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SHORT COMMUNICATION

Relative colour cues improve colour constancy in birds

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ABSTRACT

A ripe strawberry looks red to our eyes in sunlight and in the green light of a forest, although the spectrum of light reflected from its surface differs dramatically. This is caused by two effects: colour constancy and our ability to learn relative colour cues – the ripe strawberry remains relatively 'redder' than an unripe green strawberry. While colour constancy – the ability to recognize colours in shifted illumination – has been studied in many animals, the use of relative colour cues is investigated more rarely. In a previous study on chickens, we measured how large a shift in illumination their colour constancy mechanisms tolerate without reliable relative colour cues. Here, we show that chickens remain colour constant over larger illumination shifts, if they can use such relative colour cues. As relative colour cues are readily available in natural environments, we suggest that their use contributes strongly to colour constancy performance in nature.

KEY WORDS: Colour vision, Relational colour constancy, Relative colour learning, Bird vision, Novel colour

INTRODUCTION

Birds use colour vision for many biologically relevant behaviours, such as finding food and evaluating potential mates (Bennett et al., 1997; Hunt et al., 2001). Bird colour vision is mediated by four types of single cone photoreceptors, equipped with oil droplets that act as long-pass filters and narrow the spectral sensitivity of each cone type, thus improving colour discrimination (Hart, 2001; Osorio et al., 1999; Vorobyev, 2003) and presumably also colour constancy (Vorobyev et al., 1998).

The spectral information reaching the eyes from a given object is a function of the object's reflectance and the illumination spectrum, which changes over the course of a day and between habitats (Endler, 1993; Håstad et al., 2005; Johnsen et al., 2006). Consequently, the spectral composition of light reaching the eyes from the object will also change between different times of day and environments, meaning that the perceived colour could change. That this is not a familiar problem in our everyday life is thanks to colour constancy: the phenomenon that object colour can be recovered despite changes in the illumination (Hurlbert, 2007). Without colour constancy, colour information would be unreliable (Chittka et al., 2014). Many other animals are also colour constant (Balkenius and Kelber, 2004; Chittka et al., 2014; Dörr and Neumeyer, 2000; Neumeyer, 1998; Olsson et al., 2016). In humans, relational colour constancy, recovery of the chromatic relationship between colours in a changed illumination, is faster and more reliable (Foster and Nascimento, 1994) than absolute colour constancy, the exact recovery of the perceived colour.

Here, we tested whether learning and the use of relative colour cues also facilitates colour constancy in birds. We built on earlier experiments on chicken colour constancy (Olsson et al., 2016), but this time trained them to use relative cues, rewarding them for choosing an orange colour over a yellow unrewarded colour, thus allowing them to solve the task by choosing the relatively 'redder' colour. We quantified colour constancy performance by determining the size of the shift in illumination over which chickens could still discriminate colours.

MATERIALS AND METHODS

Animals

Twenty-four chickens, *Gallus gallus* (Linnaeus 1758), of both sexes of the Lohman White breed were obtained as eggs (Gimranäs AB, Herrljunga, Sweden), hatched in a commercial incubator (Covatutto 24, Högberga AB, Matfors, Sweden) and kept in 1×1 m boxes in groups of six to eight individuals. Water was available *ad libitum* but access to food (commercial chick crumbs, Fågel Start, Svenska Foder AB, Staffanstorp) was restricted to afternoons and training sessions. Experiments were performed until 5 weeks of age.

Experimental arena and stimuli

Experiments were performed in a matte grey wooden arena (0.7×0.4 m), with a holding area and a presentation area separated by a movable wall. Fluorescent tubes illuminating the arena from above provided the white control illumination (Biolux L18W/965, Osram, Germany). Six test illuminations (examples in Fig. 1A; all illumination spectra available in Fig. S1) were created by adding red light (LZ4-00R100 LEDs, λ_{max} 633 nm; LED Engin, San Jose, CA, USA) and adjusting the intensities of the two light sources. The lowest intensity used, 35 cd m^{-2}, was high enough to allow for good colour discrimination (Olsson et al., 2015).

As stimuli, we used the same type of conical food containers with random patterns of 30% coloured tiles and 70% grey tiles as in previous studies (Olsson et al., 2015, 2016; Osorio et al., 1999). The tiles differed in intensity, and the range of intensities was larger for the grey than for the colour tiles. The achromatic contrast between the brightest coloured tiles of two colours was smaller than 0.1, the achromatic and the darkest contrast threshold of chickens (Jones and Osorio, 2004).

Colour differences between stimuli and between different illumination colours were calculated using the receptor noise limited (RNL) model of colour vision (Vorobyev and Osorio, 1998), and achromatic contrasts were described as Michelson contrast for the double cone (for details, see Olsson et al., 2015, 2016). The RNL model assumes that colours are coded by opponent mechanisms and discrimination thresholds are set by receptor noise. The colour difference between two spectra is given in just noticeable differences (JND), where differences >1 JND are assumed to be

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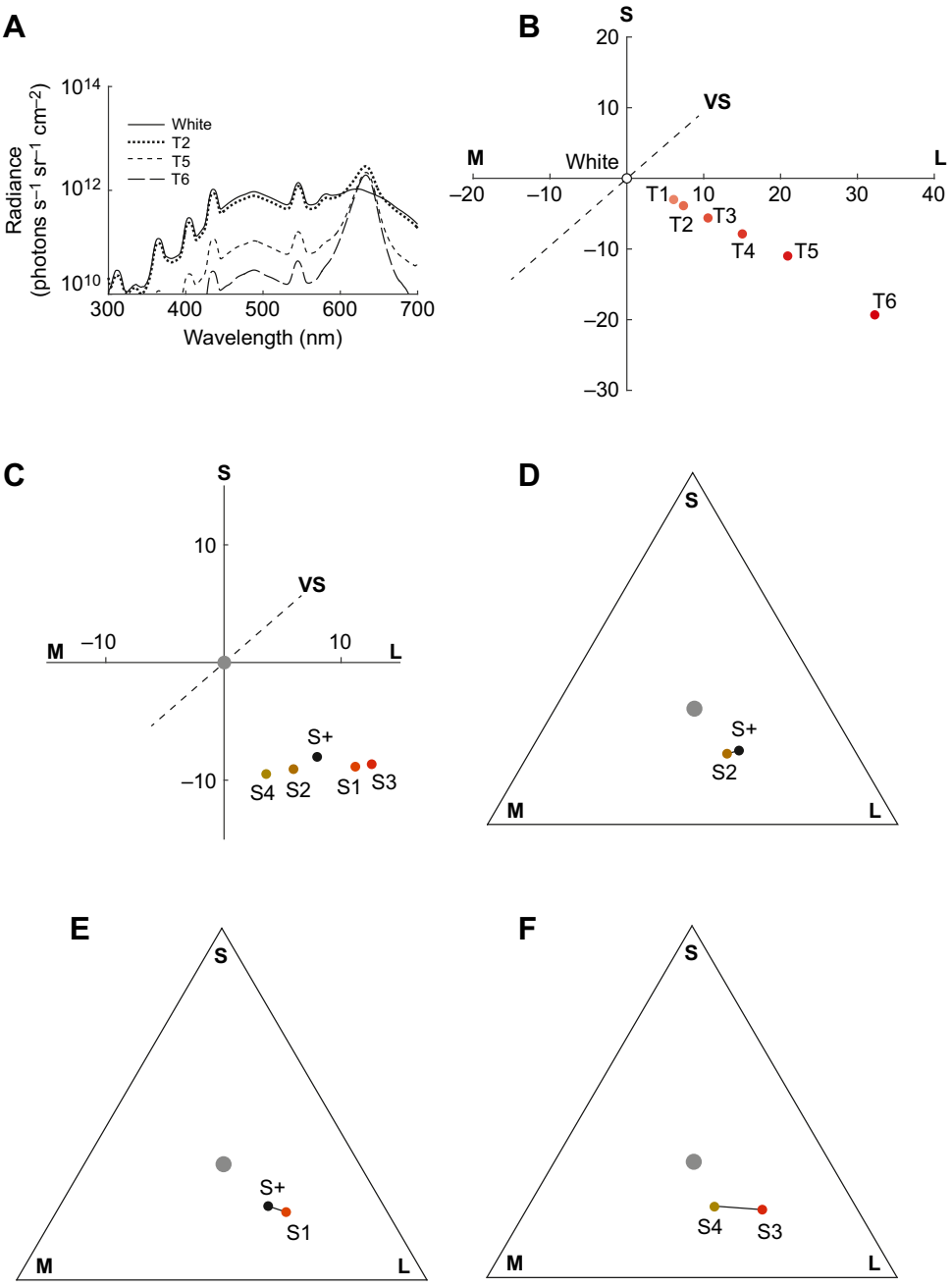

Fig. 1. Illumination and stimuli.
(A) Examples of the illumination spectra. The white illumination (control) and three of the test illuminations, as measured from a white standard placed on the floor of the experimental arena are shown. (B) Chromaticity diagram of illuminations calculated using the receptor noise limited (RNL) model (Renoult et al., 2015; Vorobyev and Osorio, 1998). (C) Chromaticity diagram (VS, S, M and L indicate violet-, short-wavelength-, medium-wavelength- and long-wavelength-sensitive cone types, respectively) of all stimuli in experiment 1, using the RNL model, for adaptation to the grey background under white illumination (grey circle). The axis units in B and C are in just noticeable differences (JND). (D) Chromaticity diagram of the stimuli used in colour constancy tests in experiment 1. (E) Chromaticity diagram of the stimuli used to test for general relative colour preference in white light. (F) Chromaticity diagram of the two unfamiliar stimuli used to test for general relative colour preference. In D–F, VS cones are excluded for illustration purposes.

discriminable. We used model parameters for the visual system of the chicken determined by Olsson et al. (2015).

Training and testing procedures

To make the data comparable, we used the same training procedure as in our previous study on colour constancy (see supplementary material in Olsson et al., 2016), following ethical approval (permit nos M6-12 and M11-14). The birds learned over 3 days, during two daily training sessions in the white control illumination, that pecking at food containers of the rewarded colour gave access to food. From the fourth day, empty food containers of the unrewarded colour were introduced, and training continued as a two-choice discrimination task. Each training session consisted of 20 trials. Within every block of 10 trials, in one randomly chosen trial, the container of the rewarded colour was also empty. With this approach, we aimed to reduce the effect of extinction in test trials.

Testing started after chickens had reached the learning criterion, a proportion of correct choices of 0.75 in two consecutive training sessions, in the control illumination. Each test session consisted of 20 trials. One randomly chosen trial out of 10 was a test trial. The illumination was changed to one of the test illuminations just before stimulus presentation (see movie in Olsson et al., 2016), thus allowing no adaptation time. In test trials, choices were never rewarded. As the remaining nine trials in the white control illumination were rewarded, training continued throughout the experiment. We started with four test sessions in the control illumination, and continued with four sessions in each red-shifted test illumination. This way, each chicken made eight choices in test trials, in each illumination.

Experiment 1

We trained chickens to discriminate a rewarded orange (S+) from an unrewarded yellow (S2) in the control illumination

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(Fig. 1A, solid line). These colours were two of the three colours used in the previous study on colour constancy (Olsson et al., 2016) and had a colour difference of 2.89 JND (Fig. 1C,D). We then tested the choice performance with these two colours in four red-shifted test illuminations (Fig. 1A, dashed lines, Fig. 1B; Fig. S1).

To test the hypothesis that the chickens used relative colour cues generally, outside of the main experiment, we performed two additional tests in the control illumination, allowing for two test trials per session, over four sessions. In the first test (Fig. 1E), we presented the rewarded colour (S+) and a new red colour (S1) that had a colour difference from the rewarded colour (3.16 JND) similar to that for the unrewarded colour (S2). In the second test (Fig. 1F), we presented the chickens with two new colours, S3 (a red shade) and S4 (yellow), with similar colour differences from the rewarded colour (S+; 4.5 and 5.3 JND, respectively). If chickens had learned relative cues, we would expect them to choose S3 significantly more frequently than S4. These test trials were also interspersed between training trials, in which the chickens were presented with S+ versus S2.

Finally, we tested whether longer adaptation time facilitated colour constancy in tests with large differences of illumination colour, something we had observed previously (Olsson et al., 2016). After 10 training trials, we allowed the chickens to adapt to a test illumination, in which they previously had failed to choose the correct colour, for 5 min, and then to make four test trials in sequence. In two such test sessions, each bird thus made a total of eight choices.

Experiment 2

In the second experiment, we tested the effect of the colour difference between stimuli on choice performance in different illuminations. We trained eight chickens to discriminate orange from yellow, four with a larger (O1+/Y−: 6.6 JND) and four with a smaller colour difference (O2+/Y−: 4.3 JND). Eight other chickens learned to discriminate green from blue, four with a large (G1+/B−: 5.9 JND) and four with a small difference (G2+/B−: 3.7 JND; see Fig. S2).

All animals were trained in the white control illumination and tested in red-shifted test illuminations. Chickens trained with the small colour differences (O2+/Y− and G2+/B−) were also tested with longer adaptation time, in the same way as described above.

Analysis

We analysed choice performances in different tests by fitting generalized linear mixed models (GLMMs) to the data, including individual identity as a random effect and illumination as a fixed effect, with a logistic link function using the lme4 package (http://lme4.r-forge.r-project.org/) in R (http://www.R-project.org/). We compared the nested models using the change in deviance and Akaike information criterion (AIC) score (Akaike, 1974), preferring the models with lower deviance and AIC score.

For each experiment, we determined the illumination shift at which chickens reached a threshold proportion of 0.7 correct choices, by interpolation from the fitted model, as in our previous study (Olsson et al., 2016). We compared colour constancy performance of chickens in experiment 1 with data from our previous experiment, in which no relative cues were available (Fig. 2A,B) (Olsson et al., 2016), as well as performance with different stimulus colour differences, with GLMMs combining the choice data from the two experiments and adding a factor variable to separate the data from the two experiments.

To evaluate whether longer adaptation time improved performance, we compared choice frequencies with and without adaptation time using the Friedman test in Matlab R2015b.

RESULTS AND DISCUSSION

In both experiments, choice performance depended on illumination colour (Figs 2 and 3). The chickens were able to significantly discriminate the colours in test illumination T3, but performance dropped in illumination T4 or T5, depending on the experiment.

Experiment 1: relative colour cues and colour constancy

The chickens trained with one constant unrewarded colour, yellow, were able to choose the rewarded orange (S+) even in a strongly shifted illumination colour (≈22 JND; Fig. 2A). This is twice the illumination shift compared with that for chickens trained and tested previously (Olsson et al., 2016), with yellow and red as alternating unrewarded colours, making relative colour cues unreliable (≈10 JND; Fig. 2A,B; GLMM, $P<0.05$). In both studies, the chickens chose between two colours in any given trial. This indicates that chickens can learn relative colour cues (i.e. choose the 'redder' colour) in colour-guided behaviours, and use them to recognize learned colours in changed illumination, for better colour constancy.

It could be argued that even chickens trained to S+ versus two alternating unrewarded colours (Olsson et al., 2016) could have used relative cues if they learned two different rules (choose the 'redder' colour in in tests with yellow, and the 'yellower' colour in tests with red). Indeed, we previously found that chickens that had never seen yellow or red as unrewarded colours during training to orange (S+) (Olsson et al., 2016), showed even less robust colour constancy when tested with alternating red and yellow in red-shifted illuminations (Fig. 3G, compare absolute learning, orange versus yellow/red and orange versus yellow). We therefore conclude that the degree to which relative colour information is available during learning has a major effect on colour constancy performance.

Relative colour preference in the control illumination

The chickens initially preferred a novel red colour (S1, Fig. 1E) to the rewarded orange (S+), but after ∼3 trials most animals chose S+ again (Fig. 2E). This change in choice behaviour can be described by a logistic regression (GLM, $P<0.05$). It appears inconsistent with the peak-shift phenomenon of learning (Mackintosh, 1997) and the assumption that chickens generalize relative colour preference. Similar results are known from other studies, in which chickens initially preferred an unrewarded novel colour to a trained colour but quickly learnt to ignore it (Zylinski and Osorio, 2013). A preference for novel stimuli is consistent with the information primacy hypothesis of foraging behaviour (Inglis et al., 2001).

To control for the preference for novel colours, we tested the chickens with two novel colours, red (S3) and yellow (S4, Fig. 1F). The chickens consistently chose the redder colour (Fig. 2F). This may suggest that they used relative colour cues other than for the trained colour pair, or that they generalized the unrewarded training colour (S2) to the novel yellow (S4). More experiments are needed to resolve this.

Experiment 2: influence of colour difference on colour constancy performance

Chickens trained to orange versus yellow chose the correct colour in more red-shifted illuminations when the colour difference between

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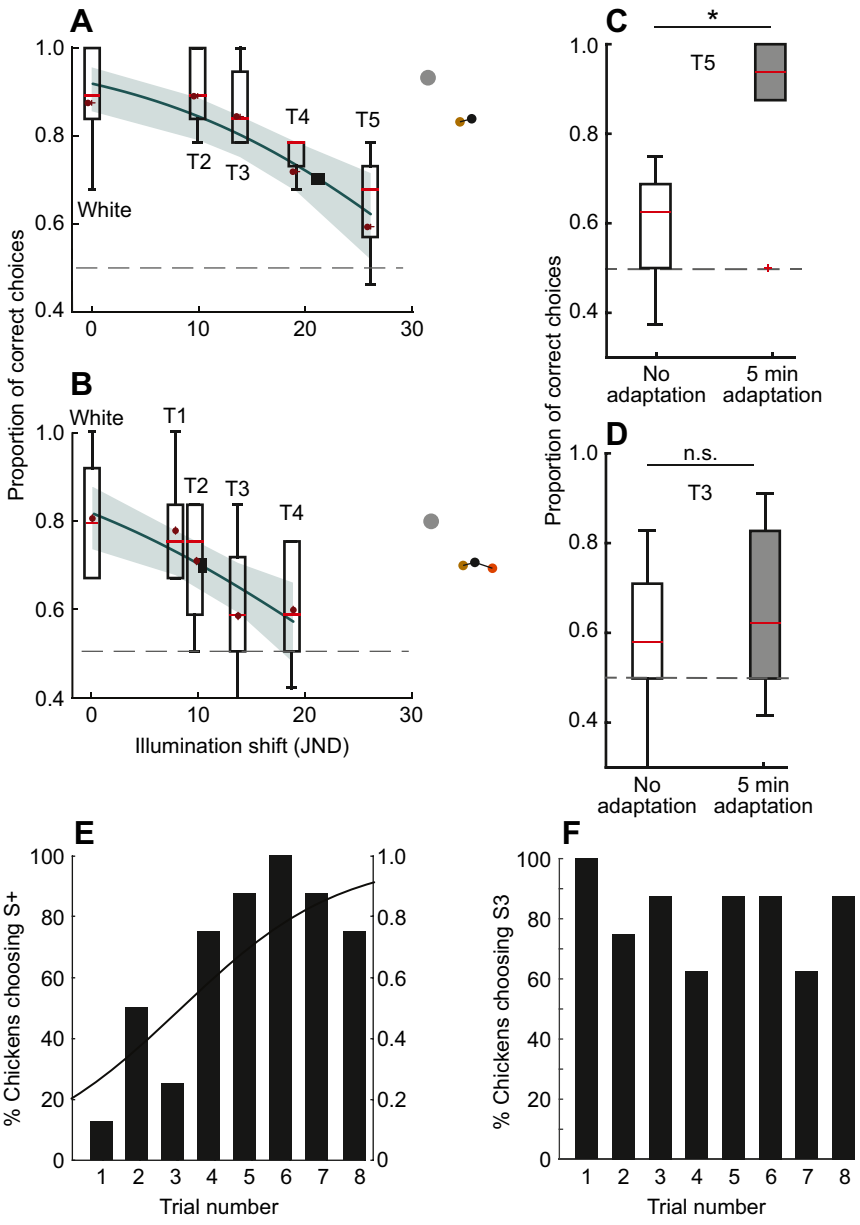

Fig. 2. Colour discrimination in different illuminations. (A) Proportion of correct choices (n=8 per individual) for chickens (N=8) in experiment 1 choosing between S+, an orange colour, and S2, a yellow colour. A GLMM including illumination shift as fixed effect and individual identity as random effect was a better fit than a model including only individual variability, by reduction in both AIC score (320.0 versus 337.6) and deviance (GLMM, P<0.05). The model (bold line) with the standard error of the fit (shaded area), the average choice frequencies at each illumination (brown dots) and the interpolated threshold (black square) are shown. The boxes signify the distribution of the data (the first and third quartiles), the bar signifies the median and the whiskers display 1.5× the interquartile range. (B) Results from a previous study, in which six chickens (each making 12 choices) were presented with S+ and alternately with either a redder (S1) or a yellower (S2) unrewarded colour (Olsson et al., 2016). (C) The proportion of correct choices improved in illumination T5 after 5 min of adaptation (Friedman's test, *P<0.05). (D) In our previous study (Olsson et al., 2016), performance in T3 did not improve after 5 min of adaptation. (E) Percentage of chickens (N=8) choosing the rewarded stimulus (S+) versus a novel redder stimulus (S1) in each of eight trials. The line represents a fitted logistic function and relates to the y-axis on the right. (F) Number of chickens (N=8) choosing the unfamiliar red colour (S3) versus the unfamiliar yellow colour (S4) in each of eight trials.

the stimuli was larger (compare Figs 2A and 3A,B; GLMM, P<0.05; interpolated threshold illumination shifts 27 and 20 JND). The choice performance of chickens choosing between green and blue did not differ significantly with colour difference (Fig. 3C,D; GLMM, P>0.05; interpolated threshold illumination shift 22 and 15 JND). In both experiments, illumination was red-shifted. This suggests that the chromatic direction of the illumination shift in relation to the colours to be discriminated may have an important influence on colour constancy performance.

The influence of adaptation on colour constancy

In both experiments, choice performance in illumination T5 improved when chickens were given 5 min adaptation time (Figs 2C,D and 3E,F; Friedman's test, P<0.05). Chromatic adaptation, one of the physiological processes underlying colour constancy, is a function of time (Werner, 2014), which explains why longer adaptation time improved colour discrimination performance (Fig. 2C). Interestingly, with the largest illumination shift (T6), the chickens in experiment 2 could not perform correct colour discrimination even after longer adaptation time (Fig. 3E). Possibly, even more time would be needed for adaptation to such a large illumination shift.

Concluding remarks

In humans, colour constancy is mediated by many mechanisms (Werner, 2014). The perceived colour of a given object has a complex relationship to the surrounding colours (Land, 1977; Brown and Macleod, 1997; Werner, 2014). This, along with chromatic adaptation (Werner et al., 2000) and memory (Granzier and Gegenfurtner, 2012), contributes to human colour constancy. Our present study shows that colour constancy builds on multiple mechanisms in the chicken, as well, and that relational colour constancy is one of them, similar to humans. The ability to use relative colour cues may be ecologically important. Ripe red berries or UV-reflective ornamental colours are likely to provide relative colour cues compared with unripe berries and UV-poorer plumage.

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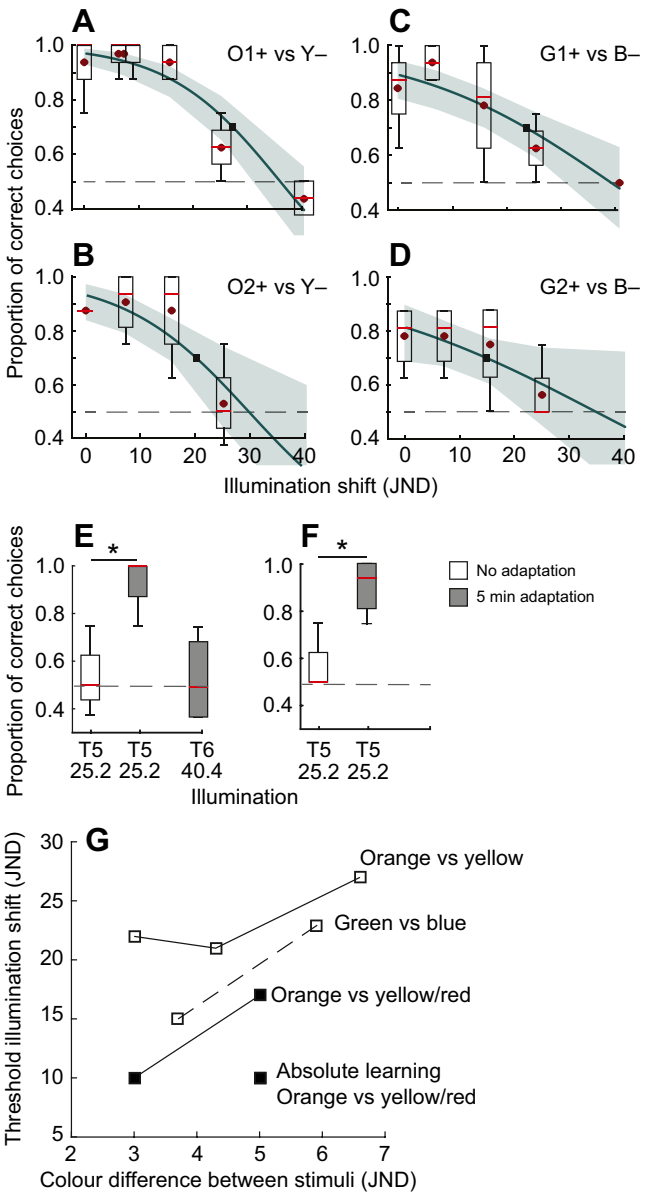

Fig. 3. Influence of colour difference on colour discrimination in different illuminations. (A,B). Proportion of correct choices (n=8 per individual) for chickens (N=4) between orange and yellow colours with large (A) and small (B) colour difference. Note that all chickens had the same choice frequency in the 'white' illumination in B. (C,D) Proportion of correct choices (n=8 per individual) for chickens (N=4) between green and blue colours with large (C) and small (D) colour difference. In all four experiments, a GLMM with illumination shift as fixed effect and individual identity as random effect fits the data better than a model including only individual identity, indicated by a reduction in AIC score (145.3 versus 189.3 in A; 122.6 versus 133.2 in B; 172.4 versus 188.2 in C; and 154.1 versus 156.1 in D) and deviance (Δ –46.04 in A; Δ –12.55 in B; Δ –17.86 in C; and Δ –4.02 in D; GLMM, P<0.05). Box details as in Fig. 2A,B. (E,F) Proportion of correct choices without and with 5 min adaptation for (E) O2+ versus Y– and (F) G2+ versus B– in two test illuminations (Friedman's test, *P<0.05). The numbers beneath the illumination name give the illumination shift in JND. (G) The interpolated threshold illumination shifts found in the experiments presented here (open squares) and in Olsson et al. (2016) (filled squares) as a function of the colour difference between stimuli.

Relative colour cues could be an important aspect of colour learning for birds as they improve colour constancy performance and thereby increase the robustness of colour-guided behaviours in natural, spectrally variable illuminations.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
A.K. had the initial idea for the study, P.O. developed and performed the experiments and analysed the data together with A.K. P.O. wrote the manuscript with contributions from A.K. Both authors approved the final version for submission.

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