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Lynn B. Martin II · Dennis Hasselquist
Martin Wikelski

Investment in immune defense is linked to pace of life in house sparrows

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Abstract The evidence for a relationship between life history and immune defense is equivocal, although the basic premise is intuitively appealing: animals that live short lives and reproduce early and rapidly should not waste resources on defenses they might never use. One possible reason for a lack of strong support for this hypothesis could be the inherent complexity of the vertebrate immune system. Indeed, different components of the vertebrate immune system vary in their relative costs and benefits, and therefore only some defenses may complement variation in species' life history. To address this hypothesis, we compared multiple types of immune activity between two populations of house sparrows (*Passer domesticus*) with distinct life histories, one from Colon, Panama, which lay small clutches over an extended breeding season (i.e., slow-living) and the other from Princeton, New Jersey, which lay larger clutches in a smaller window of time (i.e., fast-living). We expected (a) that more costly types of immune defenses would be stronger in the slow-living sparrows and (2) that the slow-living sparrows would show a greater increase in whole-body energy expenditure after immune challenge compared to their fast-living counterparts. We found that secondary antibody response to a novel antigen was more rapid and energetic investment in immune activity was greater in slow-living sparrows. However, cell-mediated immune activity was more robust in fast-living sparrows, and other measures of defense were not different between populations. These results provide partial support for a relationship between life history and immune defense in this species, but they also indicate that this relationship is not clear-cut. Further study is necessary to identify the influence of other factors, particular pathogen environment during development, on the architecture of the immune system of wild animals.

Keywords Humoral · Immunocompetence · Innate · Passerine · RMR · Trade-off

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L. B. Martin II (✉) · M. Wikelski
Department of Ecology and Evolutionary Biology,
Princeton University, Princeton, NJ, USA
E-mail: lmartin@mail.psy.ohio-state.edu
Tel.: +1-614-5389540
Fax: +1-614-4513116

D. Hasselquist
Department of Animal Ecology, Lund University, Lund, Sweden
E-mail: dennis.hasselquist@zooekol.lu.se

L. B. Martin II
Departments of Psychology and Neuroscience,
The Ohio State University, Townshend Hall,
Columbus, OH 43210, USA

Introduction

The concept of immunocompetence, or the capacity of an organism to prevent or control infection (Owens and Wilson 1999), became popular in the ecological literature when it was first characterized as a commodity that was diminished when animals elevated circulating testosterone to augment or express their sexual ornaments (Folstad and Karter 1992). To date, evidence corroborating this "immunocompetence handicap hypothesis" is equivocal (Grossman 1985; Hasselquist et al. 1999; Owen-Ashley et al. 2004; Greenman et al. 2005). Still, the conceptualization of immune defense as a malleable investment commodity attracted the interest of ecologists and evolutionary biologists (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Norris and Evans 2000) and led to many new discoveries. One of the most striking discoveries is that immune defenses are quite variable among and within species (Nelson and Demas 1996; Martin et al. 2001; Tella et al. 2002). Historically, it was predicted that the random nature of generating lymphocyte diversity would produce similar levels of defense (per unit body size) among taxa (Cohn and Langman 1990). What remains unclear now is why variability in immune defense exists; presumably all animals would benefit from defense against pathogens at all times of their lives, so why is this not seen in nature?

Mounting evidence indicates that the energetic (Demas 2004), nutritional (Lochmiller and Deerenberg 2000), and immunopathological (Råberg et al. 1998) costs of immune defense may be important. Trade-offs between immune defense and other costly activities appear common in wild species. In passerine birds, induction of immune activity often negatively affects reproductive success (Ilmonen et al. 2000; Råberg et al. 2000; Bonneaud et al. 2003), tissue growth (Martin 2005), and survival (Hanssen et al. 2004). Similarly, the large developmental costs of certain immune defenses, particularly the generation of a diverse T and B cell repertoire, have been proposed to explain why many songbird species maintain long incubation periods in nest-predator rich environments (Ricklefs 1992).

We expect that if such cost-benefit counterbalances explain why organisms can mount robust immune responses at only certain times in their lives, a similar perspective might explain how the immune systems of animals are generally organized. For instance, animals living in parasite-dense environments should invest heavily in immune defense relative to those living in parasite-poor habitats (Piersma 1997). Similarly, animals living relatively slow-paced lives (e.g., animals that mature and breed late and modestly; Wikelski et al. 2003) should invest heavily in immune defense because they are more likely to be exposed repeatedly to pathogens than their fast-living relatives (Klasing and Leshchinsky 1999). Recently, two studies found evidence for relationships between immune defense and pace of life in birds (Martin et al. 2001; Tella et al. 2002). In both cases, species exhibiting life history traits representing a slow pace of life (e.g., long-developmental period, small-clutch size, or large-body size) showed greater levels of one type of immune defense, phytohemagglutinin (PHA) induced wing-web swelling. Recently, we found that the same measure of immune defense varied between populations of house sparrows (*Passer domesticus*) living their lives at different paces. First, we found that Panamanian sparrows, which lay small clutches over a 10-month breeding season (slow-living; Martin et al., submitted), maintained similar levels of PHA responsiveness year-round. Sparrows from New Jersey on the other hand, which lay larger clutches over a shorter breeding season (fast-living; Summers-Smith 1988), showed a reduction in PHA swellings during the height of the breeding season (Martin et al. 2004). Additionally, we found that corticosterone, an immunosuppressant in vertebrates when maintained at high levels for extended periods, did not affect PHA swelling in the slow-living sparrows. However, it did suppress PHA swelling in the fast-living birds (Martin et al. 2005). We interpreted this difference to indicate that slow-living sparrows do not decrease immune investments even when under chronic stress. Although the findings of these studies are intriguing, their reliance on a single immunological measure can only indicate that life history–immunology relationships probably exist. The related possibility, that the pace of life of animals directly shapes their immunological portfolios, remains unresolved.

In this study, we attempted to address this possibility. Specifically, we wanted to determine whether all or only some types of immune activity varied between the same two populations of house sparrows investigated earlier. In other words, we wanted to determine if a slow pace of life was related to greater overall immunocompetence. We relied on the immune-defense component model of Schmid-Hempel and Ebert (2003) to identify the appropriate techniques to use to test this hypothesis. This heuristic model recognizes that immune defenses are complex and can be (a) maintained at a certain level irrespective of the disease environment (constitutive), (b) activated only in response to a disease challenge (inducible), (c) targeted against a particular pathogens (specific) and/or (d) generally responsive to a variety of different threats (non-specific). Further, the Schmid-Hempel and Ebert model (2003) indicates that defenses vary in terms of the resources necessary to develop, use, and/or maintain them (Lochmiller and Deerenberg 2000; Klasing 2002), and each defense type differs in its ability to control a given pathogen challenge (Kaufmann et al. 2002). Finally, the model notes the hierarchical but redundant organization of immune defenses. It recognizes that some defenses may be obsolete if strong defenses are in place upstream, but it also acknowledges that the same endpoint can often be achieved by multiple immunological strategies. In Table 1, we list the types of defenses we measured in our study. In addition to comparing these immune defenses outright, we measured energy expenditure in response to one type of immune challenge in slow and fast-living sparrow populations (Martin et al. 2003). This comparison allowed us to directly test whether slow-living birds invest more energy in defense than their fast-living counterparts over the short term.

Materials and methods

Field sites and study species

The house sparrow is a small (~25 g), granivorous passerine found on every continent but Antarctica, in most cases because of human introduction (Summers-Smith 1988). Although house sparrows are not native to the western hemisphere, populations exhibit many of the latitudinal life history clines of indigenous species, including a decrease in clutch size (Summers-Smith 1988), an increase in the length of the breeding season (Summers-Smith 1988), and a decrease in rate of energy turnover (Kendeigh and Blem 1974; Kendeigh 1976) towards the equator. Birds in this study were from a North-temperate site (Princeton, New Jersey, USA: 40°21′N, 74°40′W), and a Neotropical site (Colon, Panama: 9°1′N, 80°1′W; see Martin et al. 2004 for details). In 2003, we characterized the reproductive life history of the Panamanian (slow-living) population and

Table 1 Measures of immune function compared between fast and slow-living house sparrows

Immune defense	Challenge used	Response measured	High response indicates
Constitutive			
Natural antibodies/ Complement	Rabbit red blood cells (RRBCs)	Agglutination of RRBCs Lysis of RRBCs	High surveillance for extracellular parasites
Induced, specific			
T-cell memory	Keyhole limpet hemocyanin (KLH) Intact, heat-killed *E. coli*	Swelling of wing patagium	Strong capacity to respond to previously encountered antigens
Antibody proliferation (B cell)	Keyhole limpet hemocyanin (KLH)	Primary antibody response	Strong capacity to recognize and make antibodies for a novel antigen
		Secondary antibody response	Strong capacity to respond to previously encountered antigens
Energy investment			
Cost of acute phase response	Phytohemagglutinin (PHA)	Change in resting metabolic rate	Large investment in acute phase response

found that these sparrows lay smaller clutches (3.3 eggs/clutch; New Jersey: clutch size = 4.6) and breed over much of the year (10 months; Martin, unpublished data), traits that are distinct from all temperate North American populations studied to date (Summers-Smith 1988). Table 2 presents a more detailed characterization of the reproductive life histories of the slow-living population (Martin et al., submitted) and a population at the same latitude as Princeton, New Jersey (North 1973). To date, we have not characterized the reproductive life history of the fast-living population we have studied, but we have no reason to suspect that it would differ dramatically from other populations at the same latitude (Summers-Smith 1988).

For the duration of all immune assays, birds were held in captivity and receivedad libitum mixed seeds and water every day and boiled, mashed chicken eggs and/or live mealworms (*Tenebrio molitor*) every third day. Photoperiod and temperature were held at ambient levels of each latitude during the natural antibodies, KLH, and RMR comparisons (see below); during *E. coli* DTH challenges, all birds were held on long photoperiods (14L:10D) at 25 ± 2°C and 40 ± 5% R.H. (see additional details below) in climate-controlled rooms in Princeton, NJ. For all immune assays, individual sparrows were used only once unless otherwise noted. We took this approach in case prior immune activation affected subsequent immune responsiveness. All assays were conducted between the months of February and August, as both populations have been found to breed during this period. Generally, we included similar numbers of males and females in each comparison, but because of low sample size, we did not have sufficient statistical power to detect significant effects of sex on our results. Further, we used only birds that showed no signs of molt, as feather growth can affect some types of immune activity in passerines (Martin 2005) or vice versa (Sanz et al. 2004).

We chose the house sparrow as a model species for several reasons. First, by using a single species, we could eliminate phylogenetic artifacts inherent to interspecific studies, and we could standardize our immunological methods (Kreukniet et al. 1994). Second, by using a granivorous human commensal, we could reduce the impact of uncontrollable factors, such as diet (Lochmiller and Deerenberg 2000), on our results. Last, by conducting all experiments in a similar window of time, we could be reasonably sure that immunological differences identified in this study were not due solely to time of year or stress (Nelson 2004; Martin et al. 2004, 2005). We were aware from the outset that our approach (comparing two populations) would not enable us to be sure that immunological differences detected were solely due to life history variation (sensu Garland and Adolph 1994). Still, given (a) the extensive literature on physiological and life history variation in North American house sparrows and (b) the many insightful two-species/two-population comparisons in the past (Weathers and Greene 1998; Klein et al. 1999; Ghalambor and Martin 2000), we felt that our approach would be a useful first step to determining whether and how animals' life histories shape their immune systems.

Table 2 Reproductive life history characters of two populations of house sparrows

Location	Latitude	Breeding season (months)	Clutch size (mean)	Clutches year^{-1}	Eggs female^{-1} year^{-1}	Incubation (days)	Nestling (days)	Hatching (%)	Fledging (%)
Slow-living	9	10	3.4	3.2	10.8	10.5	16.2	0.84	0.86
Fast-living[a]	43	4	5.0	1.5	7.4	11.7	15.4	0.51	0.61

[a]Data from North 1973

Constitutive defenses

Natural antibody levels and lysis activity

We used a rabbit red blood cell (RRBC) agglutination/lysis assay to characterize natural antibody (NAb) levels (predominantly IgM) and complement/NAb-mediated lysis capacity of plasma (Matson et al. 2005). In February–April 2003, we captured wild birds and took 50 µl blood samples from each animal; plasma was then stored at −20°C until assay (January 2004). In each assay, we randomized samples among assay runs and then added 25 µl of plasma from each sparrow and an equal volume of saline to six columns of a flat-bottom 96-well plate. To the remaining two columns, we added saline and a positive control (RRBC-activated chicken plasma collected on heparin: #ES1032P, Biomeda). We then performed a serial dilution of the plasma and the positive standards to the remaining wells on the plate. To the filled plate, we then added 25 µl of a 1% RRBC suspension (washed cells collected on citrate and reconstituted in Alsevers: #wrb100, Hemostat) to all wells except the last row; these wells contained only saline and served as negative controls.

Once a plate was filled, we covered it with Parafilm, placed it on a shaker for 2 min, incubated it in a warm water bath (37°C) for 90 min, then tilted the plate at a 45° angle for an additional 20 min to allow for potential separation of the blood cell pellet. After this period, a photograph of the plate was taken using a scanner connected to a desktop computer. Seventy minutes later, this procedure was repeated. Photographs were taken at these time points in particular to enable to score both agglutination and lysis in the same plasma sample (Matson et al. 2005). Maximum agglutination was scored as the concentration of plasma in which the blood cell pellet remained completely intact after 20 min of tilting. Lysis was scored (90 min post-incubation photo) as the plasma dilution at which >75% RBCs had ruptured. This and all other assays (except KLH antibodies: see below) were conducted blind to treatment by the same person (L. Martin).

Inducible defenses

Cell-mediated immunity (delayed-type hypersensitivity (DTH))

We used two substances to assess T-cell mediated immunological memory, keyhole limpet hemocyanin (KLH) and heat-killed *Escherichia coli* bacteria. Although KLH is usually favored for measuring antibody responses, many substances that possess a diverse complement of epitopes (i.e., antigenic sites) can be used to assay T-cell memory (Turk 1967). We chose these two substances particularly because pilot studies in our lab and previous work from other labs (Smith et al. 2005) indicate that many substances can induce swelling (i.e., T-cell mediated memory). For KLH, birds were captured from the wild (Feb–May 2003) and held in captivity in pairs in cages for 24 h prior to initial injection. To induce hypersensitivity to KLH, we injected 100 µl of a 1 mg ml^{-1} KLH (Sigma H7017) in 0.9% pyrogen-free saline solution into the wing web of each bird 3× over the course of 2.5 months at equal intervals. For the *E. coli* assay, we used the fast-living birds from the preceding KLH assay (after a 3-week recovery period) and slow-living sparrows from a prior common garden experiment (Martin et al. 2004). For *E. coli*, we injected the wing web of each bird with approximately 10 k bacteria suspended in 50 µl saline (Lee et al. 2005). Injections were prepared using dehydrated bacteria (ATCC# 11303) that were re-suspended in broth, incubated at 37°C for 24 h, spun down, washed twice in saline, diluted 1:10, and fixed in 10% formalin overnight. Just prior to all injections and 24 and 48 h after the third injection, we measured wing-web swelling with a Teclock pocket thickness gauge (model: SI-510) to the nearest 0.1″ and later converted these measures to metric units. To ensure that we were measuring T-cell mediated immunological memory, we measured swelling 24 and 48 h after all three injections in the *E. coli* group. We were unable to do the same for the KLH groups because of time constraints (imposed by concurrent field work). For graphical clarity, we report only 24-h swellings; differences in swelling at 48-h intervals were similar.

Humoral immunity (antibody responses)

We used KLH to induce primary and secondary antibody responses in the two sparrow populations; these assays were conducted from May to June 2003. First, we injected the wing web of all birds (KLH groups from above experiment) with 100 µl of a 1 mg ml^{-1} KLH in pyrogen-free saline. This approach enabled us to measure cell-mediated and humoral immune activity in the same bird simultaneously. To assess the primary antibody response to KLH, we took small blood samples (50 µl) from the brachial vein of each bird just before the first injection and 5, 10, and 15 days post-injection; to assess the secondary antibody response, we injected KLH into all birds again (12 days after last blood sample) then took additional blood samples just prior to and 3, 6, and 9 days after this second injection. After collection, plasma was removed from all blood samples, briefly stored on ice, and frozen at −20°C until antibodies were measured (December 2003).

We used a previously developed ELISA assay to measure KLH antibodies in our sparrows (Hasselquist et al. 1999). Briefly, 96-well plates were coated with KLH, plasma from house sparrows was added to each well (in duplicate), then incubated with an anti-red-winged blackbird antibody (produced in rabbits), and a goat–anti-rabbit antibody labeled with peroxidase. All samples in these assays were coded and measured by a person unaware of the aim of this study.

Energy invested in immune defense

Cost of an acute phase response

We measured the energetic cost of one type of immune response to directly determine if investment in immune defense varied between the two populations of sparrows. In February 2003, we captured birds ($n = 7$; three male and four female birds) from the wild in Colon, Panama, and that same night we placed them in 1 l respiratory chambers and measured rates of oxygen consumption. We used these data to calculate resting metabolic rate (RMR, see Martin et al. 2003 for details), the rate of energy expenditure of post-absorptive birds during this quiescent phase (night) under thermoneutral conditions (T_a: 28–30°C; relative humidity: 80%; Aschoff and Pohl 1970). For the next three nights after capture, we measured energy expenditure of each bird to establish its baseline RMR, as we have found that house sparrow RMRs tend to decrease over the first several nights in captivity (Martin et al. 2003).

After this period of acclimation, we injected the wing webs of all birds with 100 µl of 1 mg ml^{-1} phytohemagglutinin (PHA-P: Sigma L9017) in pyrogen-free saline and measured RMR for four additional nights (Martin et al. 2003). We chose to use PHA to induce systemic inflammatory immune activity, as it increases resting metabolic rate (Martin et al. 2003) and circulating levels of acute phase proteins (Matson et al. 2002) in temperate house sparrows and induces local macrophages and basophils to secrete IL-1 and TNF-α, two fever-inducing cytokines, in chickens (Stadecker et al. 1977). We used RMR the night prior to injection as the baseline rate of energy expenditure of each bird. We did not measure RMR of a control (saline-injected) group of sparrows simultaneously (sensu Martin et al. 2003) because our metabolic system was committed to other projects. Also, data for the third night post-PHA injection became corrupted for slow-living birds and could not be used in population comparisons. For fast-living house sparrows, we used data from a previous study on Illinois house sparrow metabolic responses to PHA challenges (February 2000; Martin et al. 2003). We chose the particular measurement paradigm outlined above because the similar methodologies allowed us to compare data from this study to data collected from fast-living birds. In that study, however, only adult males were used.

Data analysis

Prior to performing statistical comparisons, all data were tested for normality and equality of variances using 1-sample Kolmogorov–Smirnov and Levene's tests, and histograms of each variable were examined visually. When data were parametrically distributed, we used either independent sample T tests, ANOVA in conjunction with simultaneous Bonferroni post hoc tests, or repeated-measures general linear models (GLM) for statistical comparisons. When data were non-normally distributed, we transformed them. If transformations were unsuccessful, we used non-parametric Kruskal–Wallis or Mann–Whitney U tests. When sample size was low for statistical comparisons, we performed power analyses and report observed power estimates (β). We used SPSS v 10.0 (1999) for all statistical comparisons, setting $\alpha = 0.05$.

Results

Constitutive defenses

Natural antibody levels and lysis activity

Neither NAb levels ($t_{33} = 0.31$, $P = 0.76$) nor lysis activity ($U = 127.5$, $P = 0.40$) was significantly different between house sparrow populations (slow-living: $n = 20$; fast-living: $n = 15$); sex of individuals also had no effect on either measure. However, both measures were comparable to those of a population from St. Louis, MO, USA (Matson et al. 2005).

Inducible defenses

Cell-mediated immunity (DTH)

To ensure that we were measuring DTH and not non-specific local inflammation, we compared swelling induced in birds after three different injections of *E. coli* bacteria spread over the course of several weeks (slow-living: $n = 14$, fast-living: $n = 11$). Swelling induced soon after (< 48 h) a single injection would indicate a non-specific inflammatory response (i.e., predominantly non-T-cell mediated); swelling induced after multiple injections, however, would indicate cell-mediated memory orchestrated by T-cell clonotypes receptive to antigens of *E. coli* (Turk 1967). Initial and second injections induced very little swelling in either population, indicating weak or no non-specific inflammation (Fig. 1). However, after the third injection, fast-living sparrows exhibited significantly larger swellings than they did after being injected once or twice [Fig. 1: $F_{2,23} = 12.4$, $P < 0.001$; Bonferroni post hoc test, third injection versus first ($P = 0.001$) and second injection ($P < 0.001$)]. This result did not hold in slow-living sparrows, however, as swellings did not increase significantly over the three injections ($F_{2,23} = 1.5$, $P = 0.23$). Due to time constraints, we were unable to perform a similar test for induction of DTH by KLH. However, we sensitized animals to this antigen in the same manner as *E. coli*, and thus assume that any swelling after three injections represents (predominantly) cell-mediated hypersensitivity and not non-specific inflammation.

To determine if cell-mediated immune activity differed between sparrow populations, we compared

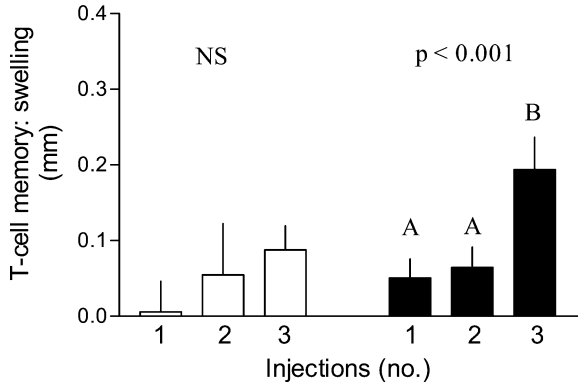

Fig. 1 T-cell memory as expressed by wing-web swelling in response to repeated injections of killed *E. coli* bacteria in slow-living Panamanian (*open bars*, $n = 13$) and fast-living New Jersey (*filled bars*, $n = 11$) house sparrows (*Passer domesticus*). *Bars* depict means + 1SE. *P* value from repeated-measures ANOVA; *letters* indicate group membership according to simultaneous Bonferroni post hoc test. *NS* indicates no significant change in amount of swelling after multiple injections

swellings between populations 24 h after the third injection of either *E. coli* or KLH. We found that swelling induced by killed *E. coli* tended to be greater in fast-living sparrows (Fig. 2a: $F_{1,23} = 4.14$, $P = 0.054$; $\beta = 0.494$). We also included sex as a covariate in this model, but found that it strongly reduced statistical power but did not change the outcome of the prior comparison ($F_{1,23} = 2.04$, $P = 0.155$; sex: $F_{1,23} = 0.010$, $P = 0.74$, $\beta = 0.062$). Swelling induced by KLH, however, was distinctly greater in fast-living birds (Fig. 2b; $F_{1,25} = 26.0$, $P < 0.001$); sex of individuals, however, did not affect this result ($F_{1,25} = 14.991$, $P < 0.001$; sex: $F_{1,25} = 2.38$, $P = 0.137$, $\beta = 0.314$).

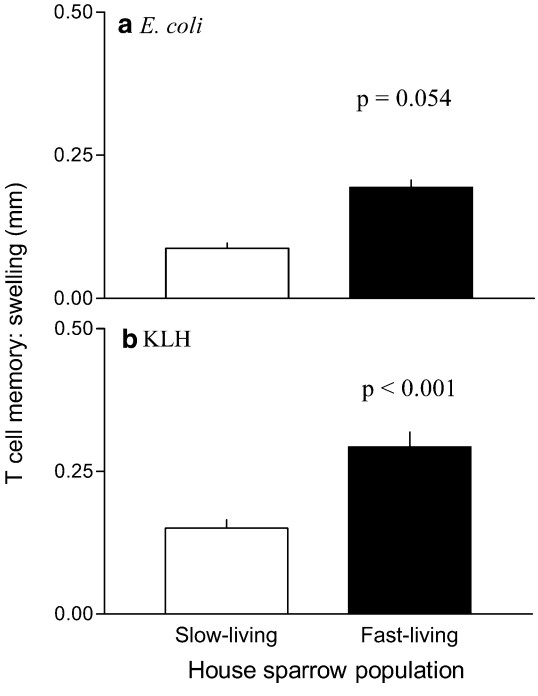

Fig. 2 T-cell memory as expressed by wing-web swelling to **a** killed *E. coli* bacteria (slow-living: $n = 13$; fast-living: $n = 11$) and **b** keyhole limpet hemocyanin (KLH; slow-living: $n = 14$; fast-living: $n = 11$) in slow versus fast-living house sparrows. *P* values from independent samples T test. *Bars* are means + 1SE

Humoral immunity (antibody response)

To assess differences in the humoral arm of the immune system between populations, we compared primary and secondary antibody responses to KLH (slow-living: $n = 13$; fast-living: $n = 11$). Neither sparrow population showed a significant primary antibody response to KLH (Fig. 3a: $F_{1,22} = 0.007$, $P = 0.94$). Therefore as expected, there was no significant difference in the primary response between populations ($F_{1,22} = 0.58$, $P = 0.45$) nor was sex significant ($F_{1,22} = 0.001$, $P = 0.976$, $\beta = 0.050$). Both populations, however, showed significant secondary antibody responses to KLH (Fig. 3b: $F_{1,24} = 33.2$, $P < 0.001$). Although there was no overall effect of population on the secondary response ($F_{1,24} = 0.26$, $P = 0.874$), the time-course of the response varied with latitude ($F_{1,24} = 5.7$, $P = 0.03$). Inclusion of sex in the model, however, only resulted in a weakening of the

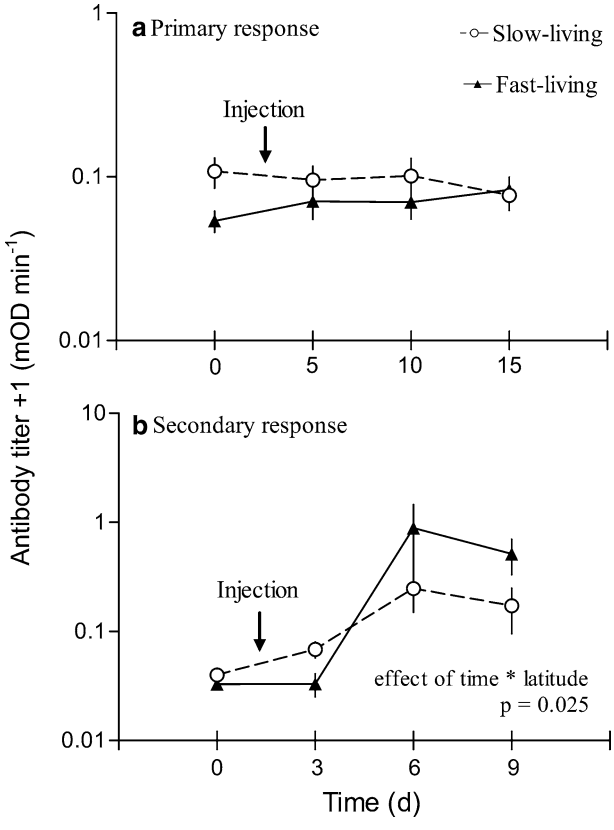

Fig. 3 **a** Primary and **b** secondary antibody response to KLH (mOD per min + 1) for slow-living ($n = 14$) and fast-living ($n = 11$) house sparrows (means ± 1SE). *P* values from repeated measures ANOVA (see Materials and methods). Note the logarithmic scale used

latitudinal effect ($F_{1,22}=3.817$, $P=0.064$; sex: $F_{1,22}=0.040$, $P=0.843$, $\beta=0.054$). Figure 3b suggests that slow-living birds tended to produce antibodies more rapidly but reach lower maximum titers; fast-living birds tended to produce more total antibodies, but they took longer to do so. When we compared antibody titers in each population at the earliest time point of the secondary response (3d post-injection), we found that slow-living sparrows indeed produced significantly more antibodies compared to fast-living sparrows (Fig. 3b; $t_{24}=2.52$, $P=0.02$). Contrastingly, when we compared antibody levels after 9 days when fast-living sparrows appeared to have higher titers, we found only a marginally significant difference ($t_{24} = -1.87$, $P=0.07$).

Correlations between induced immune responses

To determine if birds that mounted strong primary antibody responses also had strong secondary responses to KLH, we performed Pearson correlation analysis between the maximum primary antibodies and maximum secondary antibodies of each bird. We found no significant relationship, however, in either population (slow-living: $P=0.56$; fast-living: $P=0.34$). To determine if a strong cell-mediated response (DTH) was related to a strong humoral response (antibody titer) in the same bird, we performed Pearson correlation analysis on maximum KLH-induced swelling and maximum levels of KLH-specific antibodies during the secondary response. Again, we found no significant relationship within individuals in either population (slow-living: $P=0.56$; fast-living: $P=0.80$).

Energy invested in immune defense

At capture, slow-living house sparrows were significantly lighter than fast-living sparrows (slow-living: 23.4 ± 1.8 g, $n=7$; fast-living: 28.4 ± 2.3 g, $n=7$; $t_{12}=-4.5$, $P=0.01$). Because of this difference, we divided the RMR of each bird by its mass the night prior to metabolic measurement and compared these values between groups. We found that both sparrow populations showed a decrease in RMR over the first 3 days of measurements (prior to immune challenge) followed by a general increase after PHA challenge (cubic model: $F_{1,14}=10.9$, $P=0.006$). Slow-living sparrows, however, showed a different metabolic response to PHA challenge than fast-living birds; RMR of slow-living birds tended to increase and remain elevated for all 4 days post-challenge, whereas RMR for fast-living birds did not increase until 48 h after challenge and remained elevated for only the following 24 h (Fig. 4a; see also Martin et al. 2003). Overall, latitude of origin had a significant effect on RMR over the course of the experiment ($F_{1,14}=8.58$, $P=0.01$). To ensure that this result was not a consequence of different sexes being measured in slow-living birds but only males being measured in fast-living birds, we compared metabolic rate between male and female slow-living house sparrows. We found no effect of sex on RMR ($F_{1,7}=0.03$, $P=0.87$; $\beta=0.052$).

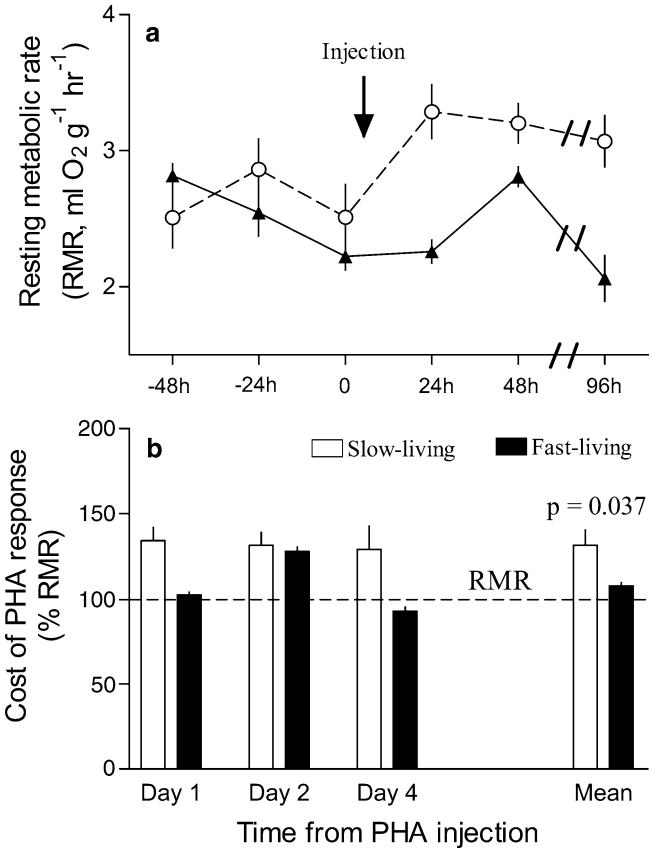

Fig. 4 **a** Resting metabolic rate (RMR, ml O_2 g^{-1} h^{-1}) before and after phytohemagglutinin (PHA) injection into wing-web and **b** cost of the PHA response, expressed as percentage of RMR in slow-living (*open symbols*) and fast-living (*solid symbols*) house sparrows (means ± 1SE). *Diagonal lines* (//) in (**a**) indicate missing data due to failure of data recording during experiment. In **b** *P* value is from independent samples *T* test; $n=7$ in each group

To compare the relative proportion of the energy budget that each population expended on immune activity, we calculated the percent increase in RMR on the 3 days post-immune challenge (using RMR of the evening prior to the challenge as a baseline), and compared the average of these values between populations. We found that slow-living sparrows showed a greater mean increase in energy expenditure to PHA challenge compared to fast-living birds (Fig. 4b: $t_{12}=2.4$, $P=0.037$). Also in all birds, PHA induced significant wing-web swelling as it did in several studies from our lab (Martin et al. 2004, 2005; Greenman et al. 2005); data are not shown here for brevity.

Discussion

Poultry breeders have been attempting to engineer disease-resistant strains of chicken and turkeys for many years without success (Kreukniet et al. 1994; Parmentier

Table 3 Comparisons of immune activity between two populations of house sparrows

Immune defense	Greater in	Relative cost
Natural antibody levels	Neither	Low
Complement activity	Neither	Low
T-cell memory	Fast-living	Low
Antibody proliferation	Slow-living	High
Energy expenditure	Slow-living	High

et al. 1996). They are beginning to recognize that their difficulties may stem from the nature of the immune system itself. Just like in the endocrine or nervous systems, different components of the vertebrate immune system have unique roles and subsequently impart distinct costs and benefits (Lochmiller and Deerenberg 2000; Råberg et al. 2002). In this study, we sought to determine if the pace of life of house sparrows influenced the organization of their immune systems. In order to do this, we felt it was critical that the complexity of the immune system be taken into account. Table 3 summarizes the immunological differences we found in our sparrow populations. Although our data show that immune activity is variable between populations, they only partly support our prediction for stronger immune defenses in the slow-living house sparrows. The lack of differences in NAb levels and lysis activity and the unexpected result for stronger cell-mediated immunity in the fast-living sparrows indicates that other factors besides life history must shape the immune portfolios of these birds.

The costs and benefits of immune defense

Inducible defenses

Specific, inducible defenses include primary and secondary antibody proliferation (mediated by B cells) and cytotoxic and helper T-cell activity (Elgert 1996). The advantages of these defenses lie in their specificity and low costs of use once they are generated (Råberg et al. 2002); disadvantages lie in their high developmental costs (Klasing and Leshchinsky 1999), predilection for autoimmune damage (Graham 2002), and long latency prior to utility (Elgert 1996). In terms of B-cell mediated immune function (humoral defense), secondary but not primary antibody responses were different between populations. Specifically, slow-living birds produced antibodies more rapidly than fast-living birds, but total levels of antibodies tended to be greater at the height of the response in fast-living birds. Rapid production of antibodies is important for checking secondary infections (Elgert 1996)—the sooner antibodies target (opsonize) pathogens, the less likely pathogens are to take hold.

Such immunological differences may be complementary of life history variation. Specifically, one way in which slow-living birds might generate antibodies quicker than fast-living birds is to possess more lymphocytes receptive to the large number of epitopes of an antigen such as KLH (Cohn and Langman 1990; Harris and Markl 1999). This endpoint could be achieved during ontogeny via generation of a diverse B (and/or T) cell repertoire, which would promote responsiveness to a greater number of components of the antigen in adults (Ricklefs 1992; Klasing and Leshchinsky 1999). Such a diversification would be expensive, however, because of the random nature of lymphocyte receptor generation (Cohn and Langman 1990; Klasing and Leshchinsky 1999). Still, one might expect that if such differential B-cell diversification did take place, more rapid antibody production would manifest during the primary, not secondary, antibody response (Klasing and Leshchinsky 1999). Further, it is unclear how much a delay in the production of antibodies would hinder the control of a secondary infection in fast-living birds anyway. Perhaps by generating high antibody titers late in an infection, fast-living birds could compensate for delayed early antibody production without dramatic consequences.

In terms of cell-mediated immunity, we found that delayed-type hypersensitivity to two different antigens was generally stronger in fast-living birds. Alone, these results would seem to indicate that fast-living sparrows are better at eliminating intracellular infections or controlling tumors than their slow-living brethren (Turk 1967). In conjunction with previous findings though, specifically that PHA-induced wing-web swelling is weaker in fast versus slow-living sparrows in March–April (Martin et al. 2004), such a generalization becomes less viable. Work in other taxa has shown that DTH responses often vary depending on the substances used to induce the response. In these house sparrows, different substances (KLH, *E. coli*, and PHA) may have activated different immune cell populations leading to different amounts of swelling in each population (Turk 1967). In light of these inconsistencies among DTH responses, it is difficult to say which population actually possesses greater cell-mediated immunocompetence. Greater *E. coli* and KLH-induced DTH responses in the fast-living population may represent greater T-cell mediated immune defense, or they may represent a greater predisposition to allergic reaction in that population (Turk 1967). This inconsistency suggests that the typical "more-is-better" interpretation of some DTH swelling responses may be unjustified.

Constitutive defenses

Constitutive immune defenses are continuously mobilized defenses that include physical barriers to infection as well as circulating immune cells and cell products (Elgert 1996). The main benefit of these defenses is that they are (usually) able to restrict the entry of pathogens, but their costs have yet to be calculated. Neither NAb levels nor lysis activity was different between sparrow

populations in this study. This lack of a difference may indicate that constitutive defenses in general are not different between populations. More likely though, this lack of variation arises as a consequence of the short period of time populations have been separated (~150 years, Summers-Smith 1988; Ridgely and Gwynne 1989). Some immune defenses, particularly germ-line encoded ones such as natural antibodies (Parmentier et al. 2004), may not have had sufficient time to diverge.

Energy invested in defense

Slow-living birds showed a much greater increase in resting metabolic rate (RMR) after injection with PHA than did fast-living birds. Whole-body increases in energy expenditure are probably due to the induction of acute phase responses by PHA in both populations. Typically, PHA is used to induce only local immune activity in birds (Stadecker et al. 1977; Greenman et al. 2005). However, some studies have used it to induce acute phase responses (Matson et al. 2002; Martin et al. 2003). Although it is not clear what differences in energy expenditure indicate in terms of the strength of acute phase responses per se, it is apparent that slow-living sparrows make larger energetic investments in this type of immune activity than fast-living birds (sensu Lee et al. 2005).

Why does immune activity vary between populations?

It is apparent from the above data that relationships between life history and immune defense in animals are unlikely to be simple. In a life history context, immune defense has been predicted to be greatest in animals: (a) with a long lifespan (Klasing and Leshchinsky 1999), (b) with long development times (Ricklefs 1992), (c) with a slow pace of life (Ricklefs and Wikelski 2002; Martin et al. 2004), or (d) of large body size (Tella et al. 2002; Nunn 2002). None of these generalizations incorporate specific predictions about components of the immune system themselves, but our approach of looking for immunological variation in two populations of house sparrows with pre-existing differences in life history characters represents a natural experiment for testing of our "pace of life" hypothesis. We found some support for our initial predictions: energy expended after induction of an acute phase response was significantly greater in slow versus fast-living house sparrows, and slow-living sparrows had more rapid antibody responses than fast-living birds. Other immune measures, particularly cell-mediated immune activity, varied counter to our expectations indicating that slow-living sparrows are not generally "more immunocompetent" that their fast-living relatives.

One important factor that may determine the nature of populations' immune defenses is pathogen exposure during development. In our house sparrow populations, ecto-parasite and blood parasite levels are similar during the early and late breeding season, but during the non-breeding season, no blood parasites are detectable in fast-living birds (Martin et al., unpublished manuscript). If we assume that this pattern of parasite infections is representative of other threats to house sparrows in both places, we can interpret some of our immunological data. For instance, more efficient antibody-mediated responses in slow-living birds may represent a strategy to control persistent repeat infections using the most economical and targeted means of defense (Klasing and Leshchinsky 1999). However, if cost-minimization due to high probability of infection was the sole driving factor influencing defense portfolios, one would not expect slow-living sparrows to show such strong acute phase (energetic) responses because these are the most costly and damaging types of defenses (Klasing and Leshchinsky 1999). Slow-living animals (with presumably long lifespan) could be jeopardizing their survival probabilities combating infections in this way. This inconsistency for strong acute phase responses in the face of supposedly persistent disease threats may indicate that other pathogens, including bacteria and viruses, must follow different seasonal trajectories than the blood and ecto-parasites mentioned above.

Another possible influence on the immunological patterns we found involves simple shortcomings of methodology (Adamo 2004). Kreukniet et al. (1994) discovered that chickens that had strong tissue swelling responses to PHA had weak in vitro cellular proliferation to the same challenge. These contrary results show that even when immunological methodologies are well-developed, as they tend to be in domestic fowl, interpretation can still be contingent upon the assay used. Pre-existing differences between individuals or populations in comparisons like ours may also obscure immunological uniqueness. For instance, there are two approaches to conducting studies like ours. One option is to solely study free-living animals. This practice allows one to measure traits of interest in animals without potential confounds of captivity stress. The shortcoming of this approach is that one cannot usually take repeated measurements of the same individuals over short time scales, particularly because individuals can also be stressed by repeated capture and because it may be difficult or impossible to recapture net-shy species like house sparrows. More importantly, one cannot guarantee that wild animals do not also experience natural stressors (e.g., temperature, predation, food limitation) that may have confounding effects on physiological traits themselves. Captivity allows one to make repeated measures and standardize diet and other environmental conditions (e.g., light, ambient temperature), but it may also induce stress, potentially in different manners or degrees in different populations of animals. Indeed, stress can have large effects on the type and intensity of immune responses (Sapolsky et al. 2000). In house sparrows, we have already found that PHA-induced

wing-web swelling can be influenced by exogenously administered corticosterone (Martin et al. 2005). Furthermore, it is becoming clear that single measures of immune activity are often state dependent. That is, if an animal is growing feathers, going through maturation, developing reproductive organs, or partaking in reproductive behaviors, immune activity may change (Greenman et al. 2005; Martin 2005). For this reason, we did not include birds in heavy molt in this study, and we conducted all of our comparisons during the breeding seasons of both populations. Still, variation among individuals in breeding state within each population may have affected some immune measures. If this effect was sufficiently large, however, we would not expect to find any differences in immune activity in a life history context.

Conclusion

In a descriptive study like this one, it is impossible to know whether immunological variation between two populations is due to particular factors of interest (Garland and Arnold 1994). Nevertheless, the extensive variation seen in many physiological and life history characteristics of North American house sparrows (Kendeigh 1976; Summers-Smith et al. 1988) strongly suggests that immunological differences detected in this study are partly adaptive and perhaps genetic (Martin et al. 2004). Moreover, because the immune system is arguably one of the most complex phenomena in biology, studies like this one can provide useful background for future, more controlled work in understanding the forces that determine the architecture of species' immune systems. Although substantial molecular understanding exists with regard to the form and function of the vertebrate immune system, we have gained little integrative, adaptive perspective on how it works. To continue to make progress in this direction, we suggest keeping the Schmid-Hempel and Ebert (2003) immune defense component model in mind when designing future studies. Furthermore, researchers should try to directly characterize disease-resistance in populations when possible (Adamo 2004), appreciating that these studies, too, may be limited in their scope. Indeed, it is unclear whether the ability of a species to combat or prevent one disease would be representative of its ability to fight all diseases. Lastly, one should recognize that prophylactic behavioral defenses, such as responsiveness to novel foods, environments, and/or objects (Martin and Fitzgerald 2005), may be important first lines of defense in some cases. Ultimately, such a multi-tiered approach will lead us to a robust, evolutionary understanding of the vertebrate immune system.

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