Black bib size is associated with feather content of pheomelanin in male house sparrows

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Dear Editor,

Pigments constitute the basis of most visual signals involved in animal communication. Melanins are the most common pigments, often representing the basis of signals that are honest (i.e. signallers transfer reliable information about their genotypic quality to receivers; McGraw, 2008). The black chest bib of male house sparrows *Passer domesticus* is a melanin-based plumage patch (Figure 1) that constitutes one of the most intensively studied animal signals (Anderson, 2006). Birds develop the bib not only during the breeding season but all year round, larger bibs being likely associated with higher melanocyte numbers. Male house sparrows displaying larger bibs are dominant in aggressive interactions with other males, have better body condition and, in some populations, achieve higher lifetime reproductive success (reviewed in Nakagawa et al., 2007). This has been explained through the handicap principle, that is low-quality house sparrows do not display large bibs because their production represents costs that these birds cannot afford. In particular, bib expression depends on testosterone levels, which in turn suppresses the immune system, and immunosuppression costs would be only affordable by high-quality males (i.e. the immunocompetence handicap hypothesis; González et al., 1999).

However, the signal content of house sparrows' bibs is assumed to be intimately related to their constitutive pigments (i.e. melanins), so signal expression (i.e. bib size) is likely to depend more primarily on the mechanisms that control overall melanin biosynthesis than on testosterone level, although testosterone clearly affects the deposition of melanins in feathers (e.g. Haase et al., 1995). Two main chemically distinct forms of melanin are identified in vertebrate animals: eumelanin, a polymer of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxilic acid (DHICA) units, and pheomelanin, a polymer of benzothiazine and benzothiazole derivatives (García-Borrón and Olivares Sánchez, 2011). The environmental conditions under which producing pheomelanin and eumelanin is costly differ for each pigment (Galván and Alonso-Alvarez, 2009), and the genetic and physiological bases of melanogenesis are relatively well known (García-Borrón and Olivares Sánchez, 2011). Thus, understanding how the melanin composition of the house sparrow's bib affects its size might be very useful to understand why this signal is honest.

To our knowledge, a chemical analysis of house sparrow bib melanins has never been carried out, and it has been assumed that variation in bib characteristics depends on its content of 'melanin' in general terms or mostly on eumelanin content (McGraw, 2008). Thus, it has been assumed that the honesty of the signal should lie in producing more melanin (which would correspond to more melanocytes, assuming that melanin production per melanocyte is independent of bib size), but here, we aim at determining the content of eumelanin and pheomelanin in bibs separately, considering that the mechanisms of synthesis of the two chemical forms differ and that this may help understanding the honesty of bib size. We therefore analyse the associations between the size of the bib of male house sparrows (Figure 1) kept in captivity during the course of a experiment (in which the levels of the antioxidant glutathione, GSH, were manipulated in some birds while others were controls) and the content of pheomelanin and eumelanin in their feathers. To measure pheomelanin, we used the specific markers 4-amino-3-hydroxyphenylalanine (4-AHP) and thiazole-2,4,5-tricarboxylic acid (TTCA), and to analyse eumelanin, we used the specific marker pyrrole-2,3,5-tricarboxylic acid (PTCA) (see Data S1 for experimental and analytical procedures).

The general linear model (GLM) exploring the relationship between final bib size (see Data S1) and TTCA concentration showed a negative and significant effect of TTCA levels ($b = -6.02$, $t = -2.73$, $F_{1,36} = 7.47$, $P = 0.009$; Figure 2), in addition to the effects of experimental treatment ($F_{1,36} = 3.75$, $P = 0.061$) and tarsus length ($F_{1,36} = 0.01$, $P = 0.933$). The model with 4-AHP showed a marginally non-significant effect of this variable on bib size ($b = -2.09$, $t = -1.84$, $F_{1,37} = 3.27$, $P = 0.079$). The relationship between bib size and PTCA levels was not significant ($b = 1.13$, $F_{1,37} = 0.16$, $P = 0.686$). Therefore, the bib size of male house

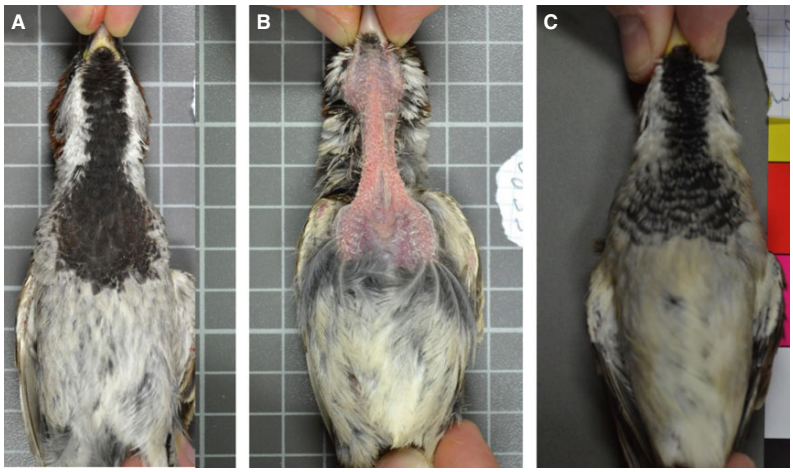

Figure 1. Male house sparrows used in the experiment showing (A) the initial bib just before the beginning of the experiment, (B) the bib after all its feathers and neighbouring feathers were plucked in the beginning of the experiment and (C) the final bib with newly grown feathers at the end of the experiment.

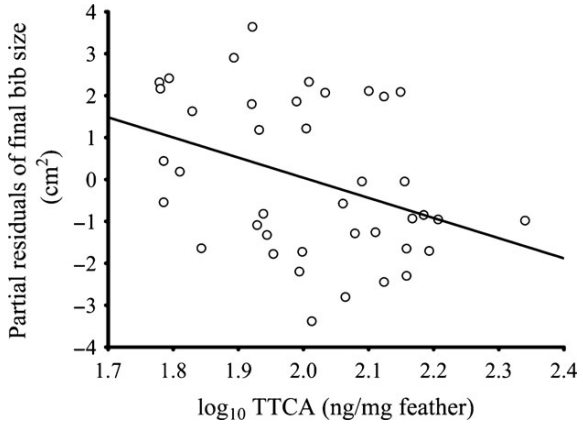

Figure 2. Relationship between bib size and pheomelanin levels in bib feathers (TTCA levels) of male house sparrows. The ordinate axis depicts the residual size of the bib patch, calculated as the residuals of a GLM with bib size as the response variable, treatment as a fixed factor and tarsus length as a covariate, but not including TTCA levels. Thus, the ordinate axis values show the variation in bib patch size that is not explained by neither treatment nor tarsus length, so that the association with TTCA levels is not affected by those confounding factors. The line is the regression line.

sparrows was negatively correlated with the levels of pheomelanin (TTCA) in its feathers and unrelated to the levels of eumelanin. The total content of eumelanin is considerably greater than the total content of pheomelanin, but the latter is more variable among individual birds than the content of eumelanin (see Data S1).

Our results suggest that, in male house sparrows, developing large bibs means producing small amounts of pheomelanin. This may seem counterintuitive because some authors have assumed, but never proved, that bib size variability depends on eumelanin levels in the bib feathers as the black colour of the bib may be conferred by eumelanin and not by pheomelanin, which produces lighter colours than eumelanin (McGraw, 2008). However, pheomelanin and eumelanin are produced under different conditions, and thiols that promote pheomelanin production inhibit the activity of the enzyme tyrosinase, hence reducing eumelanin production (del Marmol et al., 1993). Therefore, bib size may be either positively related to eumelanin levels or negatively related to pheomelanin levels. Our study confirms the latter. It also confirms previous observations suggesting that pheomelanin is biologically relevant even if present at low proportions in feathers whose appearance is given by the darker colours conferred by eumelanin (Galván et al., 2010). Recently, the size of a brown plumage patch in masked boobies *Sula dactylatra* has also been reported to be negatively associated to the levels of pheomelanin (4-AHP), but not related to the levels of eumelanin (PTCA) in the patch's feathers (Fargallo et al., 2014). Thus, the honesty in the signalling content of the black bib of male house sparrows should be based on the mechanisms that lead to the production of small amounts of one of its constitutive pigments, that is pheomelanin (see also Data S1).

This finding is relevant for understanding the honesty in the bib size of male house sparrows. The control of dishonesty might be mediated by the production costs of large bibs, and the only information on production costs so far proposed for this signalling system (although non-physiological costs are also possible for large plumage patches; Senar, 2006) is provided by the immunocompetence handicap hypothesis (González et al., 1999). However, to our knowledge, there is no empirical demonstration that the physiological costs derived from testosterone-mediated immunosuppression are so high that the production of large bibs is not possible for low-quality birds (i.e. those with smaller bibs), just that males with large bibs have higher levels of immunosuppression (González et al., 1999). Therefore, production costs are not enough to explain why the signalling system is not invaded by cheaters producing bluffs, that is males producing bibs disproportionally larger relative to their genotypic quality, and the evolutionary logic of the

honesty of house sparrows' bib remains unclear. Pheomelanin is produced when thiols (cysteine or GSH) are present, while eumelanin is produced when thiols are absent or below a threshold level in melanocytes (García-Borrón and Olivares Sánchez, 2011). Therefore, to achieve a low production of pheomelanin that allows the development of a large bib, it is necessary that thiol levels in melanocytes, where melanogenesis takes place, are low. In experiments not shown, we found that induced reductions of the levels of GSH led to the development of better phenotypes (i.e. larger bibs) in high-quality birds only (i.e. those which already displayed larger bibs before the experimental manipulation), suggesting that a physiological mechanism may have evolved in low-quality birds to avoid producing bibs of size not corresponding to their quality. Given the existence of specific transporters of cystine (the dimer of cysteine) that takes it into melanocytes (Chintala et al., 2005) or pump it out of melanosomes (Chiaverini et al., 2012), this may constitute a mechanism of control of signal expression. If the genetic basis of variation in the quality of male house sparrows was actually variation in the expression of genes coding for those transporters (*Slc7a11* and *CTNS*; Chintala et al., 2005 and Chiaverini et al., 2012), then birds could develop bibs of size according to their quality and production costs would not be necessary to explain the honesty of the signal. That is, the expression of these genes may be linked to the quality of the birds, so that the physiological state of low-quality birds may prevent them from producing large bibs even if those birds would decide to invest in the production of large bibs. Indeed, this opens a new perspective for the evolution of one of the most intensively studied systems of honest communication.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1 Experimental procedure.