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Evolutionary biology

Female plumage colour influences seasonal oxidative damage and testosterone profiles in a songbird

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Across diverse taxa, morphological traits mediate social interactions and mate selection. Physiological constraints on signal elaboration have been widely documented, but the potential for trait display to influence physiological state remains poorly understood. We tested for the presence of causal links between ventral plumage colour—a trait known to covary with reproductive performance—and physiological measures in female North American barn swallows, *Hirundo rustica erythrogaster*. Naturally darker swallows have lower levels of plasma oxidative damage. Females manipulated to display darker ventral plumage during reproduction rapidly decreased oxidative damage, adopting the physiological state of naturally darker individuals. These results support the presence of a social mechanism that links static plumage traits with the physiological state of their bearer during trait advertisement, long after the completion of signal development.

1. Introduction

Features of morphology are widely used in animal communication to advertise information about their bearer to potential mates or competitors [1]. An interesting puzzle is how these traits—many of which persist for long periods, changing little after they are developed—continue to provide current and relevant information. While physiological constraints on trait elaboration have been widely studied [2–4], another potential mechanism has been largely overlooked: that because signals influence social interactions [1,5] that can alter physiological state [6,7], trait appearance may itself produce ongoing signal–physiology links [8,9].

By experimentally darkening ventral feathers within the natural range of variation in free-living female barn swallows, we tested whether plumage colour influences aspects of physiology during the breeding season. In *Hirundo rustica erythrogaster*, birds of both sexes with darker melanin-based ventral colour have higher reproductive success independent of age [10], and experimentally darkened males gain more paternity [11]. Although melanin-based plumage has a strong heritable component [12], its elaboration can also be influenced by social status and physiological state during moult, including circulating testosterone and oxidative stress [13–15].

If signal–physiology links during the breeding season result solely from temporal consistency in the traits that constrain signal development, experimentally darkening feather colour should not influence circulating hormones or oxidative state. By contrast, if signal–phenotype links are influenced by the appearance of signals themselves, experimental darkening is predicted to induce changes in the physiological parameters that are associated with elaborate plumage.
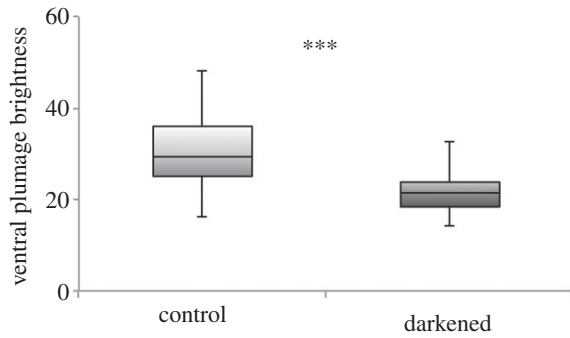
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Figure 1. Distribution of female ventral brightness following plumage manipulations. Box plots represent the median plus the 25th and 75th quartiles and range. Asterisks indicate the significance of differences among treatments; ***$p < 0.001$. Individuals with lower brightness values are darker in appearance.

2. Material and methods

(a) Experimental procedure

Female barn swallows were captured with mist nets at breeding sites in Boulder and Jefferson Counties, Colorado from April–July 2009. Captures were timed to maximize the probability of manipulating females immediately prior to or during pairing (one to two weeks prior to clutch initiation). Blood samples were taken from the brachial vein within 10 min of disturbance. Females ($n = 60$) were randomly allocated to the experimental ($n = 30$) or control ($n = 30$) groups. In the experimental group, the colour of the entire ventral plumage patch was enhanced (figure 1) using a non-toxic marker (PrismaColor, light walnut no. 3507). Control birds were treated identically to those in the experimental group except that the colouring process was mimicked using a capped marker. Previous use of an additional clear colour treatment group showed no differences between unpainted and clear colour controls [11]. The colour of melanin-based ventral breast plumage was scored using a reflectance spectrophotometer (Ocean Optics USB4000), as previously described [16]. Plumage manipulations significantly decreased ventral plumage brightness (paired t-tests: before: 31.35, after: 21.83, $t = -7.53$, $n = 28$, $p < 0.001$) and increased hue (before: 606.9, after: 622.4, $t = 1.79$, $n = 28$, $p = 0.042$), and saturation (before: 0.450, after: 0.486, $t = 1.79$, $n = 28$, $p < 0.001$). The colour of experimentally darkened birds remained within the natural range of variation in the population (less than or equal to 1.5 s.d. on all measures) when compared with 223 feather samples that were taken from non-manipulated females during the same year. Feather brightness—measured as the total amount of reflected light from 300 to 700 nm—was highly correlated with hue and saturation; thus, we used brightness alone in analyses. We were able to recapture and collect plasma samples from a similar proportion of darkened ($n = 19$) and control ($n = 17$) birds (range = 6–17 days post-manipulation).

(b) Physiological measurements

We measured plasma testosterone concentrations using an enzyme immunoassay (EIA) kit (901–065; Assay Designs, Ann Arbor, MI, USA) as described in Clotfelter et al. [17]. Briefly, steroids were extracted thrice with diethyl ether. From each extract, 100 μl was assayed in duplicate according to the EIA manufacturer's guidelines, and 100 μl was used to quantify and correct for extraction efficiency ($88.2 \pm 0.3\%$). Serial dilution of pooled barn swallow plasma extracts yielded a displacement curve parallel to the standard curve ($r^2 = 0.98$). Intra-assay variability was 2.4% and inter-assay variability was 8.1% ($n = 4$ assays).

The d-ROMs kit (Diacron International, Grosseto, Italy) was used to determine the concentration of reactive oxygen metabolites (ROMs)—primarily hydroperoxides—which derive from the

oxidation of biomolecules, providing a marker of oxidative damage. The manufacturer's instructions were followed with one addition: samples were centrifuged immediately after incubation (75 min at 37°C) [18]. Measured values were calibrated with a reference standard, and converted to millimolar of H_2O_2 equivalents. Intra-assay variability was 7.9% and inter-assay variability was 6.7%.

The total plasma antioxidant barrier (antioxidant capacity, AOC) was measured using the OXY-adsorbent test (Diacron International), which quantifies the ability of plasma antioxidants (including proteins, thiols, ascorbate, vitamin E and carotenoids) to resist oxidation by hypochlorous acid (HOCl; an endogenously produced oxidant). Test procedures were conducted according to the manufacturer's instructions. Measured values are expressed in millimolar of HOCl neutralized per millilitre of sample. Intra-assay variability was 5.6% and inter-assay variability was 8.9%.

(c) Statistical analyses

We conducted statistical analyses using SAS v. 9.2. All available samples with sufficient plasma volume were included. When plasma volume was insufficient to conduct all physiological tests, we chose a random subset for each individual. Circulating testosterone concentrations were log-transformed to meet assumptions of normality; all other measures were normally distributed. General linear mixed models ($\alpha = 0.05$) were used to test the relationships between variables prior to experimental manipulation and to examine within-individual changes following plumage manipulation. All models included breeding site as a random effect, and plasma sampling date and time of day—which did not differ among treatments—as fixed effects. Elapsed days between captures (which were marginally lower among darkened birds: $t = 2.07$, $n = 38$, $p = 0.05$) and pre-manipulation colour were also included in models testing the effect of treatment on within-individual changes in physiological parameters. Backwards elimination was used to remove non-significant predictors when $p > 0.10$, and the final models are presented here.

3. Results

Prior to plumage manipulation, treatment and control groups did not differ in any of the measured physiological parameters or in plumage colour (ROMs: $t = -1.47$, d.f. = 37, $p = 0.15$; AOC: $t = 0.20$, d.f. = 47, $p = 0.84$; testosterone: $t = -0.89$, d.f. = 32, $p = 0.38$; brightness: $t = -0.86$, d.f. = 53, $p = 0.39$). Naturally darker females had lower ROMs ($F_{1,26.5} = 11.30$, $p = 0.002$) but did not differ in AOC (brightness: $F_{1,45} = 0.03$, $p = 0.87$; sampling date: $F_{1,45} = 6.76$, $p = 0.013$), or testosterone (brightness: $F_{1,30} = 0.21$, $p = 0.65$; date: $F_{1,30} = 3.22$, $p = 0.08$; time: $F_{1,30} = 3.49$, $p = 0.07$).

Following plumage colour manipulations, darkened birds decreased both ROMs (final model: treatment: $F_{1,19} = 14.46$, $p < 0.001$; pre-manipulation brightness: $F_{1,19} = 3.03$, $p = 0.10$) and circulating testosterone (treatment: $F_{1,24} = 5.81$, $p = 0.024$), reversing the seasonal increases in both of these parameters observed in controls (figure 2). AOC was not influenced by plumage manipulation ($F_{1,21.8} = 2.55$, $p = 0.12$). In contrast to the effect of colour treatment, original plumage colour was not a significant predictor of the physiological response to manipulation in any of the models.

4. Discussion

Our results indicate that the colour of ventral plumage in female barn swallows—which is developed on Neotropical wintering
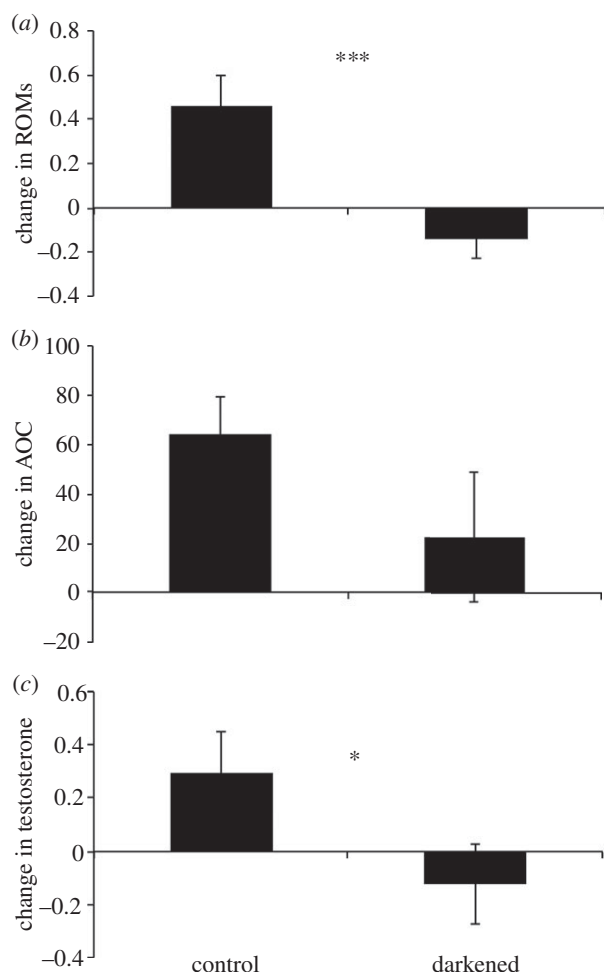
Figure 2. Within-individual changes in physiological measures following signal manipulation (means ± s.e.). Asterisks indicate the significance of differences among treatments; *$p < 0.05$, ***$p < 0.001$. (*a*) Reactive oxygen metabolites (ROMs: mM H_2O_2 equivalents), an index of plasma oxidative damage, (*b*) plasma antioxidant capacity (AOC: mM HOCl neutralized) and (*c*) testosterone (log of ng ml^{-1}).

grounds months before the breeding season—influences the physiological state of individuals during reproduction. Existing hypotheses to explain signal–physiology links focus predominantly on consistent individual differences in physiology or phenotypic condition that constrain the development or display of signal traits [2–4]. For example, melanin-based traits like the ventral plumage of barn swallows have been proposed to signal resistance to oxidative stress though several mechanisms, including the direct suppression of melanization by oxidative stress, or an allocation trade-off between antioxidant defence

and signal development [14,15,19]. While our results support the role of melanin-based plumage as a reliable indicator of susceptibility to oxidative stress [2,20], they implicate an alternative driver of this relationship: the display of darker plumage resulted in a decrease in both ROMs and testosterone during the breeding season.

The rapid within-individual decreases in ROMs and testosterone following plumage manipulations are consistent with the hypothesis that the altered social interactions of darkened birds [5,11] drive changes in physiological state [8]. Specifically, the pattern of physiological changes observed here could result from darker plumage conferring a social advantage in females, with darker females receiving fewer challenges from conspecifics during the early reproductive period. The potential for social feedback about signal quality to affect physiology has also been suggested by findings that signal manipulations influence not only the behaviour of conspecifics but also of signal-enhanced individuals [21,22]. While most studies are not designed to detect the delayed changes in behaviour or physiology that are predicted to result from social feedback about altered morphological traits, evidence from a classic signal manipulation study supports this prediction: darkened Harris's sparrows (*Zonotrichia querula*) exhibit behaviours associated with social dominance only after repeated interactions with conspecifics [5].

The signal–physiology links revealed by these experiments provide support for a social mechanism by which static signals may convey reliable information about their bearer's current physiological condition long after trait development has ceased. Future challenges include determining the role of signal-driven social interactions in generating the observed links between static signals and physiology, and exploring the role of this mechanism in the evolution and maintenance of signal-based communication.

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