

Why birds eat colourful grit: colour preferences revealed by the colour of gizzard stones

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Abstract

Colour preferences from sexual or social contexts are assumed to have arisen owing to preferences for specific kinds of food, representing a sensory bias, but once colour preferences have evolved in a sexual context, they may also be expressed during foraging. We tested whether preferences for specific body colours (i.e. plumage and soft parts) were related to colour preferences for grit ingested by birds. Birds eat grit to facilitate break down of food by the gizzard, and this function is independent of the colour of grit, but depends on the physical properties of stones. Bird species were significantly consistent in colour of grit, and grit of different colours varied in prevalence among species, even when analyses were restricted to a sample from a single locality. There were positive correlations between presence of lilac and red grit in the gizzard and presence of sexually dichromatic lilac and red colour on the body. There was a positive correlation between red grit colour and red sexually monochromatic body colour. Bird species with many different sexual colours, but not sexually monochromatic colours on their body had many different colours of grit. Males had more lilac and red grit than females, with this effect differing among species, whereas that was not the case for grit of other colours. These findings are consistent with the sensory bias hypothesis that birds express preferences for grit of specific colours and a high diversity of colours related to sexual colouration of the body, even when the colour of such grit is only visible to the individual at the moment of ingestion.

Introduction

Numerous taxa such as insects, fish, amphibians, reptiles, birds and mammals display preferences for exaggerated colours (Andersson, 1994). Greater colour intensity, large coloured patches or more coloured patches are apparently because of open-ended preferences for even more exaggerated traits (Ryan & Keddy-Hector, 1992). Although there are extensive studies of the functional benefits of such preferences, the origin of the preferences is poorly understood.

Secondary sexual traits may have evolved within or outside a sexual selection context. Fisher (1930) suggested that initially characters that were slightly exaggerated

may have been preferred and become further exaggerated, if such traits were just slightly informative about costs involved in their production. Alternatively, preferences for sexual signals may originate from preferences for similar sizes, shapes or colours in other contexts (e.g. Heisler *et al.*, 1987; Kirkpatrick, 1987). Sexual preferences may originate from a foraging context, if food items of such characteristics have become preferred because of their nutritive qualities (Rodd *et al.*, 2002). Such as preference may then also be expressed in a sexual context, and become further exaggerated, if acquisition of mates with such features is associated with a fitness advantage. This scenario predicts colour preferences being related to the colour of food, and that sexual colour preferences subsequently evolved as a consequence of pre-existing bias.

Once strong preferences for sexual colouration have evolved, we could also expect colour preferences to be expressed outside the sexual context in which they

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originally evolved. That should particularly be the case when there is little or no cost associated with the expression of such preferences. Alternatively, colour preferences in a sexual context are independent of colour of food. Gizzard stones that birds ingest to facilitate mashing up their food may constitute such an example.

Birds do not have teeth and use small stones in their gizzards for reducing the size of food items to facilitate digestion, and such grit is eventually worn down and replaced (McLelland, 1979). Grit is ingested during foraging when an individual bird searches for a stone to be ingested. Birds often move large distances to find grit, and any preference for grit of a specific colour is expressed in the moment when a stone is picked up, leaving a permanent record of the preference inside the bird. Gionfriddo & Best (1996a,b) have shown that two species of birds select grit of specific colours when given a choice. The function of grit may depend on their colour if grit provides minerals (Lee *et al.*, 2004), or if they constitute a potential detoxification agent (Gilardi *et al.*, 1999). However, such functions seem unlikely. In contrast, grit colour may reflect inherent preferences for objects with specific colour as shown by the discovery of the ruby mines in Myanmar, which is because of a ruby found in the stomach of a pheasant (Schifferli, 1985).

The objective of this study was to test whether colour of grit reflected innate colour preferences related to plumage colour. Specifically, we conducted five specific tests to examine the hypothesis that preferences for colour, expressed in choice of grit, arose prior to the evolution of plumage colour preferences. We tested (i) to which extent colour of grit is a repeatable feature of a species. (ii) Whether colour of grit is positively correlated with colour of the body, especially sexually dichromatic body colour (i.e. colour of the plumage and soft parts). We expected that this would particularly be the case for red and reddish grit because there are ubiquitous strong preferences for such colours (e.g. Møller *et al.*, 2000; Hill & McGraw, 2006). Many species have evolved exaggerated colouration in males compared with females, giving rise to the prediction that the correlation between colour of grit and colour of the body should be particularly pronounced for sexually dichromatic species. We also expected a positive correlation between presence of white and black colours in grit and on the body because many studies have shown sexual preferences for these colours in many different species (white colour preferences: Höglund *et al.*, 1990; Kose & Møller, 1999; Kose *et al.*, 1999; McGlothlin *et al.*, 2005; Bókonyi *et al.*, 2006; Hanssen *et al.*, 2006; black colour preferences: Senar, 1999; McGraw, 2006), and comparative studies of colouration in ducks also suggest that white colour is important in sexual selection (Hegyi *et al.*, 2008). (iii) If there were pre-existing biases in colour preferences, then we should expect preferences for certain colours like red to be expressed even in species without red body colour.

In contrast, if the preference for colour is influenced by sexual selection on body colour, then grit colour preferences should be most intense in the most colourful members of a taxon, especially in females that tend to be the choosy sex. (iv) If different body colours have evolved as a consequence of sexual preferences, we should expect bird species with many different colours to have grit of many different colours. (v) Finally, if grit colour preferences had evolved as a consequence of female mate preferences, we should expect females to have more brightly coloured grit than males, and that should especially be the case for sexually dichromatic body colours. Alternatively, if males had more brightly coloured grit than females, this would be consistent with body colours having evolved in a context other than sexual selection (e.g. foraging). We tested these predictions using a unique data set with information on grit from 309 individuals of 61 species of birds.

Materials and methods

Study material

J. Erritzøe opened the gizzard of 309 individual dead birds delivered by the public to him during 2000–2008. Most individuals were from Denmark, with 10% originating from other countries. Specimens derived from more than 200 different localities, and any given locality had a very diverse variety of grit owing to stones deposited during the glaciations, allowing birds to choose among grit of many different colours. After the contents of the gizzard were removed, all the food material was carefully removed and washed while leaving all grit down to the size of sand grains to sediment. This material was subsequently dried, placed in zip-lock bags and stored in the dark until further analyses were made. The analyses presented here were based on the entire sample although a second series of analyses was based on grit samples from a single locality (Christiansfeld and environs). Individual differences in grit colour among species that share a common environment must be owing to differences in colour preferences rather than differences in availability of stones of different colours.

Colour scores

We used colour scores provided by three human observers as a measure of colouration of grit and body of different bird species. Although a photospectrometer could provide detailed information about colouration of grit and plumage, the colour of grit would be strongly influenced by structural properties of stones and hence time since the grit had been ingested. Therefore, we decided to classify the colours of grit and the body into six different categories for simplicity. We explicitly tested for repeatability of this classification within and between observers. We assume in the following that any hetero-

generity in this classification will only cause random noise in the data without any consistent bias.

The colour of all grit was carefully recorded by J. Erritzøe during December 2008 to January 2009 as lilac, red, brown, grey, black or white, allowing for multiple colours to be recorded for a given individual. If any of these colours were present in the grit of an individual bird, it was given a score of 1, and if absent it was given a score of 0, with scores made blindly without prior knowledge of the hypotheses under test. To test for repeatability of colour scores of grit J. Erritzøe scored the colours of grit for 30 individuals a second time in January 2009, without reference to the first series of scores. The repeatability of colour scores was large (Table 1). We also tested for repeatability of colour scores of grit by asking an independent observer (H. Boulet) to score the colours of grit for 30 individuals in February 2009, with scores made blindly. Again, the repeatability of colour scores was large (Table 1).

A. P. Møller, independently of the colour scores of grit, scored the plumage and soft tissue of all bird species investigated using Mullarney *et al.* (2000), del Hoyo *et al.* (1992–2004) and National Geographic Society (1992) as sources. For each of the six colours listed before, every species was given a score of 1 if the colour was present or 0 if the colour was absent. Blue, green and yellow colours

were ignored because there was no grit of these colours. However, a second data set that included these colours provided qualitatively very similar conclusions for the analysis of the number of colours (the only difference being that these colours were added to the number of colours reported in Appendix S1), and only the results from the first data set are provided here. Two sets of scores were made. In the first set, colour scores were only given to the colour present in both sexes, thus reflecting sexually monochromatic colouration. In the second set, colour scores were only given to the colour present in only one of the sexes, thus reflecting sexually dichromatic colouration. We did not assess ultraviolet colouration in this study, although such a study may be revealing. Eaton (2005) has shown widespread sexual dichromatism in species considered to be sexually monochromatic by humans, although several of these cases are simple misclassifications (e.g. blue tit *Cyanistes caeruleus* and common whitethroat *Sylvia communis*), because standard handbooks provide clear criteria for sexing. However, we consider that aspects of colouration visible to both humans and birds to be of biological significance as shown by a number of different studies (e.g. Møller & Birkhead, 1994). Seddon *et al.* (2009) have shown recently that human vision reliably reflects sexual colouration in the plumage of birds. A. P. Møller recorded the two sets of colour scores a second time, without reference to the first set of scores. There was only a single difference that was attributed to an error in the second score. Hence, colour scores were highly repeatable among scoring events.

We recorded the number of different colours of grit and sexually monochromatic and dichromatic colour of birds as the sum of all the colours recorded as being present. This data set is reported in Appendix S1.

All grit is stored in the collections of J. Erritzøe.

Statistical analyses

Colour scores per species were square root arcsine-transformed to normalize data. We investigated the relationships between grit colour (and number of different grit colours) and monochromatic and dichromatic plumage colour and diet category by using partial Kendall rank-order correlations. We used nonparametric tests because of non-normal distributions of the data.

Most statistical approaches assume that each data point provides equally precise information about the deterministic part of total process variation, that is, the standard deviation of the error term is constant over all values of the predictor variables (Sokal & Rohlf, 1995). We weighted each observation by sample size to use all data in an unbiased fashion, thereby giving each datum a weight that reflects its degree of precision owing to sampling effort (Draper & Smith, 1981; Neter *et al.*, 1996). Comparative analyses may be confounded by sample size if sampling effort is important, and if sample

Table 1 Repeatability (*R*) of colour of grit and standard error (SE) of repeatability within and among individuals of different species of birds. The degrees of freedom were 29, 30 for the first two series of analyses and 29, 30 for the third series of analyses.

Stone colour	<i>F</i>	<i>P</i>	<i>R</i>	SE
Within individuals by the same observer				
Lilac†	–		1.00	0.00
Red	14.79	< 0.0001	0.87	0.06
Brown	8.90	< 0.0001	0.80	0.10
White	29.69	< 0.0001	0.93	0.03
Grey	27.21	< 0.0001	0.93	0.04
Black	12.00	< 0.0001	0.85	0.07
No. colours	14.74	< 0.0001	0.87	0.06
Within individuals by two different observers				
Lilac†	–		1.00	0.00
Red	3.00	0.0019	0.50	0.20
Brown	3.18	0.0012	0.53	0.19
White	5.17	< 0.0001	0.68	0.14
Grey	3.77	0.0003	0.58	0.17
Black	4.61	< 0.0001	0.64	0.15
No. colours	8.53	< 0.0001	0.79	0.10
Among individuals				
Lilac	12.25	< 0.0001	0.62	0.04
Red	2.40	< 0.0001	0.17	0.03
Brown	4.49	< 0.0001	0.33	0.04
White	3.45	< 0.0001	0.26	0.04
Grey	3.18	< 0.0001	0.24	0.04
Black	2.47	< 0.0001	0.17	0.03
No. colours	6.27	< 0.0001	0.43	0.04

†All scores were identical and hence the repeatability was 1.00.

size varies considerably among taxa (Garamszegi & Møller, 2009). To weight regressions by sample size in the analysis of contrasts, we calculated weights for each contrast by calculating the mean sample size for the taxa immediately subtended by that node (Møller & Nielsen, 2007). None of these weighted analyses were qualitatively different from the unweighted analyses, and we thus only report the latter.

We calculated repeatabilities using one-way analyses of variance with specimen as the factor in analyses of measurement error (or in the case of consistency among individuals of the same species, by using species as a factor), and we estimated repeatabilities and their standard errors using equations in Becker (1984). We explicitly tested whether spatial heterogeneity in samples could account for repeatability of grit colour because different sites have grit that differ in colour by restricting the analyses to 98 individuals derived from the single locality of Taps, under the assumption that all individuals had access to the same variety of grit colour.

We controlled for similarity in phenotype among species owing to common ancestry by using comparative analysis for continuous variables based on generalized least squares (GLS) models (Pagel, 1997, 1999). First, we investigated the role of phylogenetic inertia by estimating the phylogenetic scaling parameter λ that varies between 0 (phylogenetic independence) and 1 (species' traits covary in direct proportion to their shared evolutionary history; Freckleton *et al.*, 2002). We permitted λ to take its maximum likelihood value, and tested whether there was any evidence for $\lambda > 0$, which indicates that trait variation is dependent of phylogeny. Then, we combined the phylogeny scaling factor and statistical weight using a matrix formula, $Q = V + cW$, where V is the phylogeny matrix, W is the diagonal matrix of 1/weights and c is a constant (Martins & Hansen, 1997). We searched for a combination, which provided the highest maximum likelihood (Freckleton, 2009), and we calculated the phylogenetically corrected and weighted correlation between variables of interest. We fitted the weighted phylogenetic models in the R statistical computing environment, with additional unpublished functions by R. Freckleton (University of Sheffield, available upon request) for the phylogenetic GLS procedure developed for multivariate models. We present results based on the most appropriate phylogenetic and weight adjustments. For illustrative purposes, figures show the untransformed species-specific values.

The composite phylogeny used in the analyses (Appendix S2) was based on Sibley & Ahlquist (1990), combined with information from other sources (Johnson & Sorenson, 1998; Lucchini *et al.*, 2001; Donne-Goussé *et al.*, 2002; Thomas *et al.*, 2004; Jönsson & Fjeldså, 2006; Hackett *et al.*, 2008). As the information for the composite phylogeny came from different studies using different methods, consistent estimates of branch lengths were unavailable. Therefore, branch lengths were transformed

assuming a gradual model of evolution with branch lengths being proportional to the number of species contained within a clade. The results based on these branch lengths were compared with those obtained using constant branch lengths (a punctuated model of evolution). Nowhere were the results qualitatively different. In addition, the results from the phylogenetic analyses were also qualitatively similar to those found when making the calculations using the taxonomy of Sibley & Monroe (1990).

We used the sequential Bonferroni correction to assess the table-wide type I error rate (Holm, 1979; Wright, 1992). Strict application of this method results in a lack of power of single tests (Wright, 1992). Sacrifice of too much power owing to Bonferroni correction can be avoided by choosing an experiment-wise error rate higher than the usually accepted 5%. Therefore, we used 10% as suggested by Wright (1992) and Chandler (1995).

Results

Grit varied enormously in colour among bird species (Figs 1 and 2a, Table 2 and Appendix S1). The preva-

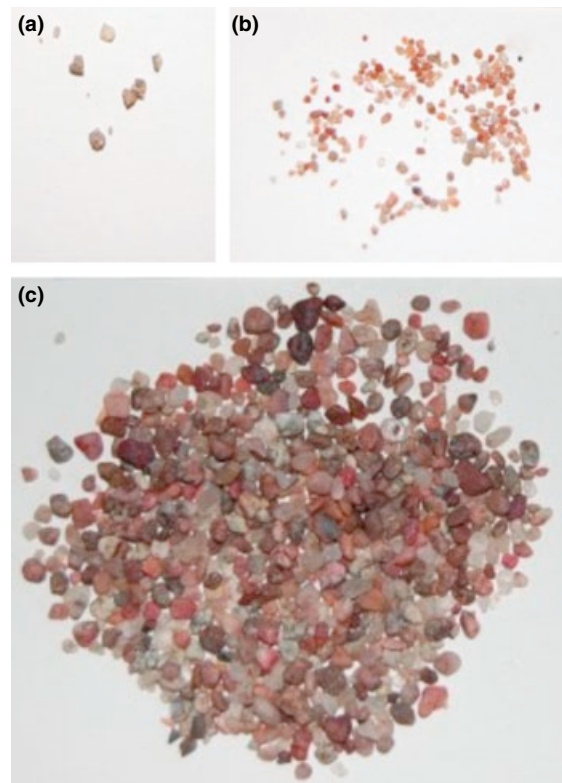


Fig. 1 Photographs of grit of different colours. (a) Corn bunting *Emberiza calandra*, a species with grit of one colour; (b) bullfinch *Pyrrhula pyrrhula*, a species with two colours; (c) black grouse *Lyrurus tetrix*, a species with four colours. (See online publication for colour version of this figure.)

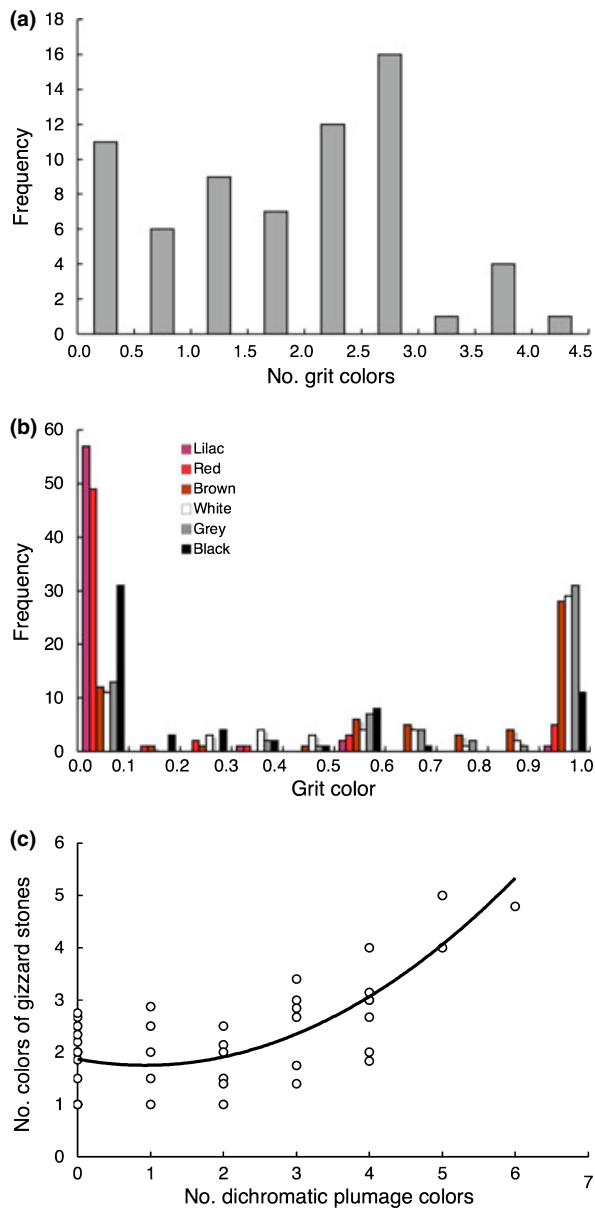


Fig. 2 Number of different colours of grit is positively related to number of different sexually dichromatic colours of plumage in 61 species of birds. The line indicates polynomial regression.

Table 2 Prevalence (proportion of species with the colour present) and mean (SE) colour score of grit in 61 different species of birds.

Stone colour	Prevalence	Mean	SE	Range
Lilac	0.082	0.044	0.020	0–0.857
Red	0.148	0.085	0.031	0–1
Brown	0.738	0.617	0.053	0–1
White	0.787	0.638	0.053	0–1
Grey	0.787	0.642	0.052	0–1
Black	0.525	0.300	0.048	0–1
No. colours		3.115	0.170	1–6

Table 3 Repeatability of colour of grit (R) among individuals of different species of birds and standard error (SE) of repeatability. The degrees of freedom were 21, 76 for the first series of analyses and 35, 215 for the second series of analyses.

Stone colour	F	P	R	SE
Analysis based on local sample				
Lilac†				
Red	1.75	0.040	0.14	0.07
Brown	2.42	0.0027	0.24	0.07
White	4.38	< 0.0001	0.43	0.07
Grey	2.14	0.0086	0.20	0.07
Black	1.36	0.17	0.07	0.06
No. colours	6.51	< 0.0001	0.55	0.14
Analysis based on total sample				
Lilac	12.25	< 0.0001	0.62	0.04
Red	2.40	< 0.0001	0.17	0.03
Brown	4.49	< 0.0001	0.33	0.04
White	3.45	< 0.0001	0.26	0.04
Grey	3.18	< 0.0001	0.24	0.04
Black	2.47	< 0.0001	0.17	0.03
No. colours	6.27	< 0.0001	0.55	0.07

†There were no lilac stones recorded in the local sample and hence repeatability could not be estimated.

lence of different colours of grit varied from rare colours like lilac and red that were found in 8% and 15% of species, to common colours like brown, white and grey that were found in 74–79% of all individuals, with mean colour score for grit varying significantly among species (Fig. 2b and Table 2). The colour of grit was significantly repeatable among individuals of a given species for all colours (Table 3). The largest repeatability was recorded for lilac (0.62), which can be considered to be large (sensu Bourdon, 2000), with all other values ranging from 0.17 to 0.33 being low to moderate (Table 3). When the analysis of repeatability was restricted to samples from a single locality, results were qualitatively similar (Table 3), with the two sets of repeatabilities being positively correlated ($F_{1, 4} = 10.80$, $r^2 = 0.73$, $P = 0.030$; Kendall $\tau = 0.83$, $P = 0.022$). Likewise, the two sets of repeatabilities did not differ significantly from each other in a paired analysis (paired t -test, $t_5 = 0.29$, $P = 0.78$).

For monochromatic body colours (i.e. colours of plumage and soft parts), there was a significant positive correlation between grit colour and colour of the body for red colour only after Bonferroni adjustment, and an analysis based on a GLS approach provided a similar conclusion (Table 4).

There were significant positive correlations between the colour of grit and sexually dichromatic colour for lilac and red across bird species when results were adjusted for multiple tests using sequential Bonferroni correction (Table 4). Analyses based on a GLS approach showed similar conclusions (Table 4).

Bird species with more dichromatic body colours also had more colours of their grit (Fig. 2c and Table 4), whereas that was not the case for monochromatic

Table 4 Kendall τ partial rank-order correlations between the colour of grit and the colour of monochromatic and dichromatic plumage in 61 different species of birds based on analyses of species-specific data and λ parameter, t -statistics and slope (SE) estimates from a generalized least squares approach. Significant results after sequential Bonferroni correction are shown in bold font.

Monochromatic plumage colour					Dichromatic plumage colour			
Stone colour	Kendall τ	λ	t	Slope (SE)	Kendall τ	λ	t	Slope (SE)
Lilac	-0.048	1.000	-0.024	-0.003 (0.118)	0.561***	0.251	6.000***	0.401 (0.067)
Red	0.468***	0.595	4.118***	0.328 (0.080)	0.627***	0.519	4.939***	0.339 (0.069)
Brown	0.020	0.000	0.466	0.045 (0.098)	0.133	0.000	1.098	0.104 (0.095)
White	0.009	0.183	1.658	0.171 (0.103)	0.125	0.126	1.051	0.106 (0.101)
Grey	0.159	0.224	1.253	0.131 (0.105)	0.048	0.164	0.607	0.069 (0.114)
Black	-0.060	0.000	-0.941	-0.088 (0.094)	0.144	0.000	0.830	0.074 (0.090)
No. colours	-0.032	0.478	0.957	0.091 (0.095)	0.294**	0.000	3.477***	0.193 (0.056)

** $P < 0.01$; *** $P < 0.001$.

colours (Table 4). A secondary polynomial fitted to the relationship for dichromatic colours provided a better fit ($F_{2,58} = 33.752$, $r^2 = 0.54$, $P < 0.0001$) than a simple linear relationship ($F_{1,59} = 44.258$, $r^2 = 0.43$, $P < 0.0001$). Analyses based on a GLS approach likewise revealed a significant positive correlation for the number of dichromatic colours, but not for the number of monochromatic colours and the number of grit colours (Table 4). This relationship was not simply caused by the fact that species with larger body mass collected more grit and therefore had more colours of grit as a coincidence. The

Table 5 Effects of species, sex and species by sex interaction on colour of grit in 61 different species of birds based on analyses of species-specific data and statistically independent linear contrasts. Significant results after sequential Bonferroni correction are shown in bold font.

Stone colour	Species	Sex	Species \times sex
Lilac	51.54***	3.23* Male: 0.040 (0.008) Female: 0.022 (0.009)	12.36***
Red	2.17**	9.28** Male: 0.032 (0.016) Female: 0.000 (0.018)	2.17**
Brown	6.05***	3.64 Male: 0.774 (0.048) Female: 0.700 (0.054)	1.36
White	4.70***	0.18 Male: 0.734 (0.051) Female: 0.778 (0.057)	1.33
Grey	3.55***	0.02 Male: 0.621 (0.061) Female: 0.556 (0.068)	0.87
Black	2.59***	2.23 Male: 0.290 (0.061) Female: 0.344 (0.068)	0.74
No. colours	8.15***	0.01 Male: 2.492 (0.092) Female: 2.400 (0.102)	1.15
d.f.	23, 166	1, 166	23, 166

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

number of grit was not significantly related to body mass ($F_{1,53} = 3.40$, $r^2 = 0.06$, $P = 0.07$), and body mass did not predict the number of colours of grit [partial $F_{1,57} = 2.33$, $P = 0.07$, slope (SE) = 0.24 (0.16)]. In contrast, the number of dichromatic body colours did predict the number of colours of grit [partial $F_{1,57} = 7.21$, $P = 0.0095$, slope (SE) = 0.15 (0.06)]. Analyses based on a GLS approach revealed similar conclusions.

An analysis restricted to the 24 species for which we had grit colour for both males and females showed consistent effects of species on colour and number of colours (Table 5). In addition, we found a significant effect of sex for lilac and red grit, but not for the other colours (Table 5). Males had higher scores than females for lilac and red grit. Finally, there was a significant interaction between species and sex for lilac and red grit, showing that the difference between sexes varied among species (Table 5). The sex difference in grit colour score was significantly positively related to dichromatic colour score of the body (lilac: Pearson $r = 0.94$, $t_{21} = 12.72$, $P < 0.0001$; red: $r = 0.58$, $t_{21} = 3.25$, $P = 0.0038$), but only significantly to monochromatic body score for red (lilac: Pearson $r = -0.07$, $t_{21} = -0.34$, $P = 0.74$; red: $r = 0.53$, $t_{21} = 2.87$, $P = 0.0092$).

Discussion

The main findings of this study of colour of grit were that bird species were consistent in their choice of grit colour, colour of grit was positively correlated with some body colours (i.e. colours of plumage and soft parts), but not for others and the number of different colours of grit was positively correlated with the number of sexually dichromatic body colours, but not the number of monochromatic colours. Finally, there was an effect of sex on grit colour, with this effect differing among species for lilac and red grit, but not for grit of other colours.

Different individuals of the same species were consistent in choice of colours of their grit, as shown by significant repeatabilities. Repeatability was high for lilac, with lower but still significant values for other colours

(sensu Bourdon, 2000). Thus, choice of colours deviated from random. We do not know whether all individuals had access to grit of all different colours. However, an analysis of grit colour from a single locality, where all individuals of all species had access to the same colours of grit, provided very similar repeatability estimates as in the entire sample. This finding implies that there were inherent grit colour preferences.

Bird species showed strong preferences for specific colours, as evidenced by the positive correlations between colour of grit and presence of the same dichromatic body colour, whereas that was not the case for several other colours. This provides evidence consistent with the hypothesis that colour preferences for grit are linked to processes that have given rise to body colours. Not only colour of grit, but also the diversity of colours varied significantly among species. Thus, individuals of a given species tended to ingest a similar number of different colours of grit, and this diversity of colours reflected the diversity of body colours. However, this pattern was only evident for sexually dichromatic colouration, but not for monochromatic colouration. This difference between sexually monochromatic and dichromatic colours is consistent with the *a priori* expectation that sexual selection is responsible for having shaped the preference for body colours, and that this preference is also expressed as a preference for colours of grit. We are unaware of any other study showing extensive evidence of colour preferences evolved in a sexual selection context that are also expressed in a nonsexual context.

We presented two alternative hypotheses for sex differences in grit colour. If grit colour preferences have evolved as a consequence of previous sexual colour preferences, we should expect the sex with the strongest mate preferences (i.e. females) to show the clearest grit colour preference. Alternatively, if colour preferences have evolved in a nonsexual context such as foraging, we should expect individuals of the sex with the strongest food preferences to express similar colour preferences for grit. The sexes differed in grit colour, with males having more red and lilac grit than females. This result is clearly inconsistent with the hypothesis that grit colour preferences arose from sexual preferences because females rather than males should then have had more brightly coloured grit. In contrast, it is consistent with the hypothesis that food colour preferences subsequently gave rise to grit colour preferences and eventually to body colour preferences by individuals of the choosy sex (i.e. females). Indeed, if food colour preferences evolved as a result of preferences for food items rich in carotenoids, males rather than females should express strong colour preferences, because males use more carotenoids for sexual signals than females.

Why did bird species show preferences for red (only in species-specific analyses) and lilac grit, but not for other

colours, and why were these colour preferences mainly related to sexually dichromatic colours? We hypothesize that these differences reflect the architecture of colour preferences among species, with the magnitude of preferences for different colours being proportional to the importance of these specific colours in sexual and nonsexual contexts. Red and lilac may resemble carotenoid-based colours that are preferred in fish, reptiles and birds (Andersson, 1994; Gray, 1996; Møller *et al.*, 2000; Hill & McGraw, 2006). White and black colours are also important in sexual signals of many different species (white colours: Höglund *et al.*, 1990; Kose & Møller, 1999; Kose *et al.*, 1999; McGlothlin *et al.*, 2005; Bókony *et al.*, 2006; Hanssen *et al.*, 2006; black colours: Senar, 1999; McGraw, 2006), and comparative studies of colouration in ducks also suggest that white colour is important in sexual selection (Hegyi *et al.*, 2008). The dichotomy between carotenoid-based colours and other colours is most likely related to the different physiological functions of pigments involved in these two categories of colouration, with lilac, red and yellow colours being derived from carotenoids having physiological functions as immunostimulants and perhaps antioxidants (e.g. Møller *et al.*, 2000; Hill & McGraw, 2006; McGraw, 2006).

In conclusion, bird species differed in colour preferences as reflected by colour of grit, demonstrating that specific preferences exist. Colour preferences for specific kinds of grit reflected sexually dichromatic body colour, implying that grit preferences and colour preferences have a common basis, and species with the greatest diversity of body colours also had the greatest diversity of grit colours, suggesting that grit colour preference and body colour preferences have coevolved. Males rather than females had colourful grit, suggesting that choice of grit with specific colours had subsequently become expressed in sexual contexts.

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References

- Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ.
- Becker, W.A. 1984. *Manual of Quantitative Genetics*. Academic Enterprises, Pullman, WA.
- Bókony, V., Lendvai, A.Z. & Liker, A. 2006. Multiple cues in status signaling: the role of wingbars in aggressive interactions of male house sparrows. *Ethology* **112**: 947–954.
- Bourdon, R.M. 2000. *Understanding Animal Breeding*, 2nd edn. Prentice Hall, Upper Saddle River, NJ.

- Chandler, C.R. 1995. Practical considerations in the use of simultaneous inference for multiple tests. *Anim. Behav.* **49**: 524–527.
- Donne-Goussé, C., Laudet, V. & Hänni, C. 2002. A molecular phylogeny of Anseriiformes based on mitochondrial DNA analysis. *Mol. Phylogenet. Evol.* **23**: 339–356.
- Draper, N.R. & Smith, H. 1981. *Applied Regression Analysis*, 2nd edn. John Wiley, New York, NY.
- Eaton, M.D. 2005. Human vision fails to distinguish widespread sexual dichromatism among sexually “monochromatic” birds. *Proc. Natl. Acad. Sci. USA* **102**: 10942–10946.
- Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Clarendon, Oxford.
- Freckleton, R.P. 2009. The seven deadly sins of comparative analysis. *J. Evol. Biol.* **22**: 1367–1375.
- Freckleton, R.P., Harvey, P.H. & Pagel, M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* **160**: 712–726.
- Gilardi, J.D., Duffey, S.S., Munn, C.A. & Tell, L.A. 1999. Biochemical functions of geophagy in parrots: detoxification of dietary toxins and cytoprotective effects. *J. Chem. Ecol.* **25**: 897–922.
- Gionfriddo, J.P. & Best, L.B. 1996a. Grit color selection by house sparrows and northern bobwhites. *J. Wildl. Manage.* **60**: 836–842.
- Gionfriddo, J.P. & Best, L.B. 1996b. Grit-use patterns in North American birds: the influence of diet, body size, and gender. *Wilson Bull.* **108**: 685–696.
- Gray, D.A. 1996. Carotenoids and sexual dichromatism in North American passerine birds. *Am. Nat.* **148**: 453–480.
- Hackett, S.J., Kimball, R.T., Reddy, S., Bowie, R.C.K., Braun, E.L., Braun, M.J., Chojnowski, J.L., Cox, W.A., Han, H.-L., Harshman, J., Huddleton, C.J., Marks, B.D., Miglia, K.J., Moore, W.A., Sheldon, F.H., Steadman, D.W., Witt, C.C. & Yuri, T. 2008. A phylogenomic study of birds reveals their evolutionary history. *Science* **320**: 1763–1768.
- Hanssen, S.A., Folstad, I. & Erikstad, K.E. 2006. White plumage reflects individual quality in female eiders. *Anim. Behav.* **71**: 337–343.
- Hegyí, G., Garamszegi, L.Z. & Eens, M. 2008. Roles of ecological factors and sexual selection in the evolution of white wing patches in ducks. *Behav. Ecol.* **19**: 1208–1216.
- Heisler, L., Andersson, M.B., Arnold, S.J., Boake, C.R., Borgia, G., Hausfater, G., Kirkpatrick, M., Lande, R., Maynard Smith, J., O’Donald, P., Thornhill, A.R. & Weissing, F.J. 1987. The evolution of mating preferences and sexually selected traits. In: *Sexual selection: Testing the alternatives* (J.W. Bradbury & M.B. Andersson, eds), pp. 96–118. Wiley, Chichester, NJ.
- Hill, G.E. & McGraw, K.J. (eds) 2006. *Bird Coloration*. Harvard University Press, Harvard, MA.
- Höglund, J., Eriksson, M. & Lindell, L.E. 1990. Females of the lek-breeding great snipe, *Gallinago media*, prefer males with white tails. *Anim. Behav.* **40**: 23–32.
- Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* **6**: 65–70.
- del Hoyo, J., Elliott, A. & Sagartal, J. (eds). 1992–2004. *Handbook of the Birds of the World*. Lynx, Barcelona, Spain.
- Johnson, K.P. & Sorenson, M.D. 1998. Comparing molecular evolution in two mitochondrial protein coding genes (cytochrome b and ND2) in the dabbling ducks (Tribe: Anatini). *Mol. Phylogenet. Evol.* **10**: 82–94.
- Jönsson, K.A. & Fjeldså, J.Å. 2006. A phylogenetic supertree of oscine passerine birds (Aves: Passeri). *Zool. Scr.* **35**: 149–186.
- Kirkpatrick, M. 1987. The evolutionary forces acting on female mating preferences in polygamous animals. In: *Sexual Selection: Testing the Alternatives* (J.W. Bradbury & M.B. Andersson, eds), pp. 263–271. Wiley, Chichester, NJ.
- Kose, M. & Møller, A.P. 1999. Sexual selection, feather breakage and parasites: the importance of white spots in the tail of the barn swallow. *Behav. Ecol. Sociobiol.* **45**: 430–436.
- Kose, M., Mänd, R. & Møller, A.P. 1999. Sexual selection for white tail spots in the barn swallow in relation to habitat choice by feather lice. *Anim. Behav.* **58**: 1201–1205.
- Lee, D.E., Hamman, M.G. & Black, J.M. 2004. Grit-site selection of black brant: particle size or calcium content? *Wilson Bull.* **116**: 304–313.
- Lucchini, V., Höglund, J., Klaus, S., Swenson, J. & Randi, E. 2001. Historical biogeography and a mitochondrial DNA phylogeny of grouse and ptarmigan. *Mol. Phylogenet. Evol.* **20**: 149–162.
- Martins, E.P. & Hansen, T.F. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* **149**: 646–667.
- McGlothlin, J.W., Parker, P.G., Nolan, V. Jr & Ketterson, E.D. 2005. Correlational selection leads to genetic integration of body size and an attractive plumage trait in dark-eyed juncos. *Evolution* **59**: 658–671.
- McGraw, K.J. 2006. Mechanics of melanin-based coloration. In: *Bird Coloration, Volume I: Mechanisms and Measurements* (G.E. Hill & K.J. McGraw, eds), pp. 243–294. Harvard University Press, Cambridge, MA.
- McLelland, J. 1979. Digestive system. In: *Form and Function in Birds* (A.S. King & J. McLelland, eds), Vol. 1, pp. 69–181. Academic Press, London.
- Møller, A.P. & Birkhead, T.R. 1994. The evolution of plumage brightness in birds is related to extra-pair paternity. *Evolution* **48**: 1089–1100.
- Møller, A.P. & Garamszegi, L.Z. 2009. Effects of sample size and intraspecific variation in phylogenetic comparative studies: a meta-analytic review. *Biol. Rev.*, in press.
- Møller, A.P. & Nielsen, J.T. 2007. Malaria and risk of predation: a comparative study of birds. *Ecology* **88**: 871–881.
- Møller, A.P., Biard, C., Blount, J.D., Houston, D.C., Ninni, P., Saino, N. & Surai, P.F. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Poult. Avian Biol. Rev.* **11**: 137–159.
- Mullarney, T., Svensson, L., Zetterström, D. & Grant, P.J. 2000. *The Complete Guide to the Birds of Europe*. Harper Collins, London.
- National Geographic Society. 1992. *Field Guide to the Birds of North America*, 2nd edn. National Geographic Society, Washington DC.
- Neter, J., Kutner, M.H., Nachtsheim, C.J. & Wasserman, W. 1996. *Applied Linear Statistical Models*. Irwin, Chicago, IL.
- Pagel, M. 1997. Inferring evolutionary processes from phylogenies. *Zool. Scr.* **26**: 331–348.
- Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401**: 877–884.

- Rodd, F.H., Hughes, K.A., Grether, G.F. & Baril, C.T. 2002. A possible non-sexual origin of mate preference: are male guppies mimicking fruit? *Proc. R. Soc. Lond. B* **269**: 475–481.
- Ryan, M. & Keddy-Hector, A. 1992. Directional patterns of female mate choice and the role of sensory biases. *Am. Nat.* **139**: S4–S35.
- Schifferli, L. 1985. Grit. In: *A Dictionary of Birds* (B. Campbell & E. Lack, eds), p. 256. Poyser, Calton.
- Seddon, N., Tobias, J., Eaton, M. & Odén, A. 2009. Human vision can provide a valid proxy for avian perception of sexual dichromatism. *Auk*, in press.
- Senar, J.C. 1999. *Plumage Coloration as a Signal of Social Status*. Proceedings of the 22nd International Ornithological Congress, Durban, pp. 1669–1686. BirdLife South Africa, Johannesburg, South Africa.
- Sibley, C.G. & Ahlquist, J.E. 1990. *Phylogeny and Classification of Birds, a Study in Molecular Evolution*. Yale University Press, New Haven, CT, and London.
- Sibley, C.G. & Monroe, B.L. Jr 1990. *Distribution and Taxonomy of Birds of the World*. Yale University Press, New Haven, CT, and London.
- Sokal, R.R. & Rohlf, F.J. 1995. *Biometry*, 3rd edn. Freeman, New York, NY.
- Thomas, G.H., Wills, M.A. & Székely, T. 2004. A supertree approach to shorebird phylogeny. *BMC Evol. Biol.* **4**: 1–18.
- Wright, S.P. 1992. Adjusted *P*-values for simultaneous inference. *Biometrics* **48**: 1005–1013.

Supporting information

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

Appendix S1 Summary statistics for colours of sexually monochromatic and dichromatic parts of birds and colours of grit in 61 species of birds. See ‘Materials and methods’ for further details.

Appendix S2 Phylogenetic relationships between the species included in the present study. See ‘Materials and methods’ for sources.

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