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Physiology

The size of a melanin-based plumage ornament correlates with glucocorticoid receptor concentrations in the skin of that ornament

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Glucocorticoid hormones such as corticosterone (CORT) play crucial roles in many physiological processes. CORT's actions are primarily mediated via binding to two receptors (glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs)) in different target tissues. CORT receptors can be independently regulated from circulating hormone titres, from tissue to tissue and even within different regions of the same tissue type. Increasing evidence has shown relationships between circulating CORT and melanin-based pigmentation in skin and feathers, yet to our knowledge, there have been no studies of CORT receptors in the skin of melanized ornaments. Male house sparrows (*Passer domesticus*) have a black, melanized bib, and evidence suggests that bib size is an important intraspecific signal. We examined the relationship between bib area and tissue sensitivity to CORT by quantifying GR and MR in bib skin and in adjacent paler-feathered belly skin (as a control tissue) at different life-history stages using radioligand binding assays. Males with larger bibs relative to their life-history stage had less GR in bib skin, but not belly skin, than males with smaller bibs. These results suggest a connection between the size of a melanin-based ornament and the underlying tissue's responsiveness to CORT.

1. Introduction

Glucocorticoid hormones such as corticosterone (CORT) play crucial roles in a number of physiological processes [1]. Two intracellular receptors bind to CORT to cause these effects: the low-affinity glucocorticoid receptor (GR) and the high-affinity mineralocorticoid receptor (MR) [2]. Because of its higher affinity, MR is thought to be more important in binding baseline concentrations of CORT, whereas GR shows increased binding at stress-induced concentrations [3]. MR is present at high concentrations in select tissues such as kidney and brain [4,5], whereas GR is ubiquitous throughout the body [4,6]. CORT receptors in different tissues can be regulated independently from hormone titres [7,8], from tissue to tissue [9–11], and even within different regions of the same tissue [12,13]. Differential regulation of receptors may allow animals to modulate CORT's effects by increasing receptor density in some tissues but not others.

Several recent studies have found associations between circulating CORT and melanin-based coloration in species ranging from fish to mammals (reviewed in [14]). Because circulating CORT concentrations, melanin coloration and a suite of several other behavioural and physiological traits could potentially be modulated by expression of a common upstream regulator (the pro-opiomelanocortin, or POMC, gene), the extent of melanin coloration could reflect a whole suite of traits. Most studies have examined baseline and/or stress-induced CORT titres; however, because CORT's effects also depend on tissue receptor density, we hypothesized there could be a relationship between the concentration of CORT receptors in skin and the extent of

melanized coloration. GR has been found in both mammalian and avian skin [4,15,16], including in the melanocytes responsible for producing melanin [15,17]. MR is also present in skin, but its distribution appears limited to sodium-transporting epithelia [18]. Therefore, we predicted that melanized coloration would correlate with skin GR, not MR.

Similar to other melanin-based ornaments [19], the black 'bib' of male house sparrows (*Passer domesticus*) is an important intraspecific signal. Bib size positively correlates with fighting ability, age and body condition [20] as well as with stress-induced concentrations of CORT during breeding [21]. We tested whether GR and/or MR in the male house sparrow bib would also correlate with the size of that ornament. We caught male sparrows at five different times of year, measured bib area using photographs and quantified GR and MR in bib skin with radioligand binding assays. We also quantified GR and MR binding in the pale-feathered belly skin directly below the bib as a control. The pale grey feathers of this region likely contain some melanin, but in general, darker-coloured feathers contain more melanin than lighter-coloured feathers [22,23]. Furthermore, feathers in this area are functionally very similar to the bib (body feathers, as compared with wing feathers) and are not known to have any signal function.

2. Material and methods

(a) Study subjects

We caught wild male house sparrows in Medford and Somerville, MA, USA during early winter (12–15 December 2010, $n = 6$), late winter (1–14 February 2011, $n = 7$), pre-egg-laying (31 March 2011–5 April 2011, $n = 6$), breeding (23–24 May 2011, $n = 6$) and late breeding (12–18 July 2011, $n = 6$). See [24] and the electronic supplementary material for additional information on verifying breeding stage in these birds.

(b) Quantifying glucocorticoid receptor and mineralocorticoid receptor in skin

Tissue preparation and receptor assays for GR and MR have been fully described and validated for house sparrow skin [4] and were based on Breuner & Orchinik [8]. These techniques were performed in the same manner for this study and are briefly summarized in the electronic supplementary material.

(c) Bib size analysis

At capture, we took two to four photos (Canon Powershot SD 1000, Lake Success, NY, USA) of each male next to a small ruler for scale to quantify bib size. We used IMAGEJ software (National Institutes of Health, USA) to set scale in each photo using the photographed ruler, then traced the area of contiguous black feathers from the midline of the beak on one side to the midline of the beak on the other and down the chest. For each individual, mean bib area was calculated from all photos ($n = 2$ –4 photos per bird, mean = 2.8 photos). All photos were scored by two different observers (coefficient of variation = 17%), and we used the mean of both sets of scores as the bib area for each male.

(d) Data analysis

Statistical analyses were run using JMP v. 10.0 (SAS Institute Inc., 2012). Because the buffy tips of some black bib feathers wear down as time from moult progresses, bib area tends to increase over time in all males [25,26]. Indeed, we found significant effects of life-history stage on bib area (see Results). However, even though over time all males' bibles increased in size, there was still

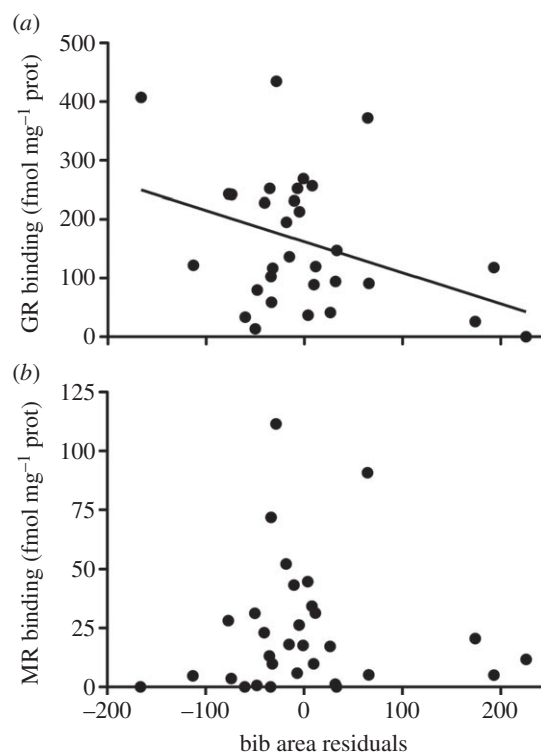


Figure 1. The relationship between (a) GR and (b) MR binding in the bib skin of wild male house sparrows caught at five different life-history stages ($n = 6$ at each stage except late winter, when $n = 7$). Because bib area varied by life-history stage (see text), we graphed (for reference only) the residuals of bib area from the mean of each life-history stage. An individual with a bib area residual of -200 has a much smaller bib than the average for his life-history stage, whereas an individual with a bib area residual of 200 has a much larger bib than average.

substantial variation in bib area within a life-history stage (see electronic supplementary material, figure S1). Therefore, we ran four different ANOVA models with bib area as a dependent variable and receptor concentration (bib GR, bib MR, belly GR or belly MR), and life-history stage as independent variables ($n = 31$ individuals in each model). Data are available at the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.2215v> [27].

3. Results

In bib skin, GR but not MR showed a significant correlation with bib area in male house sparrows; bib area also varied by life-history stage (figure 1; GR: full model: $F_{5,25} = 4.92$, $p = 0.0028$, bib skin GR: $F = 4.67$, $p = 0.041$, life-history stage: $F = 6.05$, $p = 0.0015$; MR: full model: $F_{5,25} = 3.37$, $p = 0.018$, bib skin MR: $F = 0.012$, $p = 0.91$, life-history stage: $F = 2.87$, $p = 0.044$). That is, males with smaller bibles than the mean for their life-history stage had significantly more GR, but not MR, in bib skin than males with larger bibles. In belly skin, neither GR nor MR showed significant correlations with bib area, although again, bib area varied by life-history stage (figure 2; GR: full model: $F_{5,25} = 3.53$, $p = 0.015$, belly skin GR: $F = 0.49$, $p = 0.49$, life-history stage: $F = 3.23$, $p = 0.029$; MR: full model: $F_{5,25} = 3.39$, $p = 0.018$; belly skin MR: $F = 0.07$, $p = 0.79$, life-history stage: $F = 3.69$, $p = 0.017$).

4. Discussion

Larger bibbed male house sparrows had relatively fewer GR in bib skin, but not belly skin, relative to smaller bibbed males.

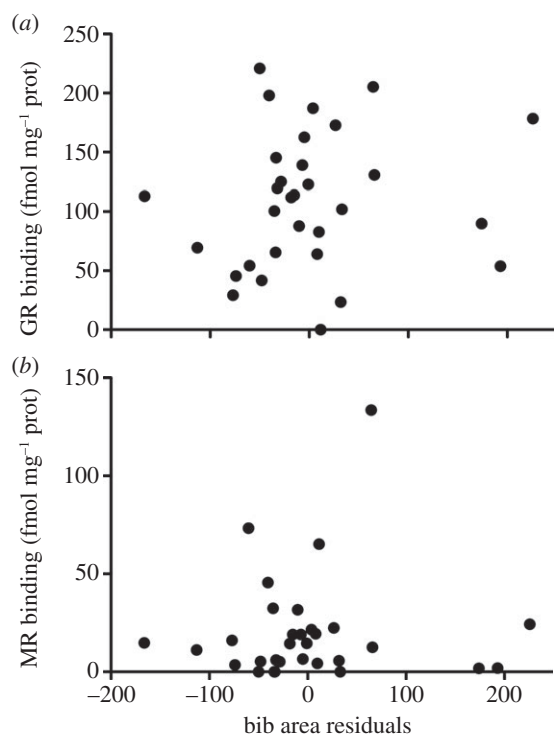


Figure 2. The relationship between (a) GR and (b) MR binding in the belly skin of wild male house sparrows caught at five different life-history stages. See figure 1 caption for sample sizes and additional information.

This suggests a connection between the size of a melanin-based ornament and the underlying tissue's responsiveness to CORT, supporting prior relationships between melanization and activity of the hypothalamus–pituitary–adrenal (HPA) axis [14,28,29]. An earlier study [21], however, found that larger bibbed male house sparrows secreted more CORT in response to a stressor compared with smaller bibbed males, suggesting that circulating CORT and skin CORT receptors are independently regulated in this species.

Alternative splicing of the POMC gene produces several products, including adrenocorticotropin hormone, which stimulates CORT release, and α -, β - and γ -melanin-stimulating hormones, which are involved in melanin production, among other functions [30]. POMC is under negative feedback regulation from CORT [30]; indeed, locally applied CORT can result in skin blanching [28], and the lack of CORT negative

feedback can cause hyper-pigmentation [29]. There is evidence that CORT's negative feedback role on POMC may be a direct effect of a GR–CORT complex, rather than owing to the induction of a repressor-like molecule [30]. The fact that we saw a relationship between male bib size and GR in bib but not belly skin, not only suggests local regulation in the skin, but also independent regulation from skin region to skin region.

These results present some intriguing implications for intraspecific signalling. There has been considerable speculation on potential mechanisms linking intraspecific signalling ornaments to internal or external states [19,31]. Given the importance of the stress response in transducing emergency events into physiological responses, and the central role CORT plays in those responses [1], it is not surprising that studies have examined CORT as a potential physiological mechanism regulating signalling ornaments [32]. As discussed above, expression of the common upstream regulator POMC could link melanization and HPA axis activity; our data suggest this could be regulated on a very local level, with the skin of dark ornaments showing reduced CORT receptor density compared with the skin of paler adjacent regions. In any case, the negative correlation between bib area and bib skin GR concentration suggests that bib size in male house sparrows reflects at least one aspect of CORT responsiveness and thus could provide information on an individual's physiology to his potential mates or competitors. Although responding to environmental stressors is essential to survival, long-term activation of the HPA axis can lead to chronic stress and pathology [33]; reduced receptor density in select tissues could be one way to mitigate these negative effects. Future studies should examine how different components of the HPA axis (circulating CORT concentrations, receptors in different tissues, etc.) might be independently regulated and related to factors such as age, body condition and stressors experienced during development.

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