ORIGINAL PAPER

Testosterone and melanin-based black plumage coloration: a comparative study

Veronika Bókony · László Zsolt Garamszegi ·
Katharina Hirschenhauser · András Liker

Abstract Despite the functional significance of melanin-based plumage coloration in social and sexual signaling, the mechanisms controlling its information content are poorly understood. The T-regulation hypothesis proposes that melanin ornaments signal competitive abilities via the effects of testosterone (T) mediating both melanization and sexual/aggressive behaviors. Using the phylogenetic comparative approach, we tested whether frontal black melanization is associated with elevated T around the time of breeding plumage development across all bird species with available T-data. We found a context-dependent relationship between melanization and T, varying with the type of ornamentation (patchy or full-black) and with the presumed taxonomic distribution of the hormonal control of plumage dichromatism. Within two taxa in which male plumage development is assumed androgen-dependent (Charadriiformes, Corvida), evolutionary increases in male melanization, and melanin dichromatism correlated with increases in T in most analyses but not within the basal lineage (ratites, Galloanseriformes) with androgen-independent male plumage. Among Passeroidea with presumably genetically or luteinizing-hormone-based male plumage, melanization and its dichromatism correlated with T only in species with <100% frontal melanization. These results were robust as we controlled for several confounding variables such as mating and parental behaviors. This study is the first to test and support the T-regulation hypothesis interspecifically, suggesting that among-species differences in melanization may have evolved in response to differences in circulating T in certain avian taxa. Our results imply that the extent of black ornamentation may serve as an honest indicator of male competitiveness in those species that evolved an appropriate hormonal basis (T dependence) for color production.

Keywords Androgens · Hormonal control · Melanin ornaments · Sexual selection · Status signals

Communicated by: K. McGraw

V. Bókony (✉)
Department of Ecology, Szent István University,
Rottenbiller u. 50,
1078 Budapest, Hungary
e-mail: vbokony@enternet.hu

L. Z. Garamszegi
Department of Biology, University of Antwerp, U.A.,
Universiteitsplein 1,
2610 Wilrijk, Belgium

K. Hirschenhauser
Konrad Lorenz Research Station,
4645 Grünau, Austria

A. Liker
Department of Limnology, University of Pannonia,
Pf. 158,
8201 Veszprém, Hungary

Introduction

Conspicuous coloration has long been the focus of research on sexual selection, and plenty of studies have demonstrated the adaptive function of color traits in both intrasexual competition and mate choice (Andersson 1994; Hill 2006; Senar 2006). Research on the proximate control of coloration has lagged behind until the last decade, despite the crucial importance of understanding the mechanisms that regulate the expression of coloration. For example, it is

difficult to see why female house finches (*Carpodacus mexicanus*) would prefer to mate with males with more red in their plumage (Hill 1990), unless we understand that the red color is derived from carotenoid pigments by a process that is sensitive to both nutritional condition (Hill and Montgomerie 1994) and endoparasitic infections (Brawner et al. 2000; McGraw and Hill 2000). Thus, redness signals individual quality through its condition-dependence.

For melanin-based coloration such as the black bib of house sparrows (*Passer domesticus*), the signaled information appears more difficult to understand. The overall picture is that melanin ornaments signal dominance rank and competitive ability in a wide range of bird species (reviews by Hill 2006; Senar 2006), and several hypotheses have been put forward to explain the proximate control of melanization (Jawor and Breitwisch 2003; McGraw 2003), but so far, empirical evidence is scarce and controversial. For instance, the expression of certain melanin ornaments is under strong genetic control (Roulin and Dijkstra 2003), while others are determined by environmental effects (Griffith et al. 1999; Fargallo et al. 2007a). Experimental studies up to now barely found any effect of nutrition or endoparasitism on melanization (Hill and Brawner 1998; McGraw and Hill 2000; McGraw et al. 2002; Poston et al. 2005), whereas resistance to ectoparasites was shown to be related to melanin-based coloration in some species (Roulin et al. 2001; Fitze and Richner 2002). Altogether, these results hardly support any common mechanism that could link melanization to aggression or intrasexual vigor.

One promising candidate, however, for mediating such a link is the level of sex hormones, most importantly testosterone (T), as proposed by the T-regulation hypothesis (Jawor and Breitwisch 2003). Increased T levels of male birds have been shown to enhance aggressive and sexual behaviors (Wingfield et al. 1987), and comparative studies have supported that high T levels evolve as a physiological basis for intense intrasexual interactions (Hirschenhauser et al. 2003; Goymann et al. 2004; Garamszegi et al. 2005). In parallel, high T levels may be prerequisites not only for intrasexual behavior but also for the production of melanin ornaments. A number of intraspecific studies demonstrated the effect of T on melanocyte function and melanization (Haase and Schmedemann 1992; Haase et al. 1995; Evans et al. 2000; Peters et al. 2000; Buchanan et al. 2001; González et al. 2001; reviews by Hill and McGraw 2003; Jawor and Breitwisch 2003; McGraw 2006; but see Roulin et al. 2004; Fargallo et al. 2007b on non-black ornaments), indicating that T may be involved in regulating melanin pigmentation in several vertebrates. If the expression of melanin ornaments was facilitated or enhanced by T, then these ornaments could reflect the hormone profiles underlying competitive abilities; hence, strong competition among males should select for such signals. In house

sparrows, for example, although T levels are low during post-breeding molt, the subtle differences among males in post-breeding T are enough to determine their bib size, and the individuals' non-breeding T levels predict their T levels later during breeding (Buchanan et al. 2001), which in turn determine their investment in intrasexual competition (Hegner and Wingfield 1987). Thus, T-dependence of bib size may well explain why larger-bibbed males are more dominant throughout the year (reviewed by Senar 2006) and why males that interact more aggressively during molt grow larger bibs (McGraw et al. 2003). Is the house sparrow's bib a unique case or may it represent a more general rule for the T-link between melanization and competitiveness?

If T is widespread involved in the regulation of plumage melanization in birds, the T-regulation hypothesis predicts an interspecific relationship between species-specific T levels and the degree of melanin ornamentation. Species under stronger selection for signaling socio-sexual competitive abilities may evolve higher concentrations of circulating T to facilitate the expression of melanin ornaments. In this study, we test this interspecific prediction of the T-regulation hypothesis using the phylogenetic comparative method. The comparative approach has been successfully used to identify factors influencing the evolution of T profiles (Hirschenhauser et al. 2003; Goymann et al. 2004; Garamszegi et al. 2005; Ketterson et al. 2005; Møller et al. 2005) and melanization (Owens and Hartley 1998; Bókony et al. 2003; Bókony and Liker 2005). Although the latter studies demonstrated the adaptive value of melanin ornaments in sexual selection and parental care, no comparative study has attempted to test interspecific associations between this specific type of coloration and its proposed regulating factors such as diet, parasites, or sex hormones. Such an approach has been urged recently for teasing out general patterns (Griffith et al. 2006) but, up to now, has been undertaken only for carotenoid ornaments in relation to diet (Mahler et al. 2003; Olson and Owens 2005).

In this paper, we examine in a taxonomically diverse set of avian species whether plumage melanization is related to T levels at the time of plumage development, as predicted by the T-regulation hypothesis. We use two measures to assess the degree of melanin ornamentation: the extent of conspicuous black badges in males and the sexual dimorphism in melanized plumage (melanin dichromatism henceforward), both of which proved targets of selection for socio-sexual signaling in many intra- and interspecific studies (Bókony et al. 2003; Bókony and Liker 2005; Hill 2006; Senar 2006). Furthermore, the comprehensive review of all previous case studies on the development of plumage dichromatism (Kimball and Ligon 1999; Kimball 2006) indicates that the hormonal control of sex-specific coloration may vary across major avian clades: The presence of

androgens during molt triggers the expression of male breeding plumage in some groups (shorebirds and corvids), while they have no effect in other taxa (ratites, waterfowl, and weavers) in which other hormones are responsible for sexually dichromatic plumages. Under the T-regulation hypothesis, a relation between T and melanization is more likely in taxa with androgen-dependent than with androgen-independent male plumage. Therefore, we also investigate the relationship between T and melanization separately in taxa that are hypothesized to differ in the hormonal control of coloration.

Materials and methods

The primary selection criterion of bird species for the study was the availability of T data, without any taxonomic restriction. We collected data on non-breeding baseline T and peak T (levels A and C, respectively, sensu Wingfield et al. 1990) of male birds by careful literature search to identify all studies and species with available information on T levels published until December 31, 2006. To our knowledge, this paper reports the largest dataset on interspecific variation on T levels in wild birds. Several previous comparative studies have demonstrated that such T levels are species-specific (i.e., repeatable across different studies; see Garamszegi et al. 2005; and this study; see S1 available on-line as a supplementary material for this article) and biologically meaningful measures (Hirschenhauser et al. 2003; Goymann et al. 2004; Garamszegi et al. 2005; Ketterson et al. 2005; Møller et al. 2005). The T-regulation hypothesis predicts an interspecific association between melanization and the T levels during plumage development, but unfortunately, T data of molting birds are scarcely known. However, the species-specific T levels are positively correlated across different life-cycle periods (Garamszegi et al. 2005). Therefore, we used those available T levels that may best correspond to the periods of the growth of breeding plumage. First, post-breeding molt occurs at the time of gonadal regression or in the non-breeding season, and several studies showed that T levels typically drop after breeding and remain low during these seasons, with T at post-breeding molt often being indistinguishable from non-breeding T (Wingfield et al. 1990). Thus, we used non-breeding baseline T as a surrogate for the T level at post-breeding molt in species with a single annual molt. Second, many species undergo a second, pre-breeding molt at the onset of breeding season, coinciding with gonadal recrudescence and the elevation of T levels (Wingfield et al. 1990); thus, for these species, we used peak T levels measured in the pre-breeding period to approximate the T levels at pre-breeding molt. Direct T data for molting birds were available for 22 species only, and these molt-T levels were well correlated with our

approximate measure of "T at the development of breeding plumage" (r_s=0.56, P=0.007, n=22), which we henceforth refer to simply as T. We used T concentrations measured from blood plasma only. For *Picoides pubescens*, we calculated plasma levels from fecal T measurements according to the calibration equations given for this species (for references, see S2 available on-line as a supplementary material for this article); none of our results were changed by the exclusion of this species. We also conducted several analyses to test the reliability of our T measures, showing that our results are unlikely to be biased by variation across different assay procedures or between captive and field studies (see S1).

We measured melanization as the extent of black area on the breeding plumage (not bare parts) of the head and breast, termed frontal melanization (see Bókony et al. 2003; Bókony and Liker 2005), from color plates in field guides and handbooks that illustrated the birds in lateral view. Using the Scion Image software, we measured the proportion of black area relative to the whole frontal plumage area. This measure of melanization is highly repeatable and adequately captures the interspecific variation in the extent of black ornamentation (for details of the method and its validation among different picture sources and between lateral and frontal views, see Bókony et al. 2003). There was a strong correlation between frontal melanization measured from images and frontal melanization estimated by scoring methods based on Owens and Hartley (1998) for the head and breast (Spearman rank-correlation, r_s=0.87, P<0.001, n=133) and for the whole plumage (r_s=0.80, P<0.001, n=133). We did not consider non-black melanization, as the pigmentary basis of such colors cannot be judged by their appearance (McGraw et al. 2004), and for the time being, their status-signaling function is less evident than that of eumelanic black ornaments (reviewed by Senar 2006). Furthermore, T may affect eu- and pheomelanized plumages differently (Haase et al. 1995; Roulin et al. 2004) and may have negative effect on gray ornaments (Fargallo et al. 2007b). We focus on frontal melanization because it is highly variable across species and most likely to function in intraspecific signaling (Bókony et al. 2003; Bókony and Liker 2005; Senar 2006). Ornamental black patches are infrequent on the wings and tail compared to the frontal region, and the typical uniform melanization of flight feathers and retrices is more likely selected for resistance to wear and friction (reviewed by McGraw 2006) than for signaling.

A number of species-specific traits have been shown to influence both T profiles and melanization, including mating competition (Bókony et al. 2003; Hirschenhauser et al. 2003; Garamszegi et al. 2005; Ketterson et al. 2005), paternal care (Hirschenhauser et al. 2003; Bókony and

Liker 2005), and latitude of distribution (Burtt and Ichida 2004; Goymann et al. 2004; Garamszegi et al. 2005). Therefore, we collected data on the following potentially confounding variables: polygyny (% of polygynous males), paternal incubation and feeding (% of male contribution to incubation and to feeding offspring, respectively), and the latitudinal midpoint of breeding distribution (see S2 for data sources for pictures, molt strategies and confounding variables). Whenever possible, each value of these variables was collected for the populations in which T levels were measured. Following Liker and Székely (2005), we set the percentage of polygyny to 100% in promiscuous (lekking) species to express their strong mating competition and high mating skew, and to 0.1% in monogamous species with rare but unquantified occurrence of polygyny. In sum, we gathered data on 133 species (see S2). Although T levels are known to correlate with rates of extra-pair paternity (Garamszegi et al. 2005), such data are not available for many species in our dataset; thus, we did not control for this effect because it would have decreased our sample sizes dramatically. We also did not control for body size or its sexual dimorphism because previous studies showed that these traits evolve independently from melanization or its dichromatism (Owens and Hartley 1998; Bókony et al. 2003; Bókony and Liker 2005), and in the present dataset, we found sexual dimorphism in wing length to be unrelated to T ($r=0.03$, $P=0.834$, $n=72$).

Taxa were categorized according to the presumed hormonal control of plumage coloration following Kimball (2006) as (1) the basal lineage (ratites, Galliformes and Anseriformes) with estrogen-dependent dichromatism, (2) Charadriiformes, and (3) the Corvida clade of Passeriformes (e.g., bowerbirds, shrikes and crows) with androgen-dependent male plumage, (4) the Passeroidea clade of Passeriformes (e.g., flycatchers, finches, sparrows) with genetically or LH-controlled dichromatism, and (5) all other taxa with no information on the hormonal control of coloration. We treated Charadriiformes and Corvida separately because they represent phylogenetically distinct clades, and the T-dependence of male plumage is much more supported for the former than for the latter (Kimball 2006).

To test for the evolutionary relationships between T levels and melanization, we used phylogenetic generalized least squares (PGLS) models (Pagel 1997, 1999). The PGLS approach characterizes evolutionary changes along each branch of a phylogeny through the variance components of traits and controls for the non-independence among species by incorporating a matrix of the covariances among species based on their phylogenetic relationships (Martins and Hansen 1997; Pagel 1997, 1999). The method applies likelihood ratio statistics to test hypotheses of correlated trait evolution and also to estimate the importance of phylogenetic corrections in the models (Freckleton et al. 2002). We

conducted all analyses setting the degree of phylogenetic dependence (λ) to the most appropriate degree evaluated for each model. To represent phylogenetic relationships among taxa, we used the tapestry tree of Sibley and Ahlquist (1990) augmented by recent phylogenetic information (see S3 available on-line as a supplementary material for this article). Where branch lengths were not available from Sibley and Ahlquist (1990) for lower taxonomic levels, we set the distance between genera within families to 3.4 DT50H units and between species within genera to 1.1 DT50H units (as has been done by several comparative studies following Bennett and Owens 2002). Furthermore, because the Sibley–Ahlquist topology for songbirds was challenged by recent results (Barker et al. 2004), we repeated all our analyses using an alternative phylogeny assuming the topology for Passeriformes suggested by Barker et al. (2004) and setting all branch lengths proportional to the number of nodes (i.e., gradual branch lengths). The results of these analyses were fully consistent with the results based on the Sibley–Ahlquist topology; thus, for brevity, we report the latter only.

For statistical analyses, we used the R statistical computing environment, with additional unpublished functions by R. Freckleton (University of Sheffield) for the PGLS procedure. T values were log_{10}-transformed, and melanization, polygyny, paternal incubation, and feeding were arcsine-square-root transformed. Melanin dichromatism was expressed as the residuals from the regression between male and female frontal melanization. First, we tested the relationship of T with male melanization and melanin dichromatism in bivariate PGLS models. Secondly, to control for the effects of confounding variables, we built multivariate PGLS models for either male melanization or melanin dichromatism as dependents, containing all above-mentioned confounding variables as predictors (see S4 available on-line as a supplementary material for this article), except that chick feeding was not included for taxa with precocial young (basal lineage and shorebirds), and polygyny and incubation were not included for Corvida, as all investigated species are socially monogmous, with no paternal incubation in all but one case. To obtain the final models, we eliminated non-significant effects backwards in a stepwise manner following Grafen and Hails (2002). We also repeated all analyses after excluding species with 100% melanization because the signal function of full and patchy melanization may differ in various taxa. For example, a completely black plumage may either be a result of strong sexual selection for extensive melanization (e.g., in shorebirds; Bókony et al. 2003) or it might serve merely as a basis for the expression of sexually selected iridescent structural coloration (e.g., starlings and bowerbirds; Bennett et al. 1997; Doucet et al. 2006).

All tests were two-tailed with a 95% confidence level. Sample sizes differ across statistical analyses, as various data

were not available for some species. To determine the strength and direction of each relationship, we estimated effect sizes as correlation effect size "r" sensu Cohen (1988) and the associated 95% confidence intervals (CI). To balance Types I and II errors, we followed the recent recommendations of Nakagawa (2004) and preferred using effect sizes instead of Bonferroni correction for significance levels because the latter approach has been criticized for both mathematical and logical reasons (Nakagawa 2004; Garamszegi 2006). Because the PGLS method does not allow the graphical presentation of phylogenetically corrected data, we present figures based on raw species data. Note however that λ was small in most cases (< 0.01–0.57 in all analyses; mean, 0.03; and <0.01 for Fig. 1), similarly to previous phylogenetic analyses of T (Garamszegi et al. 2005).

Results

Using all species, including taxa with no information on the hormonal control of plumage coloration, neither male melanization nor melanin dichromatism was related to T (Table 1a), even when we controlled for confounding variables (Table 2). When we excluded all-black species, both melanization and melanin dichromatism tended to correlate positively with T, but these effects were estimated to be small and uncertain (Table 1b). The final multivariate models indicated, however, that when the confounding effect of male incubation is taken into account, species with more elevated T express greater melanin dichromatism (Table 2).

In the basal lineage with estrogen-dependent dichromatism, we found no relationship between T and melanization or melanin dichromatism (Table 1), even when we controlled for confounding variables and excluded all-black species (Table 2).

In the two taxa with androgen-dependent male plumage, both melanization and melanin dichromatism correlated positively with T in most analyses. In Charadriiformes, we found a tendency of male melanization to increase with T (Table 1), and this effect was estimated as medium to very strong in multivariate models controlling for confounding variables (Table 2). Melanin dichromatism appeared unrelated to T in shorebirds (Table 1), but notably, this avian group includes four polyandrous species with reversed sexual dichromatism and reversed sex roles. When we controlled for male incubation as a proxy for sex role reversal, we found a medium to very strong positive correlation between T and melanin dichromatism (see the final model in Table 2; Fig. 1a). Our dataset does not contain all-black shorebirds.

In the other group with androgen-dependent male plumage, the Corvida songbirds, both melanization and melanin
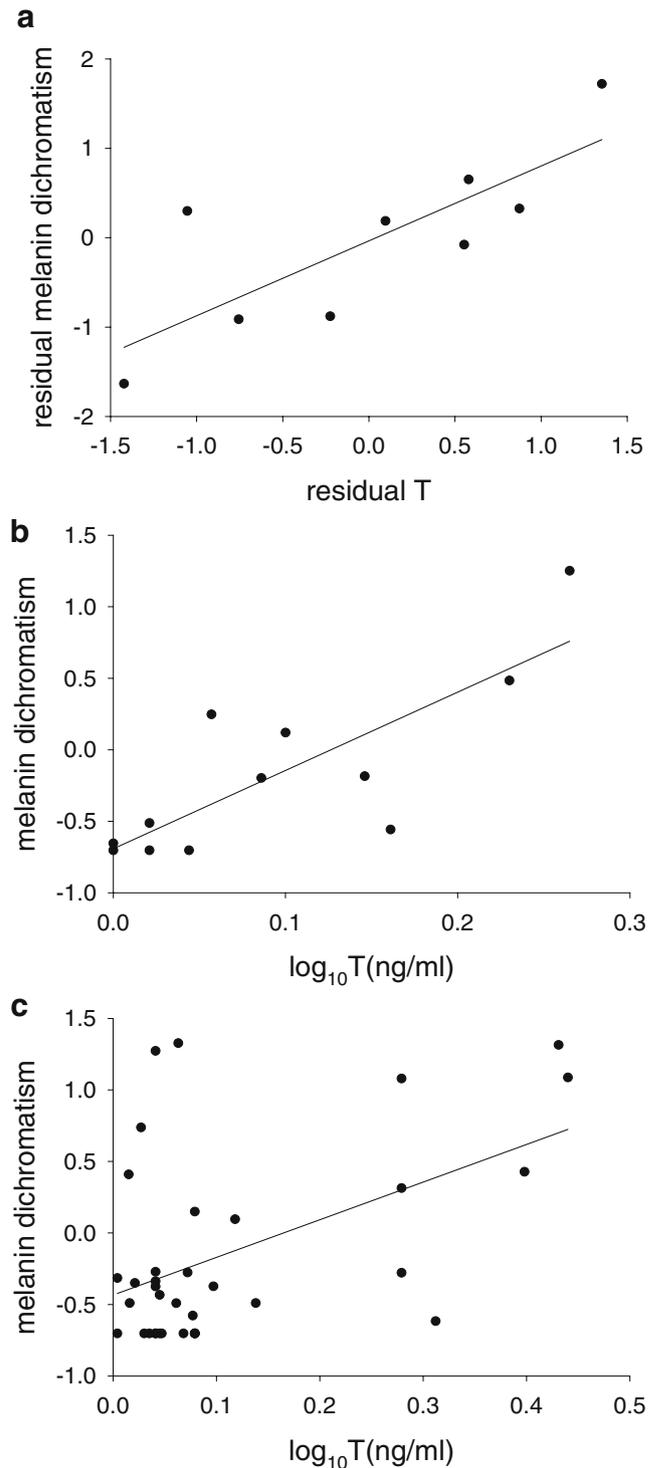
Fig. 1 Interspecific relationship between melanin dichromatism (i.e., sexual dimorphism in the extent of black plumage) and T levels (approximately at the time of breeding plumage development) in **a** Charadriiformes, both variables were controlled for male incubation to take sex-role reversal into account; **b** Corvida; and **c** Passeroidea species with <100% male melanization

Table 1 Male melanization and melanin dichromatism in relation to T levels in birds

	Male melanization			Melanin dichromatism		
	r (CI)	P	n	r (CI)	P	n
All species						
All taxa	0.01 (−0.18, 0.20)	0.903	105	0.06 (−0.13, 0.25)	0.521	105
Basal lineage	−0.07 (−0.56, 0.46)	0.817	15	0.07 (−0.46, 0.56)	0.813	15
Charadriiformes	0.61 (−0.08, 0.91)	0.079	9	0.29 (−0.46, 0.80)	0.449	9
Corvida	0.61 (0.05, 0.88)	0.037	12	0.76 (0.33, 0.93)	0.004	12
Passeroidea	0.17 (−0.14, 0.45)	0.291	42	0.29 (−0.01, 0.55)	0.062	42
Non-all-black species						
All taxa	0.16 (−0.04, 0.35)	0.121	94	0.15 (−0.06, 0.34)	0.141	94
Basal lineage	−0.03 (−0.59, 0.56)	0.938	12	0.26 (−0.36, 0.73)	0.406	12
Corvida	0.42 (−0.25, 0.81)	0.203	11	0.72 (0.20, 0.92)	0.013	11
Passeroidea	0.36 (0.05, 0.62)	0.030	37	0.49 (0.20, 0.71)	0.002	37

95% Confidence intervals (CI; lower and upper bound) for correlation effect sizes (*r*) are given for bivariate phylogenetic GLS models. Taxa with presumably androgen-dependent plumage dichromatism are marked italics. "Non-all-black species" are those with <100% melanization; note that our dataset does not contain all-black Charadriiformes.

dichromatism correlated positively with T both in bivariate analyses (Table 1a, Fig. 1b) and in multivariate models controlling for possible confounding variables (Table 2). When we excluded all-black species, melanin dichromatism was still strongly related to T, while the relationship between melanization and T was weakened (Tables 1b and 2), although it remained strong in the full multivariate model (see S4b) and also when we controlled only for chick-feeding that

Table 2 Multivariate phylogenetic GLS models of male melanization and melanin dichromatism in relation to T levels and sexual and paternal behaviors

	Number of species	Male melanization		Melanin dichromatism	
All taxa					
All species	120; 120	Polygyny	0.29 (0.12, 0.45)***	Polygyny	0.46 (0.30, 0.59)****
Non-all-black species	105; 86	Polygyny	0.19 (−0.01, 0.36)*	T	0.23 (0.02, 0.42)**
				Incubation	−0.36 (−0.53, −0.16)****
Basal lineage					
All species	15; 15	Polygyny	0.79 (0.48, 0.92)****	Polygyny	0.77 (0.45, 0.92)****
		Incubation	−0.55 (−0.82, −0.07)**	Latitude	0.55 (−0.08, 0.82)**
Non-all-black species	13; 13	Latitude	0.70 (0.25, 0.90)***	Latitude	0.51 (−0.06, 0.83)*
Charadriiformes					
All species	9; 9	T	0.80 (0.28, 0.96)**	T	0.83 (0.37, 0.96)**
		Latitude	0.83 (0.36, 0.96)**	Incubation	−0.87 (−0.97, −0.48)***
Corvida					
All species	8; 12	T	0.88 (0.48, 0.98)***	T only	0.76 (0.33, 0.93)***
		Chick-feeding	0.81 (0.26, 0.97)**		
Non-all-black species	−; 11	−		T only	0.72 (0.20, 0.92)**
Passeroidea					
All species	34; 44	Polygyny	0.52 (0.22, 0.73)***	Polygyny	0.40 (0.12, 0.62)***
		Chick-feeding	0.34 (0.003, 0.61)*		
Non-all-black species	37; 37	T only	0.36 (0.04, 0.61)**	T only	0.49 (0.20, 0.71)***

95% Confidence intervals (CI; lower and upper bound) for correlation effect sizes (*r*) are given for the predictors retained in the final models. Sample sizes are given for both models (male melanization; melanin dichromatism) in each row. For models that retained only T as predictor ("T only"), results are identical to those in Table 1. Taxa with presumably androgen-dependent plumage dichromatism are marked italics. "Non-all-black species" are those with <100% melanization; note that our dataset does not contain all-black Charadriiformes. Initial full models are presented in S4.

*$P<0.1$ (marginally significant)

**$P<0.05$

***$P<0.01$

****$P<0.001$

was a significant predictor for all Corvida [see Table 2; r (CI)=0.86 (0.29, 0.98), P=0.030]. Several Corvida species are cooperative breeders that may face strong sexual competition despite of their socially monogamous pair-bond and biparental care (e.g., refs 89, 129, 146, 148, 173 in S2); however, our results remained qualitatively unchanged when we controlled for cooperative breeding (not shown).

In the Passeroidea songbirds with genetically or LH-controlled male plumage, neither melanization nor melanin dichromatism was related to T (Table 1a), even when we controlled for confounding variables (Table 2), although melanin dichromatism tended to increase with T (Table 1a). After excluding all-black species, however, we found positive correlations of T with both melanization and melanin dichromatism (Table 1b, Fig. 1c), and T had a medium (small to strong) effect in the final multivariate models for both melanization and melanin dichromatism (Table 2).

In the full dataset, species with one vs two annual molts did not differ in the extent of melanization (mean±SE, 0.48±0.05 vs 0.55±0.08; t_{129}=0.35, P=0.729). Within taxa, all but one Charadriiformes molt twice, while all but one Corvida species molt only once per year; excluding these species from the analyses did not change the above results qualitatively (not shown). Finally, only 4 out of 56 Passeroidea species have a pre-breeding molt, and these species were not significantly blacker (mean±SE: 0.62±0.09) than their relatives with one annual molt (0.44±0.07; t_{54}=-0.68, P=0.502). Therefore, the number of molts is unlikely to have biased our results.

Discussion

Our study demonstrates that evolutionary changes in the extent of frontal black plumage and its sexual dimorphism have paralleled the changes in circulating T at molt in a number of avian groups. Instead of a general positive relationship, the association between melanization and T appears context-dependent, varying with the type of ornamentation (patchy or full black) and with the presumed taxonomic distribution of the hormonal control of plumage coloration. These results were consistent between two alternative songbird phylogenies and were unaltered or even improved when we took into account the potentially confounding effects of mating competition and parental behavior. Although some of our results clearly suffer from limited sample size, the data presented in this study include all the currently available data on T levels in birds, and we found effects corresponding to at least intermediate effect sizes (r>0.3 sensu Cohen 1988), which are likely to be important evolutionarily (Møller and Jennions 2002).

The present study is the first to provide comparative support for the hypothesis that T may be a general regulator of melanization in certain avian clades. First, in two taxa in which the development of male breeding plumage is assumed androgen-dependent, we found a positive correlation between T and the extent and dimorphism of melanization, especially when we controlled for confounding effects. Second, when we excluded all-black species, similar relationships emerged in our largest taxon, the Passeroidea. This group is special in that the most status-signaling plumage badges have been identified in Passeroidea species, mainly in the form of black patches that signal higher rank by their larger size (Senar 2006). Our findings thus support the T-regulation hypothesis in shorebirds and songbirds, suggesting that interspecific differences in black melanin signals may have evolved in response to differences in T levels. These results corroborate the idea that T regulation may be a widespread mechanism to ensure that melanin badges honestly reflect the competitive ability of individuals.

An alternative explanation for the evolutionary relationship between melanization and T may be that both variables evolve in response to social and/or sexual challenges, without any regulational link between them. According to the challenge hypothesis, T levels are responsive to social challenges imposed by rival males; thus, strongly competitive (e.g. polygynous) species maintain higher breeding baseline T levels than less competitive (e.g., monogamous) species that elevate T levels only in response to acute challenges (Wingfield et al. 1990; Hirschenhauser et al. 2003). Peak T levels are also higher in species with increased competition imposed by extra-pair matings (Garamszegi et al. 2005) and shorter breeding seasons (Goymann et al. 2004). Comparative studies have also supported that the evolution of black melanization is related to more elaborate sexual displays, polygyny, and less paternal care (Bókony et al. 2003; Bókony and Liker 2005; this study). Thus, strong sexual competition may simultaneously select both for elevated T and for increased melanization. Under this scenario, melanization should be positively related to T levels that are most sensitive to socio-sexual challenges, that is, breeding baseline T and peak T. In our dataset, however, we found no such relationships either in general or within different taxa (unpublished results). Furthermore, the relationship between T levels and melanization remained significant when we statistically controlled for the effects of mating competition. These findings may imply that the need for intrasexual signaling alone might be insufficient to provide a link between melanization and T in the absence of appropriate hormonal basis for color production (e.g., see Galloanseriformes below).

Notably, our results provide support also for the hypothesis that the hormonal control of plumage coloration is non-randomly distributed among major avian clades (Kimball and Ligon 1999). Although this hypothesis is

based on many case studies of plumage dichromatism, most of these were conducted several decades ago, using rather old methodologies for hormonal manipulations (Kimball 2006), and the number of species investigated is minute compared to the huge diversity of avian taxa. The pattern we found in this study for a specific type of plumage coloration, the extent of black melanization, fits quite well with the pattern outlined by these earlier studies, which we discuss in detail below in the light of our results and in the context of socio-sexual signaling.

In the basal lineage, including ratites, galliforms, and waterfowl, several case studies suggest that the breeding coloration of males is androgen-independent and develops in the absence of estrogens that feminize the plumage (Kimball and Ligon 1999). Consistently with these results, we found no relationship between T levels at molt and male melanization or melanin dichromatism in this group. Interestingly, androgens can be aromatized into estrogens in the feather follicles, thereby T may indirectly affect plumage coloration (Kimball 2006). This aromatization process has been suggested to control the expression of eclipse plumage in mallard (*Anas platyrhynchos*) males (Haase et al. 1995) and female-like plumage in roosters (*Gallus gallus*) with a hen-feathering mutation that increases aromatase activity in the skin (Carefoot 2002). In mallards, larger amounts of T, possibly aromatized into estrogens, cause greater loss of eumelanin in black areas of the breeding plumage, leading to a more female-like plumage (Haase and Schmedemann 1992; Haase et al. 1995). This mechanism might prevent plumage ornaments like melanization from serving as indicators of male sexual vigor and aggression in the basal lineage. In line with this idea, quite few plumage traits seem to function in male–male competition or female choice in Galloanseriformes (Kimball 2006; Senar 2006).

In many shorebirds (Charadriiformes), bright male plumage develops in the presence of androgens (Kimball and Ligon 1999); thus, a positive relationship between T and melanization may be expected. Our results corroborate this concept, as both male melanization and melanin dichromatism increased with T levels at molt. Although we had too few data to estimate effect sizes precisely, the relationships we found are probably strong ($r > 0.5$). There is limited information on the status-signaling function of melanization in shorebirds, although the two plover species investigated so far seem to fit the general picture that black ornaments reflect intrasexual competitiveness (Edwards 1982; Lendvai et al. 2004). Our findings suggest that T regulation may underlie this signal role. More experimental work would be valuable in shorebirds, especially in species with reversed sexual dimorphism in both coloration and sex roles.

In the Corvida songbirds, little is known of the hormonal control of plumage coloration, although a study on superb

fairy-wrens (*Malurus cyaneus*) indicates that male breeding plumage may be androgen-dependent (Peters et al. 2000). Our results, based on a larger number of species, provide support for the T dependence of male coloration in this group, as both male melanization and melanin dichromatism related positively to T levels at molt. Thus, black ornaments may be well suited to signal social status or sexual vigor in Corvida. Experimental work is clearly needed in more species to ascertain the relationship between T and melanization. Furthermore, the signaling role of plumage coloration, including melanin ornaments, is poorly studied in this group, but our findings suggest that it would deserve more attention.

In the Passeroidea songbirds, sexual dichromatism seems to be controlled genetically in some species and by luteinizing hormone (LH) in others. The presence or absence of LH results in breeding or non-breeding plumage, respectively, while sex steroids (androgens or estrogens) are not involved (Kimball and Ligon 1999). Therefore, any relationship between melanization and T may be unexpected in this group under the T-regulation hypothesis. However, two lines of evidence suggest that there may be more to the hormonal control of coloration in Passeroidea than those outlined above. First, the well-studied example of house sparrows, as detailed in "Introduction," demonstrates that among-male differences in melanization can be affected by among-male differences in T (Evans et al. 2000; Buchanan et al. 2001; González et al. 2001) even if plumage dichromatism itself is under genetic control. Second, as mentioned before, the most species with well-established status-signaling plumage ornaments belong to this clade (Senar 2006). These facts are paralleled by our result that the size of melanin badges (i.e., non-all-black melanization and its dichromatism) correlates with T levels at molt across Passeroidea species. Thus, T regulation might be the honesty-ensuring mechanism behind the status badges of passeroid birds, although this possibility requires further research especially on species with one annual molt such as the house sparrow.

An important limitation for interspecific studies of circulating T is that the response elicited by hormones depends not only on their plasma concentrations but also on the sensitivity of target tissues (e.g., receptor densities; Wingfield et al. 2001; Ketterson et al. 2005). Variation in tissue sensitivities may mediate several fine-scale hormone effects in shaping the interspecific differences in coloration, including the exact locality and distribution of ornament types over the plumage, e.g., the patterns of eumelanin–pheomelanin ratio (Haase et al. 1995). Tissue sensitivities may also underlie the taxonomic differences in the hormonal control of coloration (Kimball 2006). However, plasma titers of T are still considered biologically relevant at the interspecific level, given that they correlate robustly and reasonably with several

traits that are known to be T-mediated intraspecifically, such as mating competition (Hirschenhauser et al. 2003; Goymann et al. 2004; Garamszegi et al. 2005; Ketterson et al. 2005). Furthermore, the modulation of plasma-T effects by sensitivity mechanisms is thought to vary with latitude, species with longer breeding seasons towards the Equator maintaining lower plasma T than temperate species and relying more on receptor-mediated mechanisms to avoid the costs of prolonged high T (Wingfield et al. 2001; but see Roberts et al. 2004). As we controlled for latitude in our analyses, interspecific differences in tissue sensitivities are unlikely to have biased our results.

In sum, this is the first interspecific test for an association between melanin-based coloration and the T levels that birds may face at the time of plumage development, showing that such an association may underlie the signal function of melanin badges in many birds. Demonstrating the causality of the T–melanization relationship is only possible at the intraspecific level, and we hope that the patterns reported in this paper would help to focus experimental studies on the regulation and signal content of melanin ornaments in different avian groups.

References

Andersson M (1994) Sexual selection. Princeton University Press, Princeton

Barker FK, Cibois A, Schikler P, Feinstein J, Cracraft J (2004) Phylogeny and diversification of the largest avian radiation. Proc Natl Acad Sci U S A 101:11040–11045

Bennett PM, Owens IPF (2002) Evolutionary ecology of birds: life histories, mating systems and extinction. Oxford University Press, Oxford

Bennett ATD, Cuthill IC, Partridge JC, Lunau K (1997) Ultraviolet plumage colors predict mate preferences in starlings. Proc Natl Acad Sci U S A 94:8618–8621

Bókony V, Liker A (2005) Melanin-based black plumage coloration is related to reproductive investment in cardueline finches. Condor 107:775–787

Bókony V, Liker A, Székely T, Kis J (2003) Melanin-based plumage coloration and flight displays in plovers and allies. Proc R Soc Lond B 270:2491–2497

Brawner WR, Hill GE, Sundermann CA (2000) Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male House Finches. Auk 117:952–963

Buchanan KL, Evans MR, Goldsmith AR, Bryan DM, Rowe LV (2001) Testosterone influences basal metabolic rate in male house sparrows: a new cost of dominance signalling? Proc R Soc Lond B 268:1337–1344

Burtt EH, Ichida JM (2004) Gloger's rule, feather-degrading bacteria, and color variation among song sparrows. Condor 106:681–686

Carefoot WC (2002) Hen-feathering mutation HF*H may act as a eumelanising factor and modify the expression of autosomal barring. Br Poult Sci 43:391–394

Cohen J (1988) Statistical power analysis for the behavioural sciences. Erlbaum, Hillsdale

Doucet SM, Shawkey MD, Hill GE, Montgomerie R (2006) Iridescent plumage in satin bowerbirds: structure, mechanisms and nano-structural predictors of individual variation in colour. J Exp Biol 209:380–390

Edwards PJ (1982) Plumage variation, territoriality and breeding displays of the golden plover Pluvialis apricaria in Southwest Scotland. Ibis 124:88–95

Evans MR, Goldsmith AR, Norris SRA (2000) The effects of testosterone on antibody production and plumage coloration in male house sparrows (Passer domesticus). Behav Ecol Sociobiol 47:156–163

Fargallo JA, Laaksonen T, Korpimäki E, Wakamatsu K (2007a) A melanin-based trait reflects environmental growth conditions of nestling male Eurasian kestrels. Evol Ecol 21:157–171

Fargallo JA, Martínez-Padilla J, Toledano-Díaz A, Santiago-Moreno J, Dávila JA (2007b) Sex and testosterone effects on growth, immunity and melanin coloration of nestling Eurasian kestrels. J Anim Ecol 76:201–209

Fitze PS, Richner H (2002) Differential effects of a parasite on ornamental structures based on melanins and carotenoids. Behav Ecol 13:401–407

Freckleton RP, Harvey PH, Pagel M (2002) Phylogenetic analysis and comparative data: a test and review of evidence. Am Nat 160:712–726

Garamszegi LZ (2006) Comparing effect sizes across variables: generalization without the need for Bonferroni correction. Behav Ecol 17:682–687

Garamszegi LZ, Eens M, Hurtrez-Boussès S, Møller AP (2005) Testosterone, testes size and mating success in birds: a comparative study. Horm Behav 47:389–409

González G, Sorci G, Smith LC, de Lope F (2001) Testosterone and sexual signalling in male House Sparrows (Passer domesticus). Behav Ecol Sociobiol 50:557–562

Goymann W, Moore IT, Scheuerlein A, Hirschenhauser K, Grafen A, Wingfield JC (2004) Testosterone in tropical birds: effects of environmental and social factors. Am Nat 164:327–334

Grafen A, Hails R (2002) Modern statistics for the life sciences. Oxford University Press, Oxford

Griffith SC, Owens IPF, Burke T (1999) Environmental determination of a sexually selected trait. Nature 400:358–360

Griffith SC, Parker TH, Olson VA (2006) Melanin- versus carotenoid-based sexual signals: is the difference really so black and red? Anim Behav 71:749–763

Haase E, Schmedemann R (1992) Dose-dependent effect of testosterone on the induction of eclipse coloration in castrated wild mallard drakes (Anas platyrhynchos L.). Can J Zool 70:428–431

Haase E, Ito S, Wakamatsu K (1995) Influences of sex, castration, and androgens on the eumelanin and pheomelanin contents of different feathers in wild Mallards. Pigment Cell Res 8:164–170

Hegner RE, Wingfield JC (1987) Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. Auk 104:462–469

Hill GE (1990) Female house finches prefer colourful males: sexual selection for a condition-dependent trait. Anim Behav 40:563–572

Hill GE (2006) Female mate choice for ornamental coloration. In: Hill GE, McGraw KJ (eds) Bird coloration II. Function and evolution. Harvard University Press, Cambridge, pp 137–200

Hill GE, Montgomerie R (1994) Plumage colour signals nutritional condition in the house finch. Proc R Soc Lond B 258:47–52

Hill GE, Brawner WR (1998) Melanin-based plumage coloration in the House Finch is unaffected by coccidial infection. Proc R Soc Lond B 265:1105–1109

Hill GE, McGraw KJ (2003) Melanin, nutrition, and the lion's mane. Science (Lett) 299:660

Hirschenhauser K, Winkler H, Oliveira RF (2003) Comparative analysis of male androgen responsiveness to social environment in birds: the effects of mating system and paternal incubation. Horm Behav 43:508–519

Jawor JM, Breitwisch R (2003) Melanin ornaments, honesty, and sexual selection. Auk 120:249–265

Ketterson ED, Nolan V Jr, Sandell M (2005) Testosterone in females: mediator of adaptive traits, constraint on sexual dimorphism, or both? Am Nat 166:S85–98

Kimball RT (2006) Hormonal control of coloration. In: Hill GE, McGraw KJ (eds) Bird coloration I. Mechanisms and measurements. Harvard University Press, Cambridge, pp 137–200

Kimball RT, Ligon JD (1999) Evolution of avian plumage dichromatism from a proximate perspective. Am Nat 154:182–193

Lendvai AZ, Kis J, Székely T, Cuthill IC (2004) An investigation of mate choice based on manipulation of multiple ornaments in Kentish Plovers. Anim Behav 67:703–709

Liker A, Székely T (2005) Mortality costs of sexual selection and parental care in natural populations of birds. Evolution 59:890–897

Mahler B, Araujo LS, Tubaro PL (2003) Dietary and sexual correlates of carotenoid pigment expression in dove plumage. Condor 105:258–267

Martins EP, Hansen TF (1997) Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. Am Nat 149:646–667

McGraw KJ (2003) Melanins, metals, and mate quality. Oikos 102:402–406

McGraw KJ (2006) Mechanics of melanin-based coloration. In: Hill GE, McGraw KJ (eds) Bird coloration I. Mechanisms and measurements. Harvard University Press, Cambridge, pp 243–294

McGraw KJ, Hill GE (2000) Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proc R Soc Lond B 267:1525–1531

McGraw KJ, Vonnegut EA, Dale J, Hauber ME (2002) Different plumage colors reveal different information: how nutritional stress affects the expression of melanin- and structurally based ornamental coloration. J Exp Biol 205:3747–3755

McGraw KJ, Dale J, Mackillop EA (2003) Social environment during molt and the expression of melanin-based plumage pigmentation in male house sparrows (Passer domesticus). Behav Ecol Sociobiol 53:116–122

McGraw KJ, Wakamatsu K, Ito S, Nolan PM, Jouventin P, Dobson FS, Austic RE, Safran RJ, Siefferman LM, Hill GE, Parker RS (2004) You can't judge a pigment by its color: carotenoid and melanin content of yellow and brown feathers in swallows, bluebirds, penguins, and domestic chickens. Condor 106:390–395

Møller AP, Jennions MD (2002) How much variance can be explained by ecologists and evolutionary biologists? Oecologia 132:492–500

Møller AP, Garamszegi LZ, Gil D, Hurtrez-Boussès S, Eens M (2005) Correlated evolution of male and female testosterone profiles in birds and its consequences. Behav Ecol Sociobiol 58:534–544

Nakagawa S (2004) A farewell to Bonferroni: the problems of low statistical power and publication bias. Behav Ecol 15:1044–1045

Olson VA, Owens IPF (2005) Interspecific variation in the use of carotenoid-based coloration in birds: diet, life history and phylogeny. J Evol Biol 18:1534–1546

Owens IPF, Hartley IR (1998) Sexual dimorphism in birds: why are there so many different forms of dimorphism? Proc R Soc Lond B 265:397–407

Owens M (1997) Inferring evolutionary processes from phylogenies. Zool Scripta 26:331–348

Pagel M (1999) Inferring the historical patterns of biological evolution. Nature 401:877–884

Peters A, Astheimer LB, Boland CRJ, Cockburn A (2000) Testosterone is involved in acquisition and maintenance of sexually selected male plumage in superb fairy-wrens, Malurus cyaneus. Behav Ecol Sociobiol 47:438–445

Poston JP, Hasselquist D, Stewart IRK, Westneat DF (2005) Dietary amino acids influence plumage traits and immune responses of male house sparrows, Passer domesticus, but not as expected. Anim Behav 70:1171–1181

Roberts ML, Buchanan KL, Evans MR (2004) Testing the immunocompetence handicap hypothesis: a review of the evidence. Anim Behav 68:227–239

Roulin A, Dijkstra C (2003) Genetic and environmental components of variation in eumelanin and phaeomelanin sex-traits in the barn owl. Heredity 90:359–364

Roulin A, Riols C, Dijkstra C, Ducrest A-L (2001) Female plumage spottiness signals parasite resistance in the barn owl (Tyto alba). Behav Ecol 12:103–110

Roulin A, Müller W, Sasvári L, Dijkstra C, Ducrest AL, Riols C, Wink M, Lubjuhn T (2004) Extra-pair paternity, testes size and testosterone level in relation to colour polymorphism in the barn owl Tyto alba. J Avian Biol 35:492–500

Senar JC (2006) Color displays as intrasexual signals of aggression and dominance. In: Hill GE, McGraw KJ (eds) Bird coloration II. Function and evolution. Harvard University Press, Cambridge, pp 87–136

Sibley CG, Ahlquist JE (1990) Phylogeny and classification of birds: a study in molecular evolution. Yale University Press, New Haven

Wingfield JC, Ball GF, Duffy AM Jr, Hegner RE, Ramenofsky M (1987) Testosterone and aggression in birds. Am Sci 75:602–608

Wingfield JC, Hegner RE, Dufty AM Jr, Ball GF (1990) The 'challenge hypothesis': theoretical implications for patterns of testosterone secretion, mating systems and breeding strategies. Am Nat 136:829–846

Wingfield JC, Lynn SE, Soma KK (2001) Avoiding the 'costs' of testosterone: ecological bases of hormone-behavior interactions. Brain Behav Ecol 57:239–251