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IMMUNE ACTIVITY IN TEMPERATE AND TROPICAL HOUSE SPARROWS: A COMMON-GARDEN EXPERIMENT

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Abstract. We hypothesized that Neotropical passerines would invest more in costly immune function relative to north-temperate passerines, due to differences in their respective life histories. We further hypothesized that latitudinal variation in immune activity would persist in common-garden conditions. To test these hypotheses, we compared immune function, measured via phytohemagglutinin (PHA)-induced wing-web swelling in both wild House Sparrows (*Passer domesticus*) and House Sparrows kept under common-garden conditions for 18 months. We found that wild Neotropical sparrows had relatively stable immune responses across the year, whereas wild north-temperate sparrows showed substantial seasonal variation in immune activity, having lower responses than Neotropical birds during the early breeding season and significantly higher responses than Neotropical birds during the late and nonbreeding seasons. Latitudinal differences in immune responses were not related to mass, sex, or body condition, but were influenced by mass change 24 hours after immune challenge. Under common-garden conditions, birds from both populations first decreased (after five months) and then increased (after 18 months) their nonbreeding immune responses relative to wild values, indicating condition dependence in the PHA response. Relative differences in the PHA response between the populations, however, were maintained in captivity: after 18 months in common gardens, north-temperate sparrows exhibited stronger nonbreeding immune responses than Neotropical sparrows.

Key words: *clutch size; common garden; House Sparrow; immunocompetence; life history; Neotropical;* Passer domesticus; *passerine; PHA; phytohemagglutinin; trade-offs.*

INTRODUCTION

Many Neotropical passerines lay smaller clutches, but have longer breeding seasons, longer embryonic development periods, and higher survival rates than their north-temperate counterparts (Martin 1996, Johnston et al. 1997, Ricklefs 1997, Brawn et al. 1999, Böhning-Gaese et al. 2000). Although these tendencies are not ubiquitous, they exist among related species (Murray 1985, Yom-Tov 1994, Johnston et al. 1997, Bohning-Gaese et al. 2000) and among populations within species (Summers-Smith 1988, Baker 1995, Gwinner et al. 1995, Young 1996, Ricklefs 1997). Such recurring patterns suggest that common factors constrain the life history strategies of passerine birds across latitudes, independent of their evolutionary history (Ricklefs 1970, Wikelski and Ricklefs 2001). For more than 50 years, researchers have concentrated on differences in food availability and predation pressure to explain these life history differences (Moreau 1944, Murray 1985, Skutch 1985). Although some experimental evidence supports these food and/or predation hypotheses (Young 1996, Ghalambor and Martin 2001), it is unclear if they alone are sufficient to explain the worldwide latitudinal pattern of avian life history variation.

We hypothesize that trade-offs among different physiological systems, particularly immune activity vs. reproduction, may also influence latitudinal patterns in avian life histories (Wikelski and Ricklefs 2001, Ricklefs and Wikelski 2002). Ricklefs (1992) and Møller (1998) hypothesized that birds that live in the tropics would exhibit enhanced immune function because of a greater likelihood of infection by parasites. However, because immune function and reproduction are costly (Monaghan and Nager 1997, Klasing and Leshchinsky 1999, Norris and Evans 2000, Martin et al. 2003), investments in immune function may fluctuate seasonally as well as latitudinally. Specifically, we predict that immune defense in adult birds may be low during the breeding season in north-temperate birds because they lay larger clutches than Neotropical birds, due to a relatively smaller window of time of favorable climate. We predict that Neotropical birds may be able to allocate more resources per unit time to immune defense because a benign climate allows them to spread a larger number of small clutches over a longer breeding season.

This hypothesis for seasonal and latitudinal variability in immune activity is supported by several pieces of evidence. First, it is becoming clear that birds

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PLATE. 1. Two of the authors, L. Martin (right) and M. Pless (left), catching House Sparrows in mist nets in the Zona Libre, Colon, Panama. Photo credit: M. Wileski.

cannot maximize immune defense at all times of the year because of the high costs of using the immune system. Several studies have shown that immune responses are energetically (Martin et al. 2003) and nutritionally demanding (Lochmiller et al. 1993, Alonso-Alvarez and Tella 2001), and are often traded off with sexual ornament elaboration (Zuk and Johnsen 1998) and parental feeding effort (Ilmonen et al. 2000, Fargallo et al. 2002). Second, two studies have provided circumstantial evidence that tropical passerines do, in fact, invest more in immune function. Ricklefs (1992) found that intracellular blood parasite load was lower in tropical passerines relative to related north-temperate species (but see Booth and Elliot 2003). Similarly, Møller (1998) found that circulating leukocyte densities were higher and spleens were larger in tropical birds compared to related temperate species.

To directly examine investments in immune defense as a function of latitude and season in passerines, we measured in vivo phytohemagglutinin-induced immune activity of a recent invader of the neotropics, the House Sparrow (*Passer domesticus*), from one Neotropical (Colon, Panama) and one north-temperate (New Jersey, USA) population during multiple life history stages. To determine whether any differences in immune activity that we detected were fixed traits (evolved or ontogenetic differences) or simply flexible responses to different environmental conditions, we compared immune responses from wild birds to responses of birds held in common gardens. If differences among populations were indeed fixed, we would expect populations to continue to exhibit different immune activity when held in identical, benign conditions.

METHODS

Field sites

Zona Libre, Colon, Panama (9°1′ N, 80°1′ W).—The Zona Libre is a large commercial district in the coastal city of Colon at the northern edge of the Panama Canal. All of our work is conducted in France Field, a large complex of complete or in-construction warehouses with very little natural habitat. Like few other places in Panama, House Sparrows are abundant there (see Plate 1).

Belle Mead Co-op and Princeton Shopping Center, Princeton, New Jersey, USA (~40°21′ N, 74°40′ W).—The Belle Mead Co-op is a farm supply store 15 km north of the Princeton University campus. This area consists of three commercial buildings and one barn used to mill seed. The Princeton Shopping Center is a small, open-air shopping mall 2 km north of the Princeton University campus. Most birds included in this study were captured at Belle Mead (early and late breeding season); only sparrows during the nonbreeding season came from the Princeton Shopping Center. We initially intended to obtain all birds from the same location, but sparrows from the Belle Mead Co-op became too net-shy to obtain sufficient sample sizes.

Study organism

Neotropical House Sparrows exhibit life histories similar to those of native tropical species, laying smaller clutches, on average (2–3 eggs in the neotropics vs. 4–5 in the northeastern United States) and more clutches per year (four clutches vs. two) over a longer breeding season (200+ days vs. 110) (Summers-Smith 1988, Baker 1995). Adult survival and fledgling growth rates for Neotropical sparrows are currently unknown. The first record for the House Sparrow in Panama occurred in 1977, yet confirmed breeding was not documented until the mid-1980s (Ridgely and Gwynne 1989). Although no systematic study has been performed, it is believed that Panamanian sparrows arrived from Costa Rica (Ridgely and Gwynne 1989). Many introductions have taken place throughout South America, but few birds have been recorded in Colombia (Summers-Smith

1988). This lack of record, the lack of preferred habitat along the Colombia/Panama border, and the higher densities in cities closer to the Costa Rican border (David and Santiago) suggest that Panamanian sparrows arrived from North America (Ridgely and Gwynne 1989).

Immune challenge

We used the phytohemagglutinin (PHA) wing web technique to assess in vivo immune activity of House Sparrows (Smits et al. 1999). PHA is a plant lectin that nonspecifically stimulates mitogenesis and trafficking of many cell types, particularly T-cells. After injection with PHA, T-cells secrete a variety of cytokines, which stimulate local tissue infiltration by granulocytes, lymphocytes, and other immune cells (Goto et al. 1978, McCorkle et al. 1980). We quantified immune activity by subtracting the thickness of the left wing web patagium before injection with PHA from the thickness of the same wing web 24 hours after injection; a strong response was represented by a large swelling (Smits et al. 1999, Martin et al. 2003). For all challenges, we injected 100 μL of a 1 mg/mL solution of crystalline phytohemagglutinin (PHA-P) purified by affinity chromatography (Sigma L9017) in cell-culture-grade saline solution (Sigma P3813; Sigma-Aldrich, St. Louis, Missouri, USA) into the left wing web (patagium) and measured swelling with either a Starrett gauge (#10; L. S. Starrett, Athol, Massachusetts, USA) or a Teclock pocket thickness gauge (Model SI-510; Penn Tool, Maglewood, New Jersey, USA); adjusted readings from either gauge were indistinguishable. After all web measurements, each bird was weighed to the nearest 0.1 g. In five cases, we could not inject sufficient PHA solution in the wing web and thus did not include those birds in the analysis.

Field study

Seasonal definitions.—To examine seasonal variability in PHA responses between latitudes, we divided the year a priori into three categories: early breeding (March–April 2002), late breeding (July–August 2001), and nonbreeding (October–November 2002). The majority of breeding occurs from January to August in Panamanian sparrows, and from April to August in New Jersey sparrows (L. B. Martin, *personal observation*). Thus, the breeding season is much shorter at northern latitudes. However, House Sparrows will breed as early as February and as late as November in the north-temperate zone (Summers-Smith 1988). Thus, our categorizations represent both the most distinct life history stages that House Sparrows from both latitudes experience and the best characterization of life history stages that both populations share.

We are aware that these categorizations of breeding condition are of the populations, not individuals. However, we did not want to laprotomize birds or take a blood sample for reproductive hormones because this treatment may have confounded the PHA response of birds. Further, we did not assess external morphological characters, such as the presence of a brood patch or the size of the male's black bib, because these measures can result in misleading characterizations of breeding condition (e.g., the presence of a brood patch only indicates that a female has bred before, not if it is in breeding condition). Similarly, cloacal protuberance length, which is often indicative of male reproductive status (testosterone levels), was not measured because birds from different latitudes may have different circulating testosterone levels; protuberance length therefore may not mean the same thing in both populations. However, we feel that our categorizations are conservative enough to characterize differences in life history stage in populations across seasons. In fact, if birds were so asynchronous in terms of breeding status, there should be no significant latitudinal difference in PHA responses due to high within-population variance in breeding condition.

Bird capture and care.—We captured birds using mist nets, sexed them by plumage, and classified each as an adult or first-year juvenile (Summers-Smith 1988). Panamanian birds were captured in March 2002 (early-breeding), July 2001 (late-breeding), and October 2002 (nonbreeding). New Jersey sparrows were captured during April 2002 (early-breeding), late July–early August 2001 (late-breeding), and November 2002 (nonbreeding). At capture, we measured tarsus length and marked each bird with unique combinations of metal and plastic colored leg bands.

We brought birds into captivity and kept them in male–female or juvenile–juvenile pairs in small wire cages for no more than 24 hours prior to immune challenges. While birds were in captivity, we provided them with water, identical finch seed mix, and boiled chicken egg ad libitum; all birds eagerly took food and water at every stage of the experiment. We maintained photoperiod and temperature at levels representative of ambient conditions at each latitude. We were forced to keep birds in captivity during immunological measurements instead of releasing and recapturing them 24 hours later, due to problems with learned net-shyness in this species.

Common-garden comparisons

Housing.—We captured 18 House Sparrows (nine males and nine females) for export from Panama to New Jersey in late July 2001. However, because our quarantine facility was not completed until the beginning of October 2001, the sparrows were not exported immediately. For the period of August–October, we housed birds in a single aviary on the roof of the Smithsonian (Tupper) Building in Panama City, where they were provided daily with an ad libitum diet of finch seed mix, vitamin supplements, water, and boiled, mashed chicken eggs. In October 2001, we imported birds from Panama to our USDA-APHIS-certified quarantine facility (Moffett Animal Facility) at Princeton

University. During the quarantine period, all birds were tested for Newcastle's disease virus and avian influenza. All birds tested negative for these viruses, and no birds died or showed clinical signs of other diseases during the quarantine period. While they were in quarantine, we fed birds ad libitum diets of no-waste finch seed mix (Kaytee, Chilton, Wisconsin, USA), mealworms (*Tenebrio molitor*; Fluker Farms, Port Allen, Louisiana, USA), vitamin supplements (Daily Supplement 3; Golden West Bird Products, Mission Hills, California, USA), dried insect larvae (Bag O' Bugs, Golden West Bird Products), fresh oranges and salad greens, gravel, and crushed oystershells. In October 2001, before we imported birds from Panama, we captured 36 sparrows (equal numbers of males and females) from the Belle Mead Co-op in New Jersey, fed them diets identical to those of Panamanian birds, and maintained them in free-flight outdoor aviaries at Princeton University to ensure that time and experience in captivity were similar between populations.

Just before the end of the quarantine period (mid-November 2001), all birds (New Jersey and Panama) were dusted for ectoparasites (Sergeant's flea and tick powder: pyrethrin, 0.1%; Sergeants, Omaha, Nebraska, USA) and treated with an antibacterial medication (Sulfanox; Belle Mead Farmer's Co-op, Belle Mead, New Jersey, USA) to attempt to control existing differences in parasite loads between populations. All birds were then released into one of two large indoor aviaries in the Moffett Animal Facility. The floor of each aviary was covered with fine playground sand, allowing us to mimic natural conditions by minimizing disturbances (bare (tile) floors would have necessitated continuous cleaning). We provided multiple perches (wooden dowels) throughout each aviary and hung wooden nest boxes (>1 box per pair of sparrows) throughout the room. Food (diet as previously described), fresh nest material (dry hay and clean goose down), water, and diet supplements were provided in plastic dishes on the floor of the aviaries and were changed daily. We cleaned aviaries twice per week, but never disturbed birds for more than 30 minutes at a time.

For the duration of the experiment, both aviaries were maintained on an identical photoperiod, representative of Princeton, New Jersey (to encourage breeding in both groups for a separate experiment). Ambient temperature and relative humidity, however, varied between aviaries. We maintained one aviary at a temperature of 29.1° ± 1.7°C, mean ± 1 SD) and relative humidity of 26.1 ± 7.6%; this represented our "Neotropical" aviary. All Panamanian sparrows and half of the sparrows from New Jersey were housed in this aviary. The other aviary had seasonally variable climatic conditions, more characteristic of the New Jersey climate, with a temperature of 22.5° ± 4.1°C, mean ± 1 SD; minimum temperature 17.2°C, maximum temperature 30.0°C) and relative humidity of 32.4 ± 11.0%; mean ± 1 SD); this represented our "north-temperate" aviary. The remaining New Jersey sparrows and an additional group of New Jersey sparrows that was never injected with PHA (a control group for another experiment) were kept in this aviary.

We attempted to maintain relative humidity at higher levels in both aviaries, using portable humidifiers. However, we were unsuccessful because air exchange rates in the rooms were too high to allow buildup of moisture, even when we reduced them to minimum allowable settings. Further, we are aware that the design of our experimental treatments (one room with one climatic treatment) creates problems with independence of individual data points, a confound inherent in most common-garden studies. At the same time, however, splitting birds among multiple rooms would have created other problems because "room" would then have become a covariate in statistical tests. All experimental protocols were approved by the Princeton University Animal Care Committee and comply with the principles of animal care (National Institutes of Health Publication 86–23) and current U.S. laws.

Immune challenges

We used the same methodology as that for wild birds to assess immune function in our captive birds. We measured immune function in captive birds for three treatment groups (Panama origin–Panama climate; New Jersey origin–New Jersey climate; New Jersey origin–Panama climate) during two consecutive nonbreeding seasons in captivity. The first PHA response was measured after birds had been in captivity for five months (December 2001); the second was made after 18 months (December 2002–January 2003). We did not measure PHA activity in captive birds during all three seasons because we wanted to minimize disturbances that might have affected their reproductive output, a variable that we did not want to alter. At each measurement period, we captured birds from aviaries and maintained them in pairs (male–female) in wire cages to minimize the stress associated with the process of recapturing birds from aviaries. While in cages, birds were provided with the same high-quality diet as in aviaries. As with wild measurements, we measured the thickness of the wing web and body mass before and 24 hours after the PHA challenge.

Statistical analyses

We first checked that our data met assumptions of parametric statistics by using Levene's tests and visual analysis of histograms. Histograms never revealed nonnormal distributions of data, nor did Levene's test identify unequal variances among treatment groups. Thus, we used univariate ANOVA models to examine seasonal variation in the PHA response alone and in conjunction with covariates; simultaneous Bonferroni post hoc tests were used to determine where significance occurred. We used t tests to compare PHA responses and change in mass 24 hours after injection between

populations. In all parts of this study, we attempted to use PHA-naïve birds, because it is not clear what effects prior PHA challenges have on secondary or tertiary responses. A very few birds, however ($N = 7$), were included in both the wild and common-garden study, and thus were injected multiple times with PHA. Comparisons of the responses of these birds relative to naïve birds revealed no significant effects of prior challenge on the immune response after 18 months. We used ANCOVA to compare body condition (ratio of body mass to tarsus length) between groups; we found no difference between populations. We used SPSS Version 10 (SPSS 1999) for all statistical tests, setting our α value at $P = 0.05$. We chose not to use sequential Bonferroni adjustments for data used in multiple statistical tests due to the excessively conservative nature of this correction that often results in misclassification of biologically relevant results (Moran 2003).

Results

Comparisons of PHA responses in wild birds

We found that PHA-induced swelling responses were different in wild birds from Panama than in wild birds from New Jersey (Fig. 1). An ANOVA model with latitude as the main factor and season as a covariate showed that season and the interaction between season and latitude were significant (full ANOVA model, $F_{3,74} = 12.4$, $P < 0.001$; latitude of origin, $F_{1,74} = 0.29$, $P = 0.594$; season, $F_{1,74} = 12.4$, $P < 0.001$; interaction term, $F_{1,74} = 38.8$, $P < 0.001$). New Jersey birds exhibited substantial seasonal variation in PHA responses ($F_{2,32} = 52.16$, $P < 0.01$), with immune activity being lowest in the early breeding season, highest in the late breeding season, and intermediate during the nonbreeding season (as detected by simultaneous Bonferroni post hoc comparisons); Panamanian birds exhibited weak (but statistically significant) seasonal variation ($F_{2,41} = 3.50$, $P = 0.04$). We found that Panamanian sparrows had significantly different swelling responses to PHA compared to New Jersey birds during all three seasons (Fig. 1). In the early breeding season, swelling was higher in Panama ($t = 7.09$, $P < 0.001$). In the late breeding season and nonbreeding season, however, swelling was higher in New Jersey (late season, $t = -4.556$, $P < 0.001$; nonbreeding season, $t = -2.293$, $P = 0.029$).

We compared several other traits of birds from the two populations to determine if they were related to differences in immune responses. Mass varied seasonally in both populations, but this pattern was distinct for each population (for Panama, $F_{2,41} = 19.8$, $P < 0.001$; for New Jersey, $F_{2,32} = 5.90$, $P < 0.01$). An ANOVA model showed that mass, body condition, and sex had no effect on seasonal or latitudinal differences in immune responses (for mass, Panama, $F_{1,41} = 0.3$, $P = 0.57$; New Jersey, $F_{1,32} = 0.001$, $P = 0.97$; for body condition, Panama, $F_{1,41} = 0.007$, $P = 0.99$; New Jersey, $F_{1,32} = 1.1$, $P = 0.31$; for sex, Panama, $F_{1,41} = 0.6$, $P = 0.43$; New Jersey, $F_{1,32} = 1.3$, $P = 0.25$).

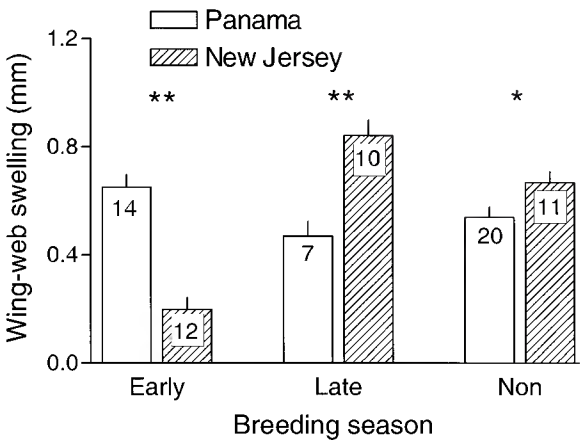

FIG. 1. Comparisons of immune function measured by PHA-induced wing-web swelling in House Sparrows from New Jersey, USA, and Colon, Panama. At both latitudes, early breeding is March–April, late breeding is July–August, and nonbreeding is October–November. Significant differences between populations by independent t tests are indicated by asterisks. Numbers inside bars indicate sample sizes. Error bars depict means + 1 SE.
$^*P < 0.05$; $^{**}P < 0.01$.

We found that change in mass 24 hours after PHA challenge also varied seasonally in both populations (ANOVA for Panama, $F_{2,32} = 13.1$, $P < 0.001$; for New Jersey, $F_{2,41} = 8.8$, $P = 0.001$). Interestingly, this variable was important in explaining differences in the PHA responses. An ANOVA of immune response by latitude with season and 24-hours mass change as covariates showed that mass change and its interaction with latitude of origin, but not season, explained immune response differences (full model, $F_{1,74} = 11.7$, $P < 0.001$; 24-hours mass change, $F_{1,74} = 8.8$, $P = 0.004$; interaction between origin and mass change, $F_{1,74} = 6.8$, $P = 0.001$; interaction between season and mass change, $F_{2,74} = 2.7$, $P = 0.10$). Generally, except for the early breeding season in New Jersey, birds that lost more mass in 24 hours had lower PHA responses; birds that gained mass had higher responses. When we compared mass change between populations in all three seasons, however, we found no consistent pattern. In the early breeding season, Panamanian birds gained marginally significantly more mass than the north-temperate birds ($t = 2.02$, $P = 0.055$). In the late breeding season, north-temperate birds gained mass whereas Neotropical birds lost mass ($t = -8.14$, $P < 0.001$). In the nonbreeding season, the trend reversed; Neotropical birds gained mass whereas north-temperate birds lost mass ($t = 3.239$, $P = 0.003$).

Comparisons of the nonbreeding PHA response in common-garden experiments

Short term.—After five months in captivity, immune response differences between latitudes disappeared (t

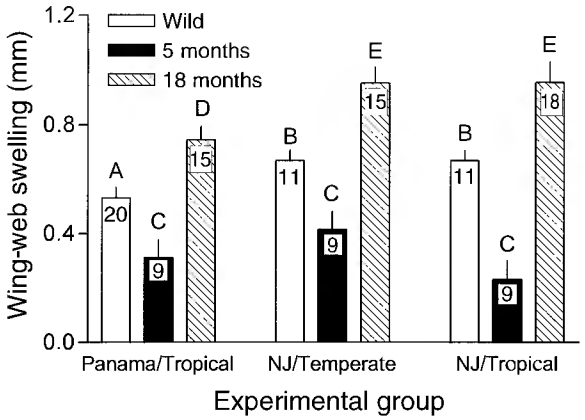

FIG. 2. PHA-induced wing-web swelling during the nonbreeding season in wild and common-garden-housed House Sparrows from New Jersey, USA, and Colon, Panama. Open bars represents wild values, solid bars represent five-month captive values, and hatched bars represent 18-month captive values. Labels for experimental groups indicate latitude of origin and the climate in which they were held. Different letters above the bars indicate significant differences between groups ($P < 0.05$) based on a variety of statistical tests (see *Methods: Statistical analyses*). Numbers inside bars indicate sample sizes. Histogram bars depict means + 1 SE.

$= 0.82$, $P = 0.423$). Birds from both latitudes significantly decreased their nonbreeding immune responses relative to responses of wild birds at the same time of the year (Fig. 2). An ANOVA between captive and wild birds from each population separately, with mass as a covariate, showed that mass was important in explaining immune response differences in 5-month captive vs. wild birds (for Panama, full model, $F_{3,29} = 5.77$, $P = 0.004$; mass, $F_{1,29} = 5.40$, $P = 0.029$; interaction, $F_{2,29} = 2.54$, $P = 0.124$; for New Jersey, full model, $F_{3,20} = 14.95$, $P < 0.001$; mass, $F_{1,20} = 5.00$, $P = 0.40$; interaction, $F_{2,20} = 3.65$, $P = 0.074$). This result suggests that the low mass of 5-month captive birds prior to challenge led to reduced immune responses in all groups relative to wild birds. It is not clear why mass was so low at this time, because all birds took food readily and appeared in good health.

Long term.—After 18 months in captivity, common-garden birds from both latitudes had nonbreeding immune responses that were increased significantly above wild values (Fig. 2; for Panama, $t = -3.43$, $P = 0.002$; for New Jersey, $t = -3.09$, $P = 0.005$). Mass did not significantly differ between captive and wild birds from either latitude. However, change in mass 24 hours after PHA was different for Panama birds; captive birds gained mass after challenge, whereas wild birds lost mass after challenge ($t = 2.74$, $P = 0.010$); New Jersey birds were not significantly different. Interestingly, although PHA responses of both groups increased after 18 months in captivity, the degree of difference in wild responses between the two populations was conserved. Responses of captive birds from Panama were still lower than those of captive birds from New Jersey after 18 months in benign conditions (Fig. 2; $t = 2.346$, $P = 0.025$). As in the wild, this difference in immune response was not related to mass, sex, condition, or 24-h mass change.

Effects of ambient climate on the nonbreeding PHA response in common gardens

In order to determine if lower nonbreeding PHA responses in the neotropics were due to exposure to prolonged high ambient temperatures, we held a group of New Jersey birds in the Neotropical aviary and measured their immune responses after five and 18 months. After five months in captivity, the nonbreeding PHA response of birds in this group was lower than wild New Jersey nonbreeding values (Fig. 2; $t = -5.85$, $P < 0.001$). However, it was not different than the other two captive groups at five months ($F_{2,26} = 1.26$, $P = 0.279$).

After 18 months in captivity however, nonbreeding immune responses in this group remained indistinguishable from those of captive New Jersey birds held under New Jersey ambient conditions (Fig. 2; $t = 0.03$, $P = 0.984$), but were significantly higher than those of captive Panama birds housed in the same aviary (Fig. 2; $t = -2.86$, $P = 0.012$). Like the other two groups, these birds exhibited a decrease in mass after five months ($t = -4.84$, $P < 0.001$) relative to wild birds, although there was no increase in mass after 18 months relative to wild birds ($t = 1.47$, $P = 0.154$). As before, condition, sex, and 24-h mass change had no effect on this outcome.

DISCUSSION

Immune defenses are critical to the survival and well-being of most wild organisms, yet the quantitative extent of variability in immune activity among wild animals remains poorly understood. We found that in House Sparrows, one type of immune defense shows extensive latitudinal and seasonal variation. Surprisingly, however, this variation appears to be only partly influenced by parasite threat.

In our Neotropical sparrow population, blood parasite prevalence is relatively stable over the year, but in our north-temperate birds, parasite prevalence is greatest in the late breeding season and diminishes to undetectable levels in the nonbreeding season (M. I. Pless, L. B. Martin, and M. C. Wikelski, *unpublished manuscript*). Thus, if parasite threat alone drives investments in immune function (and blood parasite prevalence accurately represents other parasite threats), north-temperate sparrows should have weak immune responses in the nonbreeding season and strong responses in the breeding season, whereas Neotropical sparrows would have similar immune function year-round. Our results supported these predictions for the neotropics, but not for the north-temperate zone: wild north-temperate House Sparrows indeed had seasonally variable immune responses, but immune activity was

lowest in the early breeding season, highest late in the breeding season, and intermediate in the nonbreeding season.

Why do populations differ in immune function?

Temporal asynchrony in peaks of parasite threat and immune activity may occur for several reasons. First, it may be that climatic variability and the life history strategies that it requires mandate certain immune investment strategies irrespective of parasite threats. North-temperate birds are forced to confine their breeding activity to a shorter window of time, which may lead them to lay fewer, larger clutches compared to Neotropical birds (Cody 1966). The laying of such large clutches may prohibit high investments in immune defense when breeding activity is greatest, especially if resource availability (e.g., protein) is low. Later in the year, when the demands of reproduction are reduced or food availability increases, immune function may improve. A similar hypothesis has been proposed to explain the weak immune responses and high infection rates seen in many other temperate vertebrates during the breeding season (Nelson and Demas 1996, Bentley et al. 1998; but see Møller et al. 2003). Birds at tropical latitudes may be able to allocate ample resources to immune function year-round. Unlike north-temperate birds, they can spread smaller increments of reproductive effort over a longer period of time, allowing them to increase their investments in immune function (Cody 1966, Ricklefs 1970, Hau 2001).

Alternatively, latitudinal differences in immune activity may arise because of differences in endocrine physiology between populations. Androgens, particularly testosterone, have been shown to reduce immune activity in some (but not all) passerines (Evans et al. 2000, Casto et al. 2001). In north-temperate passerines, androgen titres are often very high in male birds at the inception of the breeding season (Hegner and Wingfield 1986, Wingfield and Monk 1992), whereas tropical passerines typically maintain low levels of androgens year-round (Wikelski et al. 2003). Differences in circulating androgen levels, however, can only explain differences in immune activity in males, not in females. We found no difference in immune activity as a function of sex in our study. Thus, it may be that other hormones, such as corticosterone, an avian stress hormone, play a more important role in influencing latitudinal variability in immune function. These hormones can have strong influences on the type and degree of immune activity (Sapolsky et al. 2000) and often vary seasonally and latitudinally in birds (Silverin and Wingfield 1998, Romero 2002). Unlike steroids, however, circulating levels of these hormones can be high in both males and females (Romero 2002).

Still, differences in hormone levels and life history cannot alone explain why PHA swellings were greater in our north-temperate sparrows during two out of three seasons, a result contradictory to our initial predictions. We suggest that high PHA-induced swelling responses during the late and nonbreeding seasons in our north-temperate sparrows may not indicate increased immunological competence inasmuch as they indicate an increased emphasis on a different type of immune activity. Recently, we showed that although north-temperate House Sparrows had greater PHA-induced swelling during the nonbreeding season (November), Neotropical sparrows showed a more robust humoral response to PHA, as measured by increased lymphocyte infiltration into wing web tissue (L. B. Martin, J. Lewittes, K. C. Klasing, and M. C. Wikelski, *unpublished manuscript*).

Is latitudinal variation in immune function in sparrows plastic or fixed?

Recurrence of latitudinal patterns in physiological and life history characters among and within species suggests that birds are optimized to local conditions (Roff 1992). However, phenotypic plasticity, physiological acclimatization, or condition dependence can also explain latitudinal variation in phenotypes, especially among populations (Ricklefs and Wikelski 2002, Piersma and Drent 2003). Two experimental techniques can be used to distinguish between these four possibilities: reciprocal transplants and common-garden experiments; we chose the latter.

In terms of condition dependence, we found that captive birds that lost mass over the course of the experiment generally had low immune responses; birds that gained mass had strong responses (see also Alonso-Alvarez and Tella 2001). Additionally, we found that time in captivity affected the PHA response. After five months in captivity, immune responses in all groups were very low, but after 18 months in captivity, immune responses were higher than wild values. For the duration of the experiment, birds were maintained on similar environmental conditions and diets, suggesting that differences in immune activity were due to time in captivity alone. Furthermore, all birds were tested while in nonbreeding condition, so reproductive status would not confound differences in immune function.

Latitudinal differences in immune function in wild birds in our study, however, appeared to be more than condition-dependent differences. When we compared nonbreeding season PHA responses of both populations of birds in common-garden conditions, we found that relative differences seen in the wild were maintained in captivity. After five months in captivity, PHA responses did not differ between our three common-garden sparrow groups, but this was because all populations showed very low responses, probably due to low body mass in all birds. However, after 18 months in captivity, irrespective of the climatic conditions in which they were held, north-temperate sparrows showed significantly higher responses than Neotropical birds, as they did in the wild at this time of year. Un-

fortunately, we were not able to add a Neotropical origin–temperate climate group to our study because we were limited in the number of birds that we could export. Additionally, we were forced to use a north-temperate photoperiod for both populations, as we were concerned that north-temperate House Sparrows might not reproduce if they were not given sufficient photostimulation. Differential sensitivity to photoperiod may influence immunological differences between our sparrow populations (Demas and Nelson 2003), but we were unable to test this factor. Still, our results show that immune activity indeed varies latitudinally in passerines, and our results suggest that this variation is more than just acclimations by each population to different environments.

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