# Sexual Dichromatism in the European Magpie Pica pica. Not as Black and White as Expected

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# Sexual dichromatism in the European Magpie *Pica pica*. Not as black and white as expected

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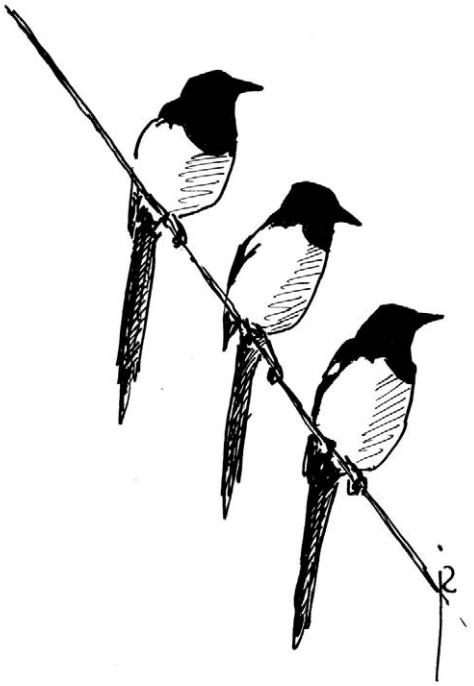

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Reflectance spectrometry, in the avian visible range, is currently a standard methodological approach for plumage colour assessment in birds. However, the method generally employed, whereby only one observation and illumination angle is used, can fail to reveal important colour characteristics. This study shows how the sexual dichromatism of the European Magpie *Pica pica* can be overlooked by the use of single angle spectrometry, rather than multiple angle spectrometry, and stresses the need to adopt more refined methods for plumage colour assessment. The results of this study may not only have implications in avian colour classification but also in many related fields of biology.

Key words: sex differences, dimorphism, spectrometry, plumage, bird, colour

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## INTRODUCTION

Perception of colour results from the combination of the capabilities of the visual system of the observer and the reflectance spectra from the observed object (Endler 1990). Colour perception is different among animals with different visual systems. Like humans, birds have three single cone types sensitive in the 'human visible range': long-wave sensitive (LWS) cones, with a maximal sensitivity (λmax) around 560–570 nm, medium-wave sensitive (MWS) cones with λmax around 500–510 nm, and short-wave sensitive (SWS) cones with λmax around 430–450 nm. In addition, most birds, with the exception of some nocturnal species (Bowmaker & Martin 1978), have a violet sensitive (VS, λmax around 400–420 nm) or an ultraviolet sensitive (UVS, λmax around 360–380 nm) single cone (Kreithen & Eisner 1978, Goldsmith 1990, Jacobs 1992, Maier & Bowmaker 1993, Bowmaker *et al.* 1997, Hart *et al.* 1998, Hart *et al.* 1999). These four classes of single cones are the basis for a potentially tetrachromatic visual system in birds (Goldsmith 1994, Maier &

Bowmaker 1993) and allows them to see in the ultraviolet (UV) range, in addition to the 'human visible range'.

The light reflected from an object's surface can be approximated as a linear combination of two reflection components: diffuse and specular reflections. In a perfect diffuser (Lambertian surface – see Lambert 1760) the incoming light ray penetrates the surface and is reflected in every direction by the same amount. Studies have shown, however, that the diffuse reflection becomes angle dependent when the surface has high macroscopic roughness (Oren & Nayar 1995). Moreover, to date no perfect matte surfaces (Lambertian) have been found in nature (Matusik *et al.* 2003, Oren & Nayar 1995). In the specular reflection the light is directly reflected at an interface between the air and the surface and has a spike in the perfect mirror direction with relatively weak intensity spread (a lobe) around the perfect mirror direction. Plumage surfaces are far from uniformly smooth and perfect matte surfaces and therefore angular dependence (change in reflectance spectra with change in illumination and observation angles) is expected (Lambert 1760, Oren & Nayar 1995) in any feather, independently of its colour origin. Colour assessment is therefore a much more complicated task than it may seem and factors such as dimensions and geometry of the surface corrugations, local reflectance properties, specular highlights (Klinker *et al.* 1987, Koenderink *et al.* 1999), not to mention the more complex dynamical properties (e.g. during display movements), need to be taken into consideration.

In the last decade, numerous plumage reflectance spectrometry studies considering the avian visual system have been published. As one of the many implications of this advance, some species previously classified as monomorphic have been proven to be dimorphic when the UV part of the spectrum was considered (Andersson *et al.* 1998, Hunt *et al.* 1998, Cuthill *et al.* 1999, Mahler & Kempenaers 2002, Perrier *et al.* 2002, Mays *et al.* 2004). Spectrometry is considered an accurate method to assess plumage colouration and has the advantage of being independent from the colour perception system of the observer (Endler 1990, Cuthill *et al.* 1999). Most plumage reflectance spectrometry studies exploit only one angle geometry (illumination/observation) and, with the exception of iridescent coloured feathers (Finger & Burkhardt 1994, Cuthill *et al.* 1999), the need for the use of more angle geometries to characterise plumage colour has rarely been stressed. Osorio & Ham (2002) made an extensive study in spectral reflectance and directional properties of both iridescent plumage and other structurally coloured feathers in several avian species. In their study, the use of different assessment angles influenced the feather's spectral characteristics. The effect of the feathers' structural components, especially the effect of layer thickness on oblique rays, and the effect of surface unevenness that works out differently on differently oriented rays, can be expected in every coloured feather type. Furthermore, the birds' body shape (elongated approximated to spherical shape) itself influences the specular curve (Lu *et al.* 2000). Hence, when a bird looks at another bird it is not looking at a flat, uniformly coloured surface but it samples colour in several different angle geometries simultaneously (Fig. 1). These arguments alone could be enough motivation for the use of more than one angle for the assessment of plumage colour. Santos *et al.* (2007) demonstrated the significance of the effect of different angle geometries in the spectrometric assessment of plumage colouration. They demonstrated the insufficiency of the use of single angle spectrometry in the plumage colour assessment in non-iridescent plumage.

Ideally, colour quantification of any anisotropic surface (of unequal physical properties along different axes) should be done by measuring the bidirectional reflectance distribution function (BRDF) (Nicodemus *et al.* 1977). This mathematical function indicates the fraction of the radiant power arriving from each incoming direction that is reflected in each outgoing directions. Only when previous knowledge about the surface's BRDF exists, can few parameters, which characterize a class of BRDF's, be elected to be used for colour assessment.

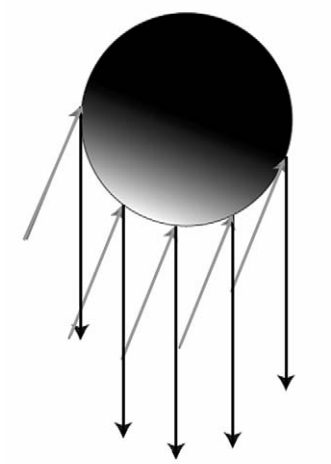

**Figure 1.** Simplified illustration of a bird's shape illuminated and observed from several directions. In natural situations, when a bird looks at another bird's plumage it simultaneously assesses a variety of angles of illumination and observation due to the shape of its body surface. The bird's body surface can be considered as an approximately convex spherical shape. When the bird is illuminated from a particular direction, many local angles of incidence are established (light grey arrows) and, at the same time, as many local angles of reflectance (dark grey arrows). In this particular case the shading representation was assumed to be perfectly diffuse or Lambertian (for which the Lightness is independent from the viewing angle or, in other words, the BRDF is a constant). It is, therefore, in natural conditions, an 'impossible scenario'.

Several species, in first instance considered as monomorphic, have later been classified as sexual dimorphic when the UV part of the spectrum was considered (Andersson *et al.* 1998, Cuthill *et al.* 1999, Perrier *et al.* 2002, Mennill *et al.* 2003). For example, the Long-tailed Finch *Poephila acuticauda*, a species initially classified as monochromatic using single angle spectrometry by Langmore and Bennett (1999), has later been classified by Santos (2005) as dichromatic by multiple angle spectrometry. Multiple angle spectrometry is an attempt to measure a subset of data, in a single plane of incidence, of the ideal surface characterisation BRDF.

In this study, we challenged the sexually monomorphic status of the European Magpie *Pica pica* (Birkhead 1991). We investigated differences between colour parameters calculated from reflectance spectra using different angle geometries in both iridescent and in non-iridescent body regions. Based on these results we tested for sexual dichromatism for each angle geometry and body region separately. Further, we defined several models based on multiple angle geometries that can be used to find 'concealed' sexual dichromatism and to predict the sex of a particular bird. Moreover, we tested one of the designed models in a new set of birds and predicted their sex with great accuracy.

## METHODS

### Animals

In total, thirty-one European Magpies where captured, using a Larsen Magpie trap with two trapping cages, under a Dutch LASER licence (FF/75A/2003/093). Twenty-one of these birds were yearlings and ten were adults. The birds were captured just before the breeding period, from November 2003 through January 2004, near Utrecht in The Netherlands. The distinction between yearlings and adult magpies was based on the patterns of the wing primary feathers, colour of the iris and nictitating membrane and colour of the mouth cavity. Young magpies go through a partial moulting process a few weeks after fledging. Their primary wing feathers only moult in the next summer of life and their colour patterns are different from those of adults. Adult wing primaries have a relatively small black tip and the first primary feather is sickle shaped, whereas yearlings have a relatively large black tip and the first primary is less curved. The adult's iris is dark brown, the nictitating membrane is greyish with an orange spot and their mouth cavity is darkly coloured. First year birds' irises are greyish and their nictitating membranes are blackish (Linsdale 1937).

### Plumage colour assessment

A selection of ten body regions (crown, back, bib, scapula, wing – secondary remiges, upper-breast, mid-breast, lower-breast, abdomen and tail –

rectrices) was made, based on their importance during sexual display (Birkhead 1991). These regions are, to the human observer, considered as black (crown, back, bib, upper-breast, mid-breast), white (scapula, lower-breast, and abdomen) and iridescent (wing – secondary remiges, tail – rectrices). From each body region ten spectra were collected, over an area of approximately 2 mm diameter, whereby the probe was replaced on the same spot for every recording at the plumage surface without pressing. Measurements were made, on a single plane of incidence, using an AVS-USB2000 spectrometer (Avantes B.V., The Netherlands) and a DH-2000 Deuterium/Halogen light source (Avantes B.V., The Netherlands). On each body region, two illumination angles were used, 45° and 90°, and four different observation angles, 30°, 45°, 90°, and 135°, all relative to the plumage surface. The illumination/observation angles geometries 45°/45° and 90°/90° were made with a bifurcated fibre optic probe (FRC-7 UV400-2, Avantes B.V., The Netherlands) with a black plastic sheath fixed to the fibre end. This allowed the same distance and position to be kept in all the measured body regions and prevented any outside light influence. All other angle geometries were achieved with a special fibre holder (AFH-15 angled fibre holder, Avantes B.V., The Netherlands) held on the same orientation in relation to the main bird plane (Fig. 2). Reflectance spectra were measured, between 320–700 nm, as a percentage of reflected light in relation to a white reference (98% reflective polytetrafluorethylene WS-2, Avantes B.V., The Netherlands) and a dark standard (light source off and probe placed against black velvet). White and dark references were taken before every new angle geometry and session of measurements. All the measurements were made randomly to the order in which body regions and geometries were measured and blindly to the birds' sex.

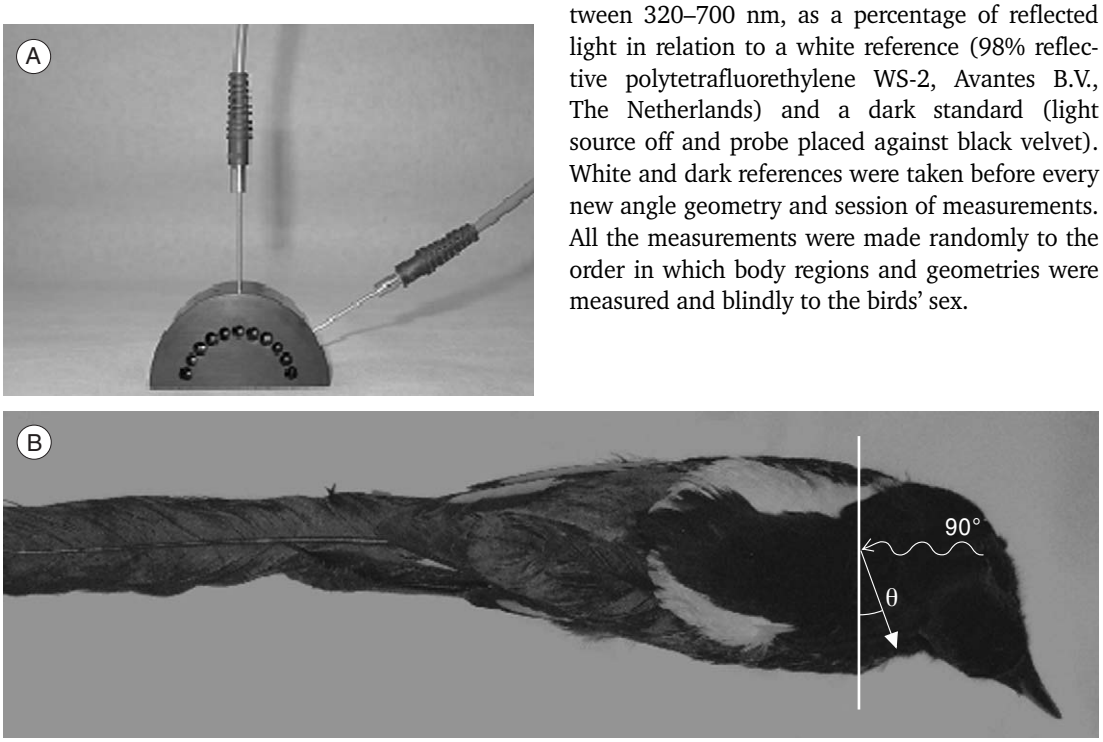


**Figure 2.** Multiple angle spectrometric measuring technique. Angle fibre holder (A) and positioning of the holder on the birds' plumage (B). The angle fibre holder is a mechanical device with 15° angle steps that holds the illumination and the observation fibre optical probes in position at a fixed distance (of 2 mm) from the measuring surface preventing any external reflection/illumination. The orientation of the angle holder was the same in every measurement and the holder was positioned on the plumage surface without pressing. The orientation of the holder was transversally to the birds' main plane (B). The undulating line represents the incident illumination beam (in this case at 90°, relative to the plumage surface) and the arrow represents an example of observation positions ($\theta = 30°$).

**Sex determination**

After all the measurements, the sex of each bird was established by DNA analysis (PCR amplification of the CHD-Z and CHD-W genes) following the procedure outlined in Kahn *et al.* (1998). Approximately 10 $\mu$l of blood was collected from the *cutanea ulnaris* vein with a capillary tube into a tube with 1 ml Cell Lysis Solution, Gentra Systems, Inc. DNA was isolated using a commercial kit from Gentra Systems, Inc. Approximately 32 ng of DNA was used in PCR reactions containing the universal primers described by Kahn *et al.* (1998): 1237L/1272H. PCR conditions were 94° for 2 min; followed by 30 cycles of 94° for 30 sec, 50° for 1 min, and 72° for 2 min; and a final extension step for 10 min at 72°. Amplification products were analysed by electrophoresis on a 2% agarose gel and marker VI (Roche, Almere, The Netherlands) was used as the molecular weight standard. Identification of one (male) or two bands (female) in the electrophoresis gel indicated the birds' sex. Whenever there were doubts in the scoring of gel bands a new test was performed and in every case the second analysis confirmed the initially predicted sex.

**Data analysis**

Each reflectance spectrum was composed of 1206 data points with 0.315 nm intervals. The original data were compressed, reducing the spectral resolution by a factor of ten (mean of ten reflectance values) to 102 data points, to facilitate calculations. Reflectance data were summarised in several parameters, both in the UV (320–400 nm) and in the total spectrum (320–700 nm). Lightness (total and UV) was defined as the light reflected by plumage surface and was calculated as the sum of percent reflectance values from the considered range (R). Colour intensity (total and UV) was defined as the maximum reflectance reached in the considered range ($R_{max}$). Hue (total and UV) was defined as the wavelength at peak reflection in the considered range ($\lambda R_{max}$). Contrast or colour amplitude (total and UV) was defined as the difference between the maximum and the minimum reflectance in the considered range ($R_{max}$–$R_{min}$) and UV Chroma was defined as the reflectance sum over the UV range divided by the total reflectance ($R_{320–400}/R_{320–700}$) (e.g. Endler 1990, Andersson 1999, Keyser & Hill 1999, Doucet 2002, Montgomerie 2006).

**Statistical analysis**

Medians of the nine colour parameters (for each bird and each body region) were used in a repeated measures multivariate analysis to test for angle geometry differences. The Wilks' Lambda test was elected for significance testing. To test whether males and females can be distinguished based on plumage colouration, separate logistic regression models were constructed for each body region and each angle geometry. The significance of the models was tested with the Likelihood ratio test. To create a more accurate and practical model to predict the sex of this species based on plumage colouration, multiple logistic regression models were established by combining parameters from different angle geometries and body regions.

Results were evaluated for *alpha* of 0.05. A potential concern is that the large number of tests performed (10 body regions, 8 angle geometries and 9 colour variables) increases the probability of a Type I error (false-positive results). In order to reduce the probability of this error a correction for multiple testing can be applied (Feise 2002). However, the use of corrections factors such as the Dunn Šidák correction has the clear disadvantage of increasing the probability of a Type II error (i.e. not identifying sexual dichromatism when in fact it is present) (Rothman 1990, Perneger 1998, Beynen *et al.* 2001). We compromised between the above-mentioned drawbacks by also considering an *alpha* of 0.001. Statistical analysis was conducted using SPSS 11.0 (SPSS Inc., Chicago USA).

Ultimately, a new group of ten adults was used to test one of the described models for sex determination. The sex of these birds was predicted based on the calculation of the contrast in the total spectrum of the abdomen measured at 45°/90°. The election of this particular model to be tested was based on the fact that, after applying an extremely stringent *alpha* (0.001) the abdomen seemed to be one of the most promising body regions to investigate sexual dichromatism.

## RESULTS

To the human eye, European Magpies have a black and white plumage. Some of the black plumage areas are highly glossy and iridescent. Reflectance spectra from the black-bluish plumage areas were characterised by a single peak in the visible part of the spectrum. Although there was a considerable UV reflection in all body regions measured, no spectral peak was found in the UV. In the white coloured areas, reflection spectra were elevated across all wavelengths reaching a maximum of 109%, followed by 105% for the abdomen and lower-breast, respectively (i.e. 'whiter' than the white standard). The scapula showed the highest reflectance, both in the UV and in the visible part of the spectrum.

In most body regions, the reflectance spectra of feathers changed significantly when the angle geometry was changed (Table 1). The UV Chroma was least affected by the change in angle geometry. Angular dependence was evident in all colour para-

**Table 1.** Repeated measures multivariate analysis testing for an effect of angle geometry in spectrometrically established colour parameters in the plumage of the European Magpie. L: Lightness/1000; CI: Colour intensity; H: Hue; C: Contrast; Ch: Chroma. UV [UV range]: 320–400 nm; total [Total range]: 320–700 nm. Significance was assessed using the Wilks' Lambda test. Lambda ranges between 0 and 1, with values close to 0 indicating the group means are different and values close to 1 indicating the group means are not different (equal to 1 indicates all means are the same).

| | Colour parameters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | L total | | L UV | | CI total | | CI UV | |
| Body region | Wilks' λ | *P*-value | Wilks' λ | *P*-value | Wilks' λ | *P*-value | Wilks' λ | *P*-value |
| Crown | 0.069 | 0.0000 | 0.065 | 0.0000 | 0.102 | 0.0000 | 0.092 | 0.0000 |
| Back | 0.015 | 0.0000 | 0.013 | 0.0000 | 0.022 | 0.0000 | 0.017 | 0.0000 |
| Bib | 0.108 | 0.0001 | 0.196 | 0.0002 | 0.130 | 0.0002 | 0.138 | 0.0002 |
| Scapula | 0.078 | 0.0000 | 0.118 | 0.0001 | 0.080 | 0.0000 | 0.086 | 0.0000 |
| Wing | 0.006 | 0.0000 | 0.016 | 0.0000 | 0.008 | 0.0000 | 0.017 | 0.0000 |
| Upper-breast | 0.035 | 0.0000 | 0.044 | 0.0000 | 0.037 | 0.0000 | 0.066 | 0.0000 |
| Mid-breast | 0.045 | 0.0000 | 0.029 | 0.0000 | 0.038 | 0.0000 | 0.033 | 0.0000 |
| Lower-breast | 0.054 | 0.0000 | 0.112 | 0.0001 | 0.048 | 0.0000 | 0.080 | 0.0000 |
| Abdomen | 0.090 | 0.0000 | 0.129 | 0.0002 | 0.080 | 0.0000 | 0.119 | 0.0001 |
| Tail | 0.026 | 0.0000 | 0.034 | 0.0000 | 0.032 | 0.0000 | 0.032 | 0.0000 |

| | Colour parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | H total | | H UV | | C total | | C UV | | UV Ch | |
| Body region | Wilks' λ | *P*-value | Wilks' λ | *P*-value | Wilks' λ | *P*-value | Wilks' λ | *P*-value | Wilks' λ | *P*-value |
| Crown | 0.113 | 0.0000 | 0.486 | 0.1738 | 0.057 | 0.0000 | 0.01S | 0.0000 | 0.307 | 0.0195 |
| Back | 0.079 | 0.0000 | 0.296 | 0.0161 | 0.045 | 0.0000 | 0.009 | 0.0000 | 0.166 | 0.0007 |
| Bib | 0.135 | 0.0002 | 0.257 | 0.0077 | 0.088 | 0.0000 | 0.030 | 0.0000 | 0.585 | 0.3659 |
| Scapula | 0.205 | 0.0023 | 0.546 | 0.2816 | 0.235 | 0.0048 | 0.151 | 0.0004 | 0.239 | 0.0052 |
| Wing | 0.031 | 0.0000 | 0.091 | 0.0000 | 0.013 | 0.0000 | 0.044 | 0.0000 | 0.056 | 0.0000 |
| Upper-breast | 0.208 | 0.0025 | 0.181 | 0.0011 | 0.006 | 0.0000 | 0.005 | 0.0000 | 0.111 | 0.0001 |
| Mid-breast | 0.094 | 0.0000 | 0.334 | 0.0298 | 0.041 | 0.0000 | 0.013 | 0.0000 | 0.165 | 0.0007 |
| Lower-breast | 0.177 | 0.0010 | 0.650 | 0.2488 | 0.148 | 0.0004 | 0.085 | 0.0000 | 0268 | 0.0097 |
| Abdomen | 0.192 | 0.0016 | 0.518 | 0.0721 | 0.151 | 0.0004 | 0.108 | 0.0001 | 0234 | 0.0047 |
| Tail | 0.326 | 0.0265 | 0.197 | 0.0081 | 0.046 | 0.0000 | 0.053 | 0.0000 | 0.168 | 0.0008 |

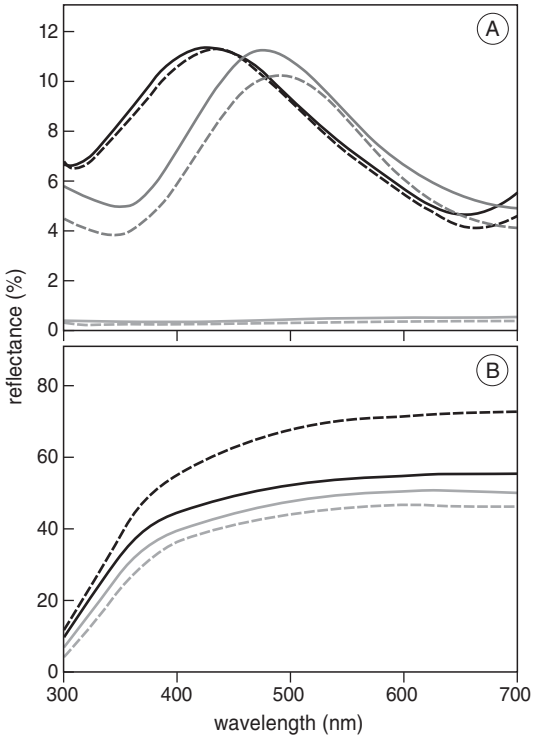


**Figure 3.** Example of spectrometric results using different angle geometries in iridescent (A, wing) and non-iridescent (B, abdomen) body regions of European Magpies. Solid lines correspond to the females ($n$ = 10) and the dashed lines correspond to the males ($n$ = 11). Each line is the average of the medians of ten measurements. In A (wing) sexual dichromatism is seen with the 45°/30° (grey lines) but not with the 45°/45° (light grey lines – with lower reflection) or with 45°/135° (black lines) geometry. In B (abdomen) the 45°/90° (black lines) shows sexual dichromatism but not the 45°/45° (light grey lines).

meters for the iridescent coloured body regions (e.g. Fig. 3A). In the white coloured (scapula, lower-breast, and abdomen) angular dependence was most noticeable in brightness and in the colour intensity (e.g. Fig. 3B). For all body regions, 45°/30° showed the highest reflection, with exception of the wing and the tail, which showed the highest reflection at 90°/90° and 90°/135°, respectively. The 45°/45° angle geometry showed the least reflection in both the UV and total spectrum.

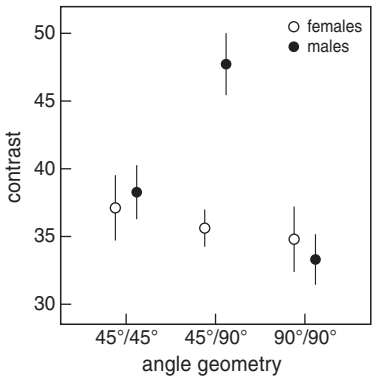


**Figure 4.** Contrast (or colour amplitude) of the white abdomen of the European Magpie assessed with three different reflectance angle geometries for females ($n$ = 10) and males ($n$ = 11). Contrast was calculated as the difference between the maximum and the minimum reflectance in the total spectrum (320–700 nm). The whiskers represent the standard error of the mean.

In most angle geometries males showed higher values for lightness in the total spectrum than females (data not shown). However, in some angle geometries and body regions the situation was reversed (e.g. at 45°/30° in the lower, mid, and upper breast, tail, scapula and wing; at 90°/90° in the mid breast and tail). Females showed higher UV chroma than males in most angle geometries and in most body regions (scapula is an exception). However, in the 45°/135°, 45°/90° and 90°/90° this feature was reversed with the males showing higher UV chroma than the females.

Sexual dichromatism in plumage was exposed in most body regions (except the scapula) and in most angle geometries (except 90°/45°) by reflectance spectrometry in group of 21 yearling European Magpies. Nevertheless, the degree of sexual dichromatism varied between the different angle geometries (Table 2).

This species could be classified as either sexually mono- or dichromatic, depending on which angle geometry and body region was used in the plumage spectrometry assessment (Fig. 3). For example, the abdomen would be classified differently using the three most commonly used angle geometries (45°/45°, 45°/90° and 90°/90°) (Fig. 4).

**Table 2.** Results of logistic regression analyses testing for sexual dichromatism in the plumage of the European Magpie, using different angle geometries. L: Lightness (R) divided by 1000; CI: Colour intensity ($R_{max}$); H: Hue ($\lambda R_{max}$); C: Contrast ($R_{max}$–$R_{min}$). UV [UV range]: 320–400 nm; total [Total range]: 320–700 nm. OR (odds ratio) is the value by which the odds of the event change when the independent variable increases in one scale unit. *P*-value represents the significance of the change in –2 log likelihood for the included variable. In the cases marked with an * there is more than one factor used for the model of sex differentiation. Note that, unlike the coefficients in a linear regression model, logistic regression results should not be interpreted as the rate of change in the expected value of the dependent variable, but as the change in the probability of one Y=1 (being a male) for any particular X.

| Angle geometry | Body region | Model variables | Mean ± SD | | OR | *P*-value | Overall % correct sex |
|---|---|---|---|---|---|---|---|
| | | | Females ($n$ = 10) | Males ($n$ = 11) | | | |
| 45°/30° | Back | L tot | 1.39±0.231 | 1.72±0.38 | 33.21 | 0.02151 | 61.9 |
| | Wing | CI uv | 7.28±1.28 | 5.82±1.07 | 0.29 | 0.00609 | 81 |
| 45°/45° | Back | L uv | 89.97±20.54 | 70.36±16.60 | 0.00 | 0.02078 | 60 |
| 45°/90° | Abdomen | C tot | 35.70±4.24 | 47.78±7.66 | 1.61 | 0.00007 | 85.7 |
| | Up-breast | CI uv | 1.87±0.40 | 2.23±0.38 | 12.94 | 0.03339 | 61.9 |
| 45°/135° | Lo-breast | C uv | 19.78±2.94 | 23.65±2.85 | 1.63 | 0.00428 | 76.2 |
| | Tail | *uv Ch | 0.15±0.02 | 0.14±0.02 | 0.00 | 0.00588 | 90.5 |
| | | *C uv | 1.50±0.30 | 2.36±0.62 | 767.21 | 0.00000 | |
| 90°/30° | Back | *uv Ch | 0.21±0.06 | 0.14±0.09 | 0.00 | 0.02024 | 71.4 |
| | | *C tot | 1.00±0.19 | 1.33±0.41 | 577.29 | 0.01242 | |
| | Abdomen | C tot | 30.86±7.49 | 37.93±6.54 | 1.16 | 0.02535 | 66.7 |
| | Lo-breast | C tot | 32.65±5.37 | 39.24±7.88 | 1.18 | 0.02652 | 71.4 |
| | Crown | H tot | 343.51±10.18 | 424.97±113.85 | 1.03 | 0.01148 | 71.4 |
| | Tail | Uv Ch | 0.15±0.03 | 0.12±0.05 | 0.00 | 0.02394 | 66.7 |
| 90°/90° | Back | L uv | 0.18±0.01 | 0.16±0.09 | 0.00 | 0.00044 | 90.5 |
| 90°/135° | Back | uv Ch | 0.19±0.03 | 0.16±0.03 | 0.00 | 0.00360 | 71.4 |
| | Bib | uv Ch | 0.20±0.01 | 0.18±0.02 | 0.00 | 0.02299 | 66.7 |
| | Up-breast | uv Ch | 0.22±0.02 | 0.19±0.03 | 0.00 | 0.00127 | 71.4 |
| | Mid-breast | Uv Ch | 0.22±0.02 | 0.19±0.03 | 0.00 | 0.01364 | 66.7 |
| | Crown | Uv Ch | 0.20±0.01 | 0.17±0.02 | 0.00 | 0.00141 | 71.4 |

At an *alpha* = 0.05, the tail and the back revealed the most predictable differences between males and females. In the tail, logistic regression analysis could separate males from females with 90.5% certainty based on the calculation of two parameters: contrast in the UV and UV chroma, using the 45°/135° geometry (Table 2). Many differences between male and females proved to be in the UV part of the spectrum, and were therefore hidden to the human eye. After applying a more stringent *alpha* of 0.001 the major findings of differences between angle geometry assessments persisted. Moreover, sexual dichromatism in the abdomen at 45°/90° (contrast total) and in the back at 90°/90° (UV lightness) also remained significant (Table 2).

Logistic regression models combining different parameters, body regions and angle geometries predicted sex up to 100% accuracy in this group of animals (Table 3 illustrates some of the possible combinations).

One of the multiple logistic regression models that was significant at P smaller than 0.001, was applied to a new group of ten new adult birds. The

**Table 3.** Examples from combined multiple logistic regression models for sex prediction of the European Magpie per body region (10 females +11 males). L: Lightness (R) divided by 1000; CI: Colour intensity ($R_{max}$); C: Contrast ($R_{max}$–$R_{min}$); UV Ch – UV chroma ($R_{320–400}/R_{320–700}$). UV [UV range]: 320–400 nm; total [Total range]: 320–700 nm. OR is the value by which the odds of the event change when the independent variable increases in one scale unit. The high value of some OR indicates possible collinearity between the variables of the model. *P*-value represents the significance of the change in –2 log likelihood for the included variable.

| Body region | Angle geometries (variables) | OR | *P*-value | Overall % correct sex predictions |
|---|---|---|---|---|
| Back | 45°/45° (L uv) | 2.46 | 0.0487 | 100 |
| | 90°/90° (L uv) | 0.07 | 0.0001 | |
| | 45°/45° (L uv) | 49.29 | 0.0001 | 100 |
| | 90°/135° (uv Ch) | 0.00 | 0.0001 | |
| Bib | 90°/135° (uv Ch) | 0.00 | 0.0015 | 85 |
| | 45°/90° (C uv) | 1.50E+04 | 0.0053 | |
| Upper-breast | 45°/90° (CI uv) | 15.82 | 0.0459 | 81 |
| | 90°/135° (uv Ch) | 0.00 | 0.0017 | |
| Mid-breast | 45°/90° (uv Ch) | 3.63E+19 | 0.0119 | 85 |
| | 90°/135° (uv Ch) | 0.00 | 0.0009 | |
| Crown | 90°/90° (C uv) | 9.39E+24 2. | 0.0015 | 90 |
| | 45°/90° (C total) | 59E+11 | 0.0001 | |

model included the abdomen with a 45°/90° angle geometry and the contrast in the total spectrum (from Table 2). With this model we were able to sex correctly eight out of nine birds. One of the birds could not be sexed using this model because the contrast of the abdomen fell outside the median ± SD intervals of either males or females from the bigger sample.

## DISCUSSION

This study demonstrates that the European Magpie has sexually dichromatic plumage characteristics that are invisible to the human eye. Furthermore, we show that the commonly used angle geometries 45°/45° and 90°/90° may fail to reveal these sex differences. Therefore, we argue that multiple angle spectrometry is superior to the most commonly used single angle assessments of plumage colour.

The plumage of the European Magpie is composed of iridescent and non-iridescent regions. The importance of multiple angle assessment in iridescent feathers has been reported previously (Finger & Burkhardt 1994, Cuthill *et al.* 1999), but the biological importance of feather structure in non-iridescent colours has received little attention. Nevertheless, Santos *et al.* (2007) showed a strong effect of angle geometry in spectrometric assessment of non-iridescent plumage and stressed the advantage of using multiple angle geometry for plumage colour assessment. Angular dependence (change in reflectance spectra with angle geometry) is a characteristic of any rough non-lambertian (perfect matte) surface (Lambert 1760, Oren & Nayar 1995) but in behavioural ecology studies plumage surface has been generally treated as a uniform smooth surface. Due to the unfeasibility of plumage surface characterisation by BRDF, we made use of multiple angle geometry in a single plane of incidence. The significant differences of

colour parameters between most angle geometries found in this study were to be expected since plumage surface is not a perfectly diffuse scatterer (Lambert 1760). Some colour parameters such as the hue, were less influenced by the change in angle geometry in non-iridescent coloured plumage regions. Nevertheless, colour differences are not limited to hue.

This study follows a series of exploratory studies performed by us in other avian species in which the influence of the angle geometry in the reflectance spectra, and therefore in plumage colour characterisation, was shown. Moreover, the plumage of some bird species previously classified by single angle spectrometry as being monochromatic, revealed sex differences when their plumage was assessed with other angle geometries (e.g. Santos 2005). Whether the birds in question are actually able to perceive these differences and use this variation to identify the sex of other individuals is a subject for future work. Nevertheless, such misclassifications may result in misinterpretations of results (such as a theory of strategic concealment of sexual identity proposed by Langmore and Bennett (1999) after classifying the Long-tailed Finch as sexually monochromatic). Our results show that the European Magpie may be subject to such misclassification using the commonly used angle geometries.

The fact that some body regions reveal more clearly the differences between males and females may be related to their role in courtship displays. In this study the back showed the highest potential for revealing sex differences. This is in accordance with its active role in sexual displays (Birkhead 1991). The tail and the wing were also expected to show sex differences but these could only be detected by a few angle geometries. The fact that yearlings do not have the definitive adult flight feathers may be an explanation for this relatively poor 'signal' in the wing body region.

Several of our models were able to predict the sex of this species with high accuracy (up to 100%) based on few body regions and angle geometries. The fact that the presented models were based on juveniles' plumage, where the sexual signals are expected to be weaker than in adults, underlines the sensitivity of this colour assessment method. At the same time, it raises awareness for the presence of sexual signals at this stage of life of this species. Our finding of sexual dichromatism in juvenile plumage is in accordance with the fact that these animals can breed in their first year of life (Birkhead 1991).

A potential concern in the evaluation of our results is that the large number of tests performed (10 body regions, 8 angle geometries and 9 colour variables) increases the probability of Type I error. With respect to the effect of angle geometry, nearly all results are highly significant, which dispels the concerns with multiple testing and Type I errors. Regarding sexual dichromatism, our general conclusion would remain valid at an *alpha* of 0.001 for the abdomen and back at three angle geometries (45°/90°, 45°/135° and 90°/90°). If the especially stringent correction were to be applied (for all body regions, angle geometries and colour variables tested using the Dunn Šidák correction), an *alpha* of 0.000071 would have to be used to maintain the experiment-wide error at 5%. Even then, we would be close to being able to separate males from females in the abdomen at 45°/90°. In order to overcome this possible shortcoming of our established sexing method we tested it with a new set of birds. The selection of these colour parameters was based on the most stringent *alpha*. The contrast in the total spectrum of the white abdomen at 45°/90° seemed to be a promising variable for confirmation of the results. We were able to predict sex in eight out of nine new birds. Although ideally the new dataset of birds to be used for the cross validation of our model should also have been juveniles (as our model was based on juvenile plumage), we wanted to assess if our findings could be extrapolated to adulthood where sex differences are expectedly bigger. The fact that we successfully classified the adult plumage boosts confidence in our model and mitigates the statistical drawback mentioned above.

Our discovery of cryptic sexual dichromatism in the European Magpie using multiple angle spectrometry highlights the importance of this tech-

nique in the study of avian signals (e.g. Endler & Théry 1996). It is important to note that our measurement of a subset of data of the BRDF was only performed in one plane of incidence and assessment. Viewing the bird from the side, or with illumination from any other angle relative to the axis of the bird, will only increase the variation observed, which strengthens our claim about the inadequacy of a single measurement geometry.

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## SAMENVATTING

Mannetjes en vrouwtjes van de Ekster *Pica pica* zien er door mensenogen hetzelfde uit. Aangezien vogels in tegenstelling tot mensen ook in het UV kunnen zien, is het mogelijk dat Eksters zelf wel verschil zien. In dit artikel wordt deze mogelijkheid onderzocht met behulp van spectrometrie. Bij deze techniek wordt een smalle bundel wit licht op een veer geschenen en het spectrum van het weerkaatste licht geanalyseerd. In tegenstelling tot de meeste spectrometriestudies in vogels gebruiken de auteurs meerdere hoeken waaronder het licht op de veer wordt geschenen en weer wordt opgevangen. De resultaten laten zien dat de mannetjes en vrouwtjes van de Ekster inderdaad van elkaar verschillen, maar dat de verschillen niet onder alle lichthoeken even opvallend zijn. Er wordt daarom gepleit voor het gebruik van meer verfijnde methodieken in spectrometriestudies. (KK)

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