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Contrasting adaptive immune defenses and blood parasite prevalence in closely related *Passer* sparrows

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Abstract Immune system components differ in their functions and costs, and immune defense profiles are likely to vary among species with differing ecologies. We compared adaptive immune defenses in two closely related species that have contrasting inflammatory immune responses, the widespread and abundant house sparrow (*Passer domesticus*) and the less abundant tree sparrow (*Passer montanus*). We found that the house sparrow, which we have previously shown mounts weaker inflammatory responses, exhibits stronger adaptive immune defenses, including antibody responses, natural antibody titers, and specific T-cell memory, than the tree sparrow. Conversely, tree sparrows, which mount strong inflammatory responses, also mount stronger nonspecific inflammatory T-cell responses but weaker specific adaptive responses. Prevalence of avian malaria parasite infections, which are controlled by adaptive immune defenses, was higher in the geographically restricted tree sparrow than in the ubiquitous house sparrow. Together these data describe distinct immune defense profiles between two closely related species that differ greatly in numbers and distributions. We suggest that these immunological differences could affect fitness in ways that contribute to the contrasting abundances of the two species in North American and Western Europe.

Keywords Immune defense strategies · Malaria parasites · Life history · Inflammation · Natural antibodies · Invasive birds · Cell-mediated · House sparrow · Interspecific comparison

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Introduction

Growing literature support the notion that variations in immune defenses have broad ecological relevance (Norris and Evans 2000; Rolff and Siva-Jothy 2003; Zuk and Stoehr 2002). However, in ecological studies, immunological measurements are still often limited to a single index, which provides only one piece of the puzzle. Different types of immune defense vary in the magnitude and currency of their costs (e.g., energetic, nutritional, developmental) and benefits (e.g., more or less specialized protection, immediately available or inducible) (Klasing and Leshchinsky 1999). It is expected that organisms balance these costs and benefits differently, in a manner dependent upon life history context and selective pressures from pathogens (Klasing and Leshchinsky 1999; Moret 2003). As ecologists strive to integrate immunology, ecology, and evolution, considering the balance between different immune components will help us to interpret the patterns we see within and between species.

Immune defense comprises several independent and interrelated functions. Conventionally, the immune system is divided into innate and adaptive immunity. Innate defenses are constitutive or rapidly inducible, and unlike adaptive defenses do not develop "memory" of specific antigens (Janeway et al. 1999). Therefore, innate defenses are most important against first exposures and quickly growing infections. However, powerful innate immune responses such as the systemic inflammatory response are also costly in terms of nutrients, energy, and behavioral changes (Klasing et al. 1987; Powanda and Beisel 2003), and can be damaging or fatal if overly vigorous or misdirected.

The adaptive immune system is subdivided into antibody-mediated (B-cell) and T-cell-mediated components. In contrast to the inflammatory response, antibody-mediated immunity is not as costly to use once in place, but has greater developmental costs due to the time and nutrients necessary to produce a diverse B-cell repertoire that will allow recognition of a wide variety of antigens (Klasing and Leshchinsky 1999). Humoral immunity includes immediately available, nonspecific "natural" antibodies (mostly IgM), and highly specific induced antibody responses (in birds, IgY). Natural antibodies can act as a first line of defense against viruses and bacteria (Ochsenbein et al. 1999), and induced antibody responses are most effective against prolonged or repeated exposures to pathogens, including bacteria, viruses, and blood parasites (Mims et al. 2001).

T-cell-mediated defenses provide important protection against infections by viruses and intracellular bacteria (Mims et al. 2001), and like specific antibody responses, T-cell memory provides increased protection against second exposures to pathogens (Janeway et al. 1999). The costs of developing the T-cell repertoire are not as well-understood as those of B-cell development, but a similar process of diversification and deletion of self-reactive cells is involved (Janeway et al. 1999). Mounting T-cell-mediated defenses can also be expensive because they are often associated with the secretion of inflammatory cytokines (Janeway et al. 1999), which stimulate local and sometimes systemic inflammatory responses. The immune response assayed by the often-used phytohemagglutination (PHA) test (Smits et al. 1999) is an example of an inflammatory T-cell response (Adler et al. 2001).

As a step toward a more comprehensive understanding of the relationship between immune system components and how they vary between species, we studied two closely related European passerine birds, the house sparrow (*Passer domesticus*) and the tree sparrow (*P. montanus*). These two species are similar in several important ways: both have broad and overlapping native distributions across Eurasia, and are gregarious, largely sedentary, and socially monogamous breeders (Summers-Smith 1988). Both species are human commensals and so likely encounter many of the same pathogens and experience similar selection pressures on their immune defenses. House and tree sparrows also differ in some traits that might co-vary with immune defenses: though not rigorously tested, there is evidence that tree sparrows have lower adult survival rates (34–44%) (Barlow and Leckie 2000) than do house sparrows (~57%) (Dobson 1990; Lowther and Cink 1992). In contrast, tree sparrows appear to have higher average reproductive rates (5.9 young fledged per pair per year) than house sparrows (4.5) (Summers-Smith 1988). The two species also differ significantly in numbers: the widespread and abundant house sparrow numbers around 150 million in North America and between 60 and 130 million in Europe, compared with 25,000 tree sparrows in North America and 26–48 million in Europe (BirdLife International 2004).

In a previous study we showed that North American house sparrows had no measurable metabolic, behavioral, or reproductive responses to inflammatory challenges, while tree sparrow responded with decreased metabolic rates during daylight hours, decreased locomotor activity, and decreased egg production. These results led us to conclude that tree sparrows mount stronger inflammatory responses than house sparrows, and that the fitness consequences of this strategy, such as compromised reproductive output, could influence their current population sizes (Lee et al. 2005).

Here we ask whether tree and house sparrows show complementary differences in adaptive immune defenses. Because tree sparrows appear to have a more robust inflammatory response than house sparrows (Lee et al. 2005), and are perhaps less likely to encounter pathogens more than once during their shorter lifetimes, we expected that they would rely less heavily on specific, adaptive immune defenses than house sparrows. To test this prediction we compared antibody-mediated defenses and both specific and nonspecific T-cell-mediated immune responses in the two species.

As indices of antibody-mediated defense we measured natural antibody levels and specific antibody responses to two different challenges. We expected both of these measures to be greater in house sparrows if they do in fact rely to a greater degree on adaptive immunity. To contrast nonspecific and specific T-cell-mediated defenses, we compared the T-cell response to PHA, which bypasses specific antigen recognition and is associated with systemic

inflammation (Adler et al. 2001), with antigen-specific T-cell memory to keyhole limpet hemocyanin (KLH), which tends not to be inflammatory (Falcone and Bloom 1997). We predicted that tree and house sparrows' nonspecific T-cell-mediated inflammatory responses to PHA would differ in the same direction as the nonspecific inflammatory response we reported earlier (Lee et al. 2005), with house sparrows having weaker responses compared with tree sparrows. Conversely, we expected that, like specific antibody responses, specific T-cell memory responses to KLH would be greater in house sparrows.

Finally, we measured the prevalence of malaria blood parasites in sympatric populations of house and tree sparrows to assess the species-level relationship between infection and immune parameters. Low-level blood parasite infections are commonly reported in passerine birds (Booth and Elliott 2002; Waldenstrom et al. 2002), and the ability to clear such infections in mammals is linked to antibody-mediated immunity (Taylor-Robinson 1995). Therefore, if house sparrows do have stronger adaptive immune defenses, they would be expected to have lower blood parasite prevalences than tree sparrows. Here we show that several immune system components and blood parasite infections differ in predicted ways between two closely related passerine bird species with differing distributions and life history parameters.

Methods

Bird capture and housing

We used mist nets to capture house and tree sparrows from sympatric populations in and around St. Louis, MO, USA (36°N, 90°W) and Meredosia, IL, USA (40°N, 90°W) during July 2002 and January 2003. Birds were individually housed during all captive experiments except in Experiment 1, during which they were housed in pairs due to a limited number of cages. Birds had ad libitum access to food (Kaytee Supreme Wild Bird Seed, Kaytee Products Inc., Chilton, WI, USA, supplemented with hard-boiled chicken eggs and, in captive short-day birds only, a chick starter mash from Agway Feed and Nutrition, Minneapolis, MN, USA) and water throughout the experiments. We measured humoral and cell-mediated immune defenses of wild-caught birds during the breeding season (photoperiod 14L:10D, "long-day" birds), and captive birds experiencing short-day conditions (10L:14D, "short-day" birds) after the completion of fall molt.

Experiment 1: primary antibody responses of long-day birds to KLH

We captured house (n=9, four females and five males) and tree sparrows (n=7, four females and three males) in July 2002 and took a blood sample for measurement of baseline antibody levels within 48 h. We injected each bird intraperitoneally with 10 μg of keyhole limpet hemocyanin (KLH, Sigma H7017, Sigma-Aldrich, St. Louis, MO, USA) in 50 μl of saline (a dose of approximately 0.45 μg KLH/g body weight). This dosage is lower than but comparable to that used in other published studies (e.g., 25 μg injected into 20 g tree swallows *Tachycineta bicolor* (Hasselquist et al. 2001)). To determine the time course of the primary antibody response, we took blood samples at 9, 12, and 15 days after injection. In all experiments we took approximately 50–100 μl of blood per sampling, which falls within the American Ornithologists' Union guidelines of not more than 2% of the body weight of a bird within a 14-day period (Gaunt and Oring 1999).

Experiment 2: primary and secondary antibody responses of short-day birds to KLH

House (n=14, seven males and seven females) and tree sparrows (n=14, eight males and six females) captured in Illinois and Missouri in July 2002 (but not used in previous experiments) were transported back to our laboratory in Princeton, NJ and held under natural light cycle and constant temperatures (25 °C). Two months after all birds had completed fall molt (January, photoperiod 10L:14D), we injected eight individuals of each species (four male and four female tree sparrows, three male and five female house sparrows) intraperitoneally with KLH (using a higher dosage of 4 μg KLH/g body weight because tree sparrow responses were low in Experiment 1) and seven of each species with saline (controls). We took baseline blood samples before injection, and then sampled on days 5, 10, 15, and 25 post-injection for measurement of the primary antibody response. Six weeks after the first injection, we injected all birds with KLH (n=14 of each species) and sampled blood before and 3, 6, and 9 days after injection. All birds received the second KLH injection so we could make certain that the responses of previously sensitized birds were higher than those of birds being simultaneously challenged with KLH for the first time.

The average primary antibody response to KLH peaked on day 10 (data not shown), so we combined these data with the primary response data from control birds that received only the second KLH injection.

Therefore, primary antibody responses are shown on days 0, 5/6, and 9/10.

Experiment 3: antibody responses of short-day birds to sheep red blood cells

We injected short-day house (n=15, eight females and seven males) and tree sparrows (n=16, seven females and nine males) not used in Experiment 2 with 50 µl of a 10% suspension of sheep red blood cells (SRBC, Sigma R3378) in saline, and five individuals of each species with saline alone. Because in Experiment 2 no primary antibody response was detectable by day 5, we took blood samples on days 0, 10, and 15 following the first injection. Eight weeks following the first round of injections, we injected all birds a second time with SRBC, and took blood on days 0, 3, 6, and 9 to measure the secondary antibody response.

Antibody assays

We used an enzyme-linked immunosorbent assay (ELISA) to measure KLH-specific antibodies (Hasselquist et al. 1999). Briefly, 96-well plates (Costar, Cambridge, MA, USA) were coated with KLH antigen, and plasma samples were added to each well and incubated overnight at 4 °C to allow the KLH antibodies in the sparrow plasma to bind to the antigen. Plates were then washed to remove unbound plasma components, and a secondary rabbit anti-Red-winged Blackbird KLH Ig antiserum (diluted 1:1,000; recognizes both IgY and IgM) that binds to the bound sparrow antibodies was added to each well. Following 1 h of incubation at 37 °C and a wash, peroxidase-labeled goat-anti-rabbit serum (1:2,000 dilution; Sigma #A6154) was added to the wells to label the bound anti-KLH Ig antibodies. After a secondary incubation (45 min at 37 °C) and a final wash, 2,2-azino-bis-3-ethylbenzthialzoline-6-sulfanic acid (ABTS; Sigma A1888) and peroxidase were added to the wells, and the plates were then immediately transferred to a Molecular Devices (Sunnyvale, CA, USA) V_{max} kinetic reaction ELISA reader. The peroxidase allows detection of the magnitude of the antigen-bound sparrow antibodies, which is proportional to a change in color in each well. Plates were read every 30 s for 12 min using a 405 nm wavelength filter. Antibody concentrations were calculated according to the slope of substrate conversion over time in units of 10^{-3} optical densities (OD) per minute (m_{OD} min^{-1}), with a higher slope indicating a higher titer of anti-KLH antibodies in the sample. All samples were run in duplicate, and the average of the two readings was our measure of antibody levels in the serum. The coefficient of variation between replicates was 1.1±0.3%. This assay has been used with success in house sparrows (Martin et al. 2006b) and other passerine species (Hasselquist et al. 2001; Ilmonen et al. 2000). All samples in these assays were coded and measured by a person unaware of the aim of this study.

We measured antibody responses to SRBC using the hemagglutination method described by Fairbrother and Fowles (1990). Briefly, plasma was heat-treated at 56 °C for 30 min to inactivate complement, then incubated at 37 °C for 60 min with 0.2 M 2-mercaptoethanol (2-ME) to inactivate nonspecific antibodies (IgM); thus values reported here are for SRBC-specific antibodies (IgY) only. Each plasma sample was then serially diluted twofold (from 1/2 to 1/2,048) in a 96-well U-bottom microtiter plate, and 20 µl of a 1% suspension of SRBC in saline were added to each well. The coefficient of variation between replicates in this assay was 15.6±2.2%. Values are shown as 1/D, where D is the highest dilution at which agglutination was observed; for example, a sample showing agglutination at a dilution of 1/4 is given the value of 4.

We measured natural antibodies in plasma collected prior to challenge from house (n=24, 13 females and 11 males) and tree sparrows (n=27, 16 females and 11 males) used in Experiments 1–4 (from both long- and short-day birds) following a published method (Matson et al. 2005). The procedure for measuring natural antibodies is similar to that described above for the SRBC antibody assays: plasma is serially diluted twofold with saline in a 96-well assay plate, and incubated with red blood cells (rabbit red blood cells, Hemostat Laboratories R59169, Dixon, CA, USA) for 90 min at 37 °C. To facilitate comparison with other studies, we report the $-\log_2(D+1)$ values, where D is the highest dilution at which agglutination was observed.

Experiments 4 and 5: PHA challenges

We injected 100 µl of 1 mg/ml PHA (PHA-P, Sigma L-9017) in saline subcutaneously into the wing web of long-day (n=10 tree sparrows, seven females, three males; n=14 house sparrows, seven males, seven females) and short-day birds (n=8 tree sparrows, two females, six males; n=8 house sparrows, three females, five males) following a standardized method (Smits et al. 1999). This dosage is commonly used in studies of house sparrows (Martin et al. 2003). PHA injected subcutaneously causes edema and T-cell-mediated infiltration of the tissue by granulocytes, macrophages, and lymphocytes (Martin et al. 2006a; McCorkle et al. 1980). We measured wing web thickness to the nearest 0.025 mm at the injection site prior to injection and

then at 24 and 48 h following injection using a pressure-sensitive spessimeter (I.P.S. Tools, El Monte, CA, USA). Within-individual measurement variation averaged 3.8±0.4%.

Experiment 6: T-cell memory responses of short-day birds to KLH

We gave a sensitization injection of 50 µl of 1 mg/ml KLH in saline subcutaneously in the wing webs of short-day house (n=8, four males and four females) and tree sparrows (n=8, four males and four females), or a control injection of saline (n=4 of each species). Two weeks later we injected all birds with the same dose of KLH. This dose is similar to that used previously to elicit a T-cell memory response to KLH in house sparrows (Martin et al. 2006a, 2006b). We measured wing web thickness at the injection site 24 h following the second KLH treatment using a pressure-sensitive spessimeter. Localized swelling 24 h after injection of an antigen to which the individual is sensitized is a type IV delayed-type hypersensitivity reaction (DTH), and is considered a indication of T-cell memory of the antigen (Janeway et al. 1999; Martin et al. 2006a, 2006b).

Blood parasite screening

We screened sparrow blood samples (n=38 house sparrows, 21 females and 17 males; n=30 tree sparrows, 16 females, 10 males, 4 unknown) for avian malaria (*Haemoproteus* and *Plasmodium* spp.) following a published method (Fallon et al. 2003). Briefly, blood samples were stored in Puregene cell lysis buffer, and DNA was extracted by salt precipitation according to the manufacturer's protocol (Gentra Systems, Minneapolis, MN, USA). All samples were screened for infection using a PCR assay based on a conserved RNA-coding region of the 6 kb mitochondrial genome of avian malaria parasites (Fallon et al. 2003).

Statistical analyses

We tested for species, treatment, and sex effects on the time courses of antibody production to KLH (Experiments 1 and 2) and wing web swelling in response to PHA (Experiments 4 and 5) using repeated-measures ANOVA. When the sex factor was nonsignificant it was removed from the analysis. We used Mann–Whitney U-tests to compare house and tree sparrow primary antibody responses to SRBC, and chi-squared tests to compare the proportion responding, because the data were non-normal (Experiment 3). Secondary antibody responses to SRBC were also non-normal at individual time points, but because peak responses were normally distributed, we used an ANOVA to test for species and treatment effects on the peak response. We used t tests to compare natural antibody titers and T-cell memory responses to KLH (Experiment 6) between species. To test for effects of the different conditions (light cycle and captivity) on natural antibody titers and PHA swelling we used an ANOVA with light cycle and species as factors. We used a chi-squared test to compare the prevalence of malaria blood parasites between species. For all tests we used SPSS 10.0 (SPSS 1999).

Results

Experiments 1 and 2: antibody responses to KLH

In long-day birds, the primary antibody response to KLH differed between house and tree sparrows (time × species term: $F_{(3,42)}$=3.11, P=0.04, Fig. 1a); house sparrows showed an increase in antibody titer over time, while tree sparrows did not. When included in the model, sex had a marginally significant effect on the antibody response (sex effect, $F_{(1,12)}$=4.45, P=0.06), with males of both species tending to have higher antibody titers than females. There was a trend for this pattern to be more pronounced in tree sparrows than house sparrows (sex × species effect, $F_{(1,12)}$=3.74, P=0.08).

In short-day birds we measured both primary and secondary responses to KLH and included saline-injected control groups in the experiment. Birds injected with saline had detectable anti-KLH antibodies, but KLH treatment induced primary antibody responses that were significantly higher than titers of control birds (repeated measures ANOVA, treatment term: $F_{(1,24)}$=6.14, P=0.02). Similar to the results from long-day birds, the pattern of the primary antibody response over time differed significantly between species (time × species $F_{(2,52)}$=4.76, P=0.01, Fig. 1b), with house sparrows again showing a more rapid increase in antibodies. There was no effect of sex on the primary antibody response in either species (sex: $F_{(1,24)}$=0.07, P=0.79; species × sex: $F_{(1,24)}$=0.38, P=0.55).

Birds that were previously sensitized had significantly stronger antibody responses to KLH than those exposed for the first time, demonstrating that birds developed immunological memory to KLH (treatment term: $F_{(1,24)}$=33.88, P<0.01, Fig. 1c compared with Fig. 1b). House sparrows mounted a significantly stronger secondary antibody response than did tree sparrows (species term: $F_{(1,14)}$=10.04, P<0.01, Fig. 1c); but the pattern of the response over time did not differ between species (time × species term: $F_{(2,28)}$=0.49,

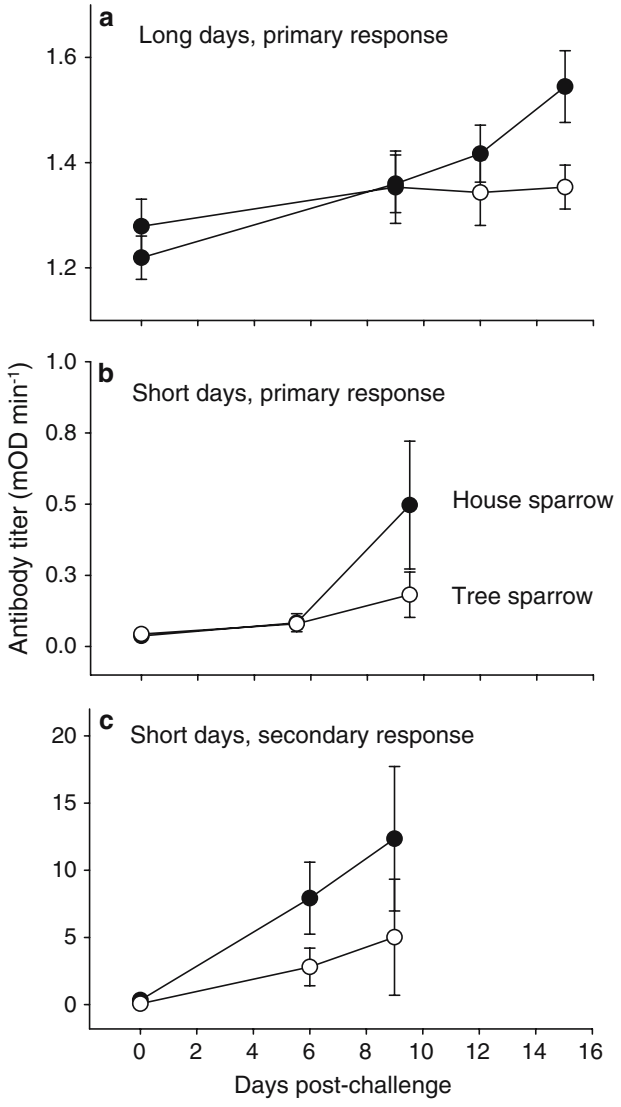

Fig. 1a–b Antibody responses (±1 SE) to KLH (keyhole limpet hemocyanin) of house sparrows (*Passer domesticus*) (*filled circles*) and tree sparrows *(P. montanus)* (*open circles*) during long days (primary responses, **a**) and short days (**b** primary responses, **c** secondary responses). Primary responses in **a** and **b** were measured in different assays and absolute values should not be compared

P=0.62). There was no effect of sex on the secondary antibody response in either species (sex effect: $F_{(1,12)}$=0.01, *P*=0.93; species × sex: $F_{(1,12)}$=0.90, *P*=0.36). Because we used different KLH doses and different between-ELISA plate standards for short- and long-day birds, the results were not comparable and we could not test for differences between them.

Experiment 3: antibody responses to SRBC

We measured anti-SRBC antibody titers in short-day birds injected with either SRBC or saline. Some saline-injected birds were able to agglutinate SRBCs at some time points, and many SRBC-injected birds mounted weak or undetectable primary antibody responses. Cross-reactive antibodies in saline-injected birds could have developed in response to the chick starter mash mixed with the birds' seed that contained mammal protein sources. Naturally occurring red blood cell agglutinins do occur in birds, and these are not always deactivated by 2-ME treatment (Matson et al. 2005). We could not compare the two species' antibody responses over time using repeated-measures ANOVA because the data were non-normal. The mean peak primary antibody response to SRBC of house and tree sparrows did not differ significantly from titers of control birds (*U*=25.50, *P*=0.28 and *U*=29.50, *P*=0.37, respectively), but more house sparrows (7 of 15: 47%) than tree sparrows (2 of 16: 13%) mounted a response that was outside of the 95% confidence interval of the mean control or "background" value (χ^2 =4.39, *P*=0.04). The number of tree sparrows mounting a primary response to SRBC was too small to allow comparison of the magnitude of responders' titers between species.

A greater proportion of tree sparrows mounted secondary antibody responses than had mounted primary responses: 5 of 15 (33%) of tree sparrows compared with 13% primary responders. The proportion of house sparrows mounting a secondary response was the same as the proportion that had mounted a primary response (7 of 15: 47%). These proportion of individuals mounting a secondary antibody response to SRBC did not differ significantly between species (χ^2=0.56, *P*=0.46). Similar to the primary responses, the secondary antibody responses to SRBC were not normally distributed, so we could not use repeated-measures ANOVA to compare the patterns of the response over time between species. Figure 2 shows the time courses of the secondary antibody responses to SRBC in house and tree sparrows. Peak titers did not differ between species (t_{29}=0.49, *P*=0.63). Numbers of individuals responding were too small to allow testing for effects of sex.

Experiments 4 and 5: PHA challenges

The pattern of the PHA-induced wing swelling over 48 h tended to differ between long-day house and tree sparrows, but not significantly (time × species term: $F_{(2,44)}$=2.78, *P*=0.07); wing web swelling tended to decline faster after 24 h in house sparrows than in tree sparrows (Fig. 3a). There was no significant effect of sex on wing web swelling in long-day birds (sex term: $F_{(1,20)}$=0.09, *P*=0.77).

In both species, responses to PHA were higher under short-days compared with long-days (photoperiod term: $F_{(1,36)}$=18.83, *P*<0.01; species × photoperiod term:

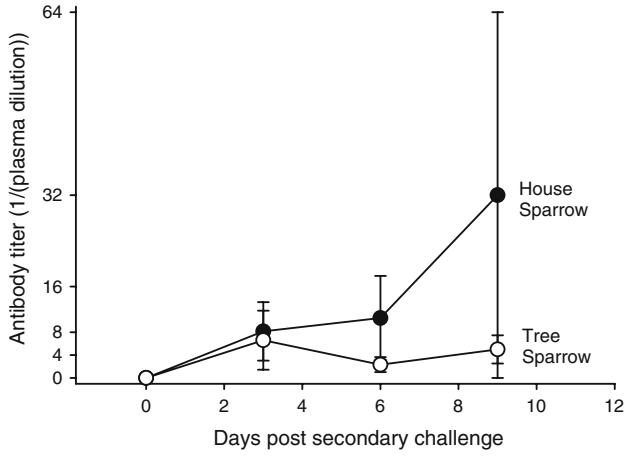

Fig. 2 Secondary antibody responses (±1 SE) to SRBC (sheep red blood cells) during nine days following challenge of house (*filled circles*) and tree sparrows (*open circles*) held on short days

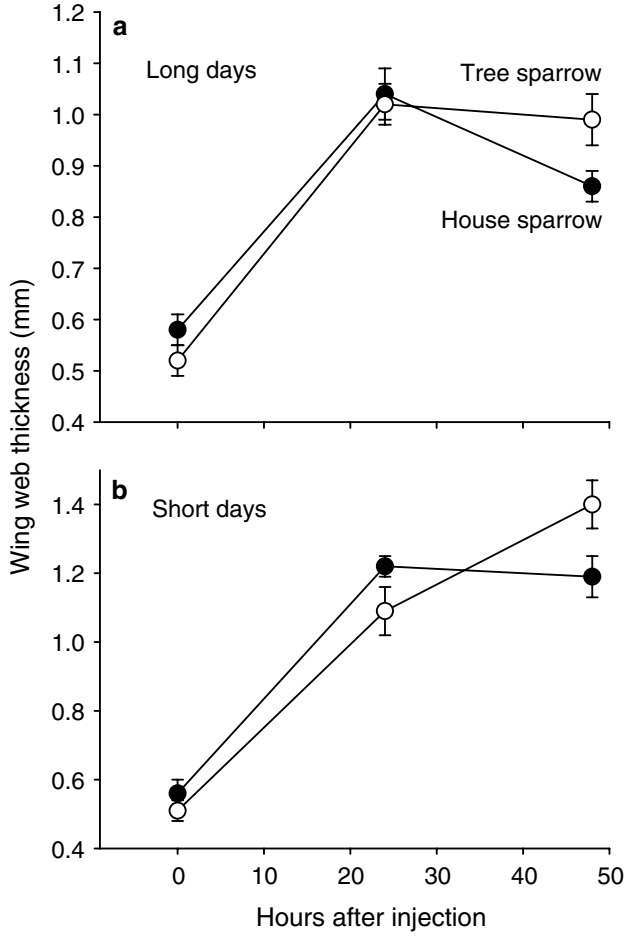

Fig. 3a–b Wing web thickness (±1 SE) in **a** long-day and **b** short-day house sparrows (*filled circles*) and tree sparrows (*open circles*) in response to PHA (phytohemagglutinin)

$F_{(2,36)}$=0.30, P=0.74, Fig. 3a,b). In short-day birds, the time course of PHA-induced swelling differed significantly between species (time × species: $F_{(2,28)}$=5.71, P<0.01); tree sparrows' swelling response to PHA continued to increase 24–48 h following injection, while the response did not change after 24 h in house sparrows (Fig. 3b). The number of female tree sparrows was too small to test for an effect of sex on the PHA response in this species, but in house sparrows males mounted stronger responses than females (sex term: $F_{(1,6)}$=6.74, P=0.04). This difference was driven by a continuing increase in swelling in males after 24 h but a decline in females (sex × time term: $F_{(1,12)}$=4.49, P=0.04).

Experiment 6: T-cell memory to KLH

Short-day sparrows previously sensitized subcutaneously to KLH mounted a significantly greater swelling response when injected again with KLH than did naïve birds, indicating that treatment birds had developed immunological memory to KLH (treatment term: $F_{(1,20)}$=4.96, P=0.04). The T-cell mediated memory response to KLH was significantly greater in house sparrows (swelling 0.15±0.03 mm) than tree sparrows (0.03±0.02 mm) (species × treatment term: $F_{(2,20)}$= 5.64, P=0.01), but there was no significant effect of sex on the T-cell memory response (sex effect: $F_{(1,12)}$=1.50, P=0.25; species × sex: $F_{(1,12)}$=1.50, P=0.25).

Natural antibodies

House sparrows had significantly higher natural antibody titers than did tree sparrows (4.24±0.28 vs. 2.88±0.39, t_{40}=2.92, P<0.01). Natural antibody titers measured in long-day versus short-day birds did not differ (ANOVA, light cycle term: $F_{(1,38)}$=0.42, P=0.52). There was no effect of sex on natural antibody titers in either species (sex: $F_{(2,37)}$=0.05, P=0.96; species × sex: $F_{(1,37)}$=0.66, P = 0.42).

Blood parasites

Malaria parasite prevalence was significantly higher in tree sparrows (60%, n=30) than in house sparrows (29%, n=38) (χ^2=6.61, P=0.010). In neither species did infection prevalence differ between sexes (house sparrows, χ^2=0.02, P=0.88; tree sparrows χ^2=2.10, P=0.15).

Discussion

Antibody-mediated defenses of house and tree sparrows

We predicted that house sparrows, which appear to invest less in nonspecific inflammatory responses (Lee

et al. 2005), should instead invest more in specific defenses capable of memory. Our results show that house sparrows mount stronger antibody responses to KLH when held in captivity under short days, and more rapid antibody responses to KLH both during long-term captivity and when wild-caught during long days, compared with tree sparrows from the same geographical region. Furthermore, a greater proportion of house sparrows mounted primary antibody responses to SRBC compared with tree sparrows, although response frequency was low, and secondary responses to SRBC did not differ between species. House sparrows also had higher levels of constitutive natural antibodies, which are relatively nonspecific in nature but, like induced antibody responses, not inherently inflammatory. Stronger antibody-mediated immunity might allow house sparrows to avoid mounting strong inflammatory responses. Additionally, if house sparrows are in fact longer-lived, B-cell memory could be favored more in house sparrows compared with tree sparrows if the likelihood of multiple challenges by the same pathogen increases with lifespan.

T-cell-mediated responses

Previously we showed that tree sparrows have stronger behavioral and reproductive responses to inflammatory stimuli compared with house sparrows (Lee et al. 2005). Here a comparison of the species' T-cell-mediated responses complements these results: tree sparrows mounted a longer-lasting nonspecific swelling response to PHA than did house sparrows, although this difference was significant only in short-day birds. Immune responses are often suppressed in animals during the breeding season (Martin et al. 2004; Nelson and Demas 1996), and if long-day birds of both species were immunosuppressed this could have contributed to the smaller interspecific differences in these compared with birds experiencing short days.

In contrast to the PHA results, house sparrows exhibited stronger T-cell-mediated memory to KLH than did tree sparrows. While PHA causes inflammation by nonspecifically stimulating many T-cell lines (Janeway et al. 1999), developing a T-cell memory response to proteins such as KLH requires antigen processing and presentation; antigens must be presented to T-cells on MHC class II receptors before T-cell clones that recognize the antigen(s) proliferate (Janeway et al. 1999). Thus, in the case of a T-cell memory response, inflammation is more specific and occurs only when an antigen is encountered a second time.

Overall, the T-cell-mediated response data are consistent with our previous results suggesting that tree sparrows rely more on nonspecific inflammatory responses than do house sparrows (Lee et al. 2005). Nonspecific PHA-induced T-cell proliferation, which is associated with systemic inflammation in birds (Adler et al. 2001), was more robust in tree sparrows compared with house sparrows, but specific T-cell memory was weaker. The lack of T-cell memory to KLH seen in tree sparrows could result from a limited T-cell repertoire arising from inbreeding or from a lesser degree of T-cell diversification during development. This could also contribute to the low anti-KLH antibody response exhibited by tree sparrows.

Blood parasite prevalence

We found a higher prevalence of malaria blood parasites in tree sparrows compared with house sparrows in North America (60% compared with 29%). These percentages are high compared with those reported for Europe, perhaps reflecting the greater sensitivity of PCR detection methods compared with visually scanning blood smears (Richard et al. 2002). Our PCR test did not allow us to distinguish between blood parasite genera; however, in Europe, house and tree sparrows have approximately equal prevalences of *Haemoproteus* parasites (13.8 and 12.3%) but *Plasmodium* prevalence is lower in house sparrows (7% compared with 13.2%) (Peirce 1981). High prevalence of infection in tree sparrows might be related to poor antibody responsiveness. In mice, control and clearance of chronic malarial infections is highly dependent on antibody-mediated defenses (Taylor-Robinson 1995). In general, weak antibody responses might render tree sparrows more susceptible to some blood parasites and viruses and result in lower survival rates. It has been shown that blood parasites can depress condition and thus survival or reproduction (Merino et al. 2000). We speculate that stronger specific immune defenses and the resulting lower disease susceptibility could in part contribute to the higher rate of population growth and invasive spread exhibited by the house sparrow in North America (Long 1981), and the greater relative abundance of house sparrows compared with tree sparrows in Europe (BirdLife International 2004). However, differences in parasite prevalence could also be due to differences in exposure, and prevalence data alone do not allow us to distinguish between these mechanisms.

Conclusions: potential ties between immunology, life history, and distributions

In combination with our previous study (Lee et al. 2005), we have shown distinct immune defense profiles and blood parasitism in two closely related passerine

species with contrasting distributions and abundances. The tree sparrow appears to invest less in specific antibody- and T-cell-mediated defenses, and rely instead on developmentally inexpensive yet functionally costly nonspecific responses. This strategy could be linked to the tree sparrow's life history: decreasing reliance on developmentally costly defenses might facilitate the production of a large number of young in a season by decreasing the time and energy or nutrient resources necessary for development of diverse B-cell and T-cell repertoires in the chick. However, mounting strong inflammatory responses could decrease reproductive success when a tree sparrow experiences an acute infection during the breeding season, as suggested by our previous study (Lee et al. 2005). Conversely, the house sparrow, which appears to be slightly longer-lived, has strong specific defenses capable of memory and a less robust inflammatory response. Strong adaptive defenses might help protect house sparrows from repeated or prolonged infections and thus facilitate its greater longevity, but house sparrows' reproductive output might be constrained by the time and energy or nutrients necessary for chicks to develop specific defenses.

The relative successes of these differing defense strategies in North America could in part explain the much greater abundance of the house sparrow compared with the tree sparrow. There is no such comparative study of the immune defenses of the two species in Europe, but there are data suggesting that immune defenses of European house sparrows might be characterized by a stronger inflammatory response than we have seen in North American populations (Bonneaud et al. 2003). If this difference is real, it could reflect differing selection pressures on defenses in the house sparrow's native and introduced ranges.

We studied single populations of only two species, so our results must be interpreted cautiously; however, the patterns we observed are largely consistent with the expectation that immune defense components should co-vary in complementary ways that are understandable in the context of species' ecologies and life histories.

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