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Brain size and the expression of pheomelanin-based colour in birds

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Abstract

Eumelanin and pheomelanin are the most common vertebrate pigments. They generate different colours and are synthesized under different physiological conditions. While pheomelanogenesis requires high levels of a key intracellular antioxidant (glutathione, GSH), eumelanogenesis is inhibited by GSH. This implies that species that present the molecular basis to produce large amounts of pheomelanin might be more limited to perform other costly processes that generate oxidative stress than species that produce eumelanin. Brain development requires large amounts of energy and antioxidants during ontogeny, so that large-brained species may be constrained in their simultaneous synthesis of large amounts of pheomelanin, but not in their synthesis of eumelanin. Here, we tested this hypothesis in a large dataset of 323 bird species. After controlling for the effects of phylogeny, latitude and sexual dichromatism, the proportion of pheomelanic plumage colour was strongly negatively related to the relative brain mass of species, whereas no relationship was found for the proportion of eumelanic colour. This indicates that the production of pheomelanin is a costly process that cannot evolve together with complex neural structures and thus with large cognitive capacity. This is the first time that the expression of melanic traits is found to correlate with another phenotypic character across species.

Introduction

Melanin is the most common pigment conferring colour to the integument of animals. In vertebrates, this pigment is mainly produced in two forms, eumelanin and pheomelanin, in melanosomes, the specialized organelles of melanocytes, often simultaneously in the same cells but one form usually prevailing over the other (Ozeki *et al.*, 1997). These pigments are synthesized by animals from the amino acid tyrosine that is oxidized to dopaquinone by the enzyme tyrosinase. Dopaquinone acts as an intermediate for the production of eu- and pheomelanin, but some biochemical differences between eumelanin and pheomelanin are reflected in the appearance of the traits coloured by them (Toral *et al.*, 2008).

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The expression of melanin-based plumage colour is intrinsically related to oxidative stress (i.e. the imbalance between the production of reactive oxygen species and the state of the antioxidant and repair machinery; Galván & Alonso-Alvarez, 2008, 2009; Galván & Solano, 2009). This is because glutathione (GSH), a tripeptide thiol found in virtually all animal cells that functions as the main physiological reservoir of cysteine (Benedetto *et al.*, 1981), and as the most important intracellular antioxidant (Anderson, 1998; Wu *et al.*, 2004), exerts a direct influence on melanogenesis. This is made by directly inhibiting the action of tyrosinase (i.e. the enzyme catalysing the first step of the process), by combating free radicals that stimulate the action of tyrosinase, and by increasing the ratio cysteine/dopaquinone (Galván & Alonso-Alvarez, 2009). This causes GSH levels to determine the pathway that is followed during the process, which can either lead to the production of eumelanin when the activity of tyrosinase is high and the ratio cysteine/dopaquinone low or to the production of

pheomelanin or even an absence of melanin synthesis under opposite conditions (Ozeki *et al.*, 1997; Galván & Alonso-Alvarez, 2009). Therefore, eumelanogenesis takes place when the levels of GSH are low and pheomelanogenesis when the levels of GSH are high, meaning that eumelanogenesis proceeds with a diminished antioxidant capacity when compared to pheomelanogenesis (Galván & Solano, 2009). The evolutionary implication of this is that species in which natural selection has favoured the development of pheomelanic traits may have a decreased capacity to combat oxidative stress when compared to species with eumelanic traits, as the maintenance of high GSH levels as required by pheomelanogenesis might be metabolically costly under adverse environmental conditions or during costly physiological processes that generate oxidative stress and thus use GSH resources (Galván & Alonso-Alvarez, 2009; Galván & Solano, 2009).

Therefore, physiological processes that produce metabolic costs can potentially be associated with the expression of melanin-based coloration, as these costs generate oxidative stress (Alonso-Alvarez *et al.*, 2004). One of these processes is brain development. Large brains are necessary for elevated cognitive capacities, but the development of complex neural structures require more resources and the investment of more time (Ricklefs, 2004). This generates developmental constraints, which in birds is translated into an association between mode of development, amount of maternal effects and relative brain size across species (Garamszegi *et al.*, 2007 and references therein). Furthermore, brain is not only costly to develop, but its maintenance has chemical and thermoregulatory requirements that may constrain the coevolution of large brains with other expensive structures (e.g. long guts; Aiello & Wheeler, 1995; Finlay *et al.*, 2001). Indeed, a positive correlation between relative brain size and basal metabolic rate has been shown among mammal species (Isler & van Schaik, 2006). Thus, large-brained individuals must be capable of continuously supplying the brain with high levels of oxygen for neuronal ion pumping, synthesis of neurotransmitters and protection from toxic compounds, which makes brain maintenance a highly oxidizing process that demands large amounts of antioxidants, in particular GSH (Bains & Shaw, 1997; Hoffman & Heinz, 1998; Sewalk *et al.*, 2001).

Brain development and maintenance may thus compete with pheomelanogenesis for GSH. Here, we test for an association between relative brain size and the expression of melanin-based coloration with a large dataset of 323 species of birds. We predict a negative correlation between the proportion of pheomelanin-based plumage colour and relative brain size, and no correlation in the case of eumelanin-based colour. In birds, senescence rate is related to breeding latitude (Møller, 2006), and oxidative stress is the main agent causing senescence (Finkel & Holbrook, 2000). As a consequence, breeding latitude may be related to the expression of melanin-based coloration. Gloger's rule predicts that animals are generally less pigmented in cool and dry environments (Mayr, 1970). Brain size is also correlated with latitude through the effect of cognitive ability on food hoarding and survival at high latitudes (Garamszegi & Lucas, 2005). Latitude is also related to the intensity of the immune response in birds (Møller *et al.*, 2006), which, in turn, is related to relative brain size (Møller *et al.*, 2005). Therefore, breeding latitude may be associated with both melanin-based coloration and relative brain size, thus representing a potential confounding variable when testing for a relationship between these traits. Sexual dichromatism is another potential confounding variable, because mono- and dichromatic species may evolve towards different optima as a result of differences in the strength of sexual selection, which may bias the analyses, and because these differences may also affect brain size. Therefore, we searched for a correlation between the expression of melanin-based coloration and relative brain mass after controlling for the breeding latitude of species and sexual dichromatism in melanin-based colour.

Materials and methods

Melanin-based plumage coloration

Using colour plates in Cramp & Simmons (1977–1992) and Cramp & Perrins (1993–1994), we obtained information on melanin-based plumage coloration of 323 bird species from the Western Palearctic. Several authors have used this method previously (see, e.g. John, 1995; Yezerinac & Weatherhead, 1995; Badyaev, 1997; Caro, 2009; Stang & McRae, 2009), and it has been shown to be a reliable method of quantifying different components of plumage colour that is even correlated with the avian perception of colour (del Val *et al.*, 2009; Seddon *et al.*, 2010). As our aim was to measure the proportion of plumage covered by melanin-based coloration, spectrophotometric techniques could not be used, as these can only be used to calculate physical measurements of coloration such as hue, brightness or chroma, but not to quantify extent of coloured areas.

Eumelanic and pheomelanic traits are generally of distinctive colours, the former being responsible for black and grey colours and the latter for yellowish, reddish, chestnut and brown colours (Toral *et al.*, 2008). Eumelanin and pheomelanin normally occur simultaneously in the tissues (Ozeki *et al.*, 1997), but the darker colours conferred by eumelanin (Toral *et al.*, 2008) make evident the lower content of this pigment in chestnut and brown colours when compared to black and grey colours (Galván & Alonso-Alvarez, 2009). Furthermore, many bird species present feather melanin contents of high purity (> 90% of either eumelanin or pheomelanin, McGraw & Wakamatsu, 2004; J.J. Negro, pers. comm.).

Therefore, we considered that black and grey plumage colours were predominantly generated by eumelanin, whereas chestnut and brown colours were predominantly generated by pheomelanin. We did not consider conspicuous yellow or red colorations assumed to be generated by other pigments (i.e. carotenoids), unless chemically identified as melanin based by Toral *et al.* (2008). Although a rough approximation to the real proportion of eumelanic and pheomelanic plumage, the assumption that black-grey colours are eumelanic and brown-chestnut colours are pheomelanic should be adequate for comparative purposes (Owens & Hartley, 1998). Indeed, this is the most appropriate method for comparative analyses, as quantifying eu- and pheomelanin contents, so that they can be compared among species, would be difficult. This is because melanin content varies between plumage patches in any given species; so, to obtain melanin content to be compared among species, these measurements should be taken for every feather of a number of individuals of each species. Measuring the melanin content of all feathers in individual birds would be practically impossible, especially in our case where we are considering 323 species of birds.

Thus, we quantified the proportion of melanic plumage parts by examining illustrations in Cramp & Simmons (1977–1992) and Cramp & Perrins (1993–1994). Illustrations of both resting and flying adult birds in breeding plumage birds were examined. The method used by Beauchamp & Heeb (2001) and Galván (2008) was followed to obtain estimates of the proportion of eu- and pheomelanic colour present in the plumage of each species, assigning scores that ranged from 0 (total lack of melanic colour) to 5 (all melanic). When a species was sexually dichromatic regarding melanin-based coloration, eumelanic and pheomelanic scores were the average obtained for males and females. When a species had different subspecies or colour morphs differing in extent or type of melanin-based coloration, we used the nominate subspecies or the most common morph, respectively. In the case of the highly polymorphic ruff *Philomachus pugnax*, we used the average colour scores of all morphs. It must be noted that eu- and pheomelanic colour patches can coexist in the same feathers, and thus the sum of both colour scores, in a species that presents both colour types, is not always necessarily 5, but higher values are also possible. A species was considered sexually dichromatic in melanin-based colour when eu- or pheomelanin-based colour scores differed in males and females. Information on eu- and pheomelanic plumage colour scores for the species used in the study is provided in Appendix S1.

Brain size

Information on brain size was obtained from data on brain mass reported by Mlikovsky (1989), Iwaniuk & Nelson (2003) and J. Erritzøe, pers. comm. Highly significant repeatabilities among studies indicate that information on brain mass can be combined across sources (Garamszegi *et al.*, 2005). Relative brain mass was obtained from the residuals of the log–log regression of brain mass against body mass. Brain mass and body mass values are given in Appendix S1.

Breeding latitude

We extracted the northernmost and the southernmost distribution limits for the breeding season to the nearest 0.1° from Cramp & Simmons (1977–1992) and Cramp & Perrins (1993–1994). Mean breeding latitude was estimated as the mean of the northernmost and the southernmost latitudes during the breeding season. We did not consider this variable for the red avadavat *Amandava amandava* because this species has experienced a huge increase in its geographical range because of anthropogenic factors. The breeding latitude of the species considered is given in Appendix S1.

Data analyses

Bird species are evolutionarily related as reflected by phylogeny, and, therefore, they should not be treated as independent sample units (Felsenstein, 1985; Harvey & Purvis, 1991). Thus, the effect of common ancestry among taxa can lead to an overestimation of degrees of freedom if phylogenetic relationships are not taken into account. We used phylogenetic eigenvector regression (PVR; Diniz-Filho *et al.*, 1998) to quantify the amount of phylogenetic signal and to correct for it in the analysis of the relationship between the expression of melanin-based coloration and brain mass. Diniz-Filho & Torres (2002) and Martins *et al.* (2002) have tested several comparative methods [Felsenstein's independent contrasts, autoregressive method, PVR, and phylogenetic generalized least squares (PGLS)] and have found that PVR yields good statistical performance regardless of the details of the evolutionary mode used to generate the data and provides similar results to other methods, with very good (i.e. low) error types I and II.

We first performed a principal coordinates analysis (PCORD) on the matrix of pairwise phylogenetic distances between the 323 bird species (after a double-centre transformation). In a second step, we selected the first 18 eigenvectors obtained by the broken-stick rule (until the increase in proportion of phylogenetic structure explained was < 0.8%) to account parsimoniously for the phylogenetic signal. The first two eigenvectors alone explained 22.3% of phylogenetic structure, but we decided to incorporate more eigenvectors in the analyses to maximize the detail of the phylogenetic structure that is considered without compromising statistical power because of the increase in the number of eigenvectors. Eigenvectors extracted from double-centred phylogenetic distance matrices are able to detect the main topological

features of the cladogram under different sample sizes or number of taxa used in the analyses (Diniz-Filho *et al.*, 1998). We found that the original matrix of phylogenetic distances between the 323 bird species and the reproduced matrix of distances estimated based on the first 18 eigenvectors were very similar (Mantel test with 999 randomized matrices to estimate significance: $r = 0.814$, $P < 0.0001$; test carried out using PopTools 3.2.3; Hood, 2010). These eigenvectors were used as additional predictor variables in a generalized linear model to control for phylogeny.

The phylogenetic hypothesis (see Appendix S2) was taken from the species-level supertree constructed by Davis (2008), with additional information from other sources for some species not covered by Davis (2008): Grosso *et al.* (2006), Voelker *et al.* (2007), Alström *et al.* (2008), Nguembock *et al.* (2009), Zuccon & Ericson (2010), and the phylogeny compiled by Møller (2006). Although, to our knowledge, there is no detailed phylogeny of *Corvus*, we considered the brown-necked raven *Corvus ruficollis* the sister group of the common raven *Corvus corax* because the former forms a superspecies with the pied crow *Corvus albus* (del Hoyo *et al.*, 2009), which was reported by Davis (2008) to be phylogenetically closer to the common raven than the rest of *Corvus* species considered in this study. As we used different phylogenies that employed different methods, we set all branch lengths equal to unity in our compiled phylogeny, thus assuming a speciational model of evolution.

We regressed the proportion of eu- and pheomelanic plumage colour on relative brain mass, mean breeding latitude, sexual dichromatism and the first 18 phylogenetic eigenvectors (EV1–EV18 hereafter). Although some dichromatic species were not assigned with an integer colour score as the values for males and females were averaged, we had a total of 11 response codes ranging from 0 to 5 that can thus be considered discrete values. Therefore, we used a generalized ordinal regression analysis with an ordinal multinomial response variable (for the response codes 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5 colour score) and a logit link function. The same was made to test for a correlation between eu- and pheomelanic plumage colour scores, but in this case breeding latitude was not included in the model. The phylogenetic signal (i.e. amount of variance or deviance exclusively explained by phylogeny) in plumage colour and relative brain mass was calculated by regressing these variables on EV1–EV18, using the above-mentioned generalized linear models for plumage colour and a general linear model for relative brain mass.

In our case, relative brain mass and breeding latitude were not significantly correlated ($b = -0.00$, $F_{1,320} = 1.94$, $P = 0.165$), thus avoiding problems of collinearity. Generalized models were checked for deviations from canonical assumptions using the overdispersion coefficients that were very low in our analyses (ranging from 1.07 to 1.11). The assumption of normality in the general linear model was checked by exploring the distribution of residuals.

When the effect of relative brain mass on plumage colour scores was significant, we corroborated the results with phylogenetic generalized least squares (PGLS; Martins & Hansen, 1997) to ensure that our results were not dependent on the comparative method used. PGLS models establish a dependence between observations by means of a variance–covariance matrix, and estimate a parameter (α) for each correlation that can be interpreted as a measure of phylogenetic signal (Butler & King, 2004). We used PGLS as implemented in Compare 4.6b (Martins, 2004), thus assuming an Ornstein–Uhlenbeck (OU) model of evolution (Butler & King, 2004). We first estimated a value of α by maximum likelihood with Compare 4.6b and then calculated the correlation coefficient between the traits at that value of α. In this case, one-tailed *P*-values are reported because we were interested in corroborating the results previously obtained with PVR.

Results

Eu- and pheomelanic plumage colour scores were strongly negatively correlated (b = −1.29, $\chi^2 = 149.34$, d.f. = 1, $P < 0.0001$). The phylogenetic signal was significant in both colour types (eumelanic: $\chi^2 = 76.46$, pheomelanic: $\chi^2 = 64.68$; both d.f. = 18, $P < 0.0001$) but slightly higher in eumelanic (7.9%) than in pheomelanic colour (7.2%). Phylogenetic signal in relative brain mass was considerably higher than in plumage colour (50.9%; $F_{18,304} = 17.50$, $P < 0.0001$).

The full model for pheomelanic plumage colour score revealed a significant negative effect of relative brain mass ($b = -3.56$, $\chi^2 = 7.30$, d.f. = 1, $P = 0.007$), but the effects of breeding latitude ($b = -0.00$, $\chi^2 = 0.05$, d.f. = 1, $P = 0.815$) and sexual dichromatism ($\chi^2 = 0.52$, d.f. = 1, $P = 0.469$) were not significant. The model without breeding latitude and sexual dichromatism was significant ($\chi^2 = 72.11$, d.f. = 19, $P < 0.0001$; 8.1% of deviance), as was the negative effect of relative brain mass ($b = -3.59$, $\chi^2 = 7.43$, d.f. = 1, $P = 0.006$; Fig. 1). The negative correlation between pheomelanic plumage colour score and relative brain mass kept significant when we considered colour scores for males ($b = -2.85$, $\chi^2 = 5.96$, d.f. = 1, $P = 0.015$) and females ($b = -3.07$, $\chi^2 = 6.91$, d.f. = 1, $P = 0.009$), separately. The whole effect of phylogeny was also significant ($\chi^2 = 64.47$, d.f. = 18, $P < 0.0001$), EV1-3, EV6 and EV10 being the main eigenvectors that contributed to this effect ($\chi^2 = 4.82$–18.86, d.f. = 1, all $P < 0.028$). The effect of the remaining phylogenetic eigenvectors was not significant ($\chi^2 = 0.03$–3.69, d.f. = 1, all $P > 0.055$).

The relationship between pheomelanic plumage colour score and relative brain mass was corroborated by PGLS

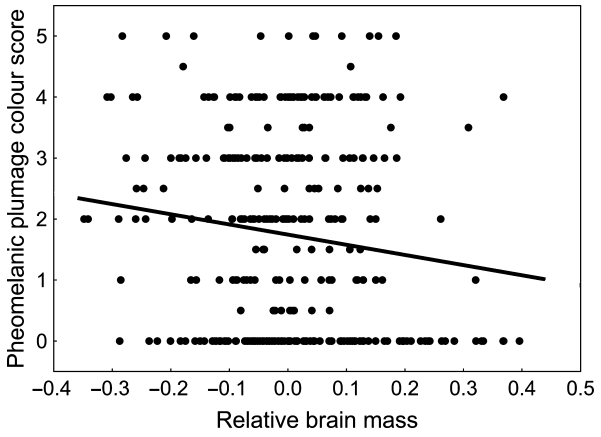

Fig. 1 Relationship between pheomelanic plumage colour score (0, no pheomelanic feathers; 5, entire body covered by pheomelanic colour) and relative brain mass (after adjusting for body mass) in 323 species of birds. The line is the best fit line.

models, when the average colour score for males and females was considered ($\alpha = 6.88$, $r = -0.11$, $n = 323$, $P = 0.024$), as well as when colour scores for males and females were separately analysed (males: $\alpha = 7.62$, $r = -0.09$, $n = 323$, $P = 0.053$; females: $\alpha = 5.81$, $r = -0.11$, $n = 323$, $P = 0.024$).

The full model for eumelanic plumage colour score was significant ($\chi^2 = 77.09$, d.f. = 21, $P < 0.0001$), but neither the effect of relative brain mass (b = −0.47, $\chi^2 = 0.16$, d.f. = 1, $P = 0.686$), breeding latitude (b = 0.01, $\chi^2 = 0.84$, d.f. = 1, $P = 0.360$) nor sexual dichromatism ($\chi^2 = 0.11$, d.f. = 1, $P = 0.734$) was significant. Thus, deviance in eumelanic colour score was only explained by phylogeny ($\chi^2 = 75.75$, d.f. = 18, $P < 0.0001$; 7.9% of deviance), mainly by EV5, EV7-8, EV10 and EV12 ($\chi^2 = 4.93$–32.43, d.f. = 1, all $P < 0.026$). The effect of the remaining phylogenetic eigenvectors was not significant ($\chi^2 = 0.04$–3.66, d.f. = 1, all $P > 0.055$).

Discussion

As predicted, the proportion of plumage coloured by pheomelanin was negatively related to relative brain mass across species independently of the breeding latitude. The large number of species (323) and broad taxonomic spectrum considered allow us to state that the relationship represents a general pattern in birds. As far as we know, this is the first time that the expression of melanin-based colour is analysed through a large phylogeny and that a significant predictor of melanin-based colour has been established.

Our results suggest that the development of large brains cannot be associated with the production of large amounts of pheomelanin. Brain development is a process consuming time, energy and antioxidants during the ontogeny, which restricts the production of complex neural structures to species with slow growth rate and large investment in maternal effects (Bennett & Harvey, 1985; Pagel & Harvey, 1988; Iwaniuk & Nelson, 2003; Ricklefs, 2004; Garamszegi *et al.*, 2007). This could also make ontogeny of large brains constrained by synthesis of pheomelanin. Pheomelanogenesis requires antioxidant resources (i.e. GSH) to proceed (Galván & Alonso-Alvarez, 2009; Galván & Solano, 2009) that may be incompatible with other processes that require energetic resources and thus generate oxidative stress (Alonso-Alvarez *et al.*, 2004) like brain development. Therefore, the negative correlation between the expression of pheomelanin-based coloration and relative brain mass may be because of a prohibitive metabolic cost of ontogeny of both traits, as avian melanocyte precursors begin synthesizing melanin as early as a few days after the beginning of embryo development (Schraermeyer, 1996; Marks & Seabra, 2001). Furthermore, GSH plays an important role in the maintenance of brain function (Bains & Shaw, 1997; Hoffman & Heinz, 1998; Sewalk *et al.*, 2001), so that natural selection may act during the adult stages of birds preventing the coevolution of large pheomelanic plumage traits and relatively large brains.

Eumelanogenesis proceeds with a diminished antioxidant capacity when compared to pheomelanogenesis, because the former is inhibited by GSH (Galván & Alonso-Alvarez, 2008, 2009; Galván & Solano, 2009). This means that eumelanin synthesis should more readily proceed than pheomelanin synthesis under environmental conditions that generate oxidative stress or coupled with costly physiological processes like brain development. However, relative brain mass was not significantly related to the proportion of plumage coloured by eumelanin, even when eu- and pheomelanic colour scores were negatively correlated. The reason for this is probably related to the fact that, whereas the synthesis of pheomelanin (for which GSH is a substrate) is traded against other costly physiological processes, the synthesis of eumelanin does not necessarily improve the performance of these processes. In other words, pheomelanin seems to act as a limiting factor, but there are no reasons that make eumelanin an enhancer of the performance of stressful processes.

We can exclude that the main result has arisen as a consequence of Gloger's rule and selection for large brains at high latitudes. Gloger's rule states that animals are less pigmented in more cool and dry environments (Mayr, 1970). Birds at high latitude may also have larger brains because many high latitude species hoard food (Garamszegi & Lucas, 2005). If Gloger's rule and larger brains had evolved at high latitudes, we should expect a negative relationship between brain size and melanin pigmentation, which is what we found. However, we did not find an association between latitude and melanic coloration, and the effect of brain size on pheomelanin-based coloration was independent of latitude, so we can discard a role of the Gloger's rule in this relationship.

Furthermore, it must be noted that avian dopaminergic neurons are joined to melanin (i.e. neuromelanin) in brain areas (substantia nigra pars compacta, SNc, and ventral tegmental area, VTA) that are physiologically and functionally similar to their mammalian homologues (Gale & Perkel, 2006). Thus, although little is known about the biochemistry and function of avian neuromelanin, this internal pigment seems to be important for the survival of neurons by protecting them from reactive quinones and toxic metals (Hearing, 2009), and a similar function should be expected in birds. In addition, the mechanism of biosynthesis of mammalian neuromelanin is still unclear, but it may be an enzymatic process mediated by tyrosinase like eu- and pheomelanogenesis (Zucca *et al.*, 2004). This, in addition to the fact that neuromelanin consists of derivative units of both eu- and pheomelanin (Zucca *et al.*, 2004), opens the possibility that its synthesis may be regulated by GSH levels like pheomelanogenesis, and thus that these processes may compete for GSH resources. Therefore, it could be speculated that, if neuromelanin has a similar role in neuron survival in birds as it has in mammals, and if its synthesis would be limited by a shared resource with pheomelanin (i.e. GSH), the negative relationship we found between pheomelanic plumage colour and brain size may partly be mediated by the specific cost of neuromelanin synthesis in the brain, which may be greater during the development of complex neural structures of large brains. This should be explored by future studies.

Our results have implications for intraspecific visual communication. Following the handicap principle (Zahavi, 1975), the content of honest signals is related to their production or maintenance costs, so that individuals of low genotypic quality pay a greater cost for a given signal expression than high-quality individuals. These costs make that trade-offs between signalling and other fitness-related physiological processes arise when resources are limited or genetic constraints exist (Zera & Harshman, 2001). The negative relationship between the proportion of pheomelanic plumage colour and relative brain mass suggests that pheomelanin synthesis is actually a costly process, which indicates that pheomelanin-based coloration has a great potential to evolve as part of honest signals of quality. Indeed, some studies have found evidence that this type of colour functions in signalling processes in different species of birds (Siefferman *et al.*, 2007; Silva *et al.*, 2008; Galván & Møller, 2009). In our specific comparative test, the production of pheomelanin-based colour would be particularly costly in those species that have large brains. Thus, because ornamental traits involved in signalling are more variable than nonornamental traits (Cuervo & Møller, 2001), it can be predicted that the intraspecific variation in the expression of pheomelanin-based plumage colour and the potential to act as signals of genotypic quality will tend to be greater in large-brained species. In these cases, the signal content of pheomelanic colour may be the possession of the genotypic capacity to synthesize pheomelanin while facing the metabolic costs of brain development during the ontogeny. As large relative brain mass is indicative of high behavioural flexibility among species (Sol & Lefebvre, 2000; Sol *et al.*, 2005) and of superior performance of fitness-related activities at an intraspecific level (Møller, 2010), pheomelanin-based coloration has the potential to signal these traits, especially in large-brained birds.

The relationship between relative brain mass and cognitive capacity suggests that bird species with relatively small brains are not able to deal with novel environmental changes (Sol & Lefebvre, 2000; Sol *et al.*, 2005). Therefore, the expression of pheomelanin-based colour could function as an indicator of the potential of species to colonize new environments, which may be useful given the current interest to find predictors of invading species (Hayes & Barry, 2008). Indeed, we already have evidence that bird species with a large proportion of pheomelanic plumage colour present a diminished capacity to colonize forest patches with an exotic vegetation composition (Galván & Rey Benayas, 2010).

Given that brain development does not only impose constraints, but is also constrained by other metabolically expensive processes (Aiello & Wheeler, 1995; Finlay *et al.*, 2001), we cannot discard the possibility that the cause–effect pattern of the negative relationship between pheomelanic colour and relative brain mass is the opposite to that we have predicted, i.e. that pheomelanogenesis imposes limits to the evolution of large brains in birds. Future studies should investigate this possibility, as well as the consequences for the evolution of life histories derived from the reduced cognitive ability that birds with a large proportion of pheomelanic plumage seem to present.

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Supporting information

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

Appendix S1 Body mass (g), brain mass (g), eumelanic and pheomelanic plumage colour score, mean breeding latitude (°N) and sexual dichromatism in melanin-based colour (0: monochromatic, 1: dichromatic) for the 323 species of birds used in the study.

Appendix S2 Phylogenetic hypothesis used in the study.

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