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Exogenous and endogenous corticosterone alter feather quality

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ABSTRACT

We investigated how exogenous and endogenous glucocorticoids affect feather replacement in European starlings (*Sturnus vulgaris*) after approximately 56% of flight feathers were removed. We hypothesized that corticosterone would retard feather regrowth and decrease feather quality. After feather regrowth began, birds were treated with exogenous corticosterone or sham implants, or endogenous corticosterone by applying psychological or physical (food restriction) stressors. Exogenous corticosterone had no impact on feather length and vane area, but rectrices were lighter than controls. Exogenous corticosterone also decreased inter-barb distance for all feathers and increased barbule number for secondaries and rectrices. Although exogenous corticosterone had no affect on rachis tensile strength and stiffness, barbicel hooking strength was reduced. Finally, exogenous corticosterone did not alter the ability of *Bacillus licheniformis* to degrade feathers or affect the number of feathers that failed to regrow. In contrast, endogenous corticosterone via food restriction resulted in greater inter-barb distances in primaries and secondaries, and acute and chronic stress resulted in greater inter-barb distances in rectrices. Food-restricted birds had significantly fewer barbules in primaries than chronic stress birds and weaker feathers compared to controls. We conclude that, although exogenous and endogenous corticosterone had slightly different effects, some flight feathers grown in the presence of high circulating corticosterone are lighter, potentially weaker, and with altered feather micro-structure.

1. Introduction

One hallmark of the stress response is the production of glucocorticoids (Romero, 2004). Glucocorticoids are produced in response to stressors, whether anthropogenic (e.g., restraint, disturbance) or natural (e.g., predator attacks, storms), and are thought to serve as one of the central regulators that orchestrates successful physiological and behavioral responses to perturbations (Sapolsky et al., 2000; Wingfield and Romero, 2001). The dominant glucocorticoid in birds is corticosterone, which elevates plasma glucose concentrations via increased protein breakdown and decreased peripheral glucose utilization (Eigler et al., 1979; Sapolsky et al., 2000). Plasma corticosterone concentrations are modulated seasonally in many birds, with concentrations lowest during the prebasic molt, when all feathers are replaced in many species (Romero and Wingfield, 1999; Romero, 2002; but see Heath et al., 2003).

Avian molt replaces worn feathers and is seasonal, with most passerines molting once or twice each year. Feather production is energetically costly, increasing basal metabolic rate 9–111% in some species (King, 1974; Lindström et al., 1993), and requires significant protein sequestration (Murphy, 1996). During molt, a large amount of protein is shed and replaced; depending on the species feathers can be 4–12% of an individual's body mass (Murphy, 1996). The quality of a bird's feathers is central to its fitness for many reasons, including predator escape (e.g., Swaddle et al., 1996; Swaddle et al., 1999) and influencing mate selection (e.g., Fitzpatrick, 1998; Ferns and Lang, 2003; Pryke and Andersson, 2005). Growth bars in feathers are wider when a bird has good nutrition, so they can indicate individual quality in both males and females. Growth bars have been correlated with reproductive success in some species (e.g., Takaki et al., 2001), and they also might be an indirect indicator of territory quality (Witter and Lee, 1995).

Feather quality and molting efficiency can influence individual energetic expenditures associated with aerodynamic efficiency (e.g., Dawson et al., 2000). Feather gaps in wings during molt decrease flight efficiency and reduce escape success from predators (e.g., Swaddle et al., 1996, 1999; Hedenström, 2003), and poor quality feathers can break, creating gaps that are not replaced immediately through molt. For instance, Tucker (1991) reported that when primaries are molted in Harris's Hawk (*Parabuteo unicinctus*), gliding performance decreases by 40%. The effects on efficiency, however, are not equal across all flight feathers. Hedenström and Sunada (1999) modeled different patterns of molt gaps and found that gaps in secondary feathers had a greater impact on flight performance than did gaps in primaries. Similar reductions in flight performance due to wing gaps have been reported in European Starlings (*Sturnus vulgaris*)

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(Swaddle et al., 1999; Williams and Swaddle, 2003) and Ruby-throated Hummingbirds (*Archilochus colubris*) (Chai, 1997).

Thermoregulation is another component of avian energetics that is influenced by feather quality. Nilsson and Svensson (1996) experimentally demonstrated that constraining the amount of time and energy Blue Tits (*Parus caeruleus*) have for fall molt results in lower over-winter survival due to thermoregulatory energy expenditure caused by lower quality feathers. Additionally, in the subsequent season, experimental birds delayed breeding and had smaller clutches compared to controls, demonstrating the potential long-term effects of poor feather quality on survival and fitness.

We proposed, therefore, that the reason seasonal corticosterone concentrations are lowest during prebasic molt is that birds down-regulate corticosterone release to avoid corticosterone's degradative effects on proteins and its inhibition in protein synthesis during feather growth. Corticosterone's proteolytic properties could have profound impacts on feathers that are 95% protein (Murphy, 1996), and feather proteins are more costly to synthesize than are muscle proteins (Lindström et al., 1993). We tested this hypothesis by measuring the effects of elevated exogenous corticosterone on feather mass as well as using a number of novel tests of morphological characters related to feather structure and strength. Although these morphological features have not, to our knowledge, been empirically demonstrated to impact feather performance, we propose that they might be related to fitness and survival of individuals through thermoregulatory and flight-performance associated costs. We then compared our results to effects from elevated endogenous corticosterone resulting from different types of stressors. Recent data suggests that endogenous corticosterone influences feather quality differently than exogenous sources (Strochlic and Romero, 2008). European starlings were selected for this study because high corticosterone levels have been shown to decrease the growth rates of feathers in this species (Romero et al., 2005).

Finally, we also tested whether exogenous corticosterone altered the regrown feathers' resistance to wear by *Bacillus licheniformis*, a feather degrading bacterium. *B. licheniformis* has been shown to breakdown feathers by using keratin as its only supply of carbon and sulfur (Burtt and Ichida, 1999). This organism and other keratinolytic bacteria are readily found on wild birds and in the environment (Wood, 1995; Lucas et al., 2003). The discovery of the anti-microbial properties of preen oil (Shawkey et al., 2003) further supports the hypothesis that these bacteria can negatively affect feather quality in birds.

2. Methods

2.1. Exogenous experiments

To simulate molt, we plucked 26 flight feathers (about 56% of the total) from 21 birds: four primaries and three secondaries on each wing, and all tail feathers (rectrices) (for details see Strochlic and Romero, 2008). The day feathers were plucked is referenced as day 0. On day 14, we subcutaneously implanted silastic capsules between the shoulder blades of each bird. Nine birds were implanted with capsules containing crystalline corticosterone (Sigma Chemical Co.). Each implant was 20 mm in length (inner diameter of 1.47 mm) with one end sealed with silicone-based glue and the other end left open to facilitate corticosterone diffusion. The remaining 12 birds received empty capsules. On day 19, we removed the implants. The exogenous corticosterone was only applied for five days for two reasons. First, that is approximately as long as we see increases in corticosterone with our implants (Romero et al., 2005). Second, we were attempting not to push the corticosterone concentrations too much into the pharmacological range, so that the duration was a compromise between a sustained increase in corticosterone while minimizing the pharmacological consequences. To determine if corticosterone implants were effective, we collected blood samples three times during the experiment: before implantation and three and five days after implantation. All surgical and blood sampling procedures follow Romero et al. (2005), and implant success was assessed via radio-immunoassay, after Wingfield et al. (1992). Once feather replacement was complete, new feathers were plucked. For each feather we determined (1) feather mass (an index of protein content because feathers are almost entirely protein), (2) rachis length, (3) vane area, (4) distance between barbs, (5) number of barbules, and for a subset of feathers (6) tensile strength and (7) stiffness of the rachis (measures of how well feathers resist breaking), and (8) hooking strength of barbicels. We also noted if (9) new feathers failed to replace plucked feathers and (10) relative breakdown of feathers due to bacterial degradation. For each bird, we used a mean value for each feather type (primary, secondary, and rectrix) for each measurement for analyses.

Rachis length and vane area were measured using Scion Image (4.0.3.2; http://www.scioncorp.com/pages/scion_image_windows.htm). Barbule numbers and inter-barb distances were measured on images taken using an Imaging Retiga 1300 digital camera mounted onto a Zeiss Stemi SVII dissecting scope. Barbule numbers were counted in a fixed area (0.13 mm^2) of each image and the distance between barbs was measured between the two center-most barbs in each image.

To determine rachis tensile strength, we used an Instron model 3366 materials tester. Individual feather rachis were glued across small card frames using a cyanoacrylate adhesive and later secured between two testing grips separated by a 15 mm gauge length, as reported elsewhere (Bonser and Dawson, 1999). The samples were pulled apart at a rate of 0.27 mm/s (Fig. 1), similar to methods reported by MacLeod (1980). All material testing data were collected and exported using Bluehill Software Ver. 2.0. Data were later analyzed by traditional mechanical testing techniques: force was normalized to measured values of the rachis cross-sectional area for a calculation of stress, while the specimen extension was normalized against the sample gauge length for calculation of strain. This normalization allowed for measurements of the intrinsic material response (i.e. microstructural behavior), rather than the contribution due to feather size. The highest stress was reported as the sample "tensile strength" while the slope of the linear portion of each stress/strain curve prior to sample failure was reported as the sample "stiffness". Feathers used in the Instron were not used in any other manipulation test.

We used air pressure to measure barbicel hooking strength in primaries and rectrices. A stream of pressurized nitrogen gas was projected through the trailing vane of each feather, and hooking strength was determined as the amount of air pressure required to disrupt the interlocking barbicels and break through the vane (Fig. 2). Air pressure was measured using a manometer.

For bacterial degradation we used the technique described by Williams et al. (1990). Briefly, 0.03 g of each feather was prepared (with duplicates for each bird and feather type) in a sterilized feather

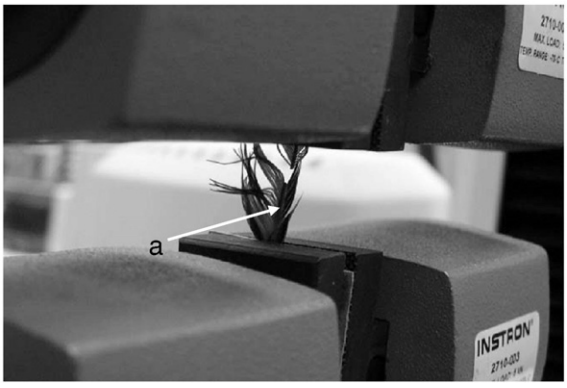

Fig. 1. Instron Model 3369 materials tester, with feather mounted between the two support grips; (a) feather rachis.