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Corticosterone suppresses cutaneous immune function in temperate but not tropical House Sparrows, *Passer domesticus*

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Abstract

Levels of corticosterone (CORT), the primary avian stress hormone, tend to vary over space and time in passerines, but why this is so remains unclear. One reason may be differential need for immune defense. Typically, sustained high levels of CORT suppress immune activity in vertebrates. Thus, animals living where parasite threats are high might maintain low levels of CORT and mount weak CORT stress responses to ensure that their immune defenses are in a high state of readiness at all times. Here, we addressed this hypothesis by comparing CORT levels in two populations of House Sparrows (*Passer domesticus*), one from the tropics (Colon, Panama) where parasite threats are high and one from the North-temperate zone (New Jersey, USA) where they are lower. Indeed, we found that House Sparrows from Panama had lower baseline and stress-induced CORT levels than House Sparrows from New Jersey. To more directly test our hypothesis, we artificially elevated CORT (via implant) in both populations of birds, expecting that cutaneous immune activity (induced by phytohemagglutinin (PHA)) would be suppressed as it is in most vertebrates studied to date. Surprisingly, we found that CORT implants did not affect immune function in Panamanian sparrows, while immune function in (non-breeding) New Jersey sparrows was suppressed. This suggests that Panamanian House Sparrows may be immunologically insensitive to CORT, in addition to maintaining low baseline and stress-induced levels of this hormone. We propose that other animals living where disease threats are high may use CORT in a similar way.

Keywords: Defense; Latitude; Life history; Phytohemagglutinin; Steroids; Stress

1. Introduction

Across their range (Bears et al., 2003; Homan et al., 2003; Romero and Wikelski, 2002; Scheuerlein et al., 2001; Wilson and Wingfield, 1994; Wingfield et al., 1983) and over time (season: Perfito et al., 2002; Wada and Shimizu, 2004; age: Schwabl, 1999), vertebrates vary in their corticosteroid responses to stress. This stress response, which helps shift the phenotype of the organism into an "emergency life history" state (Wingfield et al., 1998), often leads to behavioral and physiological changes in animals (Buttemer et al., 1991; Kitaysky et al., 2001, 2003; Pravosudov et al., 2001; Wikelski et al., 1999a) that presumably promote survival of stressful events (Sapolsky, 1992; Wingfield et al., 1998).

In passerine birds, levels of corticosterone (CORT), the primary avian corticosteroid, vary across latitudes. Typically, birds living in the arctic have more damped responses than mid-latitude congeners (Breuner et al., 2003; Silverin and Wingfield, 1998; but see Pravosudov et al., 2004). One hypothesis proposes that these low levels of CORT at high latitudes help animals complete their single reproductive attempt (Breuner et al., 2003; Pereyra and Wingfield, 2003; Pravosudov et al., 2002;

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but see Love et al., 2004; Romero, 2002). Presumably then, if time available for breeding exerts strong pressure on stress responses, tropical birds should show dramatic surges in CORT in response to a stressful event because their breeding opportunities are much less limited. So far though, no study has determined if CORT in birds living near the equator complements this pattern. In previous studies, we found that immune defense in a tropical House Sparrow (*Passer domesticus*) population (Colon, Panama) was generally stronger than one from the temperate zone (New Jersey, USA; Martin et al., 2004, in review a, in review b). We expected that because pathogen pressure tends to increase as one nears the equator (Connell, 1971; Janzen, 1970; Pless et al., in review; Ricklefs, 1992; but see Booth and Elliott, 2003) and because sustained high CORT can be immunosuppressive (McEwen et al., 1997; Sapolsky et al., 2000), tropical birds may actually maintain low levels of CORT and mount weak stress responses, contrary to the above prediction.

To directly address these competing hypotheses, we compared baseline and stressed levels of CORT and examined the effects of experimental elevation of CORT (via implantation) on the phytohemagglutinin (PHA) wing-web swelling response (Goto et al., 1978; Evans et al., 2000; Stadecker et al., 1977), an index of cutaneous immune function, in both sparrow populations. Historically, high levels of CORT were touted as obligatorily immunosuppressive (reviewed in McEwen et al., 1997), but more recent studies have shown that this relationship is actually more complex. The effects of CORT on immune function depend on the type of immune activity measured and how long CORT is allowed to take effect (Dhabhar and McEwen, 1997; Dinkel et al., 2002; Sapolsky et al., 2000). Specifically, over the short term, cutaneous immune function, the type we studied, tends to be stimulated by CORT. Over longer periods however, it tends to be suppressed (Bilbo et al., 2002; Dhabhar and McEwen, 1997, 1999). This short-term enhancement is believed to help mobilize cells to locations in the body where they would be readily available if wounding occurred (Braude et al., 1999; Dhabhar et al., 1996). Chronic immunosuppression on the other hand is thought to be useful for managing the energetic and nutritional costs of immune activity (Demas, 2004; Martin et al., 2003) or limiting autoimmune damage (Råberg et al., 1998; Romero, 2002; Sapolsky et al., 2000).

In temperate vertebrates, chronic elevation of CORT almost invariably suppresses cutaneous immune function (Dhabhar and McEwen, 1997; McEwen et al., 1997; Morici et al., 1997; Sapolsky et al., 2000). Thus, we expected that artificial elevation of CORT would result in immune suppression in both of our populations of House Sparrows. Because both corticosteroid levels (Perfito et al., 2002; Romero, 2002; Wada and Shimizu, 2004) and immune activity (Greenman et al., 2005; Nelson and Demas, 1996) can vary seasonally however, we expected that these effects might vary between the breeding and non-breeding seasons at both latitudes.

2. Methods

2.1. Study species

The House Sparrow is a small (25 g), predominantly granivorous, human-commensal passerine that is found on every continent but Antarctica (Summers-Smith, 1988). Originally introduced to multiple places in the United States in the mid-1800s, the species has spread across most of the continent and now can be found as far north and west as Alaska and as far south as Panama (Martin et al., 2004; Summers-Smith, 1988). Now, the morphology and physiology of populations vary across latitudes (Martin et al., 2004, in review a, in review b; Summers-Smith, 1988). Birds used in this study came from two places: a temperate site (Princeton, New Jersey, USA: 40°21′N, 74°40′W) and a tropical site (Colon, Panama: 9°1′N, 80°1′W; see Martin et al., 2004 for a more detailed description). To date, little is known of the population biology of either location, including whether sex ratios are maintained year-round or if populations are self-sustaining or reformed from nearby areas. Further, it is unclear if either population is migratory, although it is unlikely as most populations of this species are highly philopatric (Summers-Smith, 1988).

2.2. Circulating CORT level comparisons

To obtain baseline and stressed levels of CORT, we caught birds using mist nets between the hours of 0600 and 1100 (Rich and Romero, 2001) and took small blood samples (≈50 μl) from the alar wing vein of each animal using 26.5 gauge insulin needles and heparinzed capillary tubes. All baseline blood samples were obtained within 5 min of a bird hitting the net (Romero and Romero, 2002; Wingfield et al., 1982; Wingfield and Romero, 2001); stressed levels included samples taken from birds after they were held in cloth bags for 10 and 30 min (Canoine et al., 2002).

All blood samples were kept cool on ice (5 h max.) until they could be centrifuged and plasma removed and frozen. After the final blood sample was taken and recovery from bleeding was apparent, birds were sexed by plumage, classified as an adult or first year juvenile (Summers-Smith, 1988), weighed and marked with unique combinations of metal and plastic colored leg bands, then released. Tropical birds used in this study were captured in March 2004 (breeding) and December 2003 (non-breeding). Temperate sparrows were captured in June 2002 (breeding) and October 2002 (non-breeding; see figures for sample sizes).

2.3. CORT–phytohemagglutinin experimental protocol

For experiments, we used only naïve birds; no birds from the above stress series were included. Tropical birds used in this part of the study were captured in March 2004 (breeding) and December 2003 (non-breeding); temperate birds were captured in May 2002 (breeding) and November 2002 (non-breeding). Upon capture, birds were given individually numbered metal leg bands and held in wire cages for 3 days prior to implantation. At all times, birds were in visual and audial contact with conspecifics. During captivity, birds were provided with water, mixed seeds, and boiled chicken egg ad libitum, which they took readily. For all experiments, birds were maintained on photoperiods and at temperatures representative of ambient conditions at each locale. Sexes were equally distributed between treatment and control groups. We attempted to use only adult birds when possible, but were forced to use four juvenile birds in Panama in the non-breeding season. In this case, two juvenile birds were used in the treatment group and two in the control group.

On the day of implantation, birds were randomly assigned to treatment (CORT-implant) or control (empty-implant) groups. Immediately prior to implantation, all birds were caught from their cages, blood samples were taken within 5 min of entering the room in which they were held (Romero and Romero, 2002; Wingfield et al., 1982), and body mass was measured with a Pesola spring scale to the nearest 0.1 g. Once all birds were bled and given time to recover, one CORT-filled (Sigma C2505) or one empty silastic implant (Dow Corning; length = 10 mm, inner diameter = 1.47 mm, and outer diameter = 1.96 mm) was implanted under the skin on the rear flank of each animal (under isofluorane anesthesia—Hau et al., 2000). All silastic tubes were sealed prior to implantation on either one (New Jersey, non-breeding, Panama, both seasons) or both ends (New Jersey, breeding) with multi-purpose silicone sealant (732, Dow Corning). We chose to seal tubes on only one end after discovering that plasma CORT levels were not significantly higher in CORT-implanted versus sham-implanted birds in breeding, New Jersey birds. However, even after making this adjustment, plasma CORT levels were not always significantly increased (see below). Initially, we chose this technique because several previous studies have found that similar methods successfully resulted in elevation of circulating corticosteroid levels (DeNardo and Licht, 1993; Kitaysky et al., 2001; Kitaysky et al., 2003; Morici et al., 1997; Salvante and Williams, 2003).

CORT implants were given 3 days to take effect (Kitaysky et al., 2003). During this period, birds were only disturbed (<10 min/day) to provide food and water; bird care was performed at the same time of day every day of the experiment. On the fourth day after implantation, all individuals were again captured from cages and a blood sample was taken from each bird within 5 min of entering the room (Romero and Romero, 2002; Wingfield et al., 1982). Then, each bird was injected with phytohemagglutinin (see below) and weighed. Twenty-four hours later, body mass and wing-web thickness were measured. On one occasion (Panama, non-breeding), birds were held in captivity for different periods of time prior to implantation (1 versus 3 days) because we could not catch a sufficient number of birds in one netting attempt. In this part of the experiment, 1- and 3-day captive birds were equally distributed between empty and filled implant groups to eliminate the effects of time in captivity on our results. We failed to collect pre-implantation CORT samples from New Jersey birds during the breeding season, so we were unable to compare circulating CORT levels over the course of the experiment in sham versus CORT-implanted birds. Instead, we compared CORT 3 days after implantation. All work was approved by the Princeton University Institutional Animal Care and Use Committee (protocol number 1492), the National Environmental Authority of Panama (ANAM), and the Smithsonian Tropical Research Institute (STRI).

2.4. Immune challenge

The phytohemagglutinin (PHA) wing-web swelling technique was used to assess in vivo cutaneous immune activity of House Sparrows (Martin et al., 2004; Smits et al., 1999). PHA is lectin derived from the red kidney bean (*Phaseolus vulgaris*) that stimulates division and trafficking of many cell types, particularly T-cells (Goto et al., 1978; Stadecker et al., 1977). We quantified immune activity by subtracting the thickness of the left wing-web prior to injection from the thickness of the same wing-web 24 h post-injection; a strong immune response was indicated by a large swelling (Goto et al., 1978; Smits et al., 1999). For all challenges, 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) was injected into the left wing-web (Lindström et al., 2004; Martin et al., 2003, 2004). Swelling was then measured to the nearest 0.1 in. with a Teclock pocket thickness gauge (Model #:SI-510) and values were converted to metric units. In all cases, at least 80% of the PHA solution was injected into the wing-web of birds. Previous validations have indicated that this injection technique is sufficient to induce maximum swelling in House Sparrows (Martin, unpublished data).

2.5. Radioimmunoassay

After blood was collected, it was stored on ice until it could be centrifuged at 2000*g* for 10 min and plasma removed. Upon removal, plasma was stored at −20 °C

until radioimmunoassays were performed. For Panamanian samples, plasma was transported to Princeton, New Jersey, on ice. Hormones were extracted using dichloromethane and a standard protocol was used to quantify steroid levels in House Sparrow plasma during stress responses and before and after experimental treatments (Wikelski et al., 1999b; Wingfield and Farner, 1975). Three assays were run on plasma from this study. The first included breeding and non-breeding season field stress series and captive experiment samples for New Jersey birds and breeding season field stress series for Panamanian birds. The second assay included non-breeding experimental and field stress series for Panama. The third included breeding experiment samples for Panama. Intra-assay variation averaged 4.3%, inter-assay variation was 24%, average recoveries were 67.7%, and the average detection limit for all assays was 2.97 ng/ml. Because inter-assay variation was high, comparisons of CORT among populations or seasons in experimental birds are unadvisable. We can however confidently compare sham-implanted birds to CORT-implanted birds within populations/season however, as all of these samples were run in the same assay.

2.6. Statistical analysis

All data were tested for normality and equality of variances using 1-sample Kolmogorov–Smirnov tests, Levene's tests, and/or visual examinations of the distributions of each variable. All data were parametrically distributed except some hormone values (see below). Natural log transformations were used to improve distributions for parametric analysis. To compare CORT stress series and body mass during experiments, we used repeated measures ANCOVA with latitude of origin and season as main effects and mass, sex, and age of birds as covariates; simultaneous Bonferroni post hoc tests were used to identify significant variation between time points in stress series. To compare baseline CORT levels and body mass of birds from the stress series and CORT levels and PHA-induced swellings of our experimental birds, we used one-way ANOVA with either simultaneous Bonferroni post hoc tests or independent contrasts of particular groups of interest. For all analyses, we also calculated observed power (β) and report those results for all non-significant factors/comparisons. We used SPSS v10.0 or JMP v5.0 for statistical comparisons, setting $\alpha = 0.05$.

3. Results

3.1. CORT stress responses

Panamanian House Sparrows were lighter than non-breeding, but not breeding, sparrows from New Jersey (Fig. 1A—ANOVA: $F_{(3,35)} = 8.7$, $p < 0.001$; see graph for Bonferroni post hoc comparisons). Baseline CORT was lower in Panamanian House Sparrows (Fig. 1B—ANOVA: $F_{(6,39)}$, $p = 0.045$; latitude: $F_{(1,39)} = 10.4$, $p = 0.003$). Season ($F_{(1,39)} = 0.52$, $p = 0.424$, $\beta = 0.10$), the interaction of season and latitude ($F_{(1,39)} = 0.15$, $p = 0.705$, $\beta = 0.07$), and mass ($F_{(1,39)} = 2.8$, $p = 0.105$, $\beta = 0.37$), sex ($F_{(1,39)} = 2.0$, $p = 0.166$, $\beta = 0.28$), and age ($F_{(1,39)} = 0.43$, $p = 0.518$, $\beta = 0.097$) as covariates did not affect CORT levels.

Panamanian House Sparrows had significantly lower CORT stress responses than New Jersey Sparrows during both the breeding and non-breeding seasons (Fig. 2). Only latitude of origin (repeated measures ANCOVA: $F_{(1,33)} = 38.8$, $p < 0.001$) and mass ($F_{(1,33)} = 5.3$, $p = 0.027$), but not season ($F_{(1,33)} = 2.9$, $p = 0.096$, $\beta = 0.20$), sex ($F_{(1,33)} = 0.48$, $p = 0.493$, $\beta = 0.10$), age ($F_{(1,33)} = 0.84$, $p = 0.367$, $\beta = 0.14$), or any interactions affected the stress response of birds. When we removed season, age, and sex from the ANCOVA model, CORT responses remained different between populations ($F_{(1,35)} = 61.7$, $p < 0.001$),

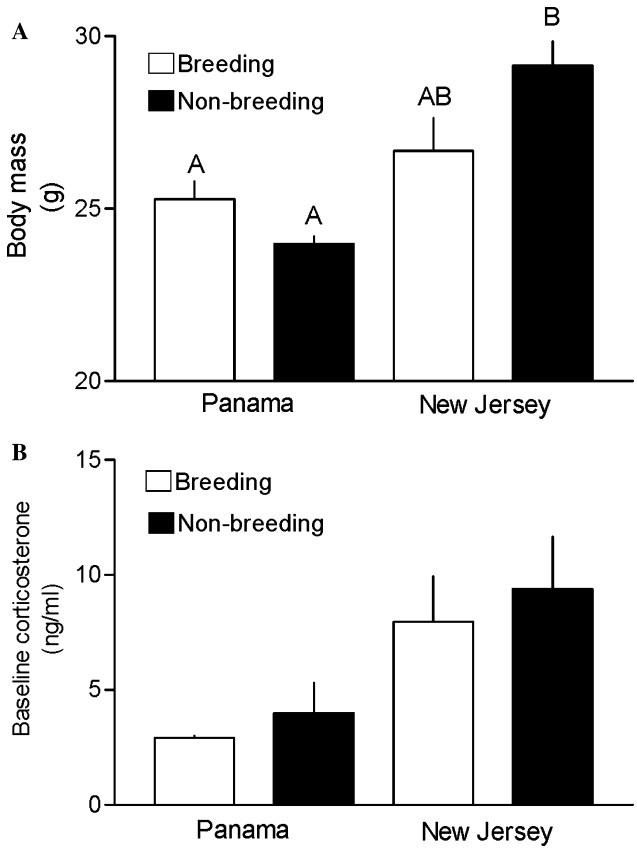

Fig. 1. Body mass (A) and baseline CORT levels (B) are higher in temperate relative to tropical House Sparrows used in stress series. Solid bars represent hormone levels during the breeding season; open bars represent levels during non-breeding. Seasons and sample sizes are same as in Fig. 3. Letters in (A) indicate group membership by simultaneous Bonferroni post hoc test. p value in (B) from ANOVA of all four groups; Bonferroni post hoc indicated no significant differences in pair-wise comparisons. Bars depict means ± 1 SE.

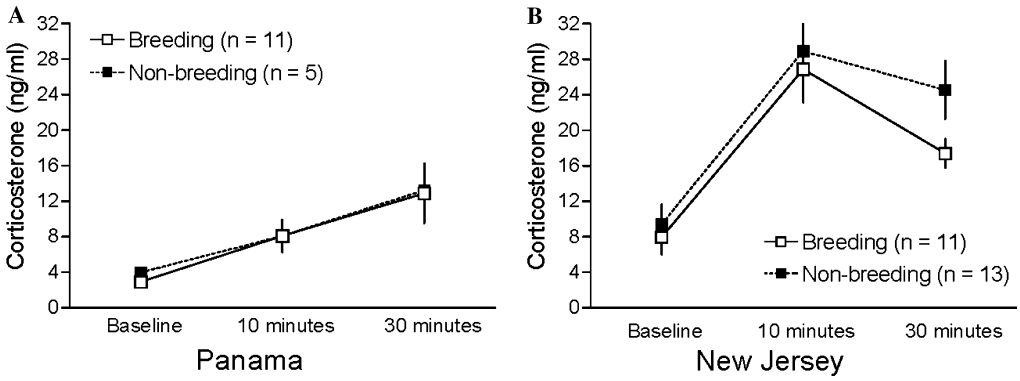

Fig. 2. CORT stress responses are more robust in wild temperate versus tropical House Sparrows. Closed symbols indicate samples taken during the breeding season (March: Panama; June: New Jersey); open symbols indicate samples taken during the non-breeding season (December: Panama; October: New Jersey). Numbers inside parentheses indicate sample sizes. Baseline corticosterone levels were collected from birds within three minutes of animals entering mist nets; all other samples were collected from the same individuals after they were held in cloth bags for the duration of time indicated on the graph. Repeated measures ANOVA indicated that tropical sparrows produced less corticosterone in response to holding in a bag, but there was no significant seasonal variation in corticosterone levels within either population (see Section 3: Stress series for details). Error bars depict means ± 1 SE.

but mass was no longer a significant covariate ($F_{(1,37)} = 2.4$, $p = 0.133$, $\beta = 0.32$).

3.2. CORT–PHA experiment

3.2.1. Effects of implants on body mass and plasma CORT

CORT-implanted birds (except Panama, non-breeding) lost mass over the course of the experiment while sham-implanted controls did not (Fig. 3: $F_{(1,39)} = 11.08$, $p = 0.02$). All three factors, the type of implantation (sham versus CORT: $F_{(1,39)} = 8.39$, $p = 0.005$), latitude of origin ($F_{(1,39)} = 30.17$, $p < 0.001$), and season ($F_{(1,39)} = 9.56$, $p = 0.004$), but no interaction terms, affected this trend.

We compared CORT levels between groups of birds immediately prior to PHA injection (3 days after implantation) to determine if our implant technique effectively elevated circulating hormone levels. We found significant variation among all sparrow groups (Fig. 4: $F_{(7,47)} = 2.95$, $p = 0.014$). Implant type ($F_{(1,47)} = 2.81$,

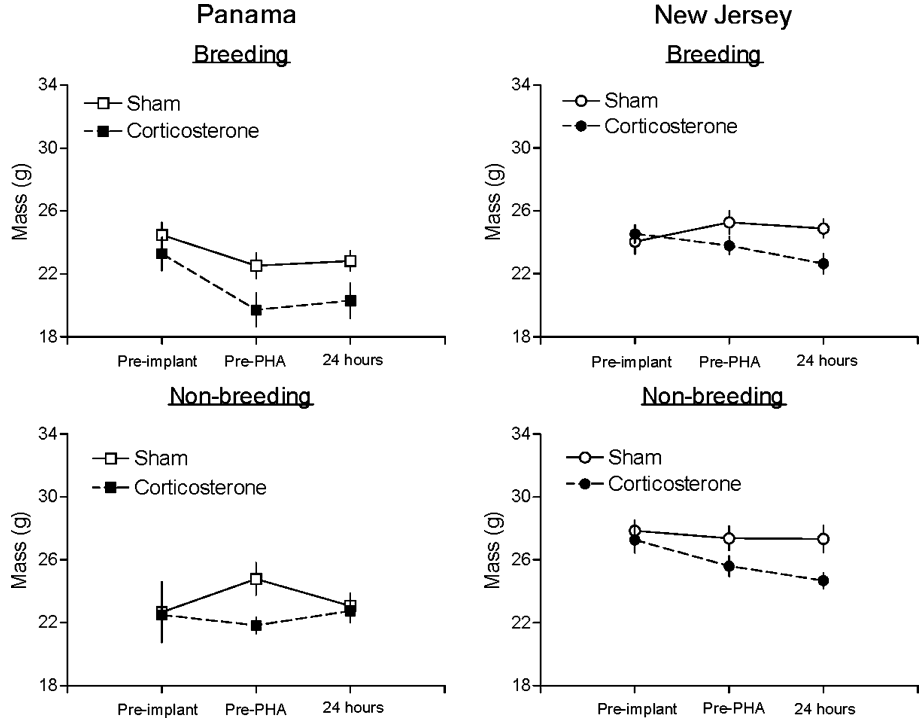

Fig. 3. CORT implantation, but not sham-implantation, tends to cause to mass loss in temperate and tropical House Sparrows during the breeding and non-breeding seasons. Bars represent means ± 1 SE.

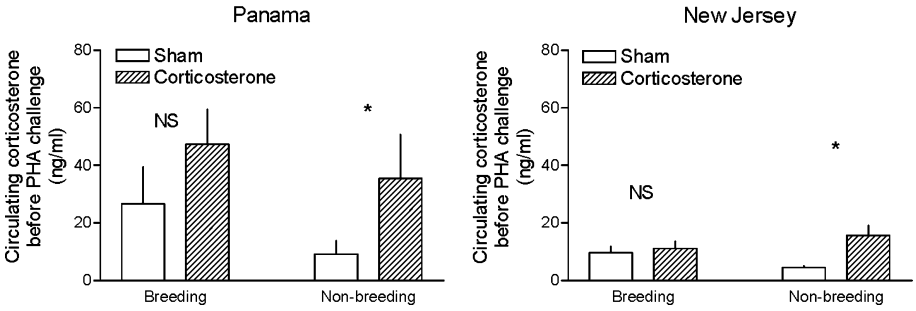

Fig. 4. CORT implantation increases circulating levels of the steroid in non-breeding, but not breeding, House Sparrows from Panama and New Jersey. Bars represent means + 1 SE. Asterisks indicate (marginal) significance ($p \leqslant 0.06$) by independent contrasts of least squares means.

$p = 0.008$), and latitude ($F_{(1,47)} = 2.67$, $p = 0.011$), but not season ($F_{(1,47)} = 0.80$, $p = 0.431$, $\beta = 0.12$) or any interaction were responsible for differences. Planned, independent contrasts showed that implants tended to increase plasma CORT levels in both populations during the non-breeding season (Panama, $F_{(1,40)} = 3.76$, $p = 0.059$ and New Jersey, $F_{(1,40)} = 3.74$, $p = 0.060$), but not the breeding season (Panama, $F_{(1,40)} = 2.15$, $p = 0.15$ and New Jersey, $F_{(1,40)} = 0.03$, $p = 0.871$).

3.2.2. Effects of implants on the PHA wing-web swelling response

The PHA wing-web response varied among our control and treatment sparrow groups (Fig. 5—$F_{(8,46)} = 11.28$, $p < 0.001$). Treatment (sham versus CORT: $F_{(1,46)} = 6.6$, $p = 0.015$), latitude ($F_{(1,46)} = 37.39$, $p < 0.001$), and season ($F_{(1,46)} = 5.6$, $p = 0.023$), but not mass ($F_{(1,46)} = 0.825$, $p = 0.369$, $\beta = 0.14$) or any interaction term explained this variation. According to simultaneous Bonferroni post hoc analyses, CORT implantation significantly suppressed PHA-induced swelling only in New Jersey birds during the non-breeding season (Fig. 5).

4. Discussion

Damped stress responses of birds living at high latitudes have been proposed to exist in part to ensure that parents complete their sole annual reproductive attempt in the face of challenging environmental conditions (Breuner et al., 2003; Pereyra and Wingfield, 2003; Pravosudov et al., 2002). This "behavioral hypothesis" (Romero, 2002) however does not explain why our tropical House Sparrows had lower stress responses than their temperate counterparts. We hypothesize that other factors that vary with latitude, specifically parasite pressure, might be more important. Indeed, we found two forms of evidence for this hypothesis in this study: (i) baseline and stress-induced levels of CORT were lower in our tropical sparrows and (ii) artificial elevation of CORT suppressed immune function in our temperate but not our tropical sparrows. Indeed, similar phenomena have been noted in some temperate vertebrates, such as the prairie vole (*Microtus ochrogaster*; Klein et al., 1996) and breeds of chickens (Siegel, 1995) and rats (Dhabhar et al., 1995). Below, we propose several reasons for why this phenomenon might exist in our tropical House Sparrows and we identify specific physiological mechanisms that could produce such differences.

4.1. Why do CORT and its effect on immune function vary with latitude?

Several hypotheses exist to explain why CORT varies over space and time (Romero, 2002). Latitudinal variation in CORT levels (and its effects on immune activity)

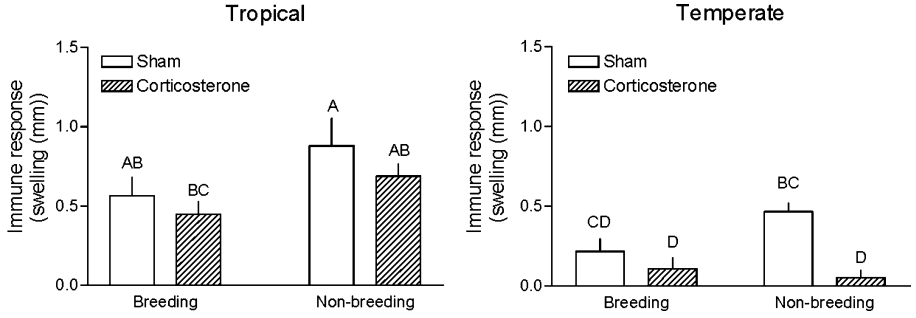

Fig. 5. CORT implantation suppresses immune activity in temperate House Sparrows during the non-breeding (but not breeding) season, but does not affect immune activity in tropical House Sparrows at either time of year. Bars represent means + 1 SE. Letters indicate group membership by simultaneous Bonferroni post hoc comparisons.

may be related to different environmental conditions (Breuner et al., 2003; Pereyra and Wingfield, 2003; Pravosudov et al., 2002). Different photoperiods may also be important, as light levels can affect both CORT (Rich and Romero, 2001; but see Breuner et al., 1999; Romero and Remage-Healey, 2000) and immune function (Bilbo et al., 2002; Greenman et al., 2005). Further, experiences during ontogeny may also matter. Stress during development, including low protein diets, can lead to weaker immune activity and exaggerated CORT responses in many species (Cronjé, 2003; but see Prendergast et al., 2004).

We propose that latitudinal variation in CORT is probably strongly influenced by the differential need of immune defense in different places and/or at different times. Indeed, differences in the abundance and diversity of parasites have been experimentally shown to influence the physiological phenotype of animals (Miller et al., 1997; Starck et al., 2001). We expect then that our tropical sparrows may maintain low baseline CORT, mount weak CORT stress responses, and be immunologically insensitive to CORT as a three-tiered means of defending themselves against the greater parasite threat that exists near the equator. This strategy may ensure that their immune defenses operate at high levels at all times (Martin et al., 2004; Romero, 2002; Sapolsky et al., 2000) in spite of the demands and/or self-inflicted damage this strategy may place on them (Råberg et al., 1998; Romero, 2002).

Conversely, our temperate sparrows may use CORT to ensure that their defenses are only activated when they can afford them (Martin et al., 2004; Nelson and Demas, 1996). Indeed, CORT levels in other wild temperate passerines complement this theory. Maximal levels of baseline and stress-induced CORT (Wada and Shimizu, 2004; Romero, 2002) are usually concurrent with breeding, the purportedly most costly time of the year for birds. Similarly, immune defenses are usually weakest during this same season in most temperate vertebrates (Greenman et al., 2005; Nelson and Demas, 1996). Although few data exist, tropical species' immune defenses tend to be seasonally invariant (Demas and Nelson, 2003; Martin et al., 2004).

4.2. How could latitudinal differences in CORT and its effects on immune function arise?

Several physiological mechanisms have been identified in vertebrates that could explain the differences we found in CORT and its effects on cutaneous immune function in this study (Dhabhar et al., 1993). El-Lethey et al. (2003) found that chronic stress increased CORT but only suppressed some immune responses in domestic chickens (*Gallus gallus*). In a previous study, we found that tropical House Sparrows invest more in immune defense (Martin et al., in revision a) and rely on different immune cell populations (lymphocytes) than their temperate counterparts when mounting PHA responses (Martin et al., in revision b). Thus, the immunological insensitivity to CORT we found in this experiment could be a consequence of the same challenge inducing different types of immune activity in each population and only one of these types of defense being sensitive to CORT (Dinkel et al., 2002; Sapolsky et al., 2000). Indeed, had we measured a different aspect of immune defense, we might have obtained a different result altogether (Schmid-Hempel and Ebert, 2003). Other immune defenses, like antibody production and acute phase (fever) responses, may not be affected by CORT in the same way as cutaneous immune function induced by PHA (Dinkel et al., 2002; Sapolsky et al., 2000).

Indeed, the PHA technique may not be an ideal model for studying the effects of CORT on cutaneous immune function because of its lack of resolution. We initially expected that the aseasonality of stress responses in our wild temperate birds would result in the lack of seasonal sensitivity to CORT. We found however that CORT suppressed immune function in the non-breeding, but not the breeding season in New Jersey sparrows. On one hand, this could accurately reflect immunological insensitivity of the skin to CORT in New Jersey birds at this time of the year. More likely though, this is a consequence of the difficulty of detecting differences in tissue swellings when the swelling in the control (sham) groups is already weak for other reasons (Greenman et al., 2005; Martin et al., 2004).

A different perspective could explain the different circulating CORT levels in our sparrow populations. One way tropical sparrows might produce less CORT in response to the same stressor is to alter the sensitivity of their adrenals to the pituitary-derived hormone, adrenocorticotropin (ACTH—Romero and Wingfield, 1998, 2001; Romero et al., 1998a) or the sensitivity of their pituitary glands to the hypothalamus-derived hormones arginine vasotocin (AVT) and/or corticotrophin releasing factor (CRF—Romero et al., 1998b). Two distinct mechanisms might underlie the immunological insensitivity to CORT we found in Panamanian sparrows. First, sparrows might vary in the diversity and density of glucocorticoid receptors they maintain. Many of the effects of CORT are only invoked when Type II (glucocorticoid) receptors are activated (Breuner and Orchinik, 2001; Dhabhar et al., 1995; McEwen et al., 1997; Romero, 2004; Sapolsky et al., 2000). Thus, tropical sparrows may avoid cutaneous immunosuppression by preventing hormone levels from ever increasing to the point that Type I (mineralocorticoid) receptors are saturated (Sapolsky et al., 2000; Spencer et al., 1991). Second, sparrow populations may differ in the abundance of binding globulins (CBGs) they maintain (Breuner et al., 2003). Although the specific function of these molecules remains unclear (Romero, 2004), CBGs could either sequester or facilitate the

activities of CORT more in one population (Breuner and Orchinik, 2000, 2002). Perhaps tropical sparrows prevent the interaction of CORT with their immune tissues/cells by rendering it functionally inert.

A difference in one or more components of the CORT response may also explain the lack of elevated circulating levels of CORT in some of our hormone-implanted birds. Typically, the implantation technique we used leads to elevation in plasma CORT (DeNardo and Licht, 1993; Kitaysky et al., 2001; Morici et al., 1997; Salvante and Williams, 2003), yet we (and others—Kitaysky et al., 2003) did not find this. We believed initially that this may have been because of the different implant techniques we used (sealing both versus one end of the silastic tube). However in Panama, we used an identical technique in both seasons; still, CORT was only elevated in non-breeding birds. Furthermore, it was clear to us that our treatment had some physiological effects: all CORT-implanted birds in both populations tended to lose mass while sham-implanted birds typically maintained or gained mass (Fig. 3). Taken together, it seems that multiple aspects of the CORT stress response probably vary seasonally, as well as latitudinally, in our birds as they do in other House Sparrow populations (Breuner and Orchinik, 2001).

In sum, it is clear from our study that latitudinal variability in CORT levels in House Sparrows occurs and is functionally significant. In Panama, it appears that the differential need for parasite defense may be more important than opportunities for breeding for determining when and how birds use CORT. Still, we cannot yet conclude that tropical House Sparrows in general have weaker stress responses or are immunologically insensitive to CORT compared to their temperate relatives. Such an argument would require explicit testing of multiple populations from tropical and temperate areas. Indeed, the differences we found could be characteristic for these two populations alone, and drawing conclusions regarding patterns for temperate and tropical birds may be premature. Future studies should attempt to: (i) determine if other House Sparrow populations (and other resident species) are similarly insensitive to CORT and (ii) identify if other components of immune defense are affected in the same way.

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