advance; all sample transport, export and storage were in compliance with USDA-APHIS and Kenyan governmental requirements.

Study time line

Immediately upon capture in the field, birds were weighed (to 0.1 g) and a small (∼100 µl) blood sample was taken from the brachial vein. This blood sample was later used to quantify *TLR-4* expression, as captivity can affect expression of this gene in house sparrows (Martin et al., 2011). Within a few hours, birds were placed individually into cages (∼33 cm^3) where they were held for the duration of the study. Throughout captivity, animals were left undisturbed except at the time of measurements, and they were provided with *ad libitum* access to tap water (treated with an anti-coccidial medication) and a mix of sorghum and red and white millet, which they consumed readily. Climate conditions and lighting tracked ambient conditions. Trials began the night of capture (∼21:00 h). Birds were randomly assigned to two cohorts because of limited respirometry equipment; one group was measured from 21:00 h until 02:00 h and the second was measured from 02:00 h until 07:00 h. For each cohort, an individual was placed singly into a respirometry chamber where it remained for 5 h to obtain resting metabolic rate (RMR) estimates. In exploratory data analyses, we queried whether metabolic rate differed between cohorts, given that it was measured at a different time of night between cohorts. As there was no detectable impact of cohort on metabolic rate, it was not included in the below analyses. After RMR measurements, birds were removed from chambers, injected with lipopolysaccharide (LPS), gavaged with ^{13}C-labeled leucine, weighed as above, and returned to their home cage. LPS was derived from *Escherichia coli* 055:B5 (L4005, Fisher, Pittsburgh, PA, USA), maintained in silanized vials at ambient temperature prior to all injections, and was injected subcutaneously over the breast muscle (1 mg kg^{-1} in 100 µl saline vehicle). Labeled leucine (20 mg of ^{13}C leucine, 99%; Cambridge Isotopes, Tewksbury, MA, USA) was administered orally in 200 µl peanut oil (McCue et al., 2011; Coon et al., 2014) within seconds of LPS injection. The next day, measurements occurred again following the same schedule, yet unlike the prior night, no new treatments (LPS or leucine) were made: the same approach was taken on a third night. On the morning of the fourth day, all birds were killed by inhalation of isoflurane followed by rapid decapitation. Organs and trunk blood were collected and stored in a liquid nitrogen-charged dry-shipper until they reached the Martin lab (FL, USA), at which point they were stored at −40°C. Sham control individuals (those given only vehicle, not LPS) were not included in the study because of equipment/time limitations.

Quantitative reverse transcription PCR (RT-qPCR) for *TLR-4* expression

We first extracted RNA from whole-blood samples using the SurePrep Leukocyte RNA Purification Kit (Fisher) (Liebl and Martin, 2013; Martin et al., 2014b). Using a spectrophotometer, these RNA extracts were then diluted to a concentration of 25–50 ng μl^{-1} before being stored at −40°C until RT-qPCR. RT-qPCR was conducted using the iTaq Universal SYBR Green One-Step Kit (Bio-Rad, Hercules, CA, USA). Each reaction contained 10 µl iTaq Universal SYBR Green reaction mix (2×), 0.25 µl iScript reverse transcriptase, 600 nmol l^{-1} each forward and reverse primers, 1 µl RNA (25–50 ng μl^{-1}) and nuclease-free water to a final volume of 20 µl. Cycling conditions and melt curve analyses were programmed according to the manufacturer's instructions specific for the StepOne Plus qPCR platform (Applied Biosystems). The *TLR-4* forward (5′- GCTCCTGTGTGTACCTGGAC -3′) and reverse (5′- ACAACACAACCACTGGGGAG -3′) qPCR primers were designed using the zebra finch (*Taeniopygia guttata*) *TLR-4*

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mRNA sequence in GenBank (accession no. NM_001142454.1). A six-point standard curve made from a homogenate of zebra finch splenic RNA (728, 227, 76, 24, 7, 2 ng μl^{-1}) was included on each plate, and all samples were run in duplicate.

Respirometry

We used an open-flow push-through respirometry system to quantify the energetic costs of inflammation. First, we estimated baseline RMR on the first night after capture (prior to LPS exposure) and then RMR on the two subsequent nights, measuring consumption of O_2 and production of CO_2 each time. Birds were measured singly, and each chamber housing a bird was measured for a 20 min period followed by a 2 min pause to clear the system before shifting measurement to a different chamber. Each respirometry chamber received 600 ml min^{-1} ambient air, allowing for constant flushing of chambers; a multiplexer (TR-RM8, Sable Systems, Las Vegas, NV, USA) controlled the chamber measured by the O_2 and CO_2 analyzer (FoxBox, Sable Systems). Exiting air from chambers passed through a water vapor analyzer (RH-300, Sable Systems) before being scrubbed of water in a column of Drierite; CO_2 concentration was subsequently measured. Following CO_2 measurement, air was scrubbed of CO_2 using Ascarite and dried using Drierite before passing through the O_2 analyzer. A baseline chamber was analyzed for 3 min at the start and end of trials and between sampling of chambers 2 and 3, and 4 and 5. RMR was calculated as the minimum 10 min mean of O_2 consumption for each individual. All birds were post-absorptive and inactive during measurements. Air temperature in each room was measured using two dataloggers (Hobo U14) and varied between 29 and 31°C, within thermoneutral conditions for this species.

^{13}C quantification

We measured the nutritional costs of inflammation by assessing assimilation of a stable isotope-labeled amino acid, leucine, into livers and spleens. As in a previous study (Coon et al., 2014), we interpreted high assimilation values as high nutritional costs of inflammation. Isotope levels in both tissue types were measured at the University of South Florida Stable Isotope Lab with a Delta V 3 keV isotope ratio mass spectrometer (Thermo Scientific, Waltham, MA, USA). Measurements were made following previously developed and validated methods for house sparrows (Coon et al., 2014). Measured ^{13}C values were recorded as $\delta^{13}C_{VPDB}$, which is the difference between the sample and an industry ^{13}C standard (Coon et al., 2014). Background (pre-capture, natural diet-associated) levels of ^{13}C in birds not exposed to isotopically labeled leucine were also measured; the mean value for all unexposed individuals from a site was then subtracted from values for all treated individuals at that site. All such unexposed individuals were adults in visibly good health. We also captured and analyzed unexposed individuals from each study site in case background isotope conditions varied among them.

Reactive oxygen metabolites

We measured collateral damage costs of inflammation in the form of reactive oxygen metabolites (ROMs) from blood using the d-ROMs test (Diacron International, Grosseto, Italy). This assay quantifies hydroperoxides, compounds that signal lipid and protein oxidative damage (Treidel et al., 2013); more ROMs are indicative of more oxidative activity in tissues and thus greater costs of inflammation. We optimized the assay by running dilution series on 10 individual house sparrow plasma samples and chose a dilution that fell at the midpoint between the calibrator and blank samples. Briefly, we diluted 1 µl of plasma in 200 µl of the provided acidic buffer solution, and then read the plate (at 490 nm on an ELx800, BioTek, Winooski, VT, USA) kinetically for 30 min (once per minute). We then calculated the rate of reaction for the calibrator (provided by the manufacturer), a blank and each sample. ROM concentration (in mmol l^{-1} of H_2O_2 equivalents) was calculated by subtracting the blank from both the calibrator and sample values and then dividing the sample value by the calibrator value and multiplying by the concentration of the calibrator. All analyses were run in duplicate, and the intra- and inter-assay coefficients of variation were 6.5% and 7.8%, respectively.

Total antioxidant capacity

We also measured a potential mitigator of collateral damage, total plasma antioxidant capacity (TAC), using the OXY-Adsorbent test (Diacron International). This assay measures the effectiveness of the blood antioxidant barrier to cope with oxidant action of hypochlorous acid (HClO) (Treidel et al., 2013); more TAC was interpreted to indicate more protection against collateral damage. We optimized the assay for Kenyan house sparrows by running dilution series on 10 individual Kenyan house sparrow plasma samples, choosing a dilution for subsequent assays that fell at the midpoint between the calibrator and blank samples. We diluted 10 µl plasma in 990 µl of distilled water and mixed 5 µl of this diluted plasma with 195 µl of the provided HClO solution. Absorbance was measured at 490 nm (ELx800, BioTek), and we calculated the rate of reaction for the calibrator (provided by the manufacturer), a blank and each sample. TAC concentration (in mmol l^{-1} of HClO neutralized) was calculated by subtracting the blank from both the calibrator and sample values and then dividing the sample value by the calibrator value and multiplying by the concentration of the calibrator. All analyses were run in duplicate, and the intra- and inter-assay coefficients of variation were 3.4% and 1.5%, respectively. We failed to collect 48 h plasma samples for TAC and ROM assays, and thus report only pre- and 24 h post-LPS exposure values.

Data analysis

All dependent variables were checked for normal distribution; the following variables required $\log_{10}$ transformations: *TLR-4* expression, RMR, TAC and ROM. We first used a general linear model (GLM) to determine whether *TLR-4* expression varied among sites. In all models, distance from Mombasa (dfM) was always a continuous predictor, as it has been in all of our prior work in this system. This approach, however, precludes all pairwise comparisons and nesting of *TLR-4* expression within sites. We then used linear mixed models (LMMs) to assess the impact of *TLR-4* expression and dfM (our surrogate for population age, where greater dfM indicates a younger population) on inflammation costs. Also, several of our analyses involved repeated measures of individuals, and linear mixed models allowed us to use individual as a random effect while simultaneously evaluating direct and interactive effects of predictors [e.g. dfM×time (pre- versus post-LPS)] on cost metrics. Some models warranted inclusion of other predictors (e.g. organ mass for nutritional costs; baseline RMR for energetic costs). Note too that degrees of freedom sometimes varied among analyses because (1) not all data were available for all individuals at all time points (i.e. RT-qPCR failed upon multiple efforts until mRNA samples were exhausted for some individuals), and/or (2) Satterthwaite approximations were sometimes required. As exploratory analyses did not reveal sex, metabolic rate, cohort or body mass as strong drivers of costs, none of these variables are mentioned in the below analyses.

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We first used separate LMMs and/or GLMs to determine influences of population age (dfM) and *TLR-4* expression on cost of inflammation [energetic (RMR), nutritional (leucine assimilation) and collateral damage (ROM)]. We also used a LMM to investigate dfM and/or *TLR-4* expression effects on TAC, a putative offset mechanism of collateral damage. We then performed separate GLMs on an integrated cost of inflammation generated via principal components analysis (PCA). To simplify the PCA (i.e. account for the dynamics of physiological responses), the total cost of each cost type was calculated. For energetic costs, this value was: (RMR 2 days post-LPS+RMR 1 day post-LPS)−RMR 1 day pre-LPS). For nutritional costs, total leucine assimilation (spleen +liver) was used. For collateral damage, the total cost was: (ROM 1 day post-LPS−ROM 1 day pre-LPS). TAC data (TAC 1 day post-LPS−TAC 1 day pre-LPS) were also incorporated into this PCA because (i) TAC partly functions to ameliorate ROM and (ii) TAC responses were inversely correlated to ROM responses (see below). Varimax rotation was used to maximize differences among principal components (PCs), and the Kaiser criterion (eigenvalue >1) was used to identify meaningful PCs. PC scores were then treated as dependent variables in GLMs with *TLR-4* expression and dfM as continuous predictors. We set α to $P<0.05$, and performed all analyses with SPSS v23.0.

RESULTS

TLR-4 expression

TLR-4 expression in blood samples at the time of capture (i.e. prior to LPS exposure) was not significantly related to dfM ($F_{2,37}=3.0$, $P=0.06$). However, there was a tendency for a pattern reversed from what we have observed previously, with *TLR-4* expression increasing with increasing population age (Fig. 1).

Energetic costs of inflammation

RMR prior to LPS exposure tracked population age (dfM: $F_{1,38}=6.0$, $P=0.02$) with RMR decreasing with increasing distance from Mombasa. However, *TLR-4* expression did not affect RMR prior to LPS exposure ($F_{1,38}=0.68$, $P=0.42$). In response to LPS, changes in RMR initially appeared modest (time: $F_{1,76}=0.18$, $P=0.67$) and not predictable by dfM (time×dfM: $F_{1,76}=1.2$, $P=0.23$; dfM alone: $F_{1,106}=1.9$, $P=0.17$). However, upon visual inspection of the time series data, RMR appeared to peak 24 h after LPS exposure and return to pre-LPS values 48 h after LPS exposure (Fig. 2A). Subsequently, the total energetic cost of LPS exposure was assessed with respect to *TLR-4* expression, dfM and RMR at the time of capture (given differences detected among sites). In that model, range-edge birds had higher energetic costs of LPS exposure than birds from the core (dfM: $F_{1,38}=20.7$, $P<0.001$; Fig. 2B). Moreover, individuals with the lowest RMR at capture also had the highest energetic costs of LPS exposure (pre-LPS RMR: $F_{1,38}=38.6$, $P<0.001$; Fig. 2C). *TLR-4* expression was not a significant predictor of total energetic costs ($F_{1,38}=0.87$, $P=0.36$).

Nutritional costs of inflammation

Exogenous, isotopically labeled leucine significantly enriched organs beyond background levels (treated versus control sparrows: $F_{1,84}=40.5$ $P<0.001$), but these effects did not differ by organ (organ×treatment: $F_{1,84}=0.05$, $P=0.82$). In terms of nutritional costs of inflammation, dfM was positively predictive of leucine assimilation into lymphoid tissues ($F_{1,65}=9.0$, $P=0.004$;

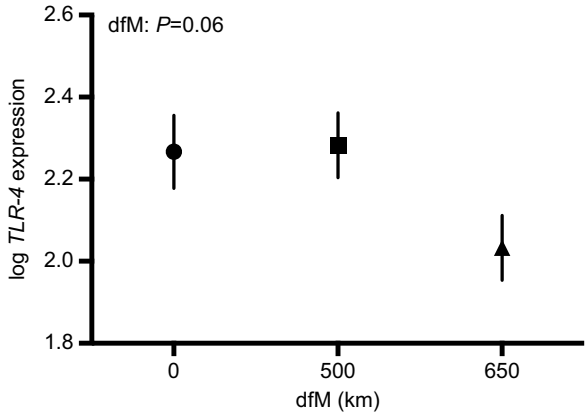

Fig. 1. *Toll-like receptor* (*TLR*) expression. Differences in *TLR-4* expression (μg^{-1} cDNA) in whole-blood samples were marginally non-significantly different among house sparrows captured from three Kenyan locations. Bars are means±1 s.e.m. dfM, distance from Mombasa (surrogate of population age).

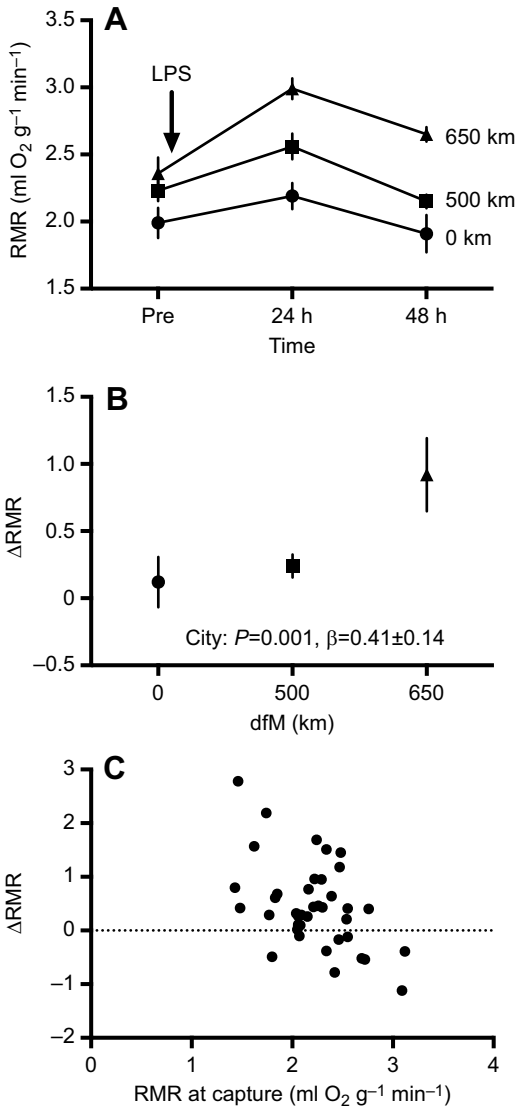

Fig. 2. Effects of lipopolysaccharide (LPS) exposure on resting metabolic rate (RMR) in Kenyan house sparrows. (A) RMR before and 24 and 48 h after LPS exposure. (B) Relationship between integrated energetic cost of inflammation (ΔRMR, 24–48 h post-LPS, i.e. area under the curves in A) and dfM. (C) Relationship between total energetic cost of inflammation (ΔRMR, 24–48 h post-LPS) and RMR before LPS exposure. Bars are means±1 s.e.m.

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Fig. 3). However, *TLR-4* expression ($F_{1,65}$=0.09, P=0.76), organ mass ($F_{1,65}$=0.12, P=0.29) and organ type ($F_{1,65}$=0.86, P=0.36) did not affect nutritional costs.

Collateral damage costs (ROM) and mitigation (TAC)

Our surrogate for collateral damage (ROM) increased in response to LPS exposure (β=0.1±0.05, where β is the slope coefficient estimate for the relationship between dfM and the dependent variable of interest; $F_{1,33.2}$=4.7, P=0.04), but this effect varied among populations (dfM×time: $F_{1,32.8}$=6.3, P=0.02). ROM declined in range-edge birds after LPS exposure whereas it tended to remain stable in the other two populations (Fig. 4A). Neither dfM alone ($F_{1,50.8,}$=2.2, P=0.15) nor *TLR-4* expression ($F_{1,35.0}$=1.7, P=0.21) affected ROM. Conversely, TAC decreased in response to LPS exposure (β=−0.06±0.02; $F_{1,36.1}$=7.4, P=0.01), but dynamics varied with dfM (dfM×time: $F_{1,,35.7}$=6.6, P=0.02). TAC tended to decline in core birds but values remained static in the other two populations (Fig. 4B). Neither dfM alone ($F_{1,57.4,}$=2.9, P=0.10) nor *TLR-4* expression ($F_{1,36.7}$=0.23, P=0.64) affected TAC. Importantly, we detected a significant negative relationship (r=−0.54, P=0.001) between ΔTAC (TAC 1 day post-LPS−TAC 1 day pre-LPS) and ΔROM (ROM 1 day post-LPS−ROM 1 day pre-LPS; Fig. 4C).

Integrated costs of inflammation

PCA indicated that two PCs captured 63% of the total variation in inflammatory costs (or mitigators thereof) among individuals. PC1 explained 37.5% of this variation and was strongly related to collateral damage (ΔROM, r=−0.86) and mitigation (ΔTAC, r=0.78) factors. PC2 explained an additional 26.0% of the variation, mostly capturing energetic (r=0.83) and nutritional (r=0.56) costs. Individual variation in collateral damage costs (PC1 scores) was predicted by dfM ($F_{1,23}$=6.0, P=0.02) but not *TLR-4* expression ($F_{1,23}$=0.08, P=0.79); birds from the newer populations were better at damage control than birds from the oldest one (Fig. 5A). dfM was marginally, but non-significantly ($F_{1,23}$=3.6, P=0.07), predictive of variation in PC2 scores. *TLR-4* expression, however, predicted PC2 variation ($F_{1,23}$=4.5, P=0.02); more *TLR-4* expression was related to lower energy and nutrient costs (Fig. 5B).

DISCUSSION

Inflammatory responses could be important in vertebrate range expansions for at least two reasons (Blossey and Notzold, 1995). First, reductions could liberate resources for traits more conducive to pressing challenges in new areas (e.g. finding food and mates and securing shelter) (Brace et al., 2015). Second, colonizers are more likely than core individuals to encounter novel threats (Dobson and Hudson, 1986), which might require range-edge individuals to maintain more robust, broadly protective defenses or succumb to unfamiliar infections (Lee and Klasing, 2004; Martin et al., 2010). At population cores, parasites should behave (relatively) as dear enemies (Prenter et al., 2004), such that generations of prior exposure would have selected hosts for low-cost types of defense (Labbé et al., 2010), parasites for reduced virulence (all else being equal), or both. Here, we found evidence for variation in costs of inflammation among Kenyan house sparrows. However, costs were linked to *TLR-4* expression, the trait previously argued to have facilitated the range expansion, in a complex way.

Population differences in inflammation costs

Only a handful of studies have evaluated the role of immunity costs in range expansions, and, to date, there has been little consensus

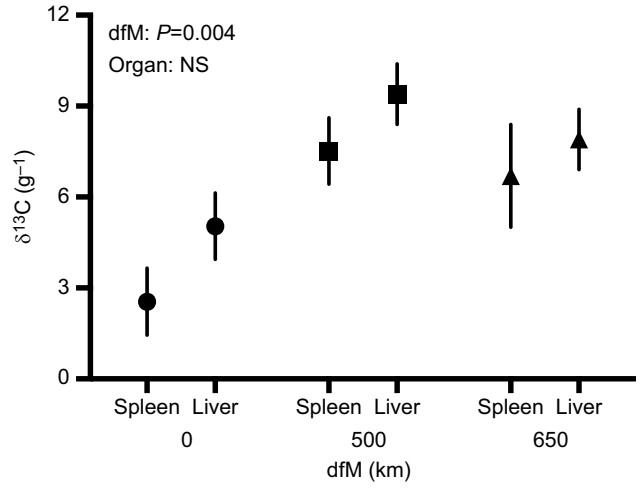

Fig. 3. Effects of dfM on leucine incorporation into organs in Kenyan house sparrows. Bars are means±1 s.e.m.

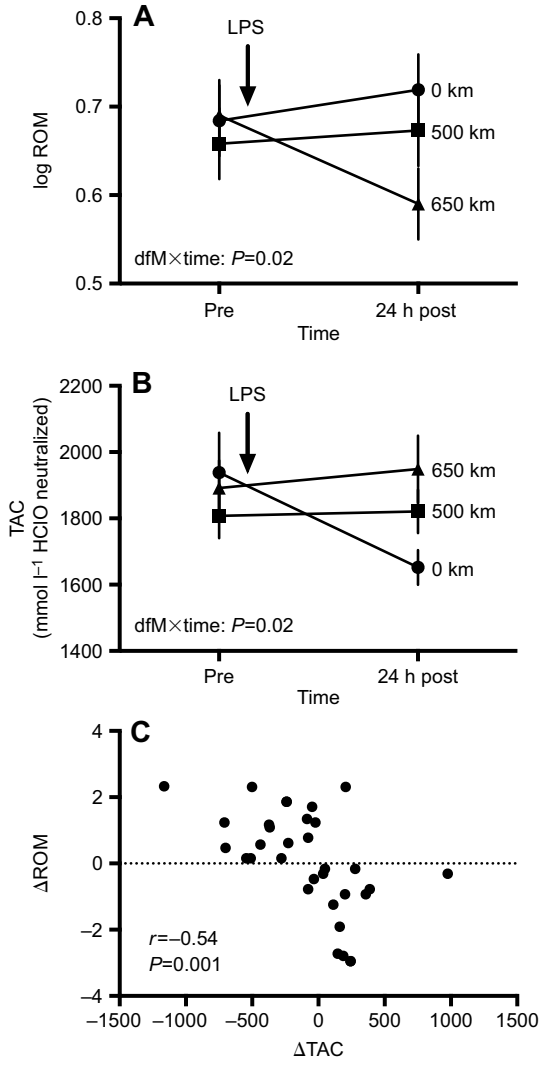

Fig. 4. LPS elicitation of collateral damage (reactive oxygen metabolite) and mitigation (total antioxidant capacity) in Kenyan house sparrows. (A) Reactive oxygen metabolites (ROM, mmol l^{-1} H_2O_2 equivalents). (B) Total antioxidant capacity (TAC). Responses in A and B vary contingent on dfM. (C) TAC and ROM responses to LPS are inversely related among individuals. Bars are means±1 s.e.m.

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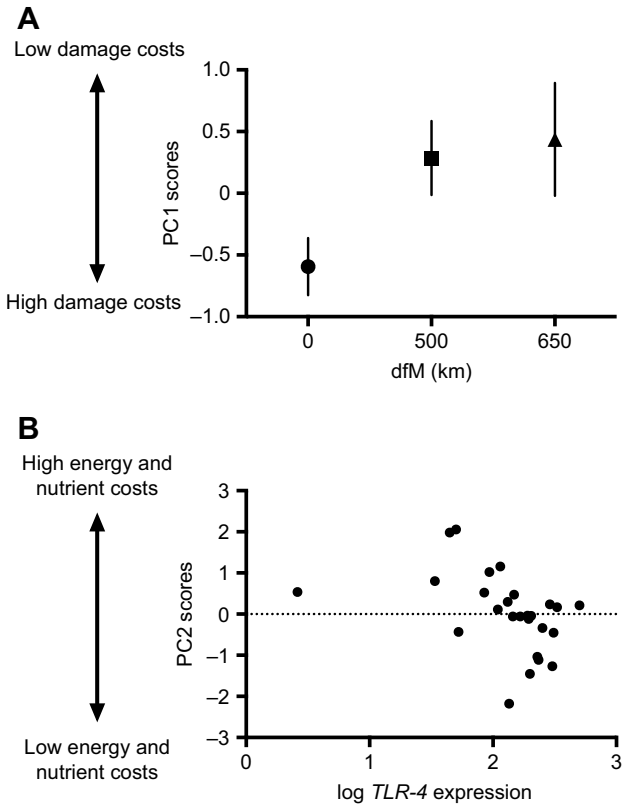

Fig. 5. Significant associations among principal component (PC) scores capturing inflammatory cost variation in Kenyan house sparrows. (A) dfM predicted oxidative damage costs and mitigation thereof (PC1 scores). (B) TLR-4 expression (μg^{-1} cDNA) from whole blood predicted energetic and nutritional costs (PC2 scores) among all sparrows.

(White and Perkins, 2012). In one comparison, songbird (*Passer*) congeners that were more or less successful at colonizing St Louis, MO, USA, over a similar time span also differed in how induced inflammation affected reproductive success. The more successful invader, the house sparrow, was unaffected by simulated microbial exposure but the same challenge halved reproductive output in the less successful species, the tree sparrow, *P. montanus* (Lee et al., 2005). An additional physiological study of the two species revealed that an inflammatory stimulus did not affect energy expenditure in the house sparrow, but it reduced it in the tree sparrow (Lee et al., 2005). Even within species, the roles of energetic costs of immunity in range expansion remain unresolved; at the southern edge of the North American range (Panama, where birds arrived in the 1980s), the cost of an inflammatory response was 7 times what it was from New Jersey house sparrows, where they have occurred for >150 years (Martin et al., 2006). In cane toads spreading across Northern Australia, smaller energetic costs of inflammation were observed at the range edge (Llewellyn et al., 2012). One reason for the inconsistencies among studies might be the ability of hosts to compensate by reducing activity or otherwise adjusting their calorie budgets; there is some evidence that Kenyan house sparrows manage their body reserves differently from a native *Passer* species when infected (Martin et al., 2010).

The second resource cost we considered was critical amino acids, and we found high deposition of leucine in lymphoid tissues in range-edge sparrows. As leucine must be obtained by foraging, cost compensation is much more challenging for critical amino acid costs than energetic costs (McCue et al., 2011). This pattern thus suggests that either range-edge birds value lymphoid tissues more than core birds or range-edge birds have a greater propensity to shunt leucine to lymphoid tissues than core birds. Unfortunately, study design constraints prevented us from resolving a key assumption of leucine assimilation: whether greater deposition at range edges is protective against infection. Future work could also better capture leucine metabolism dynamics, including assembly and export of defensive proteins (Iseri and Klasing, 2013). In another species, however, leucine assimilation scaled with parasite exposure (i.e. LPS dose), which partly supports our interpretation of more leucine assimilation as a more costly immune response (Brace et al., 2015).

Our third cost estimate involved collateral damage, and these costs of inflammation too appeared to play a role in the distribution of house sparrows in Kenya. Birds from the range edge mitigated reactive oxygen damage (ROM) without sacrificing antioxidant capacity (TAC), whereas core birds appeared to have to expend their antioxidant capacity to avoid damage (Haussmann et al., 2011). Collectively, these data indicate that populations might have diverged already in how they cope with collateral damage. However, the house sparrow, as a species, might be unique in terms of how it deals with oxidative damage during inflammation. Compared with domestic chickens (*Gallus gallus*) (Treidel et al., 2013) and prairie voles (*Microtus ochrogaster*) (Fletcher et al., 2015), two species measured in the same lab as the birds here, TAC was 10 times higher and ROM was 2 times lower prior to LPS challenge in Kenyan house sparrows. Thus, an alternative explanation for the pattern observed in Kenya is that there was strong selection for oxidative damage mitigation at the time of the initial introduction to Mombasa, and only those birds with robust TAC and/or modest ROM responses persist today. Compared with other species, Kenyan sparrows appear to maintain an exceptional collateral damage mitigation capacity.

The role of *TLR-4* expression in Kenya house sparrow expansion

We expected birds at the range edge to express high *TLR-4*, as we have observed twice previously (Martin et al., 2014a,b). However, *TLR-4* expression did not track population age significantly here; if anything, the trend was reversed from what we observed before. Several studies have found evidence of selection on *TLR* in wildlife over comparably small spatiotemporal scales (White et al., 2013). However, most of that work involved variation in the sequence of leucine-rich repeat regions of TLRs, not sequences influencing the regulation of expression (Sironi et al., 2015). Across birds, extensive evidence for episodic positive selection on TLR-4 leucine-rich repeat regions has been observed (Grueber et al., 2014). On a scale more comparable to our study, a genetic bottleneck at the time of introduction influenced variation in the *TLR-4* exon sequence in a passerine introduced to New Zealand (Grueber et al., 2013). The only study so far to consider selection on drivers of gene expression in wild animals involved the mannan-binding lectin promoter in roe deer (*Capreolus capreolus*) (Quéméré et al., 2015). In that system, there was some evidence for selection over the course of a range expansion. An altogether different perspective for our data, then, is that neutral population-genetic processes might have led to the accumulation of particular genotypes on range edges (Shine et al., 2011). Thus, the gene expression patterns we observed now and previously might not be functional, but rather artifacts of how genes have combined as birds have moved (and been moved) across the country (Schrey et al., 2014).

A second possible explanation for inter-study inconsistency in *TLR-4* expression involves plasticity, either adaptive or maladaptive

(Ghalambor et al., 2007). A similar argument was offered to explain the high incidence of spinal arthritis in cane toads at the margins of the Australian invasion (Brown et al., 2007). From this perspective, inconsistency in *TLR-4* expression among our studies might represent plastic changes in the defense portfolio of birds driven by differences in the local conditions they encounter (Gervasi et al., 2015). One potentially strong driver could be a parasite that altered gene expression patterns (Charbonnel and Cosson, 2012). Many domestic rodents alter *TLR* expression in response to microbes, viruses or other stimuli (Galic et al., 2009), and even in Kenyan house sparrows, *TLR-4* expression in circulating leukocytes can increase 3-fold in just 4 h post-LPS (Martin et al., 2011). In three-spine sticklebacks (*Gasterosteus aculeatus*), immune gene expression profiles of fish transplanted from one lake to another converged on the profile of the transplanted lake within a few months (Stutz et al., 2015). In the future, we plan to assess the relevance of lability of *TLR-4* expression in Kenya, but samples here were not prepared in the manner necessary to identify the microbes most likely to affect variation in *TLR-4* expression.

Individual predictors of inflammation costs

PCA indicated that *TLR-4* expression was related to commodity costs at the level of individuals, but in the opposite direction to that initially predicted: high *TLR-4* expression was associated with low energetic and nutrient costs. Because we used LPS to incite inflammation, we cannot resolve whether/how these costs relate to infection control, nor can we determine whether measured costs translate to (fitness) costs of ecological significance. Nevertheless, our work hints that intermediate levels of resource expenditure might be most favorable for animals (Long and Graham, 2011; Adelman, 2014): enough of an investment to limit infection but not so much as to require trade-offs with other traits. Damage costs, which appear to mitigate movement distance of individual Australian cane toads at the range edge (Brown and Shine, 2014), were not predicted by *TLR-4* expression, but an influence of site of capture suggests that damage costs warrant additional attention in future work.

Finally, an unexpected discovery offers additional insight into the importance of the energetic costs of inflammation at the level of individuals. Baseline RMR was inversely correlated to changes in RMR after LPS exposure among individuals. Subsequently, the higher RMRs of range-edge birds might cap the magnitude of induced defenses or at least their costs. A similar pattern of high RMR at a range edge was found before in Australian cane toads (Llewellyn et al., 2012) and was argued to arise because of an 'Olympic village' effect. At range edges, strong selection for rapid maturation and prolific breeding may lead to dominance of individuals with particular life histories (Moreau et al., 2011). Associations between RMR and TAC responses to LPS at the individual level too suggest that life on the range edge might have as much to do with general alleviation of oxidative damage as responses to parasites (Rollins et al., 2015).

Conclusions

Our data suggest that costs of inflammation probably have affected house sparrow range expansion in Kenya. Additional studies involving common garden experiments would be insightful, especially if they included populations of different ages and from distinct introduction events (Dunn and Hatcher, 2015). Our approach prevented us from determining whether other site traits better explained inflammatory costs. Likewise, the intriguing intra-individual correlations among costs and *TLR-4* expression coupled with inconsistencies among *TLR-4* expression patterns among years/studies leave unresolved the role of *TLR-4* expression as a driver of success in new areas (Ostfeld et al., 2014; Han et al., 2015). Going forward, it will be useful to determine how the costs and benefits of inflammation in invaders work in concert (or conflict) to mitigate the eruption and spread of parasites in natural and modified systems (Zylberberg et al., 2014; Barron et al., 2015).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: L.B.M., D.R.A.; Methodology: L.B.M., H.J.K., A.B., L.C., M.H., A.M., V.F., S.O., D.R.A.; Formal analysis: L.B.M., D.R.A.; Investigation: L.B.M.; Resources: L.B.M., D.R.A.; Writing - original draft: L.B.M.; Writing - review & editing: L.B.M., H.J.K., A.B., M.H., V.F., D.R.A.; Project administration: L.B.M., D.R.A.; Funding acquisition: L.B.M.

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Data availability

Data are available from the Dryad Digital Repository (Martin et al., 2017): http://dx.doi.org/10.5061/dryad.fn0f4.

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