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Environmental and hormonal correlates of immune activity in a cooperatively breeding tropical bird

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ABSTRACT

Because climatic patterns in temperate regions are generally predictable, species can allocate resources adaptively among competing physiological processes before environmental conditions change. In the semi-arid tropics where environments are seasonal, but highly unpredictable, allocation decisions may be more sensitive to short-term fluctuations in conditions. We asked (i) whether investments in immune function were affected by inter-annual variation in rainfall and (ii) whether corticosterone and prolactin, two hormones that modulate immune activity in other vertebrates, predict environmentally induced alterations in immune activity in cooperatively breeding superb starlings (*Lamprotornis superbus*). Superb starlings inhabit African savannas characterized by high among-year variation in rainfall, which influences their breeding life histories and hormone levels. We quantified bactericidal capacity of plasma, or bacterial killing, and prolactin and corticosterone concentrations in blood samples collected over a four year period during the dry season prior to breeding, as this is the period when reproductive roles are determined in this species and when rainfall is most variable. We found that bacterial killing was weakest in the driest year of the study, and we detected a positive relationship between bacterial killing and prolactin, but not a negative relationship with corticosterone. Together these results suggest that prolactin may mediate rainfall-induced changes in immune activity in superb starlings. This study is the first to examine relationships between prolactin and an index of constitutive, innate immunity in birds, and suggests that even species inhabiting unpredictable environments adjust their physiological priorities to environmental conditions, perhaps via prolactin.

1. Introduction

Many vertebrates exhibit seasonal variation in immune function that is driven by temporal changes in parasite threat and physiological trade-offs between immune defense and reproduction (Martin et al., 2008). However, most of our understanding of immunological seasonality comes from work on temperate species (Nelson and Klein, 2000; Prendergast et al., 2001; Møller et al., 2003), which generally experience predictable seasonal changes in climate (Wingfield et al., 2000). In contrast, few studies have examined potential immunological seasonality in free-living animals from the semi-arid tropics where environments are seasonal, but patterns of annual rainfall are highly unpredictable from year-to-year (Rubenstein and Lovette, 2007). Such unpredictable environments have profound effects on individual reproductive decisions in birds (Emlen, 1982; du Plessis et al., 1995; Rubenstein, 2007b,c), and could potentially influence investments in immune defense. Moreover, because differences in stress physiology and other life history characteristics between tropical and temperate birds may be related to the stability and predictability of different environments and habitats in the tropics (Martin and Rubenstein, 2008), it is important to examine seasonal patterns of immune function and the hormones that mediate such patterns in understudied tropical species.

Hormones play an essential role in regulating vertebrate immunity. Melatonin is an important mediator of seasonality of immune function and reproduction in vertebrates, particularly mammals (Nelson, 2004), but intra-seasonal variation in immune function is also strongly affected by two other hormones: glucocorticoids and prolactin (reviewed in Martin et al., 2008). The

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primary glucocorticoid in birds is corticosterone, which circulates at low levels in the blood and predominantly regulates energy balance (Sapolsky et al., 2000). Corticosterone (CORT) is often referred to as a 'stress hormone' because levels in the blood rise in response to a variety of abiotic (e.g., inclement weather) and biotic (e.g., changes in social rank, aggressive interactions, predation) stressors (McEwen and Wingfield, 2003). Although glucocorticoids regulate many aspects of vertebrate physiology and behavior, they can suppress immune function if elevated chronically (McEwen et al., 1997; Sapolsky et al., 2000). In contrast, prolactin (PRL) is a hormone that is typically associated with vertebrate reproduction, including egg-laying and parental behavior in birds (Riddle et al., 1935; Silverin and Goldsmith, 1983; Wingfield and Farner, 1993; Buntin, 1996). PRL may also influence adaptive adjustments in immune function in birds; PRL is immunoenhancing in many vertebrates (Yu-Lee, 2002), although such effects on free-living birds have yet to be investigated. Moreover, most studies of PRL in free-living birds have been conducted only during the breeding season when baseline levels rise in association with nesting (Wingfield and Farner, 1993). Thus, both PRL and CORT play important roles in regulating avian physiology and behavior, but they may have contrasting effects on immune function.

Here, we examine whether year-to-year variability in rainfall just prior to breeding affects how superb starlings (*Lamprotornis superbus*) (i) invest in one aspect of immune function, and (ii) how PRL and CORT might coordinate these immune investments. Superb starlings are endemic to the savannas of East Africa where rainfall varies greatly from year-to-year (Rubenstein and Lovette, 2007) and influences reproductive decisions, breeding roles, and CORT (Rubenstein, 2007b,c). Because breeding in superb starlings is also highly seasonal and tightly linked to the beginning of the rainy season (Rubenstein, 2006), there is reason to expect that PRL might also be directly related year-to-year variation in rainfall. Thus, because temporal variation in rainfall drives many of the key behavioral, hormonal, and life history traits in superb starlings, we examine immune function and its underlying hormonal correlates in the context of life history trade-offs (*sensu* Ricklefs and Wikelski, 2002). Moreover, superb starlings are cooperative breeders (Feare and Craig, 1999; Fry et al., 2000), and immune function may be particularly important in cooperatively breeding species that live in family groups compared to closely related non-cooperatively breeding species (Spottiswoode, 2008). Additionally, like CORT (Rubenstein, 2007b) and PRL (Vleck et al., 1991; Schoech et al., 1996; Brown and Vleck, 1998; Khan et al., 2001), immunity may also vary with breeding roles (i.e., breeders vs. helpers) in cooperative species (Lutermann and Bennett, 2008).

To quantify immune activity in cooperatively breeding superb starlings, we used a bactericidal, or bacterial killing, assay (BK) that provides an index of constitutive, innate immunity against Gram-negative bacteria (e.g., non-pathogenic *Escherichia coli*) (Matson et al., 2006; Millet et al., 2007). BK and hormone levels were quantified from samples collected over four years during the pre-breeding dry season, the period when rainfall is most unpredictable and when reproductive roles are determined in this species (Rubenstein, 2007b). Based upon the known immunomodulatory effects of both hormones in other taxa, we predicted that among years, BK would be (i) positively related to PRL, but (ii) negatively related to CORT. Additionally, because CORT is negatively related to pre-breeding rainfall in helpers of this species (Rubenstein, 2007b), we predicted that (iii) BK would be weakest in the driest years. However, the effects of seasonality on BK could also be independent of CORT because CORT is only related to pre-breeding rainfall in helpers and not in birds of other breeding roles (Rubenstein, 2007b).

2. Materials and methods

2.1. Study system

Superb starlings were studied from 2001 to 2005 at the Mpala Research Centre, Kenya (0°17′N, 37°52′E). Superb starlings live in mixed savanna woodland habitat (Feare and Craig, 1999; Fry et al., 2000) in groups of 10–35 (mean = 21) individuals that defend year-round territories. Birds breed during both the long (March–May) and short rains (November), and over 90% of nests have at least one auxiliary helper that aides in nestling provisioning (Rubenstein, 2007c). Breeding roles for each individual were determined during the breeding season after the long rains began using intensive 1–3 h focal observations at active nests (Rubenstein, 2007b). Breeders were defined as the social parents of the nest, helpers as all individuals that brought food to a nest excluding parents, and non-breeders/non-helpers as other group members that neither bred socially nor provisioned young (Rubenstein, 2007b). Because superb starlings are sexually monomorphic, sex was confirmed for all individuals using PCR primers (Griffiths et al., 1998) that were confirmed to work in this species using birds of known sex (Rubenstein, 2007a,c).

The main dry season (pre-breeding period) occurs between the short and long rains and generally lasts from December through February. Daily rainfall data were collected during the study using a Hydrological Services TB3 Tipping Bucket Raingauge located at the Mpala Research Centre. The amount of rainfall that fell during the pre-breeding period was calculated as the sum of the daily rainfall during December, January, and February each year. This period represented the (i) three months with the lowest average cumulative monthly rainfall, (ii) three (of four) months where the variance in mean monthly rainfall was greater than the mean value, and (iii) three (of five) months when superb starlings did not initiate new clutches of eggs (Rubenstein, 2006). There was no relationship between the amount of rain that fell during the pre-breeding and the breeding periods (Rubenstein, 2006). Pre-breeding rainfall was 20.3 mm in 2002, 91.2 mm in 2003, 137.4 mm in 2004, and 42.7 mm in 2005.

2.2. Blood sampling

Starlings were captured during the pre-breeding dry season using baited wire traps. Although PRL is typically elevated during the breeding period and may be associated with parental care in cooperative breeders (Vleck et al., 1991; Schoech et al., 1996; Brown and Vleck, 1998; Khan et al., 2001), we specifically chose to examine PRL and CORT during the pre-breeding period because in superb starlings, this period is when individuals make decisions about breeding roles and when environmental conditions are most variable (Rubenstein, 2007b,c). As one of the primary goals of this study was to examine the relationship between immune function and the hormones PRL and CORT prior to reproduction when both hormones typically increase for other reasons (Wingfield and Farner, 1993; Romero, 2002), all samples were collected prior to the first rains because breeding does not start in superb starlings until the rains begin.

Blood samples were collected within 10 min of capture (within 3 min of capture for CORT samples) from either the jugular vein using a 10 cc syringe or from the alar wing vein into capillary tubes. The number of birds captured and analyzed in each year varied; BK: 28 birds (15 males and 13 females) in 2002, 32 birds (17 males and 15 females) in 2003, 28 birds (13 males and 15 females) in 2004, and 22 birds (10 males and 12 females) in 2005; PRL: 36 birds (18 males and 18 females) in 2002, 36 birds (18 males and 18 females) in 2003, 36 birds (17 males and 19 females) in 2004,

and 27 birds (14 males and 13 females) in 2005; CORT: 33 birds (17 males and 16 females) in 2002, 35 birds (17 males and 18 females) in 2003, 36 birds (17 males and 19 females) in 2004, and 28 birds (14 males and 14 females) in 2005.

All blood samples were centrifuged at 4000 rpm for 5 min within 15 min of collection. Plasma was stored on ice for 1–4 h before transfer to a −30 °C freezer. Samples were exported on dry ice to the USA with permission from the Kenya Wildlife Service, the National Museums of Kenya Ornithology Department, and the United States Department of Agriculture, and stored at −30 °C until assay. All work was approved by the Cornell Institutional Animal Care and Use Committee (01-27).

2.3. Bacterial killing assay

Plasma samples were diluted 1:10 in CO_2-independent media under a laminar-flow conditions (Matson et al., 2006; Martin et al., 2007; Millet et al., 2007). Approximately 100 colony forming units (CFUs) of *E. coli* (ATCC#8739, Microbiologics, St. Cloud, MN) were then added to each sample (ratio = 1:10 with plasma), incubated for 2 h at 37 °C, and plated in triplicate onto tryptic-soy agar. Although other avian studies have inoculated plasma with 200 (Millet et al., 2007) to 600 CFUs (Matson et al., 2006), only 100 CFUs were used in the present study because greater numbers resulted in no BK (Martin, unpublished data). Six agar-filled Petri dishes were inoculated with either diluted bacteria alone (positive controls) or swabbed with a flame-sterilized bacteria spreader under laminar-flow conditions (negative controls) and incubated at 37 °C overnight. BK was quantified the following morning by averaging the three replicates for each individual and dividing that value by the average positive control. Mean intra-assay variation was 6%, and no negative controls contained CFUs.

2.4. Hormone assays

CORT plasma levels were determined by direct radioimmunoassay (RIA), as described previously (Rubenstein, 2007b). Briefly, aliquots of 30–60 µl plasma were equilibrated with 2000 cpm of ^{3}H-corticosterone overnight at 4 °C, extracted with dichloromethane, dried in a 40 °C water bath under nitrogen gas, and then redissolved in 550 µl of PBSG buffer. After equilibrating with buffer overnight at 4 °C, duplicate 200 µl fractions were taken for use in the RIA (Wingfield and Farner, 1975) (CORT antibody, B3-163, Esoterix Endocrinology). Additionally, 100 µl fractions were directly counted for the determination of recovery (mean recovery = 82.1%). Two 400 µl aliquots of distilled water (blanks) and six 400 µl aliquots containing either 0.15, 0.25, or 0.50 ng of non-radioactive CORT standards were taken through the whole assay procedure to estimate non-specific interference, assay accuracy, and intra- and inter-assay variation. Blanks were always below the detection limits, which were set to the detection limit for each assay (range = 0.41–0.66 ng/ml). Intra- and inter-assay variation coefficients were 11% and 10%, respectively.

PRL plasma levels were measured using a newly developed double antibody RIA described here. The assay used a highly purified recombinant European starling (*Sturnus vulgaris*) PRL (AFP1277) as the iodinated ligand, a selected rabbit anti-recombinant starling PRL (AFP2961) at a final tube dilution of 1:500,000 as the primary antibody, and the purified recombinant starling PRL as the reference preparation. Displacement curves obtained using graded dilutions of starling samples did not depart significantly from parallelism with displacement curves for the purified reference preparation. Recoveries of samples ranged from 80% to 100%, assay detection limit was 1 ng/ml, and intra- and inter-assay variation coefficients were 5% and 11%, respectively.

2.5. Statistics

Hormone data were log-transformed to improve normality. BK data could not be transformed to improve normality, so non-parametric tests were used for BK data. A Wilcoxon test was used to examine among-year variation in BK, and a Spearman rank correlation was used to examine the relationship between pre-breeding rainfall and BK. Wilcoxon and Mann–Whitney tests were used to examine the relationship between BK and breeding roles, sex, and age classes in each year. Separate tests were used for each variable in each year because BK did not meet the assumptions of normality required for multivariate models; Bonferonni correction was used to account for multiple tests. ANCOVA examined the relationship between PRL, pre-breeding rainfall, and breeding role. ANOVA examined sex differences in CORT and PRL, and post hoc independent contrasts compared differences separately in each year. A Pearson correlation was used to examine the relationship between PRL and CORT, and Spearman rank correlations were used to examine the relationships between BK and PRL and CORT. Additionally, although the BK data could not be transformed to improve normality in order to conduct a multivariate analysis, we categorized individuals into low (<80%) and high (⩾80%) groups of BK based on the natural break in the data (Fig. 1) and used multiple logistic regression to examine simultaneously the relationship between BK, PRL, and CORT.

3. Results

3.1. Bacterial killing and rainfall

BK showed a bimodal distribution with peaks around 0% and 100% (Fig. 1A). Although much of this bimodality was attributable to differences among years ($\chi^2 = 44.54$, df = 3, $P < 0.0001$), the distributions were heavily skewed in all four years with evidence of both high and low BK in three of four years (Fig. 1B). The among-year variation in BK was related to the amount of pre-breeding rainfall such that BK was lowest in the driest year and higher in wetter years ($S_r = 0.52$, $P < 0.0001$; Fig. 2). BK was not affected by breeding role, sex, or age class in any year (Table 1).

3.2. Hormones and rainfall

CORT was shown previously to be negatively related to pre-breeding rainfall, but only in superb starling helpers (Rubenstein, 2007b). In this study, PRL varied greatly among years ($F_{3,131} = 13.40$, $P < 0.0001$) and was positively correlated with pre-breeding rainfall, but similarly in birds of all breeding roles (rainfall: $F_{1,129} = 4.99$, $P = 0.027$; breeding role: $F_{2,129} = 2.20$, $P = 0.12$; interaction: $F_{2,129} = 0.19$, $P = 0.83$). Sex of birds did not affect differences in CORT in any year (year: $F_{3,124} = 0.53$, $P = 0.66$; sex: $F_{1,124} = 0.47$, $P = 0.50$; interaction: $F_{3,124} = 0.39$, $P = 0.76$), but PRL was higher in females in 2004 ($P < 0.0029$), the wettest year of the study (year: $F_{3,127} = 13.86$, $P < 0.0001$; sex: $F_{1,127} = 1.44$, $P = 0.23$; interaction: $F_{3,127} = 3.15$, $P = 0.027$). There was no relationship between PRL and CORT ($F_{1,129} = 0.08$, $P = 0.78$, $r = 0.03$).

3.3. Bacterial killing and hormones

BK was positively correlated with PRL ($S_r = 0.29$, $P = 0.0023$), but not negatively correlated with CORT ($S_r = -0.0048$, $P = 0.96$). The results of the multivariate logistic regression were similar to results from Spearman correlations: BK was positively associated with PRL, but not negatively associated with CORT (PRL: $\chi^2 = 7.19$, $P = 0.0073$; CORT: $\chi^2 < 0.01$, $P = 0.99$; Fig. 3).

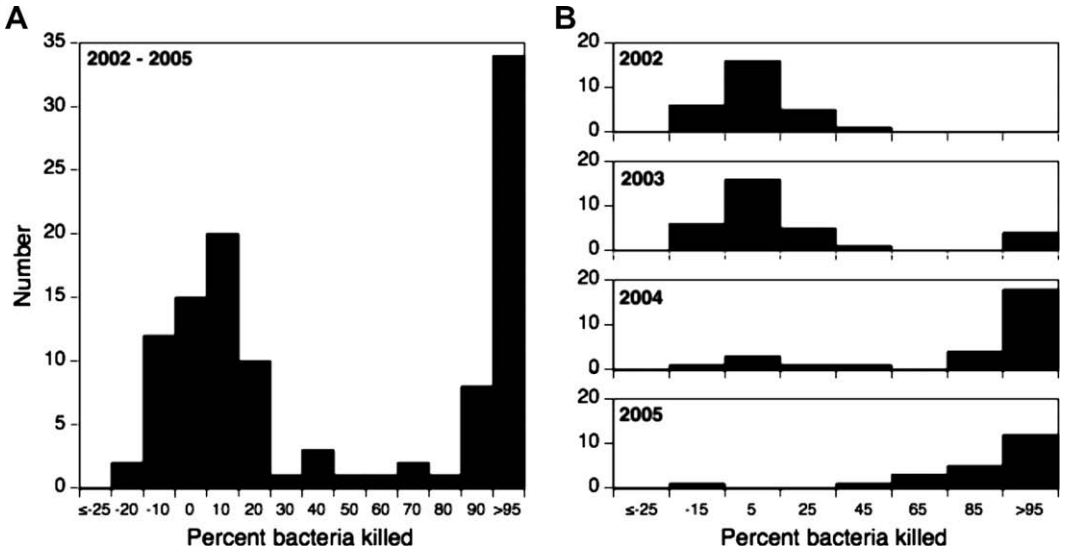

Fig. 1. Distributions of bacterial killing capacity in superb starlings for (A) all years of the study combined and (B) for each of the four years separately. There was a bimodal distribution of high and low killing bacterial killing capacity in each year of the study. Sample sizes are provided in the text.

4. Discussion

4.1. Temporal variability and immunity

Temporal variability in precipitation influences many reproductive decisions in superb starlings. Both the adoption of breeding roles (i.e., helper vs. breeder) (Rubenstein, 2007b) and the maternal control of offspring sex ratio (Rubenstein, 2007c) are affected by inter-annual variation in rainfall prior to breeding. Temporal variability in precipitation may also have influenced the evolution and maintenance of sociality in the entire group of African starlings (Rubenstein and Lovette, 2007). In the present study, constitutive, innate immunity (BK) was also related to among-year variation in pre-breeding rainfall. That is, superb starlings had increased capacity to control Gram-negative bacterial infections when environmental conditions were good, but this capacity was diminished or compromised outright when conditions were poor. Although BK also appeared to decline over time since sampling (i.e., samples from 2005 tended to have higher killing capacity than those from 2002), this trend is unlikely the result of sample degradation because the samples remained frozen continuously, except when assayed for CORT, and samples in three of four years exhibited both high and low BK. Finally, the variation in killing capacity observed within years was not explained by differences in breeding role, sex, or age class.

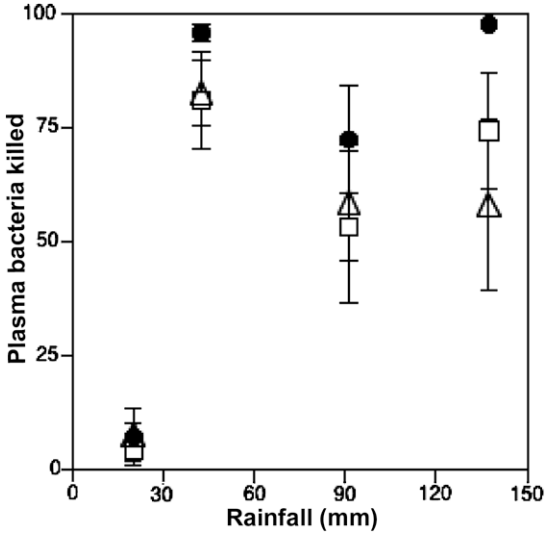

Fig. 2. Relationship between rainfall prior to breeding and bacterial killing in superb starling breeders (open squares), helpers (closed circles), and non-breeders/non-helpers (open triangles). Bacterial killing capacity was positively related to pre-breeding rainfall in birds of all breeding roles. Samples sizes were 11 breeders, 8 helpers, and 9 non-breeders/non-helpers in 2002, 10 breeders, 11 helpers, and 11 non-breeders/non-helpers in 2003, 11 breeders, 11 helpers, and 6 non-breeders/non-helpers in 2004, and 10 breeders, 5 helpers, and 7 non-breeders/non-helpers in 2005.

There are two primary hypotheses to explain seasonal variation in vertebrate immune function mediated by physiological trade-offs (Martin et al., 2008). According to the winter immunoenhancement model, animals *enhance* immune function during the non-breeding season to counteract the immunosuppressive effects of stressors during this harsh portion of the year (Nelson and Demas, 1996; Nelson, 2004). Alternatively, a second hypothesis suggests that immune function is *damped* during energetically costly times of the year, such as during reproduction (Greenman et al., 2005; Martin et al., 2008). Much of our understanding of immunological seasonality comes from work on temperate species (Nelson and Klein, 2000; Prendergast et al., 2001; Møller et al., 2003), which generally experience predictable seasonal changes in climate (Wingfield et al., 2000). Thus, both models assume that animals experience predictable seasonal changes in the environment that allow them to modify their phenotypes with some degree of certainty before the changes occur (i.e., changes in day-length). For

Table 1
Effects of breeding role, sex, and age class on bacterial killing in superb starlings in four different years

Year	Breeding role		Sex		Age class	
	χ^2	P	U	P	U	P
2002	0.29	0.87	0.61	0.43	0.11	0.74
2003	2.98	0.23	0.16	0.69	1.61	0.20
2004	1.47	0.48	0.29	0.59	3.54	0.06
2005	0.94	0.63	3.92	0.048	1.22	0.27

Statistics from Wilcoxon and Mann–Whitney tests are reported. No tests were significant after a Bonferroni correction.

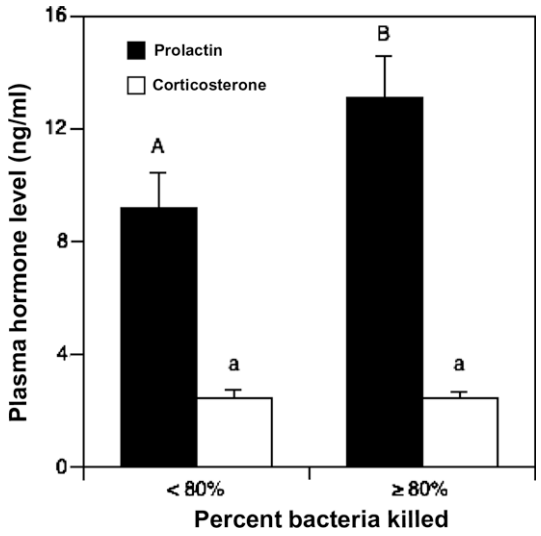

Fig. 3. Comparison of prolactin (black bars) and corticosterone (white bars) in starlings showing low (<80%) or high (⩾80%) bacterial killing capacity. Prolactin was higher in birds that showed greater killing capacity, but corticosterone did not differ between the two groups. Different letters indicate significant differences between groups ($P < 0.05$). Sample sizes are provided in the text.

species like the superb starling, which live in seasonal, but highly unpredictable savanna ecosystems, neither model is amenable to the inter-annual patterns of immune function that may occur in environments where the intensity and duration of the harsh portion of the year (i.e., non-breeding season) are unpredictable from year-to-year. Instead, for tropical species living in semi-arid environments, unpredictability in the severity and duration of the dry season is likely to influence food availability, parasite pressure, and social interactions such as within-group conflict, all of which may impact individual fitness by compromising immune function and ultimately survival.

4.2. Hormonal correlates of bacterial killing

Although superb starlings exhibited low levels or CORT and nearly undetectable levels of other steroids (e.g., testosterone, estradiol, dehydroepiandrosterone) during the pre-breeding period (Rubenstein, 2007b, unpublished data), PRL levels were relatively high in all years and comparable with levels observed during the non-breeding season in temperate birds (Wingfield and Farner, 1993), including European starlings (Ebling et al., 1982). In contrast, baseline CORT in superb starlings was much lower during the non-breeding season than in European starlings (Dawson and Howe, 1983). This suggests that PRL could serve an important function during the non-breeding season (i.e., in non-reproductive contexts) in superb starlings that may be unrelated to reproduction.

We found that the differential killing capacity across years was positively related to PRL. Thus, PRL could be related to the greater BK observed in wetter years. In rodents, PRL stimulates proliferation of natural killer cells and T- and B-lymphocytes *in vitro* (Matera et al., 1992). In birds, the effects of PRL on the immune components involved in BK (predominantly the complement protein complex; Millet et al., 2007) have not been explored. PRL promotes pro-inflammatory immune activity (Yu-Lee, 2002) and many types of leukocytes have PRL receptors (Leite De Moraes et al., 1995). Thus, PRL may be directly responsible for elevated BK by priming the immune system of superb starlings. Alternatively, PRL and immunity may respond independently to rainfall, or the immunological mediators of BK could themselves influence PRL.

Our results also indicate that CORT was not predictive of BK even though it is related to the adoption of different breeding role in this species (Rubenstein, 2007b) and it suppresses many aspects of immunity when chronically elevated in other species (Sapolsky et al., 2000). The lack of a negative correlation between CORT and BK may be related to (i) how pre-breeding CORT in this species may be driven more by the costs of social status and dominance rank than directly by rainfall and environmental conditions (Rubenstein, 2007b), (ii) the generally low baseline levels of CORT in this species (Rubenstein, 2007b), (iii) or an absence of strong modulatory effects of CORT on some aspects of immunity in tropical-dwelling birds (Martin et al., 2005). Clearly, we are only just beginning to learn how stress physiology of tropical birds may differ from that of their temperate counterparts (Martin and Rubenstein, 2008), and how this may mitigate differences in immune function.

4.3. Prolactin and helping behavior

Levels of PRL were positively correlated with rainfall, but they did not differ in birds of different breeding roles. Thus, although PRL may facilitate helping behavior in some avian cooperative breeders living at temperate latitudes (Vleck et al., 1991; Schoech et al., 1996), in superb starlings PRL does not appear to be related to the adoption of helper roles during the non-breeding season. To examine better the role that PRL plays in the adoption of different breeding roles in tropical cooperative breeders, future studies should measure superb starling PRL during the breeding season when levels rise in both breeders and helpers in temperate species (Vleck et al., 1991; Schoech et al., 1996; Brown and Vleck, 1998; Khan et al., 2001).

There were few sex differences in CORT or PRL in any of the years. However, in 2004, the wettest year of the study, PRL was significantly greater in females than in males. This result suggests that despite showing no other evidence of becoming primed for breeding (i.e., no brood patch), females may have anticipated impending good conditions for breeding by elevating PRL in response to greater rainfall during the non-breeding season. In cooperatively breeding Mexican Jays (*Aplecoma ultramarina*), even non-breeding helpers showed elevated levels of PRL a few weeks prior to breeding (Brown and Vleck, 1998). Determining how PRL changes seasonally from the non-breeding dry period to the breeding period in superb starlings will be important for better understanding what roles PRL might play during the non-breeding season, and specifically whether PRL levels during the environmentally harsh pre-breeding period might directly influence immune function.

5. Conclusions

To our knowledge, this study is the first to examine simultaneously relationships among PRL, CORT, and immune defense in free-living birds. Although correlational, our results suggest that prior to the breeding season, PRL enhances BK in a tropical passerine from an unpredictable, but seasonal environment. However, the role of CORT remains unreconciled (Martin and Rubenstein, 2008), and future work on this and other tropical species dwelling in similar semi-arid environments should explore relationships between CORT, PRL, immune defense, and temporal variability in rainfall via experimental manipulations. An historic bias towards temperate systems, which experience environmental fluctuations of different magnitudes and types from the tropics, is no longer tenable (Stutchbury and Morton, 2001). Ultimately, study of hormone-immunity-life history interrelationships in non-temperate taxa will be essential for understanding how organisms endure temporally dynamic environments.

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