LETTER

Digital imaging for colour measurement in ecological research

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Abstract

Traditional methods for colour quantification are complicated by the fact that colours change depending on illumination, and that different observers often perceive colours differently. Here we describe a new affordable method, which improves methods relying on human observers, to quantify patterns and colour variations. The procedure combines customized software with the use of digital cameras and commercial photofinishing software. The computer routines correct unavoidable illumination changes during image capturing, making all images comparable. Colours are quantified in a continuous scale of the conventional colour models developed for the human vision system, such as HSB, RGB, CMYK, or Lab, amenable for statistical analyses. We illustrate the use of this technique showing a previously unknown sexual dimorphism in the red-legged partridge, *Alectoris rufa*, undetectable with the unaided human eye. We also demonstrate that the digital system provides a finer discrimination than human observers for scoring the plumage of partridges belonging to two different subspecies. This method has potential applications in behavioural ecology, physiology, genetics, evolutionary biology, and taxonomy.

INTRODUCTION

Assessing colour patterns is critical for testing numerous ecological hypotheses (Endler 1990; Zuk & Decruyenaere 1994). In animals, colours may convey information about gender, age, readiness for breeding, or condition of the bearer (Grafen 1990; Evans & Norris 1996). In plants, colour signals may be used to indicate fruits ready for dispersal; some species also show floral colour changes to direct pollinators to rewarding and sexually viable flowers (Weiss 1991).

Colour signals may be given in the visible part of the spectrum, the ultraviolet, or both (Guildford & Harvey 1998; Hunt *et al.* 1998). Colours as perceived by humans are used in a large number of ecological studies, including those where individual traits such as condition are related to colour expression (Litvaitis 1991; Bortolotti *et al.* 1996) or species characteristics (i.e. taxonomic studies such as Johnson *et al.* 1998), as well as effects of environmental perturbations (Hill 1995). Such questions can only be addressed with objective and repeatable systems for colours assessment.

Determination of colours is difficult because colour perception is context dependent (Endler 1990). For instance, humans perceive colours differently depending on illumination. Techniques currently used for quantifying colour differences range from arbitrary rankings (e.g. dull and bright hues), matching to commercial colour standards, such as Munsell's (1976), and electronic devices including spectroradiometers (Endler 1990; Zuk & Decruyenaere 1994) and reflectance spectrophotometers, also known as colourimeters (Johnson *et al.* 1998). Although the subjectivity of ranking and matching procedures is well known (Endler 1990; Zuk & Decruyenaere 1994; Repentigny *et al.* 1997; Johnson *et al.* 1998), these methods are still widely used (Bortolotti *et al.* 1996; Repentigny *et al.* 1997) due to low cost and simplicity. Spectroradiometers and colourimeters can provide colour measures beyond the portion of the spectrum visible to humans, and are thus the method of choice when it is important to consider that other animals may have different colour sensitivities. The latter devices are expensive, however. Other limitations are that the study subjects must be illuminated with a constant and intense light source, and that the lens has to be applied directly to a flattened and size-fixed sample area. This operating mode complicates the scanning of fleshy parts, such as the iris and eye lores in live animals.

Here we describe an affordable method to quantify patterns and colour variations, which combines digital photography and computer software to control for differences in illumination. The use of digital colour cameras or software for quantifying colours is not new (e.g. Kilner 1997). Our main contribution is the combination of both digital images and methods of standardizing for different illuminations. Given that most commercial digital cameras mimic the human colour vision system, our proposed method is only valid for colours perceived by humans. To illustrate the potential applications of this system, we photographed red-legged partridges (*Alectoris rufa*). The system was used to study whether there was sexual dimorphism in redness of the eye lores of live adults. In addition, museum specimens were utilized to compare plumage scores derived from colour charts with the values provided by the digital system.

MATERIALS AND METHODS

Capturing images with a digital camera (DC)

We have used a DC with 1160×872 pixels of resolution and 256^3 colours. DCs scan images with a matrix of hundreds of thousands of microscopic photocells, creating pixels where colour is recorded as brightness values (from 0 to 255) of red, green, and blue (RGB).

Conditions during image capture still remain problematic when images are captured with the DC. To reduce environmental variability, we have scanned study subjects under artificial lights on a copy stand baseboard, with the DC placed on the copy stand column at the same height for each series of objects. We have observed, however, that the same object (e.g. a Munsell colour chip) scanned on the baseboard may still show some variation in its RGB values from picture to picture. To further standardize the images we included two control chips (standards) along with the object to be scanned.

The software

Digital images were transferred to the computer and opened with Adobe Photoshop. Portions of the image to be analysed (e.g. the eye lore in a partridge) were selected and copied to the clipboard with the "magic wand" option, while a section of each standard was retrieved separately with the "marquee" option (depending on the interest of the researcher, other select options may be used).

We have developed a program in Visual Basic (additional information and software can be found in www.ebd.csic.es/rv/index.html) that creates a new file (delimited with tabs and thus imported by most computer applications) where the R, G, and B values of each pixel

from the image in the clipboard are saved along with two identifiers, one for the photo ID and the other for an area code (e.g. standard number or body-part code).

The data gathered with the Visual Basic program have yet to be transformed using customized computer routines written in Fox-pro. In each picture we used the theoretical and observed values of the standards to calculate linear regressions for each primary colour (R, G, and B). In our case, observed R, G, and B values of the standards in the first picture of a series were used to adjust the remaining pictures. Each pixel in any of the images was then shifted according to the linear functions, and all images were therefore comparable. The assumption of linearity in the colour shifts was supported by the high similarity of the RGB values obtained for a third colour chip present in all pictures, and which served as a control. Further standardization was obtained by dividing each colour component by its luminosity, which is a function of the three primary colours. This way, indexes for amount of red (R/L), green (G/L), or blue (B/L) can be used to compare colours among individuals.

Accuracy of the procedure

Munsell colour chips are graded in roughly equal visual steps and therefore series of adjacent chips should be positioned at comparable distances by our digital procedure. We photographed four adjacent Munsell chips (5 YR:hue; 4/2, 4/4, 5/2, 5/4) five different times. As expected, values in the red coordinate were regularly spaced (Fig. 1), indicating a high discrimination of colours. The repeatability (Lessells & Boag 1987), a measure of the reliability of the multiple measurements, was very high ($R = 99.5\%$, $F_{3,16} = 1073.9$, $P < 0.0001$), indicating that the error of the system is negligible.
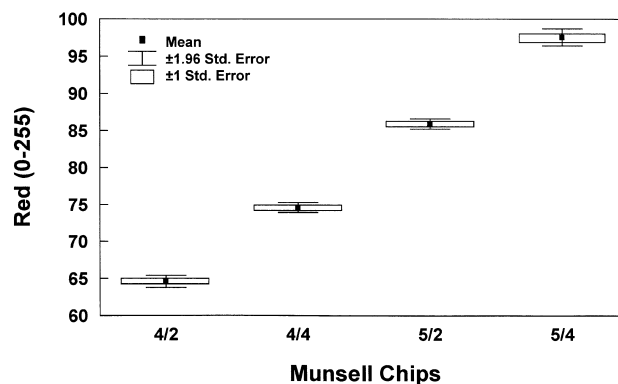

Figure 1 Mean (± 1 SE and ± 1.96 SE) red values obtained after digitalizing and transforming four adjacent Munsell Chips.

Two practical cases

We have applied the above method to study colour variation in the red-legged partridge (*Alectoris rufa*). One case relates to sexual dimorphism and the other to population differentiation.

Case 1. In the red-legged partridge, both sexes show red colouration in the exposed integuments due to carotenoid pigments. Sexes are considered to be monomorphic, but our previous research has shown age-and gender-specific patterns in plasma carotenoid concentration that vary seasonally. Adult males have significantly higher levels of plasma carotenoid than females in the mating season (i.e. February–March), and thus males might have redder eye lores at that time. On 28 February 1998, we photographed 64 adult individuals (32 breeding pairs) kept in separate breeding pens at a partridge farm in southern Spain. Images were taken with the help of an assistant who held each bird on a copy stand baseboard with attached lights (see full image in our web page).

Case 2. Two different partridge subspecies inhabit the Iberian Peninsula (Calderón 1983): *Alectoris rufa hispanica* occurs in north-western Spain and presents a generally "darker" plumage than *A. r. intercedens*, present in the rest of the country. In the zoological collections of the Biological Station of Doñana (Sevilla, Spain), we photographed the dorsal part of 12 stuffed specimens of *A. r. hispanica* collected in Galicia (northern Spain) and 13 stuffed specimens of *A. r. intercedens* collected in Andalucia (southern Spain). The same birds and plumage area were scored by five different human observers using 12 colours chips from the 5 YR hue sheet (values 5, 4, 3, and 2; chromas 1, 2, and 4). The observers were given one bird at a time and were blind to the fact that the birds belonged to two subspecies. To assess the reliability of the five measurements on the same individuals we calculated the repeatability separately for value and chroma.

RESULTS

Case 1. Sexual dimorphism. The frequency distributions of R/L values for male and female partridges overlapped to some extent (Fig. 2). Nonetheless, we found the male's lores were significantly redder (R/L, 1.73 versus 1.64; one-way ANOVA: $F_{1,58} = 10.06$, $P = 0.002$) than females' lores. Those differences are not perceptible to the human eye so we are the first in reporting this sexual dimorphism.

Case 2. Population differentiation. A total of eight of the 12 Munsell chips available for scoring were used by the five observers, although each of them used only 3–5 chips (Fig. 3). Only observer 1 correctly separated the two subspecies by assigning two consecutive "darker" chips
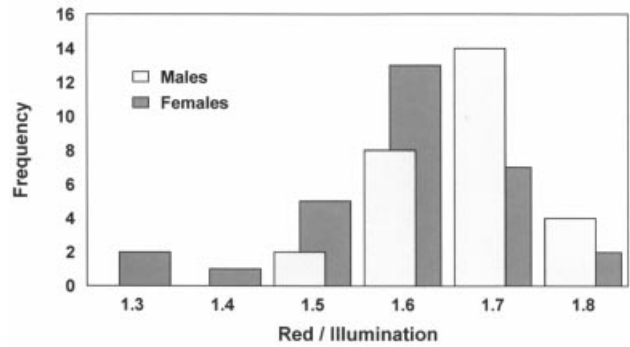

Figure 2 Frequency distribution of red/illumination values obtained from scanning with the DC the eye lore of alive male and female red-legged partridges.
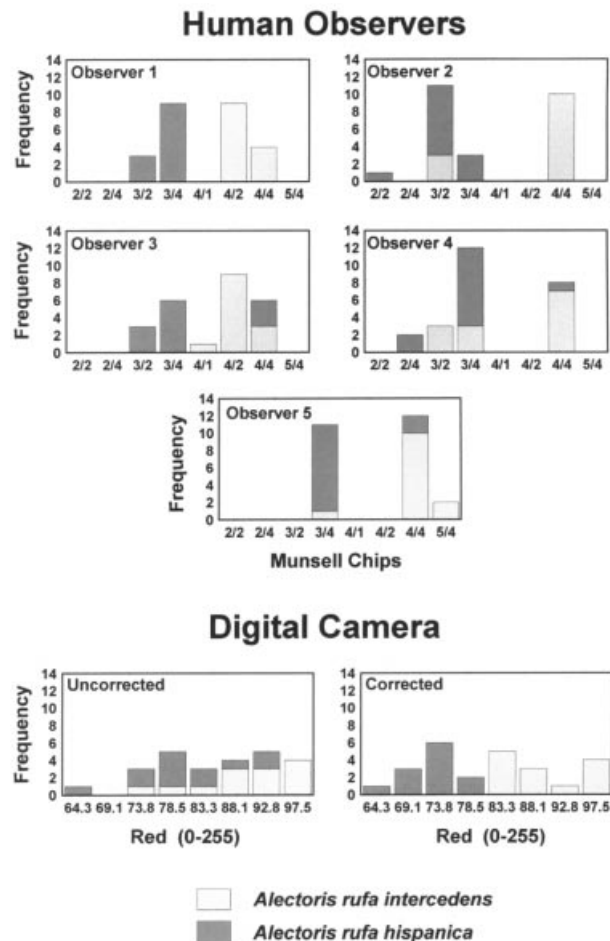

Figure 3 Categorized histograms for two subspecies of partridges, showing the results obtained by five different human observers matching stuffed birds with Munsell chips (5 YR, value/chroma), and by scaning the same areas and birds with a DC to obtain their raw (uncorrected) or standardized (corrected) red values from the RGB colour system.

(3/2 and 3/4) to *hispanica* individuals, and two consecutive "paler" chips (4/2 and 4/4) to *intercedens* individuals. The other four observers tended to classify the partridges in two groups, but the separation was not exclusive and it varied between observers. The repeatability of both "value" ($R = 53\%$, $F_{24,100} = 6.56$, $P < 0.0001$) and "chroma" ($R = 19\%$, $F_{24,100} = 2.14$, $P < 0.005$), although significantly different from zero, can only be considered moderate.

When the partridges were ranked by the original (uncorrected) R values provided by the DC, the two subspecies widely overlapped (Fig. 3, bottom left). Using the transformation with the standards, all values for individuals of the *hispanica* subspecies were lower than any value of the *intercedens* subspecies (Fig. 3, bottom right).

DISCUSSION

The method that we have used provides a fine distinction of colours and is highly repeatable. Although currently restricted to the human visible spectrum, our method constitutes an improvement of methods relying on human observers, which can differ in colour sensitivity or training. A further problem of printed colours charts is that their texture may not coincide with the one of the objects to be evaluated, complicating colour matching.

Contrary to other techniques, large objects can be scanned (e.g. a full partridge) and the digital images can be stored for further analyses of both patterns and colours. Colours are quantified in a continuous scale of the conventional colour models developed for humans (HSB, RGB, CMYK, or Lab), amenable for statistical analyses.

Even subtle differences can now be exposed and quantified with the digital camera and accompanying software. We are currently using this procedure to monitor individual colour changes in relation to season and immune status in bird populations. Here, we have shown just two simple examples using partridges. However, ecologists using other biological models, such as those studying pollinator-mediated selection in plants or carotenoid-dependant colouration in fish, may also find our method useful.

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BIOSKETCH

Rafael Villafuerte's main research interest is focused on the ecological factors affecting ranges, abundances, and physical conditions of small game species.