Rufous Common Cuckoo chicks are not always female

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Abstract

The Common Cuckoo shows two adult plumage morphs – adult male plumage is grey and adult females are either grey or, less frequently, rufous. The situation is less clear in juveniles, as both sexes exhibit variable proportions of grey and rufous colour. We thus describe the patterns related to sex-specific plumage colour variation in a central European Cuckoo population. We genetically determined sex of 91 Cuckoo chicks and using visual classification of photographs we scored juvenile plumage colouration of individual chicks into five classes based upon the increasing proportion of feathers with rufous colour. To verify these scores, we sampled chick feathers and quantified the proportion of rufous colour on individual feathers by digital image analysis. We found that juvenile females had a higher proportion of rufous colour on feathers than juvenile males. However, the difference was marginally non-significant based on visual inspection alone, and some male chicks even showed intensively rufous plumage like those of juvenile females. In contrast, we captured only grey adult males (N = 37) while 5 out of 20 adult females were rufous. The rufous colour of Cuckoo feathers considerably differed from the grey colour and the difference was larger in adults than in juveniles. We show that chicks, unlike adult females, cannot be visually assigned to either of the adult morphs. Therefore, we encourage further investigation of Cuckoo plumage colouration across the species range and to examine the process of plumage maturation. A detailed genetic analysis is necessary to understand the origin of Cuckoo feather colouration.

Keywords: avian vision; colour dimorphism; *Cuculus canorus*; molecular analysis; plumage colouration; spectral reflectance

Introduction

Colour synthesis is a very complex process that can be affected both genetically and environmentally (Galván and Solano 2016) and that may result in the coexistence of two (i.e. dimorphism, Bond 2007) or more distinct colour forms in one interbreeding animal population (Huxley 1955). This phenomenon, known as intraspecific colour polymorphism, has been observed across many vertebrate species (Galeotti et al. 2003; Gray and McKinnon 2007) and has provided key models for studies in evolutionary biology including sexual selection, speciation, and mimicry (McKinnon and Pierotti 2010). In birds, the most common plumage pigment, melanin, occurs in two forms – eumelanin and pheomelanin (Hill and McGraw 2006). The proportion of eumelanin and pheomelanin determines bird plumage colouration – lighter (yellow to red) phenotypes originate from the increased deposition of pheomelanin, while darker ones (brown to black) result from the increased deposition of eumelanin (McGraw et al. 2005; Hill and McGraw 2006).

Excluding sexual dichromatism, seasonal change and age differences, about 334 (3.5%) of all world bird species exhibit plumage colour polymorphism, including 241 species with only two colour plumage morphs (Galeotti et al. 2003). The colour polymorphism occurs most frequently in Strigiformes (owls and nightjars – 33.5% species), Cuculiformes (11.9%) and Galliformes (9.5%, Galeotti et al. 2003). Colour polymorphism has been mainly explored in adult birds, but far less attention has been paid to such a variation in juveniles. In some species, plumage morphs do not change with age and moults (e.g. Ruff *Philomachus pugnax* and Buzzard *Buteo buteo*; Lank et al. 1995; Kappers et al. 2017) whereas in others, they differ between juveniles and adults, such as in many raptors (Ferguson-Lees and Christie 2001; Roulin 2004) or cuckoos (Voipio 1953; Cramp 1985).

Obligatory parasitic Common Cuckoos *Cuculus canorus* (hereafter Cuckoos) exhibit two typical colour morphs. Adult males are grey, and females either resemble the males (some with rufous tinge on the upper breast and neck, Noh et al. 2016), or occur in a rufous morph (Voipio 1953; Payne 1967). Female polymorphism is also known in other parasitic cuckoos, but is absent in non-parasitic ones (Payne 1967). In coevolutionary arms races between cuckoos and their hosts, variable plumage colour may have evolved as a result of apostatic selection (Payne 1967) or as a counteradaptation to resemble avian predators – unknown or predator-like plumage can reduce the chance of being recognized and attacked by the hosts (Davies and Welbergen 2008; Welbergen and Davies 2011). Alternatively, the females may simply keep their plumage to adulthood as paedomorphic retention (neoteny, Trnka et al. 2015). Nevertheless, the proportions of morphs differ substantially between sites; from those where the rufous females are almost entirely absent (Thorogood and Davies 2012) to sites where they are common (Honza et al. 2006; Table 1).

Cuckoo fledglings exhibit far more varied combinations of grey and rufous colour than adults, to the point where the border between the morphs is hardly detectable (Cramp 1985). So far, only Voipio (1953) attempted to evaluate the plumage polymorphism in juvenile Cuckoos; however his study was restricted to a relatively small number of museum specimens. Contrary to Cramp (1985), Voipio (1953) distinguished three juvenile plumage types – grey-brown and rufous type for both sexes and red phase which should occur in females only. However, his material did not contain any specimen of such a red phase and the variability in the fledgling colouration has never been classified in detail. Moreover, it remains unknown whether the fledglings resembling the adult rufous morph are always females.

Here, we describe variation in the proportion of grey and rufous plumage colour in a central European Cuckoo population. We used both subjective assessments and objective digital imaging for colour categorisation, and also compare the plumage colouration between males and females in juveniles and adults. In light of the prior research mentioned above, we predict that the rufous colouration will be more frequent in females than in males. Finally, we also discuss a possible genetic background of Cuckoo plumage colouration.

Methods

Fieldwork

The study was carried out from April to July 2016 and 2017 (with some adults recaptured in 2018) in the fishpond area between Mutěnice (48°54′N, 17°02′E) and Lužice (48°50′N, 17°04′E) in south-eastern part of the Czech Republic. To find active nests with Cuckoo chicks, we systematically searched littoral vegetation with numerous territories of Eurasian Reed Warblers *Acrocephalus scirpaceus*, Great Reed Warblers *Acrocephalus arundinaceus* and Sedge Warblers *Acrocephalus* *schoenobaenus,* and also occasionally checked the adjacent sites preferred by Marsh Warblers *Acrocephalus palustris*. In April and May of the same years, we also mist-netted 57 adult Cuckoos from the same study site.

Sex determination

When the chicks were at least 6 days old, a small amount of blood (5–25 µl) was collected by tarsal venipuncture and stored in 100 µl of 96% ethanol. Chick sex was later determined in the laboratory by amplifying a part of the W-linked chromo-helicase-DNA binding gene CHD-W (unique to females) and a part of its homologue, the CHD-Z gene, linked to the Z chromosome (occurring in both sexes, Griffiths et al. 1998). After DNA extraction, the avian sex primers P2 and P8 (Griffiths et al. 1998) were used in 10 µl PCR reactions (for details of PCR conditions see Abraham et al. 2015). The PCR products were separated by electrophoresis for 45–60 min at 7–10 V/cm using 3% agarose gels stained with SYBR® Safe (Life Technologies, Invitrogen, Carlsbad, CA). Heterogametic females were characterised by a two-band profile (~350 and ~400 bp), while homogametic males by only a single band (~400 bp). The sex of adults was determined genetically following the same procedure as in juveniles.

Classification of plumage colouration

To describe and categorize plumage colouration, we photographed 77 out of the 91 Cuckoo chicks (N = 43 originating from the nests of the Eurasian Reed Warbler, N = 32 from Great Reed Warblers and 1 each from Marsh and Sedge Warblers) at the age between 9 and 18 days (median = 14). We distinguished five plumage colour classes based upon the proportion of rufous colour present on feathers on a Cuckoo back and upon the shape and size of rufous spots and stripes on individual feathers on the following semi-continuous ordinal scale: 1) Grey – rufous colour missing or present on <5% extent of contour feathers on less than one half of the back, individual rufous spots smudgy and disjunct. 2) Medium grey – rufous colour on 5–10% extent of contour feathers on the major part of the back. Individual rufous spots thin, their length exceeds the width. The spots usually do not form stripes crossing shafts of the feathers. 3) Intermediate – similar to 2, but the disjunct rufous spots are thicker, their length usually equals width. The spots are present on 11–20% extent of almost all back feathers. Sometimes, the spots may cross the whole feather, but in such case their width is similar to 2. 4) Medium rufous – rufous on 21–40% extent of all back feathers, rufous stripes lead across the feather and their width is similar to 3. Some stripes may be interrupted, but the gap thinner than one third of stripe length. 5) Rufous – rufous on >40% extent of all back feathers, uninterrupted stripes cross the feathers and their width is larger than in 3 and 4 (see Electronic Supplementary Material, Fig. S1 for sample photographs representing individual classes). These classes did not coincide with the plumage morphs in Voipio (1953; i.e. grey-brown, rufous and red) and did not depend on age when the chicks were photographed (linear regression: slope = 0.066 ± 0.068 SE, $F_{1,75}$ = 0.94, P = 0.334).

Based on this five-degree classification, one image of each chick was independently assigned to one plumage colour class by the authors (except for J. T.) and Vojtěch Brlík, Radka Poláková, Peter Samaš and Kateřina Sosnovcová (Electronic Supplementary Material, Table S1), who all have field experience with Cuckoo chicks. Each of the ten judges was blind to the nestling sex. Using R (R Core Team 2016) we calculated interclass correlation coefficient (ICC; package 'irr', Gamer et al. 2012) for testing similarity in scores given by individual judges and thus the reliability of the classification. The scores given by individual judges to each chick were highly consistent (ICC = 0.883, $F_{76,693}$ = 76.6, P << 0.001, Electronic Supplementary Material, Table S1). Finally, we averaged the obtained plumage colour scores.

We distinguished between grey and rufous colour morph in the captured adult Cuckoos. The grey morph is typical of predominantly grey upper parts without contrasting rufous colouration

(Electronic Supplementary Material, Fig. S2). The rufous morph resembles the (v) rufous juvenile plumage colour class – i.e. individuals have marked rufous stripes on the upper part of their body (Voipio 1953; Electronic Supplementary Material, Fig. S2). As the differences between the adult morphs are clear-cut, we did not apply any detailed evaluation of feathers or images of plumage colouration.

Analysis of feather colouration

Because the human classification of chick plumage coloration may be subjective, we also performed a digital analysis of feather colouration and correlated the results obtained from both approaches. We collected feathers from the upper back belonging to 87 out of the 91 Cuckoo chicks (N = 50 chicks found in the nests of Eurasian Reed Warblers, 35 in Great Reed Warblers and 1 each in Marsh and Sedge Warblers) at ages of between 11 and 23 days (median = 14). Feathers were scanned using a flatbed scanner (HP Color LaserJet Pro MFP M176n, resolution: 14028 × 10200; 1200 dpi). Because we expected a certain degree of variability in feather colouration, we always scanned up to six feathers from the same individual, performed further analyses for each feather separately and then averaged the measurements to obtain single values for each individual. Feathers were fastened by a strip of adhesive tape to a white sheet of paper and scanned with a scale. Colour measurements of the feathers were made using ImageJ version 1.51k (ImageJ 2018), by selecting four regions of interest: dark grey feather sections, rufous feather sections, light grey down feather sections and the white background of paper. In total, the mean RGB values of 442 feather sections were measured to create a model training database. These values were then used to create a neural-net multinomial logistic regression model in R (version 3.4.2), using the 'nnet' package (version 7.3–12, Venables and Ripley 2002), where feather section types (dark grey, rufous, light grey or background) were the dependent and the RGB values the independent variables. This model was able to classify 99.77% of the 442 training measurements correctly. The resulting model was converted into a JAVA ImageJ plugin script, which transformed all scanned feather images into the four categories based on pixel colour and reported the number of pixels in each group. The proportion of rufous colour on each feather was calculated as number of pixels denoting rufous colour divided by number of pixels denoting rufous and dark grey colour of the feather (see Electronic Supplementary Material, Fig. S3). This proportion did not depend on the age of the chicks (linear regression: slope = –0.763 ± 0.801 SE, $F_{1,85} = 0.91$, $P = 0.343$).

In addition, we measured spectral reflectance (300 to 700 nm) of the feathers from eight chicks (two females, six males) and two adult rufous females. We collected three feathers from the upper back of each individual. The feathers from each individual were stacked upon each other and attached to a black sheet of paper with adhesive tape. We used a reflectance spectrophotometer (USB 2000, Ocean Optics, Dunedin, FL), a deuterium and halogen light source (DT-Mini-GS, Ocean Optics), and a quartz optic fibre (QR400-7-UV/VIS-BX, Ocean Optics). The measurements were relative to a standard white reference (WS-1, Ocean Optics) and to darkness. Reference and dark calibration were made prior to the measurement. MŠ performed all measurements under standard light conditions, at the same angle (90°) and same distance from the feather sample. The reflectance of both grey and rufous colours was measured in the central part of the feather (Electronic Supplementary Material, Fig. S3). We performed and averaged three measurements of the grey part and other three measures of the rufous part of the feather sample per each individual. The average values of the grey and rufous from each individual were then averaged to obtain the overall mean reflectance spectra for each colour in juveniles and adults separately.

Results

Ranges of mean plumage colour scores were similar between juvenile Cuckoo males (mean score = 2.29 ± 0.16 SE, range: 1.0–4.5) and females (2.82 ± 0.20 SE, 1.0–5.0; Electronic Supplementary Material, Table S1), both scoring around the middle of the scale. Females were marginally non-significantly more rufous than males (Mann-Whitney-Wilcoxon W = 917, P = 0.068; Fig. 1a). This human classification of chick plumage colouration tightly positively correlated with the proportion of rufous colour measured from individual feathers using image analysis (r_S = 0.87, P << 0.001; n = 73). The proportion was significantly higher in juvenile females (0.7–46.0%, mean = 16.81% ± 2.00 SE, n = 38) than in juvenile males (0.5–38.2%, mean = 11.07% ± 1.42 SE, n = 49; Mann-Whitney-Wilcoxon W = 1197, P = 0.023; Fig. 1b).

Out of 57 captured adult Cuckoos, all 37 males belonged to the grey morph, while 15 out of 20 females were grey and 5 rufous. The rufous colour of Cuckoo back feathers considerably differed from the grey colour (Fig. 2) and the difference seemed to be larger in adults than in juveniles (Fig. 2).

Discussion

Our study presents detailed data on colour variation of Cuckoo chicks and provides a simple human visual classification tool that may help to categorize plumage colouration of Cuckoo chicks without using image analysis. Both the digital image analysis as well as visual classification revealed that most Cuckoo chicks exhibited grey or intermediate plumage colouration, but the two distinct colour morphs present in Cuckoo adults are hardly distinguishable in chicks. Regardless of the method used, female chicks were generally more rufous than males. Surprisingly, the intersexual differences in plumage colouration and in the proportion of rufous colour on feathers were quite small in chicks and some males had rather intensely rufous plumage. Yet, such male colouration was not observed in adult Cuckoos where the rufous plumage was recorded only in about a quarter of females mist-netted in our study area, which is similar to the proportion of female chicks belonging to the medium rufous and rufous plumage colour classes.

We developed a five-degree classification which covers the large variability of chick plumage colouration. Unlike Voipio (1953), who first attempted to explain patterns in Cuckoo plumage colouration, we doubt that it is possible to reliably visually distinguish any colour plumage morphs in Cuckoo chicks. Instead, individual chicks that we examined exhibited a continuous variability of rufous colour in individual feathers as well as in their plumage (see also Cramp 1985). Hypothetically, the two 'most rufous' chick plumage colour classes 4 and 5 might roughly correspond with the rufous morph in adult females (e.g. due to prevailing uninterrupted rufous stripes). In which case, around 25% of female chicks would be rufous, which corresponds with the proportion of adult rufous females in the study population. In contrast, 15% of juvenile males and no adult males would be rufous. Thus while we may possibly see a link between the colouration of juvenile and adult females, the process of male plumage maturation is probably different.

Despite Voipio's (1953) statement that the plumage of juvenile males should not be intensely rufous, together with 'textbook' images claiming that rufous Cuckoo chicks are females (but see Mann 2014), the male chicks which we investigated fell into all plumage colour classes, and some thereby resembled even the most rufous females. This striking finding could suggest that some male chicks may occasionally retain rufous colour until adulthood (Ringleben 1958; Becker 1989; Busche 2003). Although the proportion of adult rufous females in our study population agrees with their distribution across Europe (see Table 1 for summary), we did not record any adult rufous males. In Germany, adult rufous Cuckoo males should represent about 1% of rufous Cuckoos (Busche 2003) but the mechanisms underlying the origin of this male 'morph' remain unknown and may differ from those in females. We may only speculate that such males could perhaps suffer from moult disorder or eumelanin production dysfunction. Moreover, previous evidence comes only from visual observations where the sex was not genetically determined. Therefore, it is possible that even some (rufous) females may rarely exhibit male calls and thus may resemble males (Odom et al. 2014). Evidence will probably remain sporadic, because the most reliable approach – ringing projects targeting the Cuckoo – are scarce and commonly used bird monitoring schemes do not take the Cuckoo morphs into account (e.g. Gregory et al. 2007). Consequently, most observers presumably do not pay much attention to the colour and sex of individual Cuckoos.

In birds, plumage colour morphs are known to be genetically determined (Galeotti et al. 2003) and mostly follow Mendelian segregation of several alleles at a limited number of loci (Sinervo and Zamudio 2001; Roulin 2004; McKinnon and Pierotti 2010; Wellenreuther et al. 2014). Plumage polymorphism may also be governed by few genes of major effect (e.g. a supergene sensu Küpper et al. 2016). Cuckoo colour morphs have been known for a long time (Voipio 1953), but the underlying genetic mechanisms remain unknown. A high proportion of the juvenile Cuckoos within the intermediate plumage colour class (possibly heterozygous) as well as a lower proportion of grey and namely rufous Cuckoos (possibly homozygous) may suggest that Cuckoo plumage colouration follows Mendelian inheritance as well (see also Voipio 1953). However, the differences in colouration between most juvenile males and females are weak. Thus we cannot rule out the possibility that interactions among loci or between genes (e.g. non-allelic genes, duplicate genes with cumulative effects with dominance or epistatic genes – recessive or dominant epistasis) or even more complex mechanisms contribute the variation in plumage colouration (Sinervo and Svensson 2002; Carlborg and Haley 2004; Phillips 2008; Rankin et al. 2016). Rufous colouration of adult females and juveniles may further indicate that sex-specific endocrine cascades during development or frequency-dependent selection instead of the complex genetic interactions could determine the sexual differences (Rankin et al. 2016).

We have only anecdotal evidence about the plumage colouration in different stages of Cuckoo life – a rufous female chick found at the study site in 2016 (plumage colour score = 5.0) was retrapped there in spring as a rufous adult female in 2017 and also stayed rufous in 2018, when retrapped there again. Similarly, a male recaptured at the study site in 2018 was a grey chick (score = 1.9) ringed there in 2017 (Electronic Supplementary Material, Fig. S2). A possible link between the juvenile and adult Cuckoo colouration was introduced by Trnka et al. (2015) who suggested that the occurrence of the rufous female morph in adults may be caused by neoteny (paedomorphosis), i.e. by the retention of juvenile colouration in the adulthood (Gould 1977; McKinney and McNamara 1991). Such heterochronic plumage ontogeny, in which adult individuals retain juvenile like plumage, has been observed in other bird species (Foster 1987; Berggren et al. 2004). However, individual development of Cuckoo plumage remains unknown.

A potential explanation for the evolution of rufous plumage colouration in juvenile Cuckoos concerns the relation between camouflage and the risk of predation (see Wauters et al. 2004). This theory is supported by evidence that, individuals of bird species with a greater proportion of rufous plumage colour have higher relative annual survival rates (Galván et al. 2012; Galván and Møller 2013) except under adverse conditions (Karell et al. 2011). Unfortunately, we do not have enough data to test these assumptions and they remain a perspective for future research.

The Cuckoo is well-suited for the study of plumage colouration. Our classification as well as the image analysis showed that juvenile colouration of both sexes was similar. Namely, not only juvenile females, but also juvenile males may exhibit rufous colouration, which, in contrast to adult females, was not observed in adult males. However, the process of colour change during ontogeny, as well as its underlying molecular mechanisms, remains unknown. We thus encourage investigating Cuckoo plumage colouration across the species range in order to compare the patterns and processes of phenotype and genotype variation among populations. Future studies should also test for correlations with behavioural, physiological and fitness traits and explore changes in plumage colouration in particular regions over time. Such research may elucidate whether juvenile and adult colouration differs in response to factors that determine the changes in population size (Roulin 2004), and would provide valuable insights into the evolutionary history of colour dimorphism in avian brood parasites, and in animals in general.

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Compliance with ethical standards

All applicable international, national, and institutional guidelines for the care and use of animals were followed (Czech permit MUHOCJ 34437/2014 OŽP). The authors declare that they have no competing interests.

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Table 1 Proportions of adult rufous Common Cuckoo females based on published evidence. Asterisk denotes samples considering only females

Site	Coordinates	N	Rufous females (%)	Source
Germany				
East Prussia			relatively scarce	Tischler 1941
Göttingen	51°33'N 9°56'E		<30*	Arbeitskreis Göttinger Ornithologen 2007
Mecklenburg			<1	Heidecke 1981
Mindelsee	47°45'N 9°01'E		16.7	Löhrl 1979
Northern Bavaria		664	12 (4.7–23.5)	Dittrich 1982
Saxony			nowhere frequently	Heyder 1952
Sleswick-Holsatia			5–10	Berndt et al. 2003
Upper Lusatia		>300	<5	Becker and Dankhoff 1973
Entire country			<1–24	Busche 2003
United Kingdom				
Wicken Fen	52°18’N, 0°17’E		<1*	Thorogood and Davies 2012
Entire British Isles			scarce	Whitherby et al. 1949
Other countries				

Finland		8*	50	Voipio 1953
Italy			1.6–1.8	Brichetti and Fracasso 2006
Norway			rarer than grey	Schaanning 1916
Sweden			rarer than grey	Lönnberg 1929
Switzerland			scarce	Maumary et al. 2007
Other sites				
Apaj (Hungary)	47°09'N 19°05'E	30*	60	Honza et al. 2006
Fürstenfeld (Austria)	47°03'N 16°05'E	27	11.1	Sackl 1985
Lužice (Czech Rep.)	48°50'N 17°04'E	30*	20	Honza et al. 2006, this study
North-east Slovenia		103	6.7	Bračko 2017
Po Plain (Italy)		115	1.77	Quadrelli 1990
Štúrovo (Slovakia)	47°51´N 18°36´E	8*	37.5	Trnka and Grim 2013

Figure legends

Fig. 1 Proportion of (a) mean plumage colour scores in 41 male and 36 female Cuckoo chicks (1 – grey, 2 – medium grey, 3 – intermediate, 4 – medium rufous, 5 – rufous) and (b) proportion of rufous colour at individual feathers from the upper back of 49 male and 38 female Cuckoo chicks

Fig. 2 Mean reflectance spectra of grey and rufous colour on back feathers of (a) 8 Common Cuckoo chicks (2 females, 6 males) and (b) 2 adult rufous females. Bars denote standard errors of the mean

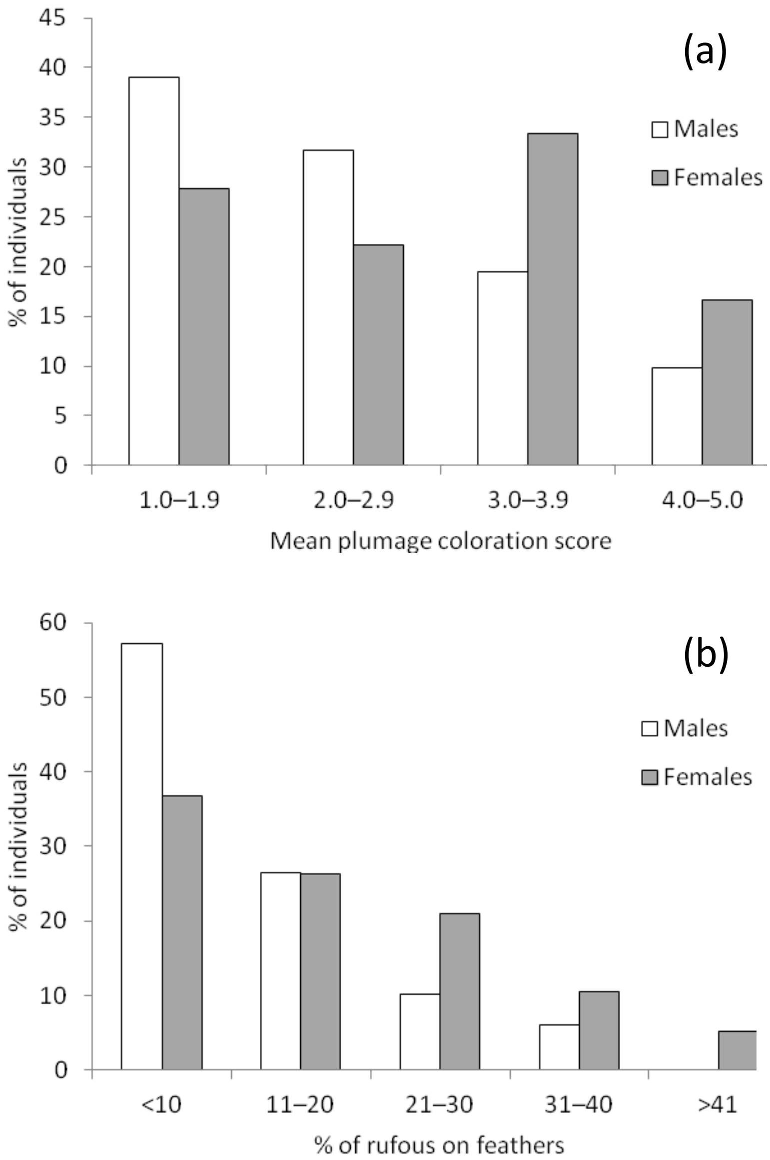
(a)
Males
Females
% of individuals
45
40
35
30
25
20
15
10
5
0
1.0–1.9
2.0–2.9
3.0–3.9
4.0–5.0
Mean plumage coloration score
(b)
Males
Females
% of individuals
60
50
40
30
20
10
0
<10
11–20
21–30
31–40
>41
% of rufous on feathers

Fig. 1

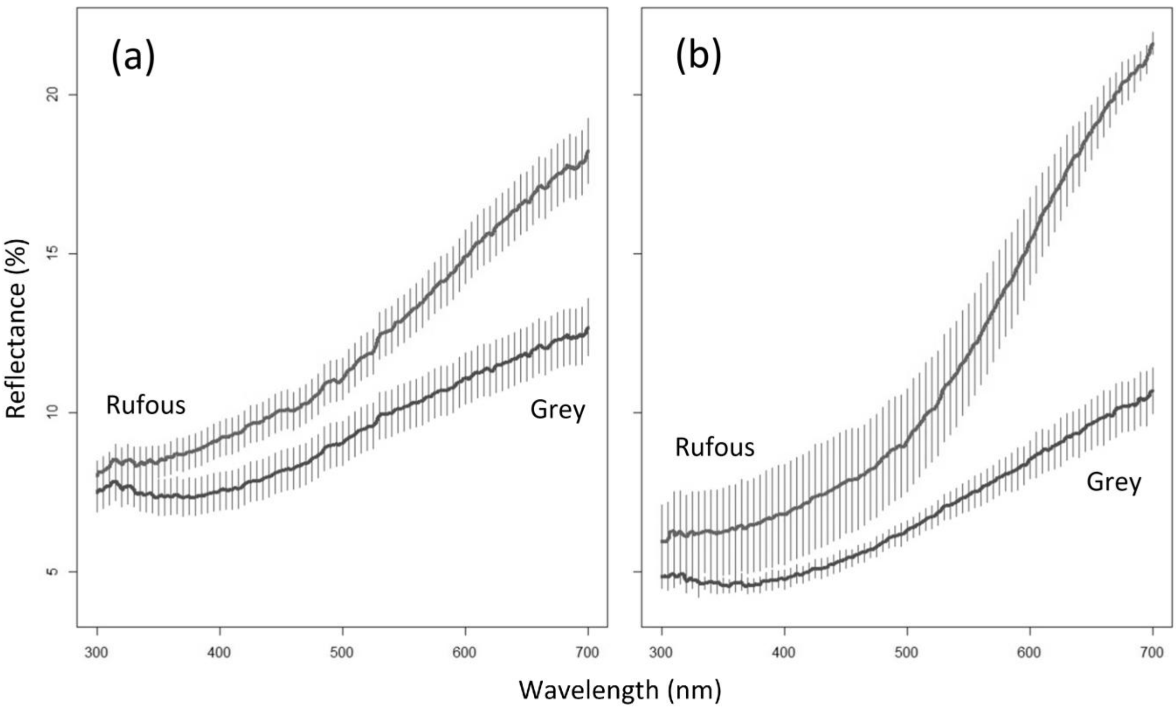
(a)
(b)
Reflectance (%)
Rufous
Grey
Rufous
Grey
Wavelength (nm)
5
10
15
20
300
400
500
600
700
300
400
500
600
700

Fig. 2