Behavioural investigation of polarisation sensitivity in the Japanese quail (*Coturnix coturnix japonica*) and the European starling (*Sturnus vulgaris*)

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Summary

Many animals have sensitivity to the e-vector of linearly polarised light, which may assist in visually mediated behaviours such as navigation, signalling and foraging. However, it is still controversial as to whether birds possess polarisation sensitivity. Several studies have found that altering the polarisation patterns of the broad visual field surrounding birds alters their intended migratory orientation. However, electrophysiological tests have failed to elicit evidence for polarisation sensitivity in birds, and the mechanism by which birds might perceive polarised light is unknown. In this experiment, we trained Japanese quail and European starlings to discriminate stimuli differing in their polarisation pattern. Although both quail and starlings were able to discriminate stimuli in which the stimulus sub-components either differed or had the same radiant intensity (the control task), they were unable to discriminate stimuli in which the e-vector orientations of the stimulus sub-components either differed by 90° or had the same angle of polarisation. The birds' successful performance on the control task, but failure to complete the polarisation task, demonstrated that they had all the necessary cognitive abilities to make the discrimination except sensitivity to angle of polarisation. We conclude that quail and starlings are unable to use polarisation cues in this foraging task.

Key words: e-vector, polarisation sensitivity, vision, foraging, avian, Japanese quail, European starling, *Coturnix coturnix japonica*, *Sturnus vulgaris*.

Introduction

Visual ecology of polarisation sensitivity

In the literature on the ecology of vision, light is often described in terms of its spectral properties but rarely in terms of its polarisation properties. Light emitted from the sun is unpolarised but becomes linearly polarised when it is scattered by small particles (for example, in the atmosphere) or when it is reflected off certain materials (Wehner, 2001). This linear polarisation can be described in two ways: first, by the e-vector angle that predominates in the photons that constitute the flux of light and, second, by how much this particular e-vector angle predominates (Nilsson and Warrant, 1999). As polarised light is common in both aquatic and terrestrial environments (e.g. Waterman, 1961), it is potentially a useful source of information to any animal that can perceive it.

Humans are able to detect the polarisation properties of light under certain circumstances (Rodieck, 1973), but phenomena such as Haidinger's Brushes are unlikely to have any ecological relevance. However, recent research has shown that some animals can perceive polarised light and that it is a source of visual information that is independent of light intensity and wavelength (e.g. Marshall et al., 1999). Many vertebrates, including fish, amphibians and reptiles, possess at least some polarisation sensitivity that may be used in activities such as orientation or foraging (Coughlin and Hawryshyn, 1995; Freake, 1999; Parkyn and Hawryshyn, 2000; Novales-Flamarique and Browman, 2001; Phillips et al., 2001). Furthermore, many invertebrates have been shown to have polarisation sensitivity, which has been implicated or demonstrated in orientation and navigation (insects, Rossel and Wehner, 1986), in communication (cephalopods, Shashar et al., 1996; Shashar and Hanlon, 1997; crustaceans, Marshall et al., 1999) and in foraging (cephalopods, Shashar et al., 1998, 2000).

It has been suggested that birds may use sky polarisation patterns as a navigational aid. Pomozi et al. (2001) used full sky imaging polarimetry to study the pattern of different angles of polarisation in different parts of the sky. These patterns were found to indicate the position of the sun and persisted beneath the clouds even when the sun was hidden behind clouds, as long as the sky was not totally overcast. Insects are able to perceive and use such skylight polarisation patterns (Labhart and Meyer, 1999) and it is generally accepted that birds are able to orientate using a sun compass (Able and Able, 1993; Munro and Wiltschko, 1995; Akesson and Backman, 1999), which may require the use of polarisation patterns in the sky when the sun is obscured (Pomozi et al., 2001).

Polarisation sensitivity may also help birds detect or see through water surfaces. Water surfaces strongly polarise light such that reflected rays have e-vectors predominately orientated in a horizontal direction, the degree of polarisation being dependent on the angle of incidence. Some aquatic insects use this cue as a 'pond-detector' (Schwind, 1991), and birds may also use similar cues to detect expanses of water. Alternatively, birds that hunt fish from above the water surface would benefit from being able to block horizontally polarised light so that they are better able to see through surface reflections. It is also possible that birds may use polarisation cues when foraging on land to detect prey items that would otherwise be camouflaged against their background. Food items, such as insect pupae, that have shiny surfaces strongly polarise the light that is reflected from them, whereas the light reflected off the soil background may be largely unpolarised (S. C. Church, unpublished data). This principle is similar to that used by cuttlefish, where polarisation vision is used to break the countershading camouflage of silvery fish (Shashar et al., 2000).

Mechanisms of polarisation detection

Many invertebrates are able to detect polarisation because of the inherent linear dichroism in their rhabdomeric photoreceptors (Wehner et al., 1975; Fent, 1986; Dacke et al., 1999). By contrast, the mechanism by which vertebrate photoreceptors might detect polarised light is less obvious. The chromophores of vertebrate visual pigments lie more or less in the plane of the disc lamellae of their photoreceptors but are randomly orientated with respect to axially incident light. As a result, their outer segments are not inherently polarisation sensitive (Land and Nilsson, 2002).

Some vertebrates achieve polarisation sensitivity by reorientation of their photopigments. The bay anchovy, *Anchoa mitchilli*, and the broad-striped anchovy, *Anchoa hepsetus*, have bifid cone photoreceptors in which the array of visual pigment-containing lamellae are orientated at right angles in the two parts of the cone, and thus they are potentially able to analyse the angle of plane polarised light (Fineran and Nicol, 1978; reviewed by Locket, 1999). Such bifid cones have also been found in the black-sea anchovy, *Engraulis encrasicholus* (Zueva and Govardovskii, 1991). The northern anchovy, *Engraulis mordax*, has been shown to be sensitive to polarisation by electrophysiological recordings from the optic nerve (Novales-Flamarique and Hawryshyn, 1998a). However, this basis for polarisation sensitivity has only been found in the Engraulidae.

In other vertebrates, a regular 'mosaic' pattern of cone photoreceptors in the retina is believed to be a requirement for polarisation sensitivity (Cameron and Easter, 1993; Novales-Flamarique and Hawryshyn, 1998a; Hawryshyn, 2000). It has been proposed that the tilt of the partition between the two members of the double cone may lead to e-vector selective reflection onto adjacent UV-sensitive cones and hence may provide a possible mechanism for polarisation detection (Novales-Flamarique et al., 1998; Hawryshyn, 2000). The distribution of double cones across the retina creates an ordered pattern that could generate a 'polarisation contrast' neural image (Young and Martin, 1984). Double cones often constitute more than 50% of the cones of bird retinas (Hart, 2001a), and birds are therefore well equipped to be polarisation sensitive if the function, or one of the functions, of double cones is the detection of polarised light.

The identification of particular retinal structures does not, however, conclusively prove that an animal has polarisation sensitivity, as visual perception relies on the neural structures further along the visual pathway as well as the responses of retinal cone cells (Jacobs, 1981; Goldsmith, 1990; Varela et al., 1993). Consequently, only behavioural tests of polarisation sensitivity are able to demonstrate conclusively whether or not the animal can perceive polarised light.

Avian perception of polarised light

If birds have polarisation vision, we do not know how they might perceive the different angles of polarised light. To maximise the information obtained from light, colour and polarisation should be analysed independently. However, in the *Papilio* butterfly species, polarisation sensitivity has been found to be directly linked to the colour vision system (Kelber et al., 2001; Horváth et al., 2002), and objects reflecting the same wavelengths but at different angles of polarisation are perceived as different, or 'false', colours. Consequently, it is possible that if birds possess polarisation sensitivity, it may be similarly linked to their colour vision system. An alternative evolutionary strategy is for species to have evolved polarisation sensitivity instead of colour vision, thus avoiding the confounding effects of colour and polarisation, and this has been suggested in the cases of anchovies and some cephalopods (Novales-Flamarique and Hárosi, 2002).

Experimentally manipulating patterns of skylight polarisation has been found to cause captive migrant birds to alter the direction of their intended migration (e.g. Helbig, 1990, 1991; Munro and Wiltschko, 1995). However, these studies test behavioural consequences of altering the polarisation pattern of the environment rather than directly investigating polarisation vision *per se*. Also, there has been some debate in the literature as to whether or not this apparent sensitivity to skylight polarisation resulted from co-varying alteration of the pattern of skylight intensity (Martin, 1991; Coemans et al., 1994). For instance, pigeons have been found to be sensitive to the orientation of polarised light (Kreithen and Keeton, 1974; Delius et al., 1976) but, when Coemans et al. (1994) repeated one of these experiments, they found that once they had removed all the secondary cues, such as intensity patterns, the pigeons did not demonstrate any directional response to the e-vector orientation of the illumination. In addition, all laboratory studies to date that have tested directly for polarisation sensitivity at either a physiological or perceptual level have failed to show that birds are sensitive to the plane of polarised light (Montgomery and Heinemann, 1952; Coemans et al., 1990; Vos Hzn et al., 1995). As a result, opinion within the scientific community as to whether or not birds can perceive polarisation is divided, and further electrophysiological and behavioural experiments are warranted.

As only behavioural tests can determine precisely what an animal perceives, we developed a behavioural test to investigate whether or not birds can see the e-vector orientation of polarised light reflected from objects. We used associative learning techniques in an attempt to train birds to discriminate target objects that differed in the polarisation of light reflected from their surfaces. A similar method was previously used successfully to demonstrate the ability of Japanese quail (*Coturnix coturnix japonica*) and European starlings (*Sturnus vulgaris*) to discriminate between different reflectance spectra (Smith et al., 2002). Quail and starlings were therefore used as model species for the present study, as their ability to learn and remember perceived differences in visual stimuli using a similar paradigm was already proven.

Starlings and quail represent phylogenetically and ecologically separate avian species. Avian polarisation sensitivity, if it exists, may be phylogenetically determined. If it is hypothesised that polarisation sensitivity is confined to migratory species then it is also of interest to test non-migratory species to demonstrate that this is the case; starlings are a migratory species whereas quail, at least those that have been artificially selected and reared in domesticated conditions, are not. Although it is important to consider migratory ability, as the only evidence that birds may be sensitive to polarisation comes from migratory studies, polarisation sensitivity may be used for other tasks such as foraging. This has been shown in other animals such as cephalopods (Shashar et al., 1998, 2000). In the present study, we tested the ability to use polarisation vision when foraging rather than in the context of navigation.

The birds were trained to discriminate a 90° difference in angle of polarisation, which is the maximum possible angular difference, using stimuli that approached 100% linear polarisation. The birds were required to differentiate stimuli that were either symmetrical or asymmetrical in terms of their polarisation pattern. The experiment was carried out under lighting conditions that were well within the photopic range of both starlings and quail and under which the same animals had successfully made colour discriminations in a similar foraging task (Smith et al., 2002). The overall intensity of the stimuli was not randomised, so that if birds have true polarisation vision the polarisation of the stimuli should be discriminable regardless of whether the mechanism of polarisation sensitivity is chromatic or achromatic. If polarisation sensitivity is perceived *via* a chromatic mechanism, the differently polarised stimuli will appear to be different colours to the birds. Alternatively, if polarisation is perceived only as intensity differences *via* an achromatic mechanism then one side of the stimulus will appear lighter or darker to the birds than the other.

Materials and methods
Quail

Japanese quail (*Coturnix coturnix japonica* L.) were obtained from Fayre Game, Liverpool, UK in January 2000, and the experiment was run between June and December 2001 when the quail were 18 months old. Four female quail were housed together in a floor pen (2.5 m×1.0 m×2.4 m high). The room was illuminated by eight 1.8 m-long, 100 W, high-frequency (>30 kHz) Durotest Truelite fluorescent lamps covered by a depolarising filter (Lee #215 white diffusion filter). The birds were kept on short day lengths (9 h:15 h L:D) to prevent them from laying eggs during the experimental period.

Starlings

Eight European starlings (*Sturnus vulgaris* L.) that had been caught as juveniles under an English Nature licence in July 2001 were housed prior to the experiment as part of a mixed-sex group in an outdoor aviary (1.8 m×0.9 m×1.8 m high) until April 2002, when they were moved into an indoor cage (1.3 m×0.65 m×1.2 m high) under artificial lighting. The lighting consisted of eight 1.8 m-long Durotest Truelite lamps powered by high frequency (>30 kHz) ballasts (Helvar, Helsinki, Finland). The lights were covered by a depolarising filter (Lee #215 white diffusion filter). The experiment was carried out between June and September 2002.

Stimuli

We tested whether the birds could learn to discriminate objects that differed in the pattern of linear polarisation of the light reflected from their surfaces. In each trial, the birds were placed in a foraging arena and presented with two types of polarisation stimuli: 'different' and 'same'. The 'different' stimuli had two sides that differed in the way in which they polarised reflected light; specifically, their angle of polarisation differed by 90°. The 'same' stimuli had the same angle of polarisation on both sides of the stimulus (Fig. 1A). These stimuli overlay food wells in the foraging arena, and food rewards were available underneath only one type of stimulus. Each bird was consistently rewarded for choosing either 'same' or 'different' stimuli to see whether, over consecutive trials, they were able to learn to recognise the rewarded stimulus type and preferentially select those stimuli. If the birds could learn a difference between the stimuli types, they must be able to see a difference between them.

To appreciate and learn the difference between the two stimulus types, the animal being tested had to be able to learn the abstract concept of 'same' *versus* 'different'. The ability to form this concept has been demonstrated in birds (Cook et al., 1997, 1999). A control task testing the birds' ability to form the same–different concept using similar stimuli to the polarisation stimuli was essential. Otherwise, it could not be determined whether failure to learn the difference between the stimuli may have resulted from an inability to perceive polarisation or an inability to make a same–different judgement. The control task therefore tested the non-visual elements of the task, i.e. whether the birds were capable of learning visual discriminations using our paradigm and whether they had the necessary cognitive ability to perform the same–different discrimination that we required them to make in the polarisation task.

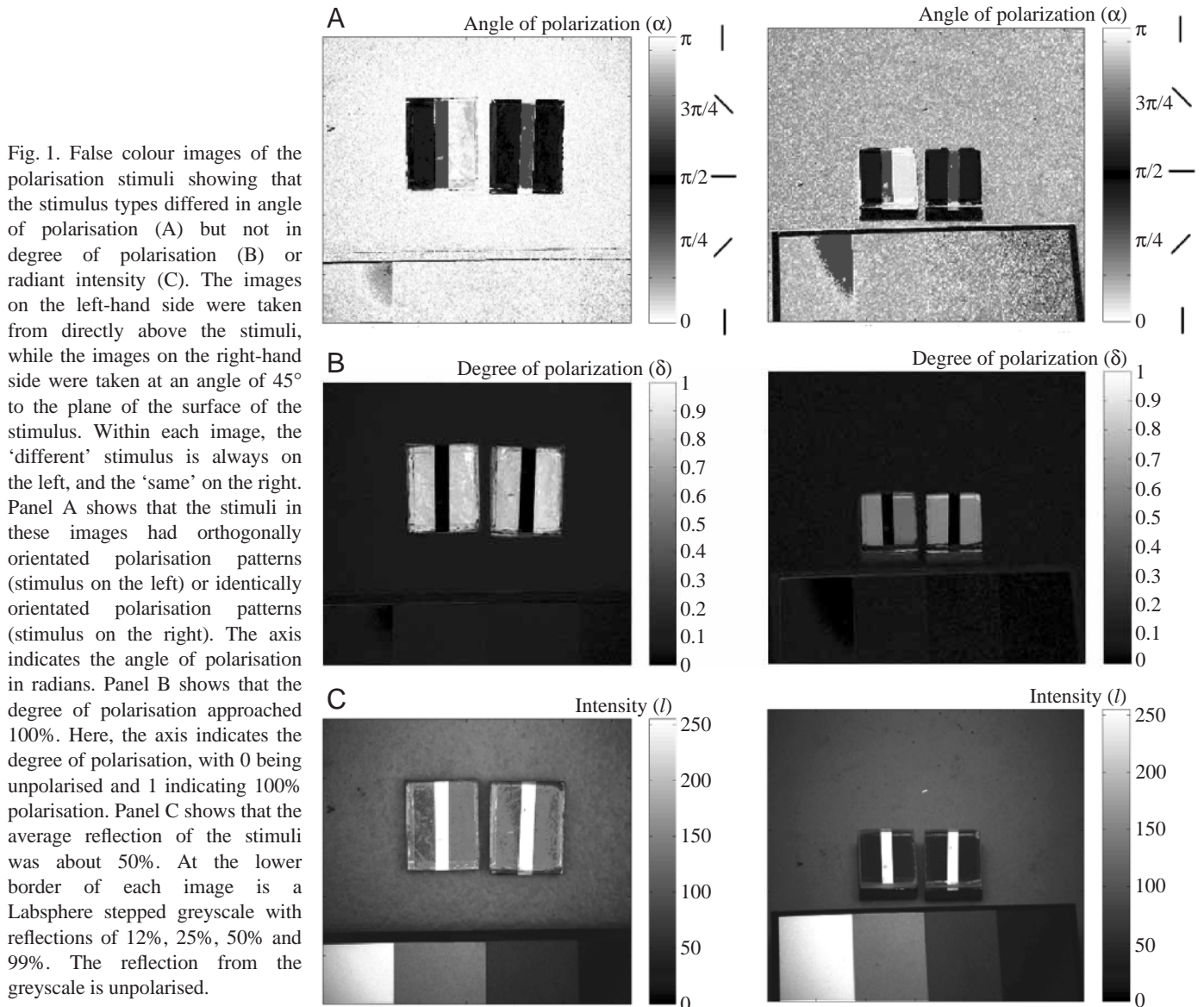As it is not known how birds may see polarisation, if they

Fig. 1. False colour images of the polarisation stimuli showing that the stimulus types differed in angle of polarisation (A) but not in degree of polarisation (B) or radiant intensity (C). The images on the left-hand side were taken from directly above the stimuli, while the images on the right-hand side were taken at an angle of 45° to the plane of the surface of the stimulus. Within each image, the 'different' stimulus is always on the left, and the 'same' on the right. Panel A shows that the stimuli in these images had orthogonally orientated polarisation patterns (stimulus on the left) or identically orientated polarisation patterns (stimulus on the right). The axis indicates the angle of polarisation in radians. Panel B shows that the degree of polarisation approached 100%. Here, the axis indicates the degree of polarisation, with 0 being unpolarised and 1 indicating 100% polarisation. Panel C shows that the average reflection of the stimuli was about 50%. At the lower border of each image is a Labsphere stepped greyscale with reflections of 12%, 25%, 50% and 99%. The reflection from the greyscale is unpolarised.

have the capacity at all, it is impossible to design a 'perfect' control. The chosen control task required the birds to classify stimuli according to whether the reflected light intensity of two sides of a stimulus was the same, or different (Fig. 2). These stimuli used spectrally neutral (grey) reflecting surfaces. This control is not perfect in that the polarisation task may be harder for the birds to learn. If the birds detect polarisation as intensity differences, the reflected angle of polarisation will change as the bird walks around the stimuli, thus causing a change in intensity as the bird moves.

The stimulus patterns (2.5 cm×2.5 cm) were attached to the top of metal blocks (2.5 cm×2.5 cm×0.6 cm; 37 g). Using metal blocks ensured that the birds could not accidentally knock the stimuli off the food wells and promoted the learning of the discrimination by increasing the cost to the bird of making a wrong choice. Each metal block was painted matt black and was laminated around the edges with clear adhesive

tape to prevent the paint from chipping, as this would have provided the birds with alternative cues with which to solve the task.

All patterns had two rectangular sub-components that were separated by a central, 0.5 cm-wide, white strip. For the control stimuli, these sub-components consisted of paper printed with various intensities of grey (Fig. 2) using a Hewlett Packard LaserJet 2100 printer. There were four types of stimulus in the control task: one 'different' (D), which consisted of one side of light grey and one side of dark grey, and three types of 'same' (S). These either had both sides of light grey (S1), both sides of dark grey (S2) or both sides of an intermediate grey (S3). Spectral reflections of the control stimuli were measured using a Zeiss MCS 501 spectrophotometer, and the mean reflections (300–700 nm) of the S1, S2 and S3 stimuli were 80%, 10% and 45%, respectively. The D target comprised strips of 80% and 10% reflection. The S1 and S2 stimuli
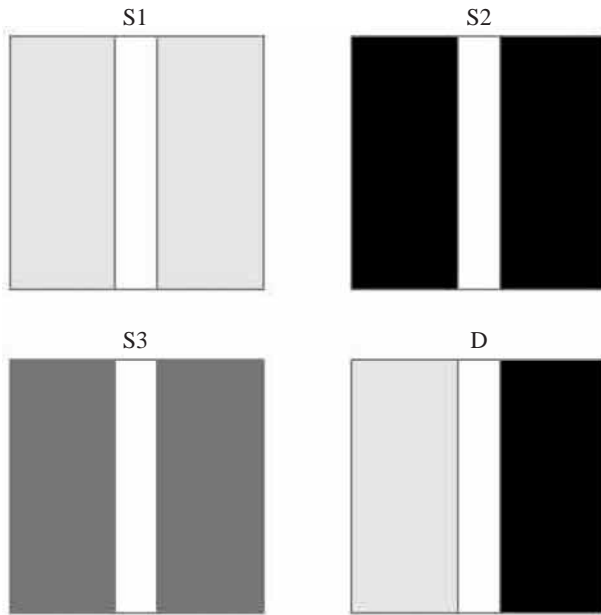
Fig. 2. Diagram of the 25 mm×25 mm control stimuli, showing the 'same' (S1–S3) and 'different' (D) patterns. Blocks of metal were covered with grey paper (as described fully in the text). S1, S2 and S3 had equal reflection on both halves of the stimuli; stimulus D had different grey levels on each half.

contained exactly the same shades of grey as the D stimuli to prevent the birds solving the task by learning to select a particular intensity. The S3 stimuli were a shade of grey that was equal to the mean intensity of the D stimuli, to prevent the birds solving the task by responding to the differences in overall intensity between the D, S1 and S2 stimuli. All the control stimuli were overlain by 3 mm-thick ultraviolet-transmitting, unstressed Perspex to protect them from damage.

The polarisation stimuli also consisted of two sub-components. The angle of polarisation of these sub-components was manipulated using filters. Instead of being covered with paper, each metal block was covered by a layer of aluminium foil, matt side upwards, to reflect the incident light equally at all wavelengths. The angle of polarisation of the reflected light was manipulated by overlaying the aluminium with two rectangular strips of linear polarising filter (Rosco #7300). The layout of the two strips was such that they

either both polarised light on each side of the stimulus with the same e-vector orientation or caused one side to polarise light in a direction orthogonal to the other side. As both sides of the stimulus were covered in polarising filter and the incident light was unpolarised, the same proportion of the light would be absorbed through both areas of filter, regardless of orientation. Intensity differences would therefore only be perceived if the animal was differentially sensitive to differently orientated, or polarised, light. The polarising filter was covered with a layer of 3 mm-thick ultraviolet-transmitting, unstressed Perspex (Fig. 3). Each stimulus was 10 mm high.

To check that the stimuli only differed in the angle of polarisation and that there were no complex interactions between the filter and the aluminium base that might create confounding intensity patterns, polarisation images were taken at both 90° and 45° to the horizontal (Fig. 1). These images show that there were no reliable intensity cues that could be used to solve the task. The stimuli were imaged at an angle of 45° to the plane surface of the stimuli, which would be the theoretical mean viewing angle of the stimuli by the birds, and at an angle of 90° (i.e. normal) to the surface of the stimulus. The latter angle is similar to the viewing direction of the birds when uncovering the food well because during this action they pecked directly on the top of the stimulus.

Experimental procedure

The procedure was similar for both tasks for quail and starlings. Birds were trained to discriminate the various types of stimuli in a foraging arena. The foraging arena was illuminated from directly above with the same light set-up as was used in the housing of the birds. The foraging arena consisted of white Conti board[TM] (55 cm×60 cm) with eight circular food wells of 1.5 cm diameter, each of which was overlain by a stimulus. Prior to starting training trials, birds were trained to push these stimuli off the food wells using behavioural shaping techniques. In each training trial, four food wells were covered by 'same' stimuli and four by 'different' stimuli. For each bird, one type of stimulus was consistently rewarded with a mealworm. Food wells underneath the other type of stimulus were left empty. We trained four quail and two starlings. Two quail and one starling were rewarded for choosing 'same' stimuli, and two quail and one starling were rewarded for choosing 'different' stimuli. There were six training stimuli of each type, and four of these
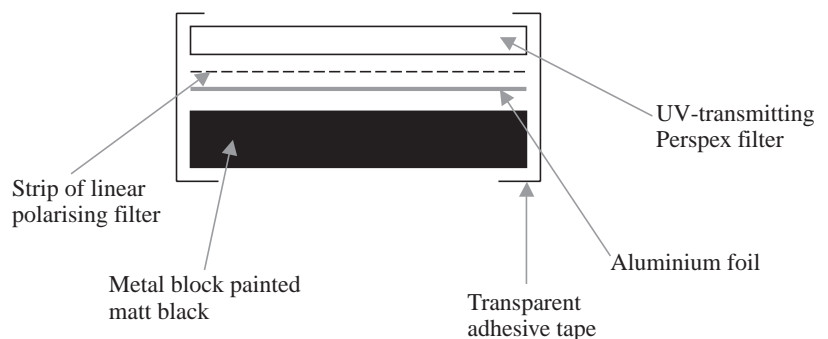

Fig. 3. Diagram of the stimuli blocks used in the polarisation discrimination (see text for dimensions).

were randomly selected for use in each trial. The location of the rewarded food wells within the foraging arena was randomised.

Quail training procedure

Birds were food deprived in a holding box (50 cm×50 cm×30 cm high) for one hour before the training trials started each day to ensure that they were motivated to search for food. Each trial was started by placing the bird in a start box in front of the arena and lasted one minute before the bird was removed from the arena. Between trials, birds were returned to the holding box, which contained water but no food, thus depriving the birds of food between trials.

Starling training procedure

An unenclosed arena is unfeasible for flying species and so, since the starlings were more settled when handling was kept to a minimum, we designed an arena in which the stimuli were moved to and presented to the bird rather than *vice versa*. For starlings, the test arena consisted of a modified cage (1.30 m×0.65 m×0.40 m high), in which two end compartments (each 0.30 m×0.65 m×0.40 m high) were separated from the main area in the middle compartment (0.70 m×0.65 m×0.40 m high) by a wire partition. Each end compartment contained a starling that had free access to both food and water and acted to prevent the test starling, which was in the middle compartment, from being socially isolated. The central compartment had a flap in the wire, through which the foraging arena could be easily passed. Starlings were presented with the foraging arena for 30 s intervals before it was removed. Between trials, the foraging arena was removed and reset, whilst the test bird remained in the main area of the test cage. The bird being tested had access to water but not to food, except for food obtained during trials.

Trial procedure

The first four stimuli that the birds chose were recorded. Each bird was considered to have made a choice when it pushed a stimulus off a food well. If the birds made no choice (i.e. no food wells were uncovered) within the trial, the trial was stopped as usual and the apparatus was reset for the next trial. These no-choice trials were recorded but were not included in the final data analysis. Each training session consisted of a block of 10 trials, with 1–5 min between each trial, and 3–4 training sessions were run each day.

The birds were trained until they achieved a mean score of 80% correct over 10 consecutive trials or until they had undergone a predetermined number of trials. The birds were not required to be 100% accurate, as in natural situations the task may change and the birds need to be able to adapt. The four quail tested had performed similar visual discrimination learning experiments before, in which they were making colour discriminations. All of the quail had learnt the colour vision discriminations within 100 trials (Smith et al., 2002), and so if the birds were still performing at random after 250 trials this was likely to be because they were unable to perceive the

required discrimination rather than that they had not had sufficient time to learn it. However, the starlings used were recently caught wild birds and were naïve to discrimination tasks. Since one of the starlings took over 400 trials to learn the control task, we extended the set number of trials to 500 for the starlings. Only if the birds were still performing at random after 500 trials would the starlings be considered unable to learn the task.

If the birds achieved the criterion of 80%, and subsequently achieved two consecutive scores of 100%, they then underwent 'probe' trials to ensure that they had not learnt to use any cue other than 'same' *versus* 'different'. Training trials were given between each probe trial to ensure that the probe trials did not affect the birds' performance. There were two types of probe trial. The first type of probe was a 'no food' probe where the birds were given a trial identical to those they had previously undergone, using the usual training stimuli, but in which no food reward was given. Correct performance would show that the birds were not using any direct cues from the food, such as olfactory cues, to solve the task. The second type of probe trial was a 'novel stimuli' probe. Here, the stimuli were identical in every way to the training stimuli but were new stimuli that had not been used during training. Correct performance on this task would indicate that the birds had learnt a general feature about the patterns rather than individual features of the training stimuli. Each bird was given three probe trials of each type, and the type of probe the bird received was alternated. There were six 'novel stimuli' probe stimuli, and four were randomly selected for use in each 'novel stimuli' probe trial.

Imaging methods

Although the different stimuli could not be differentiated by the human eye (unless looked at through a polarising filter), the stimuli were viewed and measured with an imaging polarimeter to check that there were no unintended intensity cues and that the polarisation properties of the stimuli were as designed.

To produce polarisation images it is necessary to calculate the degree and angle of polarisation of every pixel in an image. For this, it is necessary to take at least three identical images of a visual scene with a linear polariser fitted to the imaging device. We used a method modified from that of Horváth and Varju (1997) to determine whether the degree and angle of polarisation of the polarised panels of the test stimuli were as anticipated (i.e. close to 100% polarisation, with e-vectors either parallel or perpendicular to each other).

We took five images of the stimuli using the remote-controlled still-image facility of a Sony DCR-PC100E MiniDV camcorder. The camera was mounted on a tripod approximately 1 m away from the sample test stimuli, under the same lighting conditions as the behavioural experiments. Five congruent images of the stimuli were obtained through a Hoya linear polarising filter oriented at 0, $\pi/8$, $\pi/4$, $3\pi/8$ and $\pi/2$ radians to the vertical axis of the camera. A Labsphere greyscale (12%, 25%, 50% and 99% reflectance) was present in an identical position in all five images. The images were

subsequently converted to 320 pixel×240 pixel greyscale images using Adobe Photoshop 6.

Accurate estimation of polarisation parameters requires a linear relationship between signal input and output, something rarely found in digital cameras. As we knew the best-fit relationship between the measured values and the true values of the greyscale (which took the form ae^{bx}) we were able to transform our measurements to produce an approximately linear response.

The five linearized images were subsequently analysed by finding a best-fit curve of the form $I=a[\sin(\theta+b)^2]+c$ through each set of congruent pixels, where I is the intensity of a given pixel and θ is the angle of rotation of the linear polariser. The constants 'b' and 'c' influence the horizontal and vertical offsets of the function, while 'a' influences its amplitude. We used Matlab 6 to calculate the maximum (I_{max}) and minimum (I_{min}) values of this function for each congruent pixel in the five polarisation images. The degree of polarisation, δ, is then given by

$$\delta = (I_{max} - I_{min})/(I_{max} + I_{min}) .$$

Zero polarisation (when $I_{max}=I_{min}$) produces $\delta=0$, and complete polarisation (when $I_{min}=0$) produces $\delta=1$.

The angle of polarisation (α) is simply the angle at which I_{max} occurs (i.e. the dominant plane at which light is polarised). δ and α can then be represented using false colour images (Fig. 1).

The polarisation images of the stimuli confirmed that the stimuli did only differ in polarisation properties and that there were no cues available to solve the task by any animal that was not sensitive to the angle of linear polarisation (Fig. 1). This was found to be the case for images taken at both 90° and at 45° to the horizontal.

Results

As there was a low sample size, a general test of whether the birds' mean success rate was significantly different from 50% or 80% or not could not be carried out. Each bird was therefore treated individually, and non-parametric tests were carried out on the last 10 trials of each bird on each task to test whether the median was significantly different from the criterion of 80%. This tests whether each bird has learnt the individual tasks by reaching the criterion level of discrimination ability. Similar non-parametric tests were also carried out on the results of the probe trials to ascertain whether performance drops during the probe trials or whether the birds remain correct in the discrimination. We used non-parametric statistics because the data were not normally distributed as they consisted of percentages with a limited range of possible values and transformations could not normalise the data.

Quail

In the control task, each quail reached the criterion level of a mean score of above 80% correct over 10 sequential trials (means: quail 1, 84.2%; quail 2, 80.9%; quail 3, 91.7%; quail

4, 82.5%). For quail 1, 2 and 4, the scores on the last 10 consecutive training trials were not significantly different from the criterion value of 80% (see Table 1). The scores for quail 3 were significantly different from 80%, being significantly higher ($N=10$, $W=49.0$, $P=0.016$). All four quail were trained over a period of several weeks and varied in their rate of learning. Performance on the control task did not drop significantly from the criterion level of 80% when trials were either unrewarded ('no food' probe: mean score for all quail=77.8%) or when novel stimuli were used ('novel stimuli' probe: mean score for all quail=79.9%).

In the polarisation task, none of the quail achieved the criterion of a mean score of 80% correct over 10 consecutive trials within 250 trials. Also, in the last 10 training trials within the predetermined limit of 250 trials, all quail were performing at a level that was significantly below 80% (Table 1), which appeared to be completely random (means: quail 1, 52.5%; quail 2, 55.0%; quail 3, 57.5%; quail 4, 55.0%).

Starlings

The starlings were trained over a period of several months and varied in their rate of learning. In the control task, both starlings reached the criterion level of a mean score of above 80% correct over 10 consecutive trials (means: starling 1,

Table 1. *Wilcoxon one-sample tests comparing the estimated medians of the birds for the last 10 training trials and the probe trials with the criterion of 80% for both the control intensity discrimination and the polarisation discrimination*

Bird		Control experiment			Polarisation experiment
		Last 10 training trials	Probe type 1	Probe type 2	Last 10 training trials
Quail 1	Median	87.50	75.00	75.00	50.00
	W	35.00	2.00	2.00	4.00
	P	0.476	0.789	0.789	0.019
Quail 2	Median	83.50	87.50	79.25	50.00
	W	33.00	3.00	3.00	3.00
	P	0.610	1.000	1.000	0.014
Quail 3	Median	100.00*	71.00	91.75	62.50
	W	49.00	2.00	5.00	4.00
	P	0.032	0.789	0.423	0.019
Quail 4	Median	75.00	79.25	75.00	50.00
	W	27.00	3.00	0.00	0.00
	P	1.000	1.000	0.181	0.006
Starling 1	Median	100.0	75.00	100.0	50.00
	W	35.00	3.00	9.00	0.00
	P	0.476	0.584	0.201	0.006
Starling 2	Median	87.50	87.50	73.00	62.50
	W	30.00	7.00	3.00	6.00
	P	0.838	0.584	0.584	0.032

*Significantly greater than 80% ($W=49.0$, $P=0.016$).

Probe trials for the polarisation experiment were not carried out as the criterion was not reached.

87.5%; starling 2, 82.5%) and were not significantly different from the criterion value of 80% (see Table 1). Performance did not drop during the probe trials ('no food' probe: mean score for starlings=81.3%; 'novel stimuli' probe: mean score for starlings=82.4%).

In the polarisation task, neither of the starlings achieved the criterion of a mean score of 80% correct over 10 consecutive trials within the set 500 trials. By their last 10 training trials within the predetermined limit of 500 trials, the birds were performing at a level that was significantly lower than 80% (Table 1) and which, like the quail, appeared random (means: starling 1, 55.0%; starling 2, 62.5%), with neither animal showing evidence of having learnt to solve the discrimination task.

Discussion

Our results show that quail and starlings are unable to solve a foraging task using the available polarisation cues. They therefore appear unable to perceive differences in the pattern of polarised light reflected from objects. Specifically, the birds tested were unable to discriminate stimuli in which the e-vector orientations of the stimulus sub-components either differed by 90° or had the same angle of polarisation. However, all the birds were able to learn a control discrimination that tested the non-visual elements of the task, by requiring the birds to make a same–different judgement based upon differing intensities. The control was not intended to be the visual equivalent of polarisation sensitivity; rather, it was to test that (1) the animals were capable of learning visual discriminations using our paradigm and (2) that the birds had the necessary cognitive ability to perform the same–different discrimination we required them to make in the polarisation task. All the birds learned the control task and continued to perform the discrimination successfully both without reward and when using novel stimuli, showing that they had learned the discrimination based upon a general feature of the pattern rather than relying on olfactory cues or individual features of the training stimuli. Therefore, we attribute the failure of all the birds to discriminate between the two types of polarisation stimuli to an inability to perceive the angle of polarisation.

This experiment is the first behavioural polarisation discrimination task of its type to be tested on birds. Despite the evidence in the literature that the pattern of skylight polarisation may affect the migratory orientation of birds (Helbig, 1990, 1991; Munro and Wiltschko, 1995), we found no evidence to support the possibility that quail (non-migratory) or starlings (migratory) may be able to perceive the angle of linearly polarised reflections. These results are therefore consistent with the results of other behavioural tests of polarisation sensitivity in birds (Coemans et al., 1994) and electrophysiological studies, which have failed to elicit polarisation-sensitive signals from avian retinas (Vos Hzn et al., 1995).

This discrepancy between results for different methodologies may be explained by the nature of the discrimination task. Detection of polarisation patterns in the sky might be a different visual task for the animals from the foraging task presented in this experiment, as observing skylight polarisation patterns for orientation tasks rather than target discrimination may require different capabilities of the birds' visual systems. It is also possible that polarisation detection is only achievable in certain regions of the retina. As the experiments presented here study the ability of the birds to discriminate polarisation patterns when foraging, they may not be testing the same mechanisms that are required to detect skylight polarisation patterns. Foraging tasks are likely to preferentially use the dorso-temporal retina, whereas other tasks involving viewing the sky will utilise the ventral retina. Consequently, although the birds may be unable to use polarisation sensitivity in foraging tasks using object detection or recognition (e.g. for camouflage breaking when foraging), it is still possible that they are sensitive to skylight polarisation patterns.

Skylight polarisation patterns consist of a gradual change in angle of polarisation across the sky depending on the position of the sun in the sky (Pomozi et al., 2001). Insects are able to infer the position of the sun by seeing only a small area of clear sky (Labhart and Meyer, 1999), which might contain only a small variation in angle of polarisation. They must therefore be able to differentiate fine differences in angle of polarisation. Starlings and quail may be able to detect fine gradations in angle of polarisation of skylight polarisation patterns even though they are unable to solve the polarisation task, due to the unnatural situation of a 90° difference between the two angles of polarisation. However, the latter represents the greatest possible contrast between two differently polarised stimuli. The results therefore suggest that polarisation cues are not readily used for object detection by quail or starlings. Japanese quail and European starlings therefore appear unable to use polarisation sensitivity in a foraging task. This is important due to the increasingly widespread belief that birds are sensitive to linear polarisation (e.g. Able, 1996; Horváth and Pomozi, 1997).

These findings should not be generalised to all species of birds. Since the only evidence that birds may have polarisation sensitivity comes from the literature on migration (e.g. Able and Able, 1993; Munro and Wiltschko, 1995; Akesson and Backman, 1999), polarisation sensitivity may only occur in certain migrating species. It would therefore be interesting to repeat this discrimination experiment with a species in which migratory orientation has been found to be influenced by skylight polarisation.

It is also possible that other bird species in which a potential visual advantage of polarisation sensitivity is predicted, such as piscivores, may yet be demonstrated to have this ability. Polarisation sensitivity is perhaps most likely to occur in species that hunt by looking through water surfaces. Species of bird that regularly catch fish would therefore be good candidate species to test for polarisation sensitivity, as the ability to block out horizontally polarised light that is reflected off the water surface would allow aerial piscivores to see into

the water more clearly and may increase their foraging efficiency. Underwater hunters could also gain an increase in target contrast in certain visual situations (Shashar et al., 1998, 2000).

Polarisation sensitivity in vertebrates is currently thought to be dependent on the regularity in the pattern of cone mosaics in the retina. For example, fish that have fairly regular cone mosaics have been found to have polarisation sensitivity, whilst others with more irregular cone mosaics have been found not to be sensitive to the polarisation of light (Novales-Flamarique and Hawryshyn, 1998b). Hence, it may be possible to predict which species of bird are likely to possess polarisation sensitivity by studying the regularity within their cone mosaics (Hart, 2001b). As previous work on cone mosaic patterns of birds is very limited (see Hart, 2001b), this may provide an interesting area for future research.

In conclusion, Japanese quail and European starlings do not appear to be sensitive to the plane of polarised light, as they cannot perceive the different angles of target polarisation with this foraging task. It is, however, possible that other bird species may yet be demonstrated to have this ability. It is also possible that observing skylight polarisation patterns for orientation tasks may require different capabilities of the birds' visual systems than the type of target discrimination tested in this task.

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