

COLOUR VISION OF DOMESTIC CHICKS

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Summary

The colour vision of domestic chicks (*Gallus gallus*) was investigated by training them to small food containers decorated with tilings of grey and coloured rectangles. Chicks learn to recognise the colour quickly and accurately. Chicks have four types of single-cone photoreceptor sensitive to ultraviolet, short-, medium- or long-wavelength light. To establish how these receptors are used for colour vision, stimuli were designed to be distinguished only by specific combinations of receptors. We infer (1) that all four single cones are used, and (2) that their outputs are encoded by at least three opponency mechanisms: one comparing the outputs of ultraviolet- and

short-wavelength-sensitive receptors, one comparing the outputs of medium- and long-wavelength receptors and a third comparing of the outputs of short- and long- and/or medium-wavelength receptors. Thus, the chicks have tetrachromatic colour vision. These experiments do not exclude a role for the fifth cone type, double cones, but other evidence suggests that these cones serve luminance-based tasks, such as motion detection, and not colour recognition.

Key words: bird, colour, vision, behaviour, *Gallus gallus*, chick, chromatic coding, photoreceptor, cone, object recognition.

Introduction

Birds have five types of cone photoreceptor: four single cones and a double cone (Bowmaker et al., 1997). By convention, the single cones are called long (L), medium (M), short (S) and ultraviolet (UV) wavelength-sensitive (Fig. 1A) (long-wavelength-sensitive is sometimes abbreviated to 'LWS', medium to 'MWS', and so forth). Each contains a different photopigment, and the spectral sensitivities of L, M and S cones are narrowed by a coloured oil droplet which filters incoming light (Partridge, 1989; Bowmaker et al., 1997). The fifth type, double cones (D), makes up approximately half of all cone photoreceptors. These have the same photopigment as the L cones, but a different oil droplet filter gives them a broader spectral tuning (Bowmaker et al., 1997).

Chromatic coding

Colour identifies lights or objects by their intensity or spectrum, while chromatic cues distinguish stimuli of differing spectral composition, irrespective of relative intensity. This requires a comparison of signals from photoreceptors of differing spectral sensitivity, typically by chromatic opponency. An eye with n cone (spectral receptor) types takes n samples of the spectrum, so lights are represented by a point in an n -dimensional receptor space. To use this retinal information fully, and so have n -chromatic vision, subsequent neural coding must retain n degrees of freedom. Humans have three photoreceptor types and trichromatic colour vision (i.e. a three-dimensional perceptual space). For other animals, it is

helpful to distinguish the number of photoreceptor types from the number of degrees of freedom in behavioural use of colour. This number (i.e. the dimensionality of colour space) can be estimated by colour mixture experiments. If m appropriately chosen primary colours are necessary and sufficient to match any spectrum, colour vision has m degrees of freedom.

Mixture experiments establish the dimensionality of colour, but do not show how it is coded neurally. Generally, this is by chromatic and achromatic mechanisms. Chromatic coding involves subtractive (opponent) interactions between receptor signals, while achromatic coding is by additive interactions or by one receptor type. Human colour vision has a single achromatic mechanism, which leaves the remaining two dimensions of colour to be encoded by chromatic mechanisms, called red–green and yellow–blue (Jameson and Hurvich, 1955; Wyszecki and Stiles, 1982; Lennie and D'Zmura, 1988). It is likely that other animals use opponency, but there are few direct demonstrations in behaviour, except for the honeybee (Backhaus et al., 1987; Brandt and Vorobyev, 1997). Indirect evidence for chromatic mechanisms comes from threshold spectral sensitivities, i.e. detection of monochromatic light added to a white adapting field. The spectral sensitivities of several animals, including the pigeon *Columbia livia* (Remy and Emmerton, 1989) and a passerine *Leiothrix lutea* (Maier, 1992), are explained by a model which postulates that colour is coded by chromatic mechanisms (Vorobyev and Osorio, 1998). However, alternatives are not ruled out (Brandt and Vorobyev, 1997).

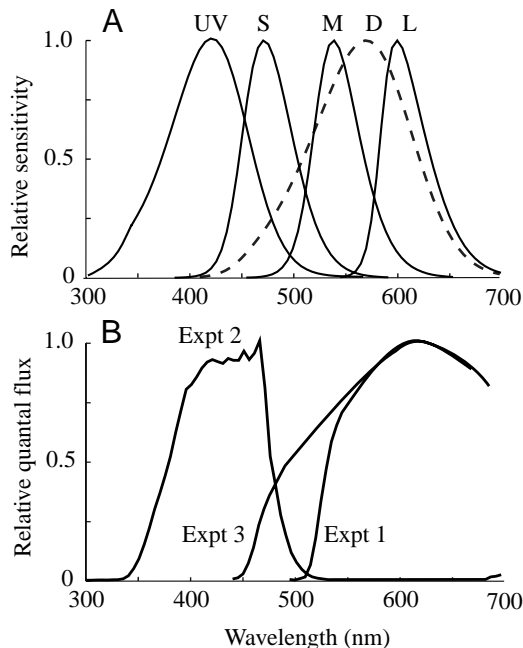


Fig. 1. (A) Spectral sensitivities of chicken photoreceptors *in vivo*, modelled from data on photopigment, oil droplet and ocular media spectral absorbance functions. See text for details. The sensitivities of each cone type are normalised to their respective maxima. (B) Spectral composition of illuminants used in experiments, normalised to their respective maxima. See also Table 1. Cones are classified as being sensitive to ultraviolet (UV), short-wavelength (S), medium-wavelength (M) or long-wavelength (L) light. D, double cones.

Tetrachromacy

Amniotes inherited four cone photopigments from fish (Bowmaker, 1991; Hisatomi et al., 1994). Two pigments were lost by mammals, but they are retained by some fish (e.g. goldfish *Carassius auratus*; Bowmaker, 1991), reptiles (e.g. turtle *Pseudemys scripta*; Goede and Kolb, 1994) and birds. All these animals have the potential for tetrachromacy. For goldfish, the mixtures of monochromatic test lights required to match various monochromatic or white standards make a convincing case that their colour vision is tetrachromatic (Neumeyer, 1992).

Tetrachromacy has not previously been demonstrated in birds. Palacios et al. (1990) and Palacios and Varela (1992) trained pigeons (*Columba livia*) to discriminate monochromatic standards from mixtures of monochromatic test lights, which were then adjusted to give the best possible match. Two monochromatic lights were needed to match standards of 590 nm or 600 nm, and also 450 nm. In the middle wavelengths, two lights could not be matched to a 520 nm standard, which implies that pigeons are at least trichromatic. This makes a good case that pigeons discriminate colours from 450 nm to 600 nm (although intensity cues are not ruled out), but does not demonstrate that they are tetrachromats.

Pigeon and *Leiothrix lutea* spectral sensitivities (see above) are predicted by a model postulating that colour vision is based

on at least three chromatic mechanisms driven by four single cones, but not the double cone (Vorobyev and Osorio, 1998; Vorobyev et al., 1998). This implies that these birds are tetrachromats, but the conclusion depends upon the model's assumption that colour thresholds are set by receptor noise in chromatic mechanisms.

Ultraviolet colour vision

The ability of birds to see ultraviolet light has recently provoked much interest, and it is clear that the ultraviolet cone signal is used for mate choice and for finding prey (Bennett et al., 1996, 1997; Burkhardt, 1996; Andersson and Amundsen, 1997; Church et al., 1998). Derimoglu and Maximov (1994) applied a conventional test for colour vision, showing that some passerines can discriminate ultraviolet-reflecting stimuli from any shade of grey, while (Bennett et al., 1996) found, in mate choice, that removing the ultraviolet is not simply equivalent to lowering the intensity. However, it remains uncertain how the ultraviolet and other receptor signals are compared or combined. A comparison of UV and S cone signals would be good for encoding spectral variation at short wavelengths. Alternatively, it would be 'reasonable' for birds to sum UV and S cone outputs to give a trichromatic eye with high sensitivity for short-wavelength light. This is (in part) because the low intensity of short-wave illumination means that the UV–S chromatic signal is relatively noisy, in which case trichromacy may be favoured (van Hateren, 1993; Vorobyev et al., 1998). Goldfish do become trichromats at low intensities, although by dropping the L cone signal (perhaps because water absorbs red light most strongly; Neumeyer and Arnold, 1989).

We describe here how domestic chicks use colour for finding food. Specific cone types and chromatic mechanisms were isolated using a combination of restricted illumination spectra (Table 1; Fig. 1) and selected object reflectances. The evidence is that all four single cone types and at least three separate chromatic opponency mechanisms are used. A role for the D cones is unlikely (Vorobyev and Osorio, 1998), but not ruled out.

Materials and methods

Animals

Male chicks (ISA-Brown) were kept in pairs under standard conditions (McKenzie et al., 1998), with experiments starting 7 days after hatching. Water and food (chick crumbs) were freely available, except that food was removed 120 min before an experimental session.

Stimuli and viewing conditions

Stimuli were printed on paper using a colour inkjet (Epson Stylus-Pro 1440 d.p.i.) and laminated with Sellotape. They were made into open-ended cones 25 mm long, 7.5 mm in diameter, and with a 12 mm equilateral triangular tab at the base that could be used as food containers. The patterns were tessellations of 6 mm × 2 mm rectangles, 30% of which were

Table 1. *Relative quantal absorptions by single photoreceptors viewing a spectrally flat surface under the three illuminants used*

| Experiment number | Illuminant | Cone type | | | | |
|-------------------|--------------|-----------|------|------|-------|------|
| | | UV | S | M | L | D |
| 1 | QH + OG530 | 0 | 0.42 | 7.22 | 11.04 | 7.90 |
| 2 | Xenon + BG12 | 6.54 | 7.28 | 0.12 | 0.01 | 0.74 |
| 3 | QH + GG475 | 0.54 | 4.74 | 8.93 | 10.87 | 9.24 |

Values are relative to those for the surface viewed under a spectrally flat illumination of approximately 10^{16} photons $\text{nm}^{-1} \text{sr}^{-1} \text{m}^{-2} \text{s}^{-1}$.

For comparison, the L cone response to the surface viewed under the quartz-halogen lamp is similar to that for a surface approximately 500cd m^{-2} viewed in direct sunlight (i.e. dull daylight).

QH, quartz halogen plus heat filter; Xenon, 150 W xenon arc; OG530, BG12 and GG475 are coloured glass filters (Schott) (see also Fig. 1).

UV, ultraviolet; S, short-wavelength; M, medium-wavelength; L, long-wavelength; D, double cones.

coloured and the remainder 'grey' (see Fig. 2A,B). Chicks resolve at least four cycles per degree (Schmid and Wildsoet, 1997). At a range of 100 mm, the side of the rectangle (2 mm) subtends 1.74° . Chicks normally selected stimuli from less than this range, and we know (D. Osorio, unpublished observations) that the pattern elements were discriminable.

The experimental arena was an aluminium box with a $0.4 \text{m} \times 0.3 \text{m}$ floor of white tissue paper. The arena was illuminated either by a 250 W tungsten-halogen lamp or a 150 W xenon arc lamp. Schott coloured glass filters restricted stimulation to specific sets of photoreceptors (Fig. 1B; Table 1): a BG12 filter gave light from approximately 350 nm to 500 nm, excluding the L and M cones; a GG475 filter excluded the UV cones; and OG530 excluded the UV and S cones.

Illumination intensities and spectra were measured using a photographic light meter and an S-1000 radiometer (Ocean Optics) calibrated using a Clarke-Berry standard lamp (JS1, National Physical Laboratory, Teddington, UK). A fresh barium sulphate surface held at 45° to the illumination had a luminance of 500cd m^{-2} under the unfiltered halogen lamp (resembling dull daylight). For the ultraviolet cone, the xenon arc + BG12 illumination produced a surface intensity equivalent to that for sunlight, giving a luminance of 1000cd m^{-2} .

Object colours produced by printer inks were measured relative to a barium sulphate standard using the S-1000 spectroradiometer with approximately coaxial illumination and with the reflecting surface at 45° to the detector. Stimuli were designed by constructing colour look-up tables in MATLAB, using cone excitation values derived as follows.

Modelling visual responses

The quantum catch of receptor type i , Q_i , to a given surface is given by:

$$Q_i = \int_{\lambda} R_i(\lambda) S(\lambda) I(\lambda) d\lambda, \quad (1)$$

where λ denotes wavelength, $R_i(\lambda)$ is the spectral sensitivity of receptor i , and $S(\lambda)$ and $I(\lambda)$ are reflectance and illumination spectra, respectively.

If receptors adapt to the (nominally) achromatic grey of the stimulus pattern, the response of cone type i , q_i , to a coloured stimulus relative to this grey is:

$$q_i = Q_i(t)/Q_i(b), \quad (2)$$

where $Q_i(t)$ and $Q_i(b)$ are quantum catches for the colour and grey, respectively.

Spectral sensitivities of chicken photoreceptors were modelled from visual pigment, oil droplet and ocular media absorptions (Fig. 1A; Govardovskii and Zueva, 1977; Partridge, 1989; Bowmaker et al., 1997). Visual pigment absorption spectra were fitted to the estimated peak using a nomogram (Maximov, 1988) with cone optical density at a maximum wavelength, λ_{max} , assumed to be 0.4. Oil droplets act as low-pass filters, and their cut-off values were fitted to published data by a hyperbolic tangent.

Modelling chromatic signals

Chromatic signals are given by opponent interactions between receptors. We use a linear model in which the chromatic signal is proportional to the difference between the receptor quantum catches. For two receptors, i and j , the chromatic signal, IJ , is given by:

$$IJ = C_{ij}(q_i - q_j), \quad (3)$$

where C_{ij} is a proportionality factor and q_i and q_j are the responses of receptors i and j , respectively. By definition (equation 2), the signal to the background is zero. The model can be extended to hypothetical chromatic mechanisms with more than two receptor inputs, e.g. $(i+j)-(k+l)$, in which signals are given by a linear combination of mechanisms with inputs from pairs of receptors. For an eye with four receptors, the signal of any chromatic mechanism is given by the combination of three arbitrarily chosen linearly independent mechanisms.

To examine how chicks use the outputs of the four single cone types, stimuli were chosen to be discriminable only by comparing specific sets of receptors. We consider three hypothetical linear independent chromatic signals, LM , SU and $(L+M)S$, defined by the formulae:

$$LM = (q_l - q_m)/(q_l + q_m), \quad (4)$$

$$SU = (q_s - q_u)/(q_s + q_u), \quad (5)$$

$$(L+M)S = [0.5(q_l + q_m) - q_s]/[0.5(q_l + q_m) + q_s], \quad (6)$$

where q_x is the response of the L, M, S and UV receptors. Proportionality factors are chosen so that chromatic signals are expressed as chromatic contrasts. This model does not predict the relative discriminability of stimuli, which depends, at least in part, on the signal-to-noise ratios in the cone mechanisms (Vorobyev and Osorio, 1998; Vorobyev et al., 1998).

Addition of luminance noise

Colour vision in animals is usually demonstrated by showing that a chromatic stimulus can be distinguished from any grey (Jacobs, 1981). Here, coloured elements were embedded in a grey background. To prevent its use, the achromatic signal was corrupted by varying the intensities (I) of coloured and grey elements with a uniform random distribution of contrast range 0.3:

$$[I_{\max} - I_{\min}] / [I_{\max} + I_{\min}] = 0.3, \quad (7)$$

where I_{\min} and I_{\max} are maximum and minimum intensities (see Fig. 2). It is desirable that coloured and achromatic elements are approximately 'isoluminant', so that chromatic signals (e.g. equations 4–6) alone distinguish coloured elements both from one another and from their background. Unfortunately, it is not possible to specify the spectral sensitivity for bird achromatic/luminance vision. The best guess is from work on motion-sensitive neurons in pigeons (Campenhausen and Kirschfeld, 1998), which are colour blind and have a spectral sensitivity similar to that of the D cones. Accordingly, tests under long-wavelength illumination (experiments 1, 3) assumed that the double cones serve luminance vision, so the (mean) grey was equal in intensity to the (mean) coloured stimuli for these cones. Even if this assumption is invalid, the small colour differences compared with luminance noise in experiments 1 and 3 (see Tables 2, 7; see Figs 2, 3) rule out any achromatic mechanism. Double cones are relatively insensitive to light at wavelengths below 500 nm (Fig. 1), and in the test of short-wavelength colour vision (experiment 2), stimuli are isoluminant for a hypothetical S+UV signal. Controls confirmed that the chicks transfer the preference for trained colours from grey of an intermediate intensity to lighter and darker greys (see Table 6).

Training and testing procedures

Chicks were housed, trained and tested as pairs because they are distressed by isolation. Four or five pairs were used for each experiment. Following food deprivation for 2 h, an experimental session lasted 2–6 h, during which the chicks were both trained and tested.

During training sessions, two types of stimuli were present; four contained a reward of a few chick crumbs, while four others were unrewarded. Rewarded stimuli were refilled at 90 s intervals on the first day and every minute on subsequent days. A training session consisted of six refills. The stimulus design means that some crumbs are easy to see in or near the container, while others lodge near the tip. Consequently, the chicks become progressively more proficient at extracting food. Experienced chicks efficiently remove crumbs by pecking at the container or by picking it up by the tip.

Tests used new empty stimuli. A test either lasted 2 min or, alternatively, scored the first 20 choices. In either case, the number of pecks on each stimulus type was recorded. Members of a pair of chicks were not distinguished. The statistics used here assume that each peck is an independent choice. To ensure that the data are not distorted by pseudoreplication, repeat

pecks at a single container or instances where a bird directly copied its partner were disregarded. The first test followed two or three training sessions, and thereafter tests and training alternated.

The accuracy of the birds' discrimination is such that stimuli were not readily discriminable by humans. To allow scoring, stimuli were marked by cutting the tip off the triangular tab. To ensure that birds did not use the cut as a cue, either stimuli used during training were not cut or, alternatively, the colour marked by a cut was reversed between training and test sessions.

Statistics

Data from separate pairs of chicks were tested for heterogeneity using a χ^2 -test (Zar, 1999, p. 500). The null hypothesis was that the data were homogeneous, and if this was not rejected ($P_{\text{het}} < 0.95$), scores for separate sets of chicks were combined. Combined data were fitted with a binomial model (Zar, 1999, p. 517), and the probability of the null hypothesis that stimuli were chosen at random was calculated using Fisher's exact test (one-tailed binomial test, Zar, 1999, pp. 543–555).

Results

We tested discrimination of food containers by the chicks to establish how cone outputs are used for colour vision. Stimuli were designed to isolate specific sets of single-cone photoreceptors (Fig. 1; Table 1). Chicks were trained and tested for up to 4 days, starting with a relatively coarse discrimination and proceeding to finer tasks. The results are evidence only that a particular pair of stimuli is discriminable and do not give perceptual thresholds.

Experiment 1: long versus medium cone signals

The peak sensitivity of the chicken L cone *in vivo* is near 600 nm and that of the M cone is near 540 nm (Fig. 1A). To show that chicks use an LM chromatic signal (see equation 4), they were trained under light that excludes UV and S cones (Fig. 1B; Table 1). Stimulus colours and the background were (on average) isoluminant to the double cones. Initially, chicks were trained three times to a rewarded colour (R_A) against a negative (U_1 ; R_A versus U_1 ; Tables 2, 3; Fig. 2). In tests (Table 3), the chicks chose the rewarded stimulus four times as often as the unrewarded stimulus. The following day, chicks were trained twice with a finer discrimination (R_A versus U_2). In two tests, the rewarded stimulus was chosen approximately twice as often as the unrewarded stimulus. After a break of 20 h, the chicks were retested on stimulus R_A versus U_2 ; there was some decline in selectivity, but the rewarded stimulus was still significantly preferred.

Experiment 2: short versus ultraviolet cone signals

The peak sensitivity of the chicken S cone *in vivo* is near 470 nm and that of the UV cone is near 420 nm (Fig. 1A). To test short-wavelength colour vision, stimuli were viewed under

Table 2. Test for a chromatic mechanism comparing L with M cone signals, experiment 1; chicks viewed colours under OG530-filtered light (see Table 1)

| Stimulus | Receptor response | | | |
|----------------|-------------------|------|------|-----------|
| | M | L | D | LM signal |
| R _A | 0.82 | 1.10 | 0.97 | 0 |
| U ₁ | 0.93 | 1.07 | 1.00 | -0.08 |
| U ₂ | 0.89 | 1.12 | 1.01 | -0.04 |
| Background | 1 | 1 | 1 | -0.15 |

M, medium-wavelength cone; L, long-wavelength cone; D, double cone.

Cone excitations relative to background for stimuli used (see Fig. 2) and LM chromatic signals (see equation 4) relative to the rewarded stimulus, R_A.

Table 3. Test for a chromatic mechanism comparing L with M cone signals, experiment 1; chicks viewed colours under OG530-filtered light (see Table 1)

| Group | R _A versus U ₁ | | R _A versus U ₂ | |
|-------------------------|--------------------------------------|----|--------------------------------------|----|
| | R | U | R | U |
| | a | 18 | 2 | 32 |
| b | 18 | 2 | 36 | 17 |
| c | 16 | 4 | 28 | 16 |
| d | 12 | 8 | 36 | 21 |
| Total | 64 | 16 | 132 | 66 |
| <i>P</i> _{het} | 0.88 | | 0.13 | |
| <i>P</i> _{NH} | 3×10 ⁻⁸ | | 2×10 ⁻⁶ | |

Choices made by four pairs of chicks trained first to R_A against U₁, and tested after three training sessions. Chicks were then trained to the finer discrimination of T against U₂, and tested after two and three further training sessions.

*P*_{het} denotes the probability of heterogeneity of the groups; *P*_{NH} denotes the probability that chicks do not discriminate the rewarded stimulus from the unrewarded stimulus (null hypothesis).

R, rewarded colour; U, unrewarded colour.

light between 350 nm and 500 nm, which excludes the L and M cones, leaving the UV, S and D cones active (Fig. 1B; Table 1). Rod sensitivity peaks near 500 nm, and we assume that the rods were bleached. Table 4 shows the excitations of the S, UV and D cones for the experimental stimuli relative to the nominally achromatic background. [Design of short-wave colours: nominally achromatic stimuli (e.g. paper and black ink) are not spectrally flat below approximately 440 nm, and paper absorbs strongly around 380–400 nm. Also, the fluorescence of magenta ink precludes its use as a pure pigment to create short-wave stimuli, because the pigment glows pink under blue (BG12) light. However, in blue (i.e. 50:50 magenta:cyan) light, the fluorescence was neither noticeable to a human observer nor measurable. Cyan, yellow and black inks do not fluoresce significantly]. The mean intensities of the achromatic background and training colours were

Table 4. Test for a chromatic mechanism comparing S with UV cone signals (experiment 2) with chicks viewing test colours under BG12-filtered light (Fig. 1; Table 1)

| Stimulus | Receptor response | | | |
|-----------------|-------------------|------|------|-----------|
| | UV | S | D | SU signal |
| UV _R | 1.45 | 1.20 | 1.05 | -0.094 |
| UV _P | 0.74 | 0.84 | 0.90 | 0.063 |
| Grey/background | 1 | 1 | 1 | 0 |

UV, ultraviolet cone; S, short-wavelength cone; D, double cone.

UV_R, ultraviolet-rich colour; UV_P, ultraviolet-poor colour.

Cone excitations relative to background for stimuli used, and LM chromatic signals (see equation 5) relative to the nominally achromatic background.

Table 5. Test for a chromatic mechanism comparing S with UV cone signals (experiment 2) with chicks viewing test colours under BG12-filtered light (Fig. 1; Table 1)

| Group | Rewarded colour | UV _R versus UV _P | | UV _R /UV _P versus grey | |
|-------------------------|-----------------|--|-----------------|--|----|
| | | R | U | R | U |
| | | A | UV _R | 42 | 16 |
| B | UV _R | 42 | 17 | 31 | 8 |
| C | UV _P | 39 | 10 | 26 | 7 |
| D | UV _P | 39 | 18 | 31 | 9 |
| Total | | 162 | 61 | 119 | 30 |
| <i>P</i> _{het} | | 0.22 | | 0.003 | |
| <i>P</i> _{NH} | | 4×10 ⁻¹² | | 5×10 ⁻¹⁴ | |

Choices made after five training sessions for two pairs of chicks trained to ultraviolet-rich (UV_R) against ultraviolet poor light (UV_P).

Each set of data is from two 2 minutes tests.

Abbreviations as for Table 3.

(approximately) equal for the summed outputs of UV and S cones.

Chicks acclimatised quickly to the short-wavelength-rich light, and four pairs were trained to discriminate an ultraviolet-rich colour (UV_R; blue to our eye) from an ultraviolet-poor colour (UV_P; green to our eye). Two pairs were rewarded on UV_R and two on UV_P. Learning was noticeably slower than for the warm colours used in experiments 1 and 3, but after five training sessions all pairs discriminated the stimuli successfully, choosing the trained stimulus 73 % of the time (Table 5). Subsequently, chicks were retrained with the same rewarded colour but with 'grey' negative stimuli. After a single training session, they chose the trained colour 80 % of the time (Table 5). There was no significant difference between the responses of chicks rewarded on UV_P from those rewarded on UV_R, excluding the remote possibility that chicks rely on the fluorescence of magenta ink in the latter (see above).

To exclude the possibility that the chicks used achromatic cues, controls were run in which 30 % of the elements in the

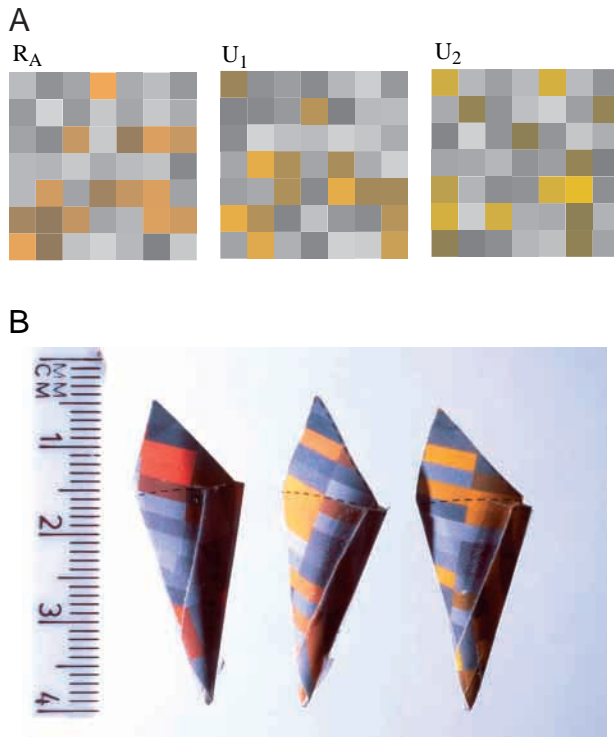


Fig. 2. (A) Stimuli used for experiment 1 (see Tables 2, 3), whose discrimination required a comparison of long-wavelength (L) against medium-wavelength (M) cone signals. 70% of the tiles are grey and 30% coloured, with their distributions and intensities randomised. Intensities vary with a uniform distribution and a contrast range of 0.3. In experiments, the tiling was similar, but of 6mm×2mm rectangles, on a conical food container. Approximately 15 tiles were visible on one face a container. Because stimuli were distributed across the 0.3m×0.4m floor of the test area, chicks seldom had the opportunity to make simultaneous comparisons. Viewed under OG530-filtered light in the experiment (Fig. 1B; experiment 1), the mean intensities of the grey background and three colours appeared approximately isoluminant to the double cones. R_A , rewarded colour; U_1 , U_2 , unrewarded colours. (B) Photograph of the experimental stimuli.

achromatic pattern (corresponding to the coloured elements on a normal stimulus) were substituted with achromatic stimuli of mean intensity either 0.5× or 1.5× the background (in all cases, luminance noise remained at a contrast of 0.3). The chicks readily transferred their ability to distinguish the medium grey from UV_P and UV_R colours to both these lighter and darker greys (Table 6).

Experiment 3: blue–yellow signals

The two previous experiments imply that chicks can compare the output of L with that of M cones and that of S with that of UV cones. Hence, all four single cones are used for colour discrimination. We now ask whether the chicks can distinguish colours by comparing the outputs of either or both of the L and M cones with that of S cones, using an $(L+M)S$ signal (equation 6). This is comparable with the human yellow–blue mechanism, which compares the blue cone signal

Table 6. Test for a chromatic mechanism comparing S with UV cone signals (experiment 2) with chicks viewing test colours under BG12-filtered light (Fig. 1; Table 1)

| Group | Rewarded colour | UV_R/UV_P versus light grey | | UV_R/UV_P versus dark grey | |
|-----------|-----------------|-------------------------------|----|------------------------------|---|
| | | R | U | R | U |
| A | UV_R | 18 | 2 | 14 | 2 |
| B | UV_R | 13 | 2 | 11 | 1 |
| C | UV_P | 16 | 4 | 16 | 2 |
| D | UV_P | 12 | 3 | – | – |
| Total | | 59 | 11 | 41 | 5 |
| P_{het} | | 0.10 | | 0.01 | |
| P_{NH} | | 2×10^{-9} | | 2×10^{-8} | |

Choices made in tests for discrimination of the trained colour against achromatic stimuli where 30% of the tiles were either on average lighter (1.5×) or darker (0.5×) than the ‘background tiles’.

One 2 min test for each condition.

Abbreviations as for Table 3.

UV_R , ultraviolet-rich colour; UV_P , ultraviolet-poor colour.

with summed red and green signals (Jameson and Hurvich, 1955; Lennie and D’Zmura, 1988). By analogy with humans, we define avian yellow as colours where $LM=0$ and $LS>0$ (see equations 4, 6). The chicken’s yellows (Fig. 3) look slightly greenish to a human trichromat.

To isolate the LS mechanism, it is necessary to prevent chicks using either LM or SU signals. To this end, the ultraviolet receptor was excluded (Fig. 1B; Table 1), while colours were chosen to lie near the line with a null LM signal (Table 7; Fig. 3). This line runs through the achromatic point [i.e. $LM=0$, $(L+M)S=0$], and in the terminology of human perception, these colours have a fixed yellow hue of varying saturation (see also Osorio et al., 1999).

Five pairs of chicks were trained to a yellow of moderate saturation (R_B) against grey (T_1 ; Fig. 3). The estimated $(L+M)S$ chromatic signal separating rewarded from unrewarded stimuli was 0.19, and the LM signal was 0.01. After two training sessions, the chicks preferred the rewarded colour (Table 8). Maintaining the same rewarded colour, the unrewarded colour was changed from grey to either a more saturated (T_2) or to a less saturated (T_5) colour; the chicks discriminated both from the training colour. They also discriminated colours intermediate between the trained and the appropriate unrewarded colours, (T_3 and T_4 ; Table 8, Fig. 3B). The fine discriminations between stimuli giving negligible LM signals is indicative of an ability to compare the S cone output with the output of either or both of the longer-wavelength single cones or possibly the output of the S cone with that of the D cone.

Discussion

The task here is recognition of food by colour. The stimuli are relatively small and manipulatable – rather like seedpods or caterpillars – and it is likely that this enhances learning

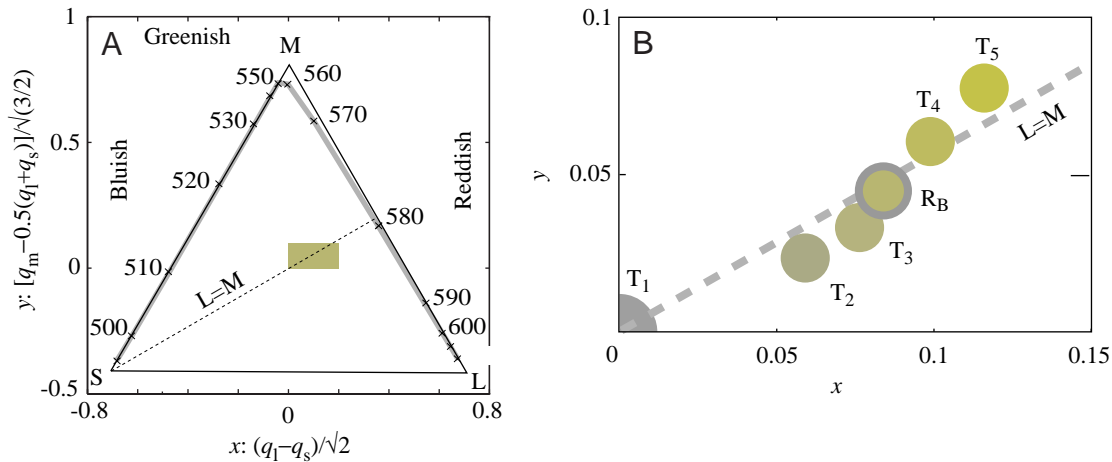


Fig. 3. Stimuli used for experiment 3 (see Tables 7, 8), whose discrimination required a comparison of short-wavelength (S) against long-wavelength (L) and/or medium-wavelength (M) cone signals. The experiment was performed under GG475-filtered light which excludes the ultraviolet cone (Fig. 1, experiment 3; Table 1). (A) Colour diagram whose three apices are defined by excitation of L, M and S cones (see legend to Fig. 1 and equations 1, 2) in isolation. The diagram gives a surface of unit radius in the three-dimensional receptor space. The monochromatic locus is plotted (grey line), with symbols plotted at 10 nm intervals from 490 nm to 620 nm; numbers give the wavelengths (nm) of most of these points. Experimental colours fell in the shaded area. q_l , q_m and q_s are responses of L, M and S cones, respectively (equation 2). (B) Shaded area enlarged, with the achromatic point at the origin. This shows colours used in the experiment (Tables 7, 8). Colours lie along the line where L and M cone signals are estimated to be equal and appeared of intensity to the double (D) cone. Since the $(L+M)S$ signals (see equation 6) are positive, the colours are comparable with a human yellow of fixed hue and varying saturation (see text). Chicks rewarded on R_B were initially trained against the achromatic colour T_1 , and they were subsequently able to discriminate R_B from the four more similar colours T_2 – T_5 (Table 8). The ability of the chicks to discriminate R_B from colours as similar as T_3 and T_4 is striking.

Table 7. Test for a chromatic mechanism comparing L and/or M with S cone signals (experiment 3) with chicks viewing colours under GG475-filtered light (Fig. 1; Table 1)

| Stimulus | Cone responses | | | | Chromatic signals | |
|----------|----------------|------|------|------|-------------------|--------|
| | S | M | L | D | LM | (L+M)S |
| R_B | 0.67 | 0.97 | 0.99 | 0.96 | 0 | 0 |
| T_1 | 1 | 1 | 1 | 1 | -0.010 | -0.188 |
| T_2 | 0.62 | 1.11 | 1.07 | 1.06 | -0.029 | 0.087 |
| T_3 | 0.66 | 1.05 | 1.04 | 1.02 | -0.015 | 0.038 |
| T_4 | 0.69 | 0.94 | 0.97 | 0.94 | 0.005 | -0.027 |
| T_5 | 0.69 | 0.86 | 0.89 | 0.86 | 0.007 | -0.070 |

T_1 – T_5 , Similar achromatic colour.

Cone excitations relative to background for stimuli used, and LM and $(L+M)S$ chromatic signals (see equation 6) relative to the rewarded stimulus R_B .

compared with more conventional operant procedures (Remy and Emmerton, 1989; Palacios et al., 1990; Maier, 1992).

The evidence makes a good but not watertight case that the chicks use tetrachromacy for object recognition. We propose that the outputs of four single cones drive at least three chromatic opponency mechanisms, namely LM, SU and $(L+M)S$ (see equations 4–6). It is theoretically possible that a single mechanism with multiple inputs, e.g. $(L+UV)$ – $(M+S)$, could account for both LM and SU colour vision. Also, a contribution from D cones cannot be excluded in experiment 3, where double cones could replace the L+M input to the $(L+M)S$ mechanism. In experiment 1, double (D) rather than L cones

Table 8. Test for a chromatic mechanism comparing L and/or M with S cone signals (experiment 3) with chicks viewing colours under GG475-filtered light (Fig. 1; Table 1)

| Group | R_B versus T_1 | | R_B versus T_5 | | R_B versus T_4 | | R_B versus T_2 | | R_B versus T_3 | |
|-----------|---------------------|----|---------------------|----|--------------------|----|--------------------|----|--------------------|----|
| | R | U | R | U | R | U | R | U | R | U |
| A | 20 | 5 | 39 | 10 | 16 | 8 | | | | |
| B | 16 | 2 | 35 | 7 | 17 | 4 | | | | |
| C | 9 | 2 | 28 | 7 | 13 | 6 | | | | |
| D | 16 | 5 | | | | | 26 | 13 | 15 | 9 |
| E | 20 | 3 | | | | | 34 | 19 | 17 | 9 |
| Total | 81 | 17 | 102 | 24 | 46 | 18 | 60 | 32 | 32 | 18 |
| P_{het} | 0.18 | | 0.11 | | 0.47 | | 0.20 | | 0.17 | |
| P_{NH} | 2×10^{-11} | | 6×10^{-13} | | 3×10^{-4} | | 2×10^{-3} | | 0.03 | |

Choices made by five pairs of chicks trained to R_B against the achromatic colour T_1 after four training sessions. Chicks were subsequently trained to R_B against either more- (T_2) or less- (T_5) saturated colours, they were then tested against these, and against intermediate achromatic colours T_3 and T_4 , respectively (see Fig. 3; Table 7).

Abbreviations as for Table 3.

might give 'DM' chromatic signals, but these are approximately half of the corresponding LM signals. The results are consistent with our model of pigeon and *Leiothrix lutea* spectral sensitivities (Remy and Emmerton, 1989; Maier, 1992; Vorobyev and Osorio, 1998), which implies that the four single cones but not the double cones are used for colour vision.

Avian ultraviolet and yellow

There is good evidence that birds use UV cone signals for mate choice (Bennett et al., 1996; Anderson, 1997) and foraging (Church et al., 1998), but this does not show whether or how UV cones signals are used (see Introduction). Experiment 2 may be the first direct demonstration that an opponent mechanism compares UV with S cone signals.

Bennett et al. (1994) emphasise that bird and human perception of colour differs. In all three experiments reported in the present study, the chicks noticed and learnt colour differences barely visible to a human (e.g. Figs 2, 3; colours in experiment 2 were completely invisible). For short-wavelength colours, the existence of separate UV and S cones mean that the chicks' advantage is not surprising. Their ability to distinguish 'yellows' of differing saturation (experiment 3) is less predictable. Owing in part to the sparsity of blue cones in the human retina (Wyszecki and Stiles, 1982), discrimination of colours that give equal *LM* signals is poor in humans. This is readily apparent because, for spectra we see as yellow, i.e. $LM \approx 0$, at the point between white and the monochromatic loci in colour space called 'Sloan's notch' (Wyszecki and Stiles, 1982), discrimination of saturation differences is poor (e.g. Fig. 3). Yellowish colours are not unusual in bird plumage. For example, differences between the yellowish-green plumages that separate various *Phylloscopus* warblers (Sylviidae) that are difficult for humans to differentiate may be much more noticeable for the birds themselves. At least for chicks, the use of colour for object recognition may be helped by their accurate memory for both hue and saturation (Osorio et al., 1999).

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