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THE INFLUENCE OF STIMULUS AND BACKGROUND COLOUR ON SIGNAL VISIBILITY IN THE LIZARD *ANOLIS CRISTATELLUS*

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Summary

Anoline lizards communicate with visual displays in which they open and close a colourful throat fan called the dewlap. We used a visual fixation reflex as an assay to test the effects of stimulus *versus* background chromatic and brightness contrast on the probability of detecting a moving coloured (i.e. dewlap-like) stimulus in *Anolis cristatellus*. The probability of stimulus detection depended on two additive visual-system channels, one responding to brightness contrast and one responding to chromatic contrast, independent of brightness. The brightness channel was influenced only by wavelengths longer than 450 nm and probably received input only from middle- and/or long-wavelength photoreceptors. The chromatic contrast channel appeared to receive input from three, or possibly four, different classes of cone in the anoline retina, including one with peak sensitivity in the ultraviolet. We developed a multi-linear regression equation that described most of the results of this study to a reasonable degree of accuracy. In the future, this equation could be used to predict the relative visibility of different-coloured stimuli in different habitat light conditions, which should be very useful for testing hypotheses that attempt to relate habitat light conditions and visual-system response to the evolution of signal design.

Key words: *Anolis cristatellus*, lizard, communication, vision, colour, motion, signal, dewlap.

Introduction

Animal signals exhibit amazing diversity, and even closely related species often have signals that differ greatly in physical form. Understanding the evolutionary forces that give rise to signal form and diversity is a major goal of studies of animal communication. In the case of visual signals, colour has received the most intense scrutiny in the literature (for a review, see Bradbury and Vehrencamp, 1998). The colours of a visual signal can serve various different functions, such as an indicator of breeding or dominance status, as an indicator of condition or as a signal of species identity. However, before a signal can serve any of these functions, it must be seen by its intended viewer, and this often occurs in a complex visual environment. Thus, one of the most critical functions of colour is to make the signal easy for the intended receiver to detect. To do this, a signal colour must efficiently stimulate the visual system of the intended viewer in the habitat light and visual background conditions in which it is normally viewed. Thus, both the sensory system of the receiver and the habitat light conditions will influence the effectiveness of any given signal colour (e.g. Endler, 1992; Endler and Théry, 1996; Lythgoe, 1979; Vorobyev et al., 1998). It has been hypothesized that diversity in the signal colours of closely related species, or in distinct populations of the same species, may have evolved because of differences in the light conditions of their different microhabitats (Endler, 1992).

To test this hypothesis, one must quantify the relationship between signal colour, the light conditions in which the signal is viewed and the response properties of the visual system. However, visual signals rarely consist solely of colour, but rather tend to be made up of complex combinations of colour, movement, form and pattern (Hailman, 1977). Each of these components may be processed in parallel by different neural pathways, each may receive input from different sets of peripheral receptors, and the different stimulus components may interact in complex ways. Thus, to understand the role that visual-system response plays in the evolution of colour patterns, it is necessary to use experimental stimuli that are similar to those experienced by the signal receiver in nature.

Anoline lizards communicate with visual displays that consist, in part, of rapid opening and closing of a colourful throat fan called the dewlap. There are approximately 300 species of anoline lizards, and dewlap colour varies considerably among them. In the most common use of the dewlap, territorial males display spontaneously from conspicuous perches to repel other males and to attract females (Fleishman, 1992). The effectiveness of this display depends largely on the efficiency with which it can attract the attention

of conspecifics, which are usually some distance away. Different species of anoline lizard occupy distinct microhabitats, and it has been hypothesized that the among-species diversity in dewlap colour evolved, at least in part, because of differences in the effectiveness of different colours in the light conditions typical of different microhabitats (Fleishman, 1992; Persons et al., 1999). Microhabitats differ in the spectral quality and intensity both of downwelling light and of light reflecting from the background vegetation against which displays are typically viewed (Fleishman et al., 1997).

Photons from any visual stimulus are captured by sets of retinal photoreceptors (cones only in the case of anoline lizards) with differing spectral sensitivity. Animal nervous systems process this input in two ways. The excitation of different classes of cone may be summed, yielding a sensation often referred to as brightness (or perceived intensity). Such a visual-system channel is referred to as an achromatic channel. Alternatively, the excitation of the different cone classes may be compared in some way. A channel that does this is referred to as a chromatic channel. Both processes are likely to occur in parallel in the visual system of any species.

Our study was carried out on *Anolis cristatellus*, whose visual physiology and anatomy are typical of most of the anoline species that have been studied (Fleishman, 1992; Fleishman et al., 1993; Fleishman et al., 1995; Fleishman et al., 1997; Persons et al., 1999; E. R. Loew, personal communication).

The anoline eye is adapted for high-acuity diurnal vision, and the retina contains four spectral classes of cone and no rods (Underwood, 1970; Fite and Lister, 1981; Fleishman et al., 1993). Fig. 1B,C illustrates the overall spectral sensitivity of *Anolis cristatellus*, and Fig. 1A illustrates the spectral response of each of the four classes of cone photoreceptor (modified by their typical oil droplet filters). These cone classes are referred to as UV (ultraviolet), S (short-wavelength), M (middle-wavelength) and L (long-wavelength) on the basis of the relative position of the wavelength of peak sensitivity.

Fleishman (Fleishman, 1986) developed an assay to test the relative effectiveness of different visual stimuli in eliciting visual attention in anoline lizards. Small moving lures were presented in the visual periphery and, if the stimulus was detected, the lizard would shift its gaze, reflexively, bringing the image of the moving stimulus onto the central fovea. The probability of detection depended on the motion pattern of the stimulus. Once attention had been shifted towards the object, the lizard examined it with foveal vision and determined whether the object was of further interest. Persons et al. (Persons et al., 1999) used this method to study the effects of the spectral quality and intensity of a moving stimulus on detection probability, and found that contrast in brightness (i.e. perceived intensity) between the stimulus flag and the background against which it was viewed was the most important factor. A difference in spectral quality between the stimulus and background increased the detection probability in an additive manner. In both these studies, the moving stimuli bore some general resemblance to natural objects (i.e. food in

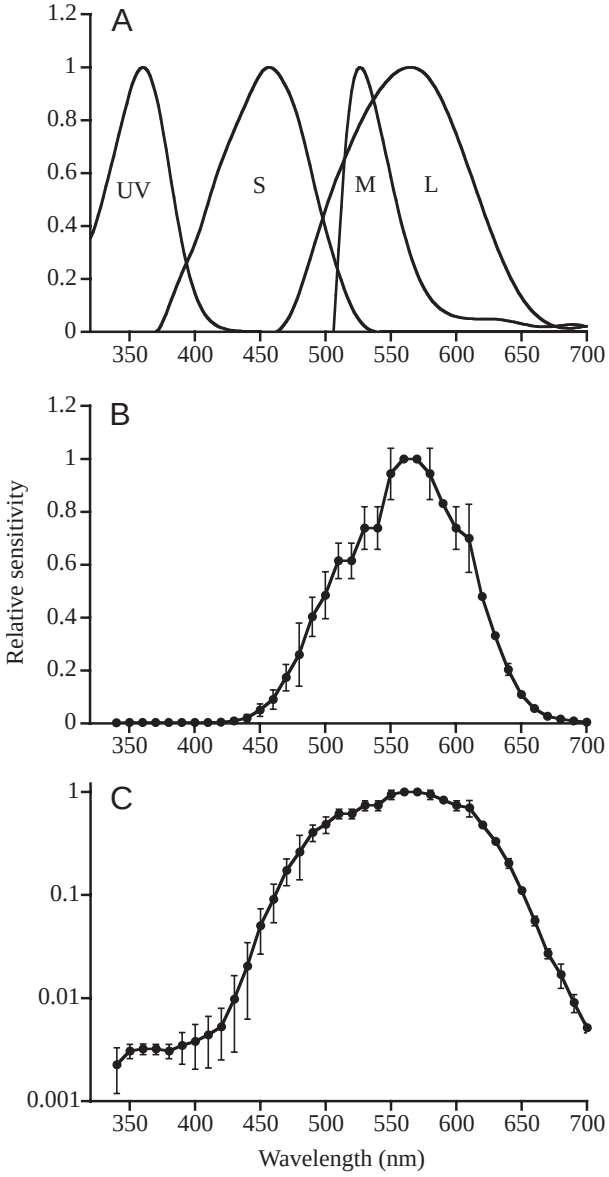

Fig. 1. (A) The spectral sensitivity function for each of four classes of cone found in the retina of *Anolis cristatellus*. Each cone class has a pigment with a characteristic absorption function and an oil droplet that filters the light approaching the cone. The sensitivity functions were created by multiplying the pigment absorption by the transmission spectrum of the oil droplet type most commonly associated with that pigment. These estimates are smoothed functions based on microspectrophotometric data provided by Dr E. Loew (personal communication). The label on the curve for each class of cone refers to the position of its peak wavelength: UV (ultraviolet), S (short), M (middle) and L (long). (B) The spectral sensitivity of *Anolis cristatellus* based on electroretinographic flicker photometry at a stimulation rate of 6 Hz (for details, see Fleishman et al., 1997). The data are plotted on a linear scale for comparison with the curves in A. (C) The spectral sensitivity function plotted on a logarithmic scale to illustrate the fact that there is measurable sensitivity at wavelengths shorter than 430 nm, although this sensitivity is two orders of magnitude lower than the peak sensitivity. Values are means ± S.D., N=3.

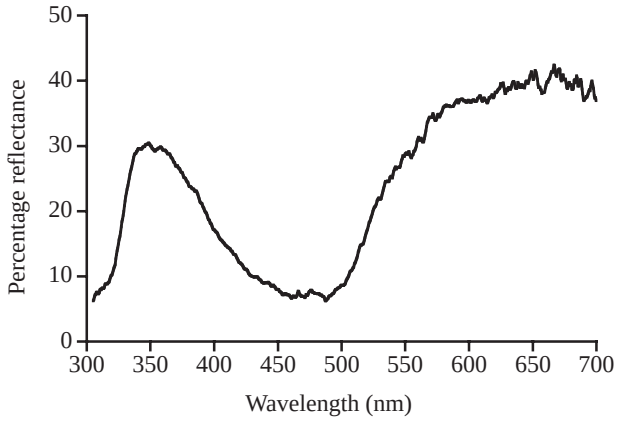

Fig. 2. A typical example of the spectral reflectance of the centre of the dewlap of a male *Anolis cristatellus*. The data were collected using a fibre-optic reflectance probe input to an Ocean Optics PS1000 spectroradiometer, with a tungsten/deuterium fibre-optic light source. The data are expressed as the percentage of light reflected relative to a white reflectance standard.

the study of Fleishman, 1986; an anoline dewlap in the study of Persons et al., 1999). However, once the animals shifted their gaze towards the stimuli, they did not treat the stimuli as the objects that they superficially resembled. Thus, stimulus detection and stimulus recognition occur as two distinct sequential steps. In earlier studies, dewlap colour has been shown to play some role in the recognition of conspecifics (Losos, 1985; Macedonia and Stamps, 1994). Here, we focus on the role of dewlap colour in making the visual signal effective in initially eliciting the attention of conspecifics.

In this study, we presented coloured, moving stimulus flags in the visual periphery and tested the probability that visual fixation would be elicited as a function of the brightness and chromatic contrast between the stimulus and its visual background. The size, motion pattern and shape of the flags roughly approximated the pattern of visual stimulus that would be produced by a natural dewlap display. Our aim was to quantify the relationship between the spectral quality of a dewlap and its effectiveness in stimulating the visual system when viewed under conditions typical of different habitats. The spectral reflectance of the dewlap of *A. cristatellus* is shown in Fig. 2 and is typical of that of most anoline dewlaps, with reflectance peaks at long and short wavelengths, the latter usually in the ultraviolet. Among-species differences in dewlap appearance arise from differences in the position of the long-wavelength peak and the amplitude of the middle- and short-wavelength reflectance. For our stimulus flags, we used spectra that covered the range of among-species variation in dewlap colour. For the visual background in our experiments, we used spectra typical of two extremes of natural anoline habitats: narrow-band green light typical of closed-canopy wet tropical forest, and broadband, grey light, typical of xeric habitats (L. J. Fleishman, unpublished data).

Our aim in these experiments was to test the response of anoline lizards to stimuli that were sufficiently general (i.e. not identical to real dewlaps) to enable us to gain a broad general understanding of how detection by the visual system is influenced by stimulus and background spectral quality and intensity. However, because of the complexity of response of the visual system, we tested responses to stimuli whose size, pattern of motion and distance from the viewer were typical of the stimulus provided by a dewlap display presented under average natural conditions. Our stimuli were not meant to be mistaken for dewlaps, but rather to test, in a more general way, visual response to the combination of variables that would be present in a natural dewlap display. We used visual fixation as an assay of response, since this directly tests the ability of different stimulus colour patterns to elicit the attention of an anoline lizard, which is a critical feature of an effective visual signal. We then related response probability to measurable physiological features of the visual system to develop a general model that could be used to predict the relative visibility of different dewlap spectral patterns under a wide range of natural habitat light conditions.

Materials and methods

Overview

In these experiments, moving stimulus flags were presented in the visual periphery of *Anolis cristatellus*, and the probability that the lizard would notice the motion and shift its gaze towards the stimulus was assessed as a function of the contrast in spectral quality and intensity between the moving flag and its visual background. At the start of each experimental trial, the direction of gaze of the lizard was drawn towards a fixed location, and the moving stimulus flag was then abruptly moved into and then out of view of the visual periphery of the lizard. We observed whether or not the lizard shifted its gaze towards the moving flag. Every stimulus combination (i.e. each stimulus/background contrast condition) was viewed by approximately 45 individuals, and the effectiveness of the stimulus at drawing attention was quantified by recording the number of individuals that responded.

Definition of terms

We use the term ‘intensity’ (or ‘radiance’) to refer to the objective strength of the stimulus in units of radiance ($\mu mol^{-1}\,s^{-1}\,m^{-2}\,sr^{-1}$, where $1\,\mu mol=6.02\times10^{17}$ photons, measured from 350 to 700 nm). To design the experiments, we needed an estimate of how intense each stimulus and background appeared to the lizard. We refer to the perceived intensity as ‘brightness’. We estimated brightness by multiplying the radiance spectrum of each stimulus (or background) by the relative spectral sensitivity function for *A. cristatellus* over the range 350–700 nm (from Fleishman et al., 1997), which was based on electroretinographic (ERG) flicker photometry with a 6 Hz flicker rate. The spectral sensitivity is shown in Fig. 1B,C. This procedure has been shown to provide a good estimate of brightness for the behavioural task used in these experiments (Persons et al., 1999). It is possible that the

actual brightness perceived by the lizard for this visual task may differ somewhat from our estimate. In the Discussion, we consider the implications of a possible difference between estimated and actual brightness.

We define 'brightness contrast' as $(B_s–B_b)/(B_s+B_b)$, where B_s is the brightness of the stimulus flag and B_b is the brightness of the background. We define 'chromatic contrast' as the difference in spectral quality between the stimulus flag and the background, independent of brightness contrast. A more formal quantitative definition of chromatic contrast, based on the visual system of *A. cristatellus*, is presented below.

A series of different stimulus and background spectra was tested in these experiments. For simplicity, we refer to these by their appearance to a human observer (e.g. white, green, red, etc.). The actual spectra associated with these different colour names are described below.

Study subjects

The experiments were carried out between 3 January and 10 July 1998 on adult male *A. cristatellus*. Twenty individuals were collected for previous experiments from Puerto Rico (for details, see Fleishman et al., 1997). Thirty-one additional males were acquired from a feral population in southern Florida by a commercial supplier (Glades Herps, Inc.). All lizards were maintained in our laboratory for at least 1 week prior to the experiments. Sample sizes for each experiment ranged from 44 to 47 individuals (most individuals were used in more than one experiment). Lizards were maintained in a cage (33 cm×19 cm×22 cm; length×width×height) under a 12 h:12 h light:dark cycle (a combination of incandescent and fluorescent lights) within a temperature- and humidity-controlled room (85 °C; 80 % relative humidity). Lizards were provided with water every other day and fed 4–5 vitamin-supplemented crickets twice weekly.

Experimental arrangement

For each set of trials, an individual lizard was placed in a five-sided Plexiglas cage with a screen top (see Fig. 3A). A 25 W incandescent light suspended 1 m above the top of the cage was the only light source, other than the stimulus and background, visible to the lizard. An opaque cardboard sheet extended over the front of the top of the cage, on the side where the stimulus and background were located, to prevent the 25 W light from illuminating either. A 38 cm branch oriented at 40 ° to the ground ran along the back wall of the cage and served as a perch for the lizard. All the walls of the cage, with the exception of the wall opposite the perch, were painted a neutral grey. The wall directly opposite the perch was transparent, and the lizard was observed with a video camera (Pulnix tm 745 monochrome camera with a zoom lens) placed 60 cm from this wall. The wall opposite, and at 45 ° to, the perch contained a small opening through which the stimulus flag and stimulus background were visible to the lizard. The entire cage and camera were surrounded by a black curtain. The lizard was observed on a video monitor from behind this curtain, and the experimenter was not visible to the lizard while behind the curtain.

Stimulus design

A stimulus consisted of the motion of a small flag into, and then out of, view of the lizard through a small opening in one side of the cage. The stimulus flag consisted of an approximately square piece of white aluminium mounted onto a Grass oscillograph pen motor by a 5 mm wide, 2.5 cm long arm (Fig. 3C). Stimulus motion was created on a computer and output through a digital/analog converter to a power amplifier that drove the motion of the pen motor. Since the stimulus was mounted on a pen motor, which produced rotational movement, the stimulus flag and viewing window were shaped to follow the rotational movement (see Fig. 3C,D).

At the start of each experimental trial, the stimulus flag was positioned below the opening, out of view of the lizard. It then rose at constant velocity for 0.083 s until 1.4 cm of the stimulus square (but not the stimulus arm) was in full view to the lizard (Fig. 3C,D). The stimulus flag stayed at this position for 0.83 s before moving down and out of view in a time of 0.083 s. To be certain that the stimulus was properly positioned relative to the point of view of the lizard, we examined the view with a mirror placed at the full range of positions of the eye of the lizard prior to each set of experimental trials. A 'lizard's-eye view' of the stimulus is shown in Fig. 3D.

The optical arrangement used to create stimulus/background spectral combinations is illustrated in Fig. 3A. The spectrum and intensity of each background and stimulus were measured with a calibrated Ocean Optics PS1000 fibre-optic spectroradiometer. A radiance probe was attached to the input end of the system, and it was placed in the position of a lizard viewing the stimuli and was sighted on either the flag or the background immediately behind the flag.

The background was created by shining a diffuse light from a 300 W xenon arc lamp onto the rear side of a piece of translucent white tracing paper. Two different background spectra were employed in the experiments. The 'white' background was created by passing the light through a neutral density filter (optical density 0.8). The green background was created by passing the light through a green filter (Kodak Wratten no. 99, 550 nm peak). The spectra of the two backgrounds are shown in Fig. 4A. The total radiance of the white and green backgrounds was adjusted so that their estimated brightness to the lizard was nearly equal. The radiance of the green background was 0.028 $\mu mol\,m^{-2}\,s^{-1}\,sr^{-1}$, while that of the white background was 0.053 $\mu mol\,m^{-2}\,s^{-1}\,sr^{-1}$.

To illuminate the stimulus flag, the light from the xenon lamp was passed through a 50/50 mirror-type beam splitter. The reflected light was focused, passed through a fused silica linearly variable neutral density filter and a coloured filter and into a liquid light guide. The diffuse cone of light emerging from the output-end of the light guide was used to illuminate the white surface of the stimulus square uniformly. The square was positioned at 45 ° to the opening of the light guide and to the face of the viewing window, as shown in Fig. 3A. The cone of output light was shaped such that it completely and uniformly illuminated the stimulus flag surface as it moved into view, but did not strike the background or the opening of the

viewing window. This produced the effect illustrated in Fig. 3D. The lizard viewed a stimulus flag that was uniformly illuminated by the light from the light guide, independent of any light from the background or from within the cage itself. Details concerning the design of the different stimuli are described in the legend to Fig. 4. For experiment 3, a modification of the optical arrangement was used to create the stimulus spectrum shown in Fig. 4D. In this case, green filtered light (from a 50 W QTH light source) was combined with ultraviolet filtered light from the xenon source using a beam combiner. This combined beam was then passed through the linearly variable neutral density filter and to the input of the liquid light guide. The optical arrangement is illustrated in Fig. 3B.

Experimental protocol

In the experiments described below, lizards were presented with different stimulus flags of differing spectral quality viewed against one of two spectral backgrounds. For clarity, a single stimulus presentation is referred to as a 'trial'. Each time a lizard was introduced to the cage, it was presented with a series of eight different 'trials', which we refer to as a 'set' of

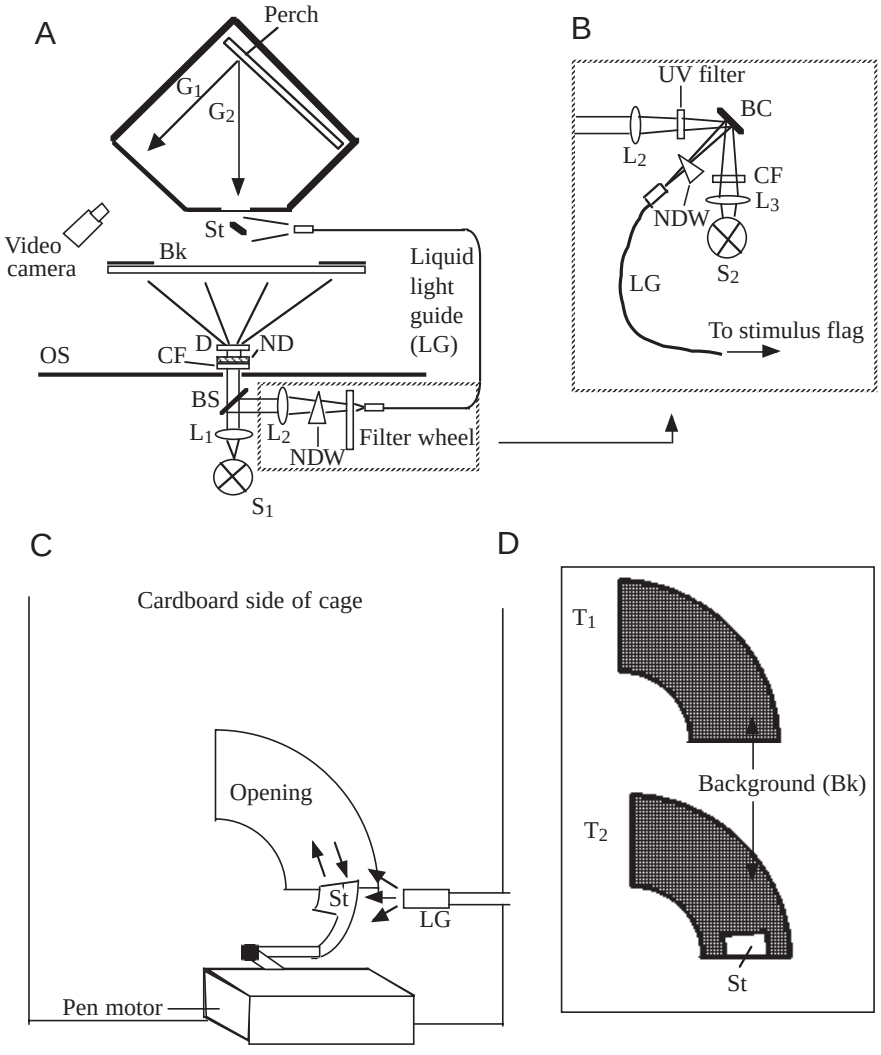

Fig. 3. (A) Diagram of the optical arrangement and cage used in these experiments viewed from above. Each lizard was placed on the perch, and its gaze was initially directed monocularly in direction G_1, where it was observed with the video camera. The stimulus flag (St) was then set into motion. If the lizard noticed the flag, it shifted its gaze to direction G_2. The stimulus flag was visible to the lizard through a small opening in the front of the cage. It was uniformly illuminated with a cone of light emerging from the liquid light guide (LG) and was viewed against the uniformly illuminated background (Bk), which consisted of a piece of white tracing paper. The light source for Bk and St was a 300 W xenon arc lamp (S_1). The output was collimated by a lens (L_1) and passed through a mirror-type beam splitter (BS). The direct light path passed through a colour filter (CF) (green background only), a set of neutral density filters (ND) and a Precision Optics holographic diffuser (D) to illuminate the background (Bk) uniformly. The second path from the beam splitter was passed through a focusing lens (L_2), through a continuously variable neutral density wedge (NDW), used to control stimulus intensity, and through a colour filter mounted in a filter wheel. The filter wheel was rotated to place different colour filters in the light path. This light was then focused into the liquid light guide and provided the irradiation of the stimulus. An opaque screen (OS) prevented stray light from striking the background. (B) In experiment 3, the portion of the apparatus enclosed by the rectangle in A was replaced by that shown here. In this case, the light path from S_1 reflecting off the beam splitter was focused by L_2 and passed through a narrow-band ultraviolet (UV) filter. Light from a second light source (S_2) was focused by a lens (L_3) and passed through a glass green-coloured filter (CF). Each of these light paths was reflected off a beam combiner (BC), and the combined light path was passed through a continuously variable neutral density wedge (NDW) and focused into the liquid light guide (LG). This arrangement was used to create a stimulus spectral pattern that was the sum of the ultraviolet and green components. (C) A drawing of the stimulus flag positioned in front of the viewing opening in front of the cage. The stimulus flag (St) was painted white, and it was uniformly illuminated by the output from the liquid light guide (LG). It was angled at 45° towards the line of sight of the lizard (G_2 in A). In this way, it could be uniformly illuminated from the side. In this drawing, the screen that forms the background for the stimulus is absent. This screen would be immediately behind the stimulus flag. (D) A 'lizard's-eye-view' of the stimulus flag and background through the viewing opening. At T_1, prior to the movement of the stimulus flag (St), the lizard saw only the uniformly lit background. At T_2, the stimulus moves up and into the lizard's view. It then quickly moves back out of view. The spectral quality and intensity of the stimulus flag and background were controlled independently.

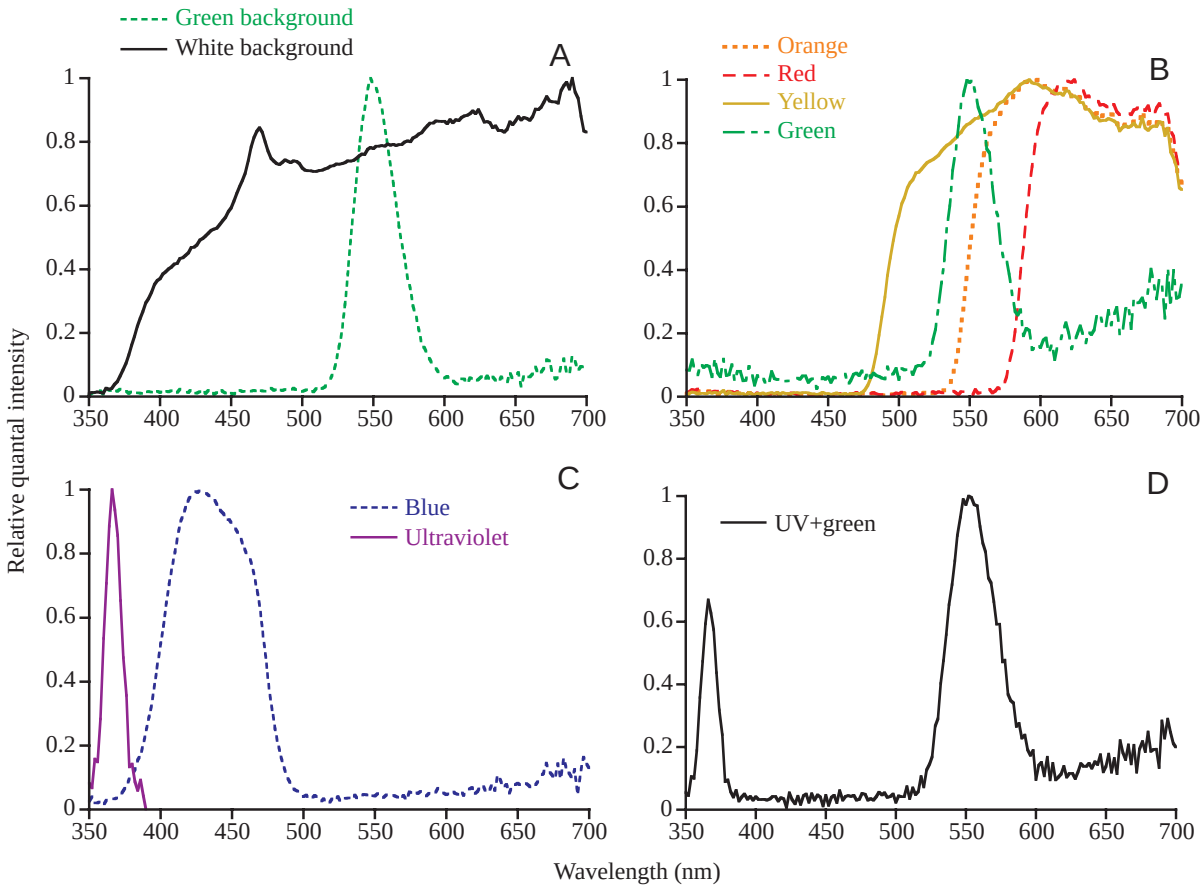

Fig. 4. The spectral radiance of each stimulus and background used in the experiments was measured using a radiance probe attached to the fibre-optic input of an Ocean Optics PS1000 fibre-optic spectroradiometer. The radiance probe was placed where the lizards sat during the experiment. Prior to each measurement, a test light was passed out through the radiance probe to identify the precise sampling location. Each spectrum is shown relative to its peak value. The intensities of different spectra were adjusted using spectrally flat neutral density filters that altered the intensity at all wavelengths equally. (A) The two background spectra employed in the different experiments. The 'green' background was created with a Kodak Wratten filter no. 99 (550 nm peak transmittance). The 'white' background was created without colour filters. Neutral density filters were used with both background spectra to make them equal in estimated brightness. The total radiance of the green background was $0.029\,\mu mol\,m^{-2}\,s^{-1}\,sr^{-1}$ (where $1\,\mu mol=6.02\times10^{17}$ photons), and the radiance of the white background was $0.053\,\mu mol\,m^{-2}\,s^{-1}\,sr^{-1}$, which was calculated to have an estimated brightness equal to that of the green background. (B) The stimulus flag spectra used in experiment 2. The four spectra used were referred to as green, yellow, orange and red on the basis of their appearance to a human observer. The green stimulus spectrum was created with a Kodak Wratten filter no. 99. The other spectra were created with glass long-pass colour filters (Oriel) with cut-on wavelengths at 495 nm (yellow), 550 nm (orange) and 590 nm (red). Neutral density filters were mounted with each colour filter to adjust them to equal brightness. They were set to be nearly equal in estimated brightness to the background at the middle intensity setting on the variable neutral density filter. (C) Spectra of the stimulus flags employed in experiment 2. The green background from (A) and the green stimulus from (B) were also employed in this experiment. The ultraviolet stimulus was created with an Oriel UV interference filter with a peak at 360 nm, while the blue stimulus was created with an Oriel glass bandpass filter with a peak at 420 nm. (D) The spectrum of the stimulus flag used in experiment 3. The green portion was created with a Kodak Wratten filter no. 99, and the ultraviolet (UV) portion was created with an ultraviolet interference filter. The two components were combined as shown in Fig. 3B.

trials (or just a 'set'). For any one set, the intensity and spectrum of the background and the spectrum of the stimulus flag were kept constant. The eight different trials making up a set consisted of seven different stimulus flag intensities and one control trial. The intensity values for the seven stimulus trials were chosen so that they created the following estimated brightness contrasts with the background: 0.84, 0.81, 0.55, 0.02, –0.28, –0.62 and –0.83 (negative values indicate that the stimulus was darker than the background). The same set of estimated brightness contrasts was used in all sets. In the control trial for each set, the starting position for the stimulus flag was moved downwards, so that at its highest position it was not visible to the lizard. Each set of trials (i.e. each stimulus flag/background spectral combination) was viewed by all the lizards (N=44–47 lizards, depending on the experiment). In the rest of this paper, we refer to a given set of stimulus trials by listing the stimulus colour followed by the background: for example red/green refers to a red stimulus flag viewed against a green background.

Trials were carried out between 07:00 and 18:00 h. At the

beginning of a set of trials, a lizard was placed in the cage and chased onto the perch with a wooden rod. Lizards were allowed to lie in either direction along the perch, but were tested only if their eyes were above a point two-thirds of the way up the perch, thus ensuring that the view of the stimulus was approximately the same for all presentations. After initial placement in the cage, the lizard was given a 10 min acclimation period before presentation of the first trial, and a stimulus was presented every 5 min thereafter. Since anoline lizards possess a pure cone retina, 10 min was sufficient to be sure that the eyes were adapted to the light levels in the cage.

Immediately prior to each stimulus presentation, a noise was produced by flicking the blind from behind with two fingers from a position immediately behind the video camera (Fig. 3A). This caused the lizard to gaze monocularly in the direction of the camera. After a 3 s delay, the stimulus was set in motion and the response of the lizard was recorded. Positive response was defined as any distinct shift of eye position in the direction of the stimulus within 5 s of completion of the stimulus motion. This was an unambiguous behaviour in nearly all cases. Immediately after the completion of the trial, the experimenter moved into the view of the lizard and tapped gently on the cage and then adjusted the stimulus flag light intensity for the next trial. This activity standardized the amount of disturbance to the lizard and helped to maintain its alertness. After 5 min, the next stimulus presentation trial was carried out. If the lizard moved off the perch during the time between trials, it was immediately chased back into position with the wooden rod, and another 5 min interval was allowed to pass before the next stimulus presentation.

A lizard remained in the cage until it had been presented with the eight different stimulus trails making up the set. An individual lizard was not presented with a new set of trials for a minimum of 10 days. We demonstrated in an earlier study (Persons et al., 1999) that lizards do not habituate to this stimulus if presented 10 or fewer times at 5 min intervals, nor is there any drop in overall response if the same procedure is repeated on the same individual after a 10 day interval.

Experiment 1: green, yellow, orange and red stimuli against a green or white background

In this experiment, each individual lizard was presented with eight different sets of trials: four different stimulus spectra (green, yellow, orange and red) were each presented against two different backgrounds (green and white). The order of presentation of the eight different sets to each individual was randomized, and at least 10 days was allowed between each set for an individual lizard. The two background spectra are shown in Fig. 4A, and the four stimulus spectra are shown in Fig. 4B.

Experiment 2: green, blue and ultraviolet stimuli against a green background

This experiment was carried out as described for experiment 1, but only three sets of stimulus/background colour combinations were employed: green/green, blue/green and ultraviolet/green. The background spectrum (green in all sets) is shown in Fig. 4A, the green stimulus spectrum in Fig. 4B and the other two stimulus flag spectra in Fig. 4C.

Experiment 3: green, and ultraviolet+green against a green background

In this experiment, two stimulus/background sets were tested: green/green and ultraviolet+green/green. The green background spectrum is shown in Fig. 4A, the green stimulus is shown in Fig. 4B and the ultraviolet+green stimulus is shown in Fig. 4D. As in the previous experiment, the spectra of the stimulus and background were constant within each set, and the stimulus flag intensity was varied to create seven different brightness contrasts.

Statistical analysis

In all three experiments, each lizard viewed sets of stimulus flag colour/background colour combinations with seven different stimulus flag intensities (plus one control), and each stimulus presentation was scored for a positive or negative response. The null hypothesis of no difference in the probability of response within a set (i.e. the effect of brightness contrast between stimulus and background for a given stimulus/background spectral combination) was tested using a Cochran's *Q*-test. To compare differences between pairs of sets, we paired the results for each brightness contrast level and tested for an overall significant difference between the sets across all brightness contrasts using a Wilcoxon signed-rank test. In all experiments, the response to control treatments was 2–3 % of total presentations, which was much less than the minimum response to any of the stimulus motion presentations. The control response is not presented in any of the graphs of the results and was not included in the statistical tests reported, so that reported significant results represent differences between actual trials and are not the result of differences between the control and other stimulus trials.

The results (probability of response) for each set of trials were also plotted against the absolute value of brightness contrast, and a least-squares linear regression line was calculated for each set. The slopes and elevations of these regression lines were compared statistically using an analysis of covariance (ANCOVA) procedure (see Zar, 1974; p. 231). As described in the Discussion, we compared the minimum response probability for each set with chromatic contrast values calculated in five different ways and calculated a linear correlation coefficient for each of these models. The correlation coefficients were then compared statistically using a procedure described in Zar (Zar, 1974; p. 241).

Results

Experiment 1

The results are summarized in Fig. 5. In Fig. 5A,B, the results for the different stimulus flag colours viewed against the green background are plotted. In general, these curves are V-shaped, with response probability increasing with the

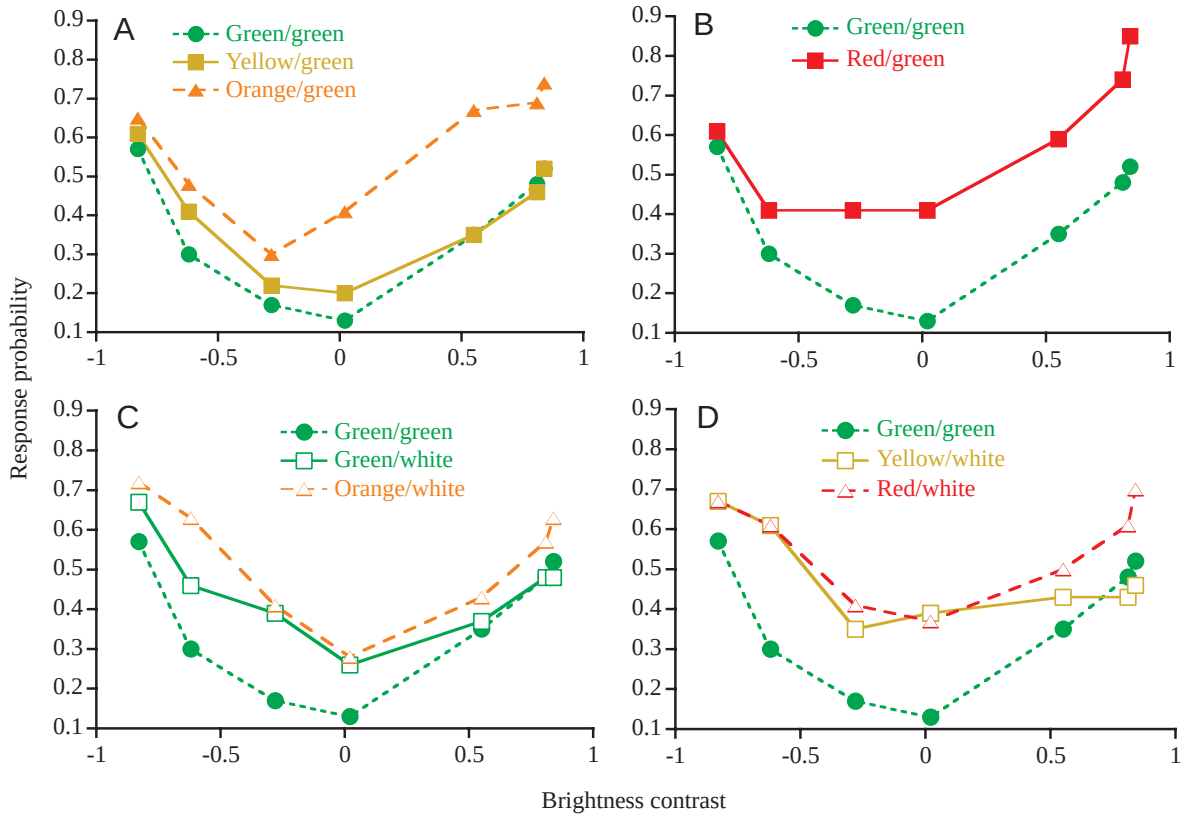

Fig. 5. The results from experiment 1. For each background/stimulus flag combination (i.e. each set of trials), the probability of response (the number of animals responding positively out of the total number of individuals tested) is plotted against the estimated brightness contrast. For clarity, the results are shown in four different plots (A–D). The results for the green/green set (green stimulus against green background) are repeated in each plot for ease of comparison. In each plot, the stimulus flag colour is given followed by the background colour. For example, red stimulus flag on green background is written Red/green.

absolute value of brightness contrast. For all eight sets of stimulus/background spectral contrast, there was a significant effect of stimulus intensity (i.e. brightness contrast) on response probability (Table 1).

If we compare the results of the yellow/green, orange/green or red/green sets with the green/green results we find that, for any given brightness contrast, the response to the green flag was generally lower, which shows that the introduction of a chromatic contrast between stimulus and background tended to elevate the response probability at any given brightness contrast level. A series of pair-wise comparisons of matched brightness contrasts for the different sets is presented in Table 2. The red/green and orange/green stimuli resulted in significantly higher response probabilities than did the green/green across all brightness contrasts. The overall response to the yellow/green set was generally greater than that to the green/green set, but the difference was not quite significant (P=0.06).

The results were similar for the white background (Fig. 5C,D), although the curves for the different stimulus flag colours were more similar than was the case for the green background. A comparison of the curve for green/green with the curve for green/white (Fig. 5C) reveals a pattern similar to that described above. When chromatic contrast was introduced (in this case by using a white background instead of a green background with a green stimulus), there was a significant overall increase in response probability across brightness contrasts.

Responses to most of the sets of trials were roughly symmetrical for positive and negative brightness contrast value. In Figs 6 and 7, for each set, response probability is plotted against the absolute value of brightness contrast, and

Table 1. *Results of tests for the significance of the effect of brightness contrast (Cochran's Q-test) for each different stimulus/background set from experiment 1*

Stimulus/background	Cochran's Q	P
Green/green	33.68	<0.001
Yellow/green	28.80	<0.001
Orange/green	34.19	<0.001
Red/green	35.65	<0.001
Green/white	20.28	<0.005
Yellow/white	16.04	<0.025
Orange/white	27.46	<0.001
Red/white	19.91	<0.005

Table 2. *Results of statistical tests for significant differences between different sets of trials* (i.e. *different pairs of stimulus/background combinations) from experiment 1*

Stimulus/background	*versus*	Stimulus/background	*P*
Green/green		Yellow/green	0.0625
Green/green		Orange/green	0.0078*
Green/green		Red/green	0.0078*
Green/white		Green/green	0.0469*
Yellow/white		Yellow/green	0.0391*
Orange/white		Orange/green	0.2344
Red/white		Red/green	0.3437
Yellow/green		Orange/green	0.0078*
Orange/green		Red/green	0.3437
Red/green		Yellow/green	0.0312*
Yellow/white		Green/white	0.2187
Yellow/white		Red/white	0.0625
Yellow/white		Orange/white	0.1094
Green/white		Red/white	0.0156*
Green/white		Orange/white	0.0078*
Orange/white		Red/white	0.0781

For each set, results were paired by brightness contrast and tested using the Wilcoxon signed-rank test.

An asterisk next to the *P* value indicates a significant difference.

the relationships are approximately linear. For each set, a least-squares linear regression line has been plotted (coloured line) together with the 95% confidence intervals. The solid black line in each figure will be explained in the Discussion. Comparing among the different sets, there is no significant difference in the slopes of the regression lines ($P>0.05$, ANCOVA; Zar, 1974), while the *y* intercepts of the regression lines do differ significantly ($P<0.05$, ANCOVA; Zar, 1974). These results are consistent with the analysis presented in the previous paragraph: within each set, response probability showed a similar dependence on brightness contrast, and the effect of stimulus/background chromatic contrast was to increase or decrease the response at each brightness contrast by a similar amount.

Experiment 2

In this experiment, we examined the response to three stimulus colours (green, ultraviolet, blue) against a green background. The stimulus flag spectra are shown in Fig. 4B,C. The results are summarized in Fig. 8. The response to the set of green/green trials was very similar to the results of experiment 1, and there was a significant effect of brightness contrast on response probability (Cochran's $Q=25.16$, $P<0.001$). The responses to the blue and to the ultraviolet

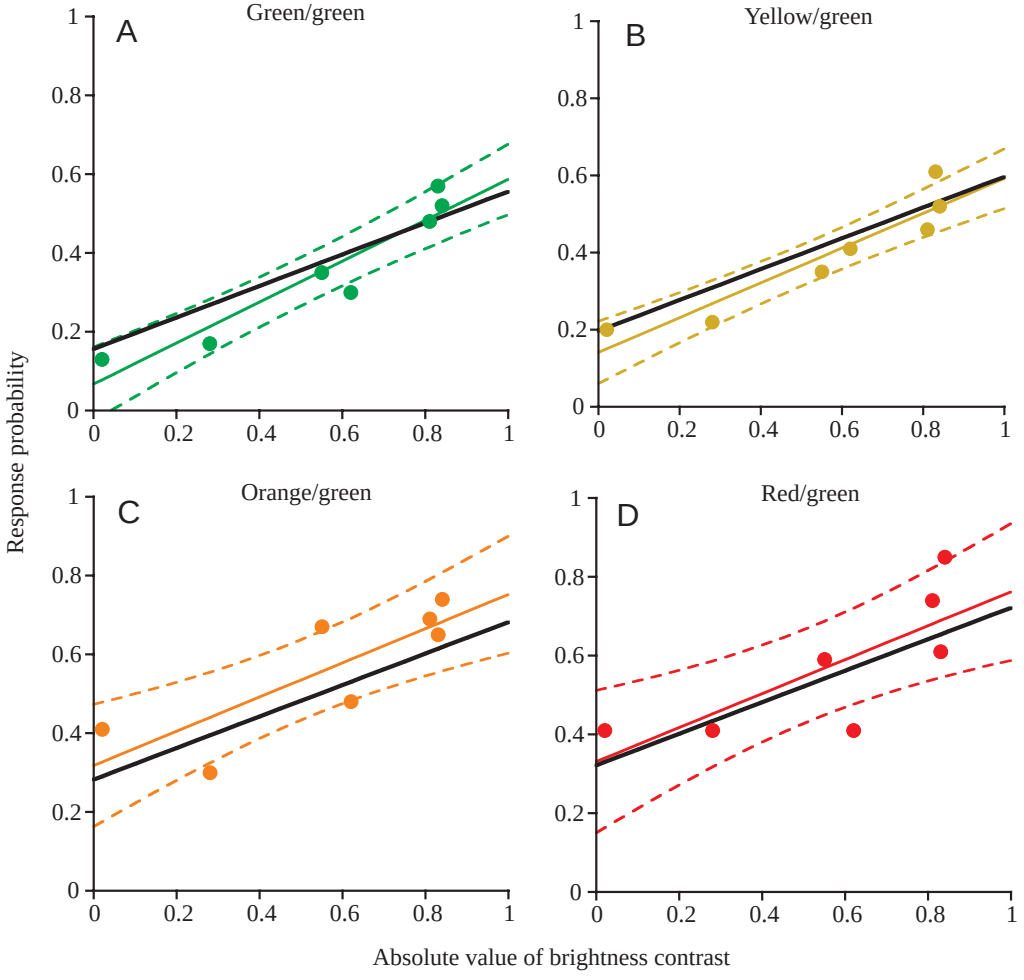

Fig. 6. Response probability *versus* the absolute value of brightness contrast for each of the sets of trials in experiment 1 using the green background. In each plot, the coloured circles indicate the actual data points. The coloured solid line is a least-squares linear regression for the actual data from each set, and the coloured broken lines are 95% confidence intervals about the linear regression line. The black line in each plot represents predicted values based on a multi-linear regression with brightness contrast and chromatic contrast as the two variables derived from all the data for experiment 1 (i.e. all sets of trials). See Discussion for details concerning this multi-linear regression. Data are shown for (A) the green, (B) the yellow, (C) the orange and (D) the red stimulus flags on the green background.

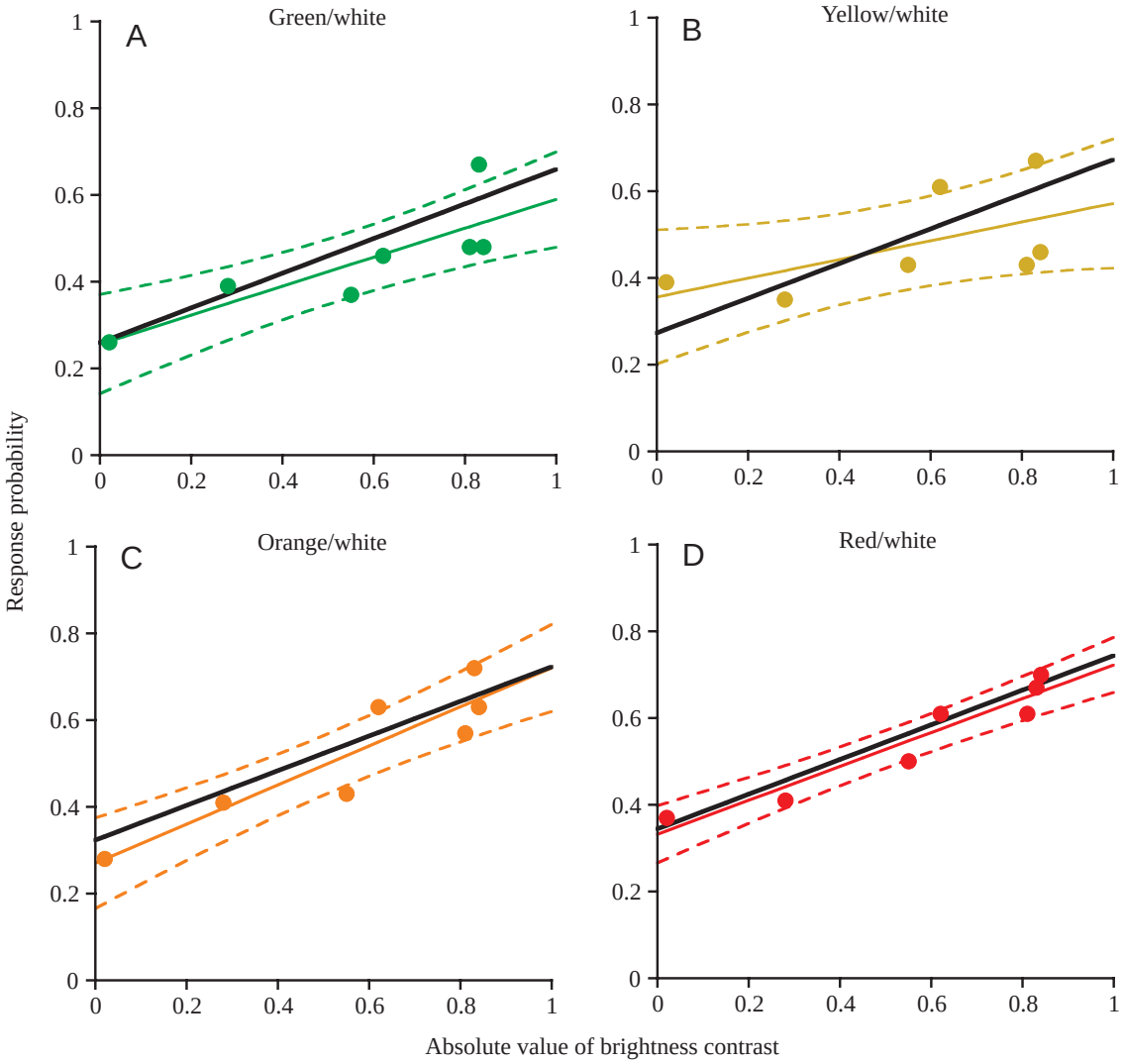

Fig. 7. Response probability *versus* the absolute value of brightness contrast for each of the sets of trials in experiment 1 using the white background. Details are as described in Fig. 6.

stimulus flags were quite different. There was no significant effect of brightness contrast on response probability for the blue stimulus flag (Cochran's Q=7.28, P>0.04) or for the ultraviolet stimulus (Cochran's Q=0.834, P>0.9). Thus, changing stimulus intensity had almost no effect on response probability. The response to ultraviolet/green was significantly greater than the response to blue/green across brightness contrasts (P=0.028, Wilcoxon signed-rank test).

Experiment 3

In this experiment, the stimulus spectrum shown in Fig. 4D was created, which was a combination of ultraviolet and green (ultraviolet+green). This experiment also included a set of green/green trials for comparison. The results are summarized in Fig. 9A. There was a significant effect of brightness contrast on response probability for the green stimulus flag (Cochran's Q=25.01, P<0.001) and for the ultraviolet+green stimulus flag (Cochran's Q=25.01, P<0.001). The response to the

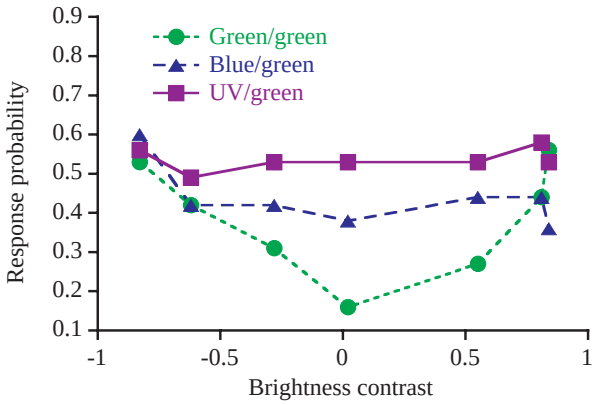

Fig. 8. The results from experiment 2. The estimated brightness contrast is plotted against the probability of response for the sets utilizing each of the three different stimulus flag spectra. UV, ultraviolet.

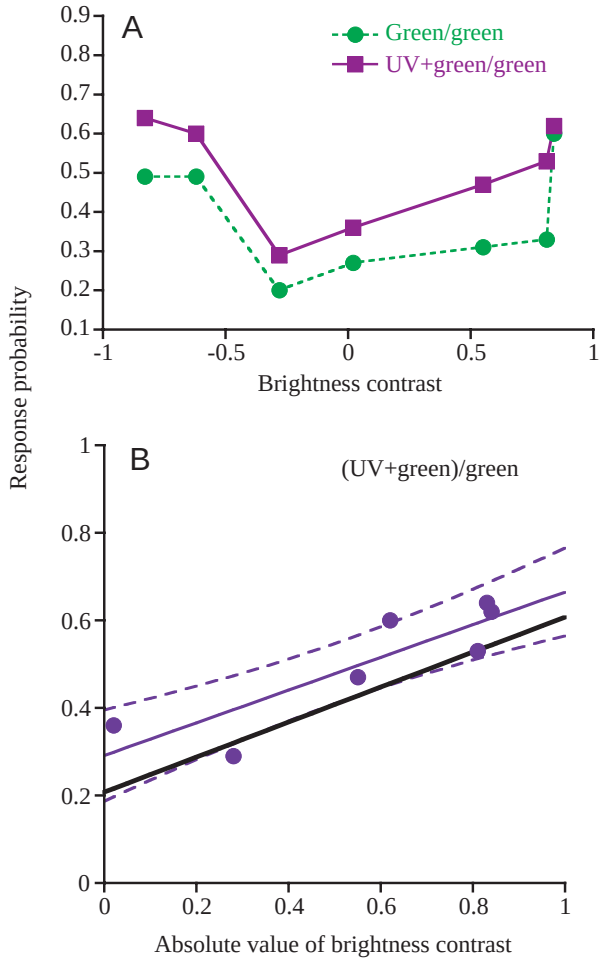

Fig. 9. (A) The results from experiment 3. The results for the two stimulus/background sets are shown. (B) The probability of response plotted against the absolute value of brightness contrast for the ultraviolet+green/green set of trials. UV, ultraviolet. The coloured circles are the actual data. The coloured line is a least-squares linear regression of this data, and the broken coloured lines are 95% confidence intervals. The black line represents predicted values based on the multi-linear regression using data from experiment 1, as described in Fig. 6 and in the Discussion.

ultraviolet+green/green set was significantly greater across brightness contrasts than the response to the green/green set (Wilcoxon signed-rank test, P=0.008). In Fig. 9B, the response probabilities for the ultraviolet+green/green set are plotted against the absolute value of brightness contrast. The relationship is approximately linear, and a least-squares regression line with 95% confidence intervals is shown on the graph.

Discussion

In experiment 1, we found that two factors contributed additively to the probability of detection of the moving stimulus flag: (i) the stimulus *versus* background brightness contrast and (ii) the stimulus *versus* background chromatic contrast. The existence of separate channels in the visual system for the analysis of brightness (or achromatic sensation) and chromatic sensation have been reported for a variety of other animal from various major groups (see, for example, Albright, 1991; Lythgoe and Partridge, 1991). For brightness contrast, it was unimportant whether the stimulus was brighter or darker than the background. As a consequence, the plots of response probability *versus* percentage brightness contrast were V-shaped. The effect of adding chromatic contrast (i.e. a spectral difference between the stimulus and background) was to elevate the V along the *y* axis.

Our brightness contrast values were based on estimated brightness, which was calculated using an ERG-determined spectral sensitivity curve. Spectral sensitivity functions are known to vary with the method of measurement and for different visual tasks (Goldsmith, 1990; Neumeyer, 1998). For this reason, we tested each stimulus flag at a range of intensities. If there had been an error in our estimate of brightness contrast for any stimulus spectral pattern, we would have expected a shift in the V-shaped curve along the *x*-axis relative to the curve for the green/green set, not an overall upward shift in response across brightness contrasts, as was observed. Our brightness estimates appear to be reasonably accurate (i.e. estimated brightness is approximately equal to actual perceived intensity) for most of our stimuli, since in most of the plots from experiment 1 the minimum response occurred at, or near, the lowest estimated brightness contrast. Results similar to ours were obtained previously (Przyrembel et al., 1995) in a study of the effect of chromatic and brightness contrast on visually mediated prey-capture responses in salamanders.

In experiment 2, we tested the response when stimuli consisted only of short wavelengths. For blue and ultraviolet stimulus flags, there was no effect of stimulus intensity on response. From experiment 1, we had concluded that the signal detection probability was based on a chromatic contrast channel and a separate brightness contrast channel. However, the majority of the quanta in the stimulus spectra in experiment 1 were at wavelengths longer than 450 nm. The blue and ultraviolet stimuli from experiment 2, which consisted primarily of short wavelengths (<450 nm), appeared not to stimulate the brightness contrast channel. Comparing the cone spectral sensitivities in Fig. 1A with the stimulus spectra for ultraviolet and blue shown in Fig. 4C, it can be seen that the ultraviolet and blue stimuli primarily stimulated the S and UV photoreceptors, while the stimuli from experiment 1 primarily stimulated the M and L photoreceptors. It appears, therefore, that the brightness channel for this behaviour receives input only from the range of wavelengths covered by the M and L photoreceptors.

Athough changes in intensity did not alter the response probability for the blue or the ultraviolet stimuli, overall there was a significantly greater response to the ultraviolet than to the blue stimuli. Two factors are likely to have contributed to the response probabilities for these stimuli. First, since the changes in intensity of these stimuli did not influence response

Table 3. *Relative stimulation values for each of four classes of cones*

	Cone class				Chromatic contrast	
Spectrum	L	M	S	UV	Green background	White background
Green background	0.400	0.482	0.064	0.053	–	–
White background	0.347	0.350	0.258	0.045	–	–
Green stimulus	0.400	0.482	0.064	0.053	0	0.241
Yellow stimulus	0.479	0.464	0.052	0.004	0.095	0.273
Orange stimulus	0.645	0.336	0.010	0.009	0.294	0.390
Red stimulus	0.703	0.247	0.024	0.026	0.386	0.439
Blue stimulus	0.066	0.065	0.803	0.080	0.912	*
Ultraviolet stimulus	0.065	0.065	0.110	0.760	0.888	*
Ultraviolet+green stimulus	0.373	0.434	0.038	0.155	0.119	*

*These stimuli were presented against a green background only.
Cone types are L (long-wavelength), M (middle-wavelength), S (short-wavelength) and UV (ultraviolet), as illustrated in Fig. 1A.
Also shown are the chromatic contrasts calculated for each stimulus/background combination used in the experiments (see Appendix).

probability, they appear not to stimulate the brightness channel. This channel would thus 'see' the stimuli as uniformly dark against the relatively bright background and, therefore, as possessing a fairly high negative brightness contrast. Second, as will be discussed below, the ultraviolet-sensitive, and possibly the blue-sensitive, cones seem to stimulate the chromatic contrast channel. The chromatic contrast may be higher for the ultraviolet than for the blue stimulus (see Table 3), which would explain the difference between the responses to the two classes of stimuli.

Since there appears to be no contribution to brightness contrast from wavelengths shorter than 450 nm, and our original estimates of brightness contrast were based on ERG responses from 350 to 700 nm, it would seem that all our original brightness contrast estimates should be in error. In our two background spectra and in all the stimuli used in experiments 1 and 3, most of the quanta in each spectrum were of wavelengths longer than 450 nm. If one examines the spectral sensitivity function used to calculate estimated brightness (Fig. 1B,C), it is apparent that sensitivity to wavelengths shorter than 450 nm is 2–3 orders of magnitude less than to wavelengths in the range 450–700 nm. This means that, for any stimulus (or background) in which a substantial proportion of the total quanta fell in the range 450–700 nm, wavelengths shorter that 450 nm made a very small contribution to the total estimated brightness. For every stimulus and background used in experiments 1 and 3, we recalculated estimated brightness including only wavelengths from 450 to 700 nm and compared the result with the original estimated brightness calculations. The difference was never greater than 1 %. The only case in which this change in the calculation of brightness had an impact was in experiment 2. If we redefine estimated brightness to include only wavelengths in the range 450–700 nm, we can no longer determine a brightness for the blue or ultraviolet stimuli of experiment 2.

The third experiment was designed to determine whether the ultraviolet stimulus, which did not appear to stimulate the brightness contrast channel, contributed to motion detection through the chromatic contrast channel. The results strongly suggest that it did, since the addition of ultraviolet to green increased the response probability (relative to green/green) by a nearly constant amount for all brightness contrasts (Fig. 9). One might ask whether the ultraviolet+green was actually a darker stimulus (as perceived by the lizard for this task) than the green alone, since the ultraviolet component was included in the original calculation of the stimulus brightness and the two different stimuli were set to equal brightness on the basis of this estimate. However, as described above, the contribution of the ultraviolet portion of the spectrum to the estimated brightness of this stimulus is very small (several orders of magnitude less than in the green portion of the spectrum). We recalculated the brightness of the two stimuli including wavelengths longer than 450 nm only and found that they differed by less than 1 %. The uniform increase in response resulting from the addition of the ultraviolet to the green stimulus must, therefore, be due to the introduction of chromatic contrast between the stimulus and background.

In summary, response probability appears to depend on the brightness contrast between the moving stimulus and the background, with brightness input only from wavelengths longer than 450 nm (which would stimulate the M and L cones), added to a chromatic contrast component. Since the L cone spectral sensitivity completely spans the spectral range of the M cone sensitivity, it is possible that only the L cone is involved in the brightness channel. This pattern of response is strikingly similar to that reported from a number of studies of motion perception in humans and other primates, in which it has been shown that the detection of motion depends on (i) a brightness (or achromatic) channel with input only from long- and middle-wavelength cones and (ii) one (or more) separate chromatic channels with input from all classes of cone found in the retina (Albright, 1991; Gegenfurtner and Hawken, 1996).

The relationship between chromatic contrast and response probability

The effect of a difference in spectral quality between

stimulus and background was to elevate response probability uniformly for any given brightness contrast. There is no simple, widely agreed upon method for quantifying the difference in appearance between two spectral patterns because this difference depends to a large degree on the neural wiring involved in colour perception of a specific visual system. In the Appendix, we present a calculation of chromatic contrast that is based on our knowledge of the spectral response of each of the four classes of cone in the anoline retina (Fig. 1A). The sensation of colour (in humans and other animals) is known to be largely a function of the ratio of stimulation of the different classes of cone, and two spectral stimuli, in general, will appear different when the ratio of stimulation of different cone classes differs. It makes intuitive sense that the extent to which two colour stimuli differ in appearance should be related to the magnitude of the difference in the ratio of stimulation of the different cone classes created by the two stimuli, although other factors (such as intensity, surrounding colours, etc.) may play some role as well. On the basis of this idea, it has been shown that one can obtain a reasonable approximation of how different two colours appear to an animal by quantifying the difference in this cone stimulation ratio (Neumeyer, 1986; Arnold and Neumeyer, 1987; Endler, 1991; Lythgoe and Partridge, 1991), and we describe such a calculation method in the Appendix. On the basis of this method, we have calculated a chromatic contrast value for each stimulus flag/background colour combination used in this study. Relative cone stimulation values for each stimulus and background spectrum and chromatic contrast values for each stimulus/background combination are listed in Table 3.

Our next step was to examine the relationship between our estimates of chromatic contrast and detection probability. For each set of stimulus trials, response probability was an approximately V-shaped function of brightness contrast. The effect of adding chromatic contrast was to shift the entire curve upwards along the *y*-axis. One way to compare the effects of chromatic contrast among the different sets is to compare the value for minimum response probability from each set (i.e. the bottom of the V). In Fig. 10, the calculated chromatic contrast value is plotted against the minimum response probability from each of the sets from experiment 1. There appears to be a linear relationship between the two variables, with a linear correlation coefficient (r) of 0.86. This suggested to us that the chromatic contrast estimate shown in the Appendix is useful for predicting the effects of spectral quality differences between stimulus and background on response probability. We do not mean to suggest that we have identified the mechanism by which colour discriminations are carried out by anoline lizards. We have, however, arrived at a method for estimating chromatic contrast that is very useful for predicting the outcome of the behavioural task employed in this study.

The chromatic contrast calculations in the Appendix are based on the relative stimulation of each of the four cone classes. However, it is possible that not all four classes contribute to this discrimination task. To explore the possibility that one of the four cone classes might not be involved, we used the method described in the Appendix and recalculated chromatic contrast for every stimulus/background combination using each possible combination of three cone classes. The results are shown in Fig. 11. We compared the result for each of these three-cone combinations with the correlation based on all four cones. Leaving out either the L or the M cone significantly reduced the correlation between chromatic contrast and minimum response probability ($P<0.05$). Removing only the ultraviolet cone from the calculation weakened the correlation somewhat, but the difference (compared with the correlation based on all four cone classes) was not significant ($P>0.05$). Removing the S cone from the calculation produced almost no change in the strength of the correlation, and the difference was not significant ($P>0.05$).

The fact that omitting the S or the UV cone from the chromatic contrast calculation did not significantly weaken the correlation between response probability and chromatic contrast may be an artefact of the stimulus combinations employed in experiment 1. Examination of the stimuli (Fig. 4B) used in this experiment shows that, while there were a number of different combinations of stimulation of the L and M cones, the UV cone was stimulated only by the white background. The S cone was also stimulated only by the white background. However, the white background also stimulated both L and M cones strongly; in other words, there was a strong correlation within the stimulus spectra themselves between S cone (or UV cone) stimulation and L plus M cone stimulation. This may explain why it is possible to have a significant

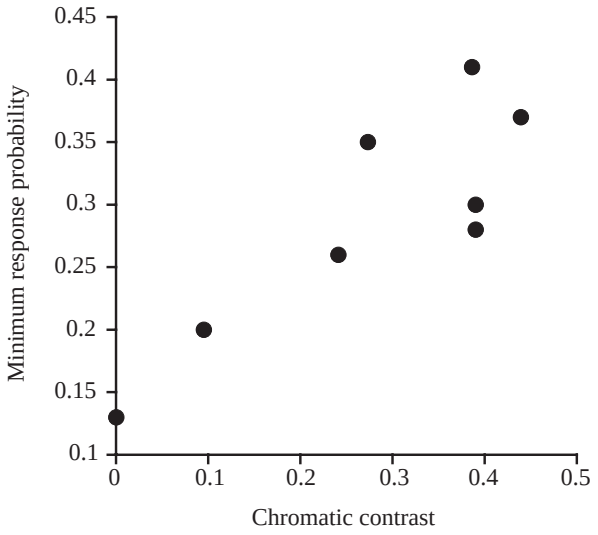

Fig. 10. The minimum response probability for each of the stimulus/background combinations (i.e. each set of trials) from experiment 1 plotted against the chromatic contrast score calculated for each combination (see Discussion and the Appendix for a description of chromatic contrast scores). The chromatic contrast values are calculated using all four cone spectral sensitivity functions. There appears to be a nearly linear relationship between these two variables, and the correlation coefficient for the two variables is 0.86.

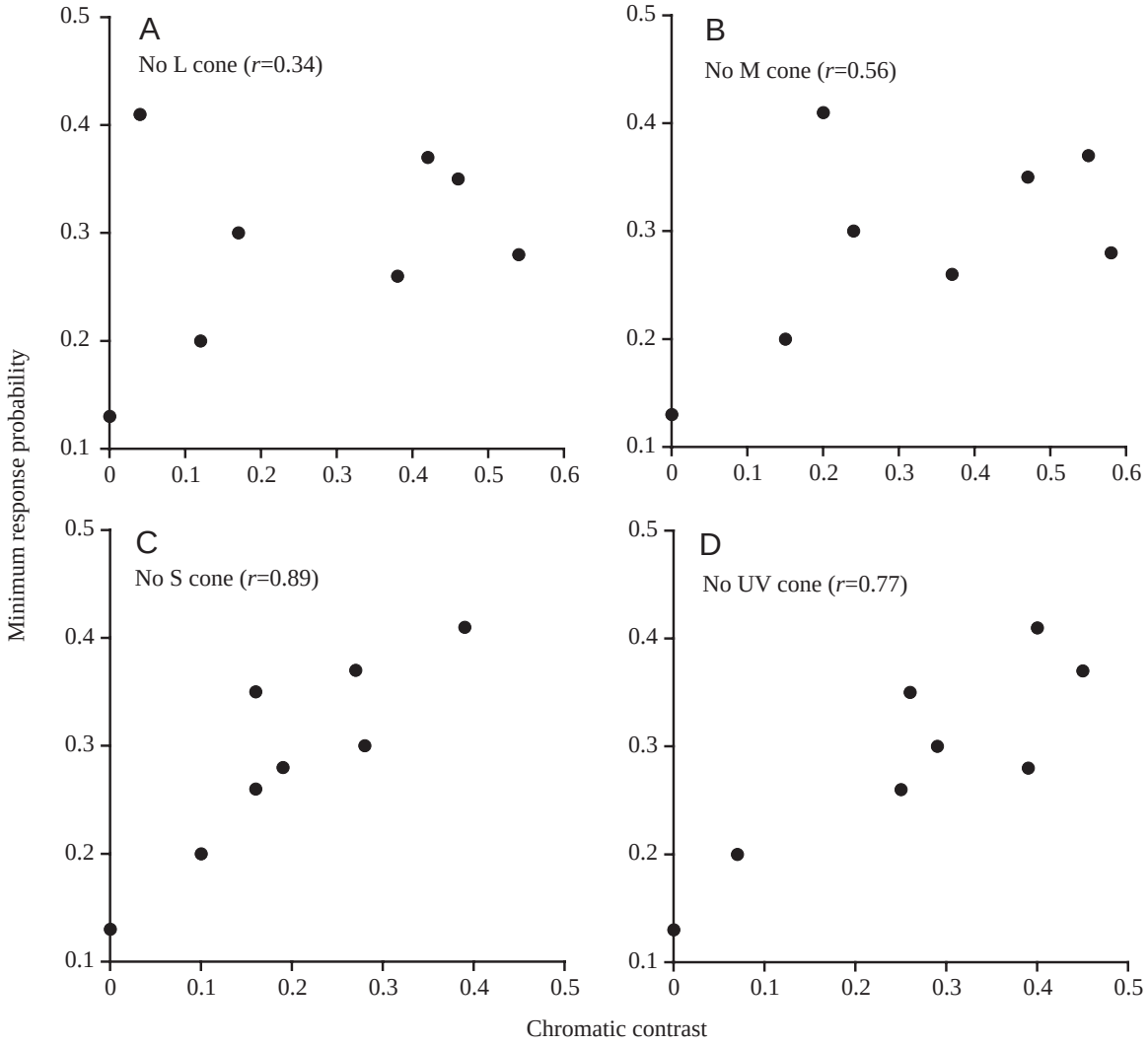

Fig. 11. The minimum response probability for each of the stimulus/background combinations (i.e. each set of trials) from experiment 1 plotted against the chromatic contrast score calculated for each combination. In this case, sets of three cone spectral response functions were used to calculate the chromatic contrast score. In each graph (A–D), one of the spectral classes of cone is not included in the calculation, as indicated at the top of each graph. The linear correlation coefficient for each plot (*r*) is shown on the graph. See Discussion for details.

correlation with chromatic contrast even when either the S or the UV cone is not included in the calculation. In the case of the UV cone, a direct test was carried out to determine whether it contributed to chromatic contrast (experiment 3), and it was shown to be important. Unfortunately, we did not carry out a similar test for the S cone.

We can tentatively conclude that the L, M and UV cones contribute to chromatic contrast for this task. Inclusion of the S cone in our calculations did not improve our prediction of response probability. However, given the limited set of spectra tested, we cannot say with confidence whether the S cone contributes to this discrimination task. In the analysis that follows, we take the conservative approach of including all four cone classes in chromatic contrast calculations.

A predictive model for detection probability

As seen in Figs 6, 7 and 9B, there is an approximately linear relationship between the absolute value of brightness contrast and response probability for a given stimulus flag/background colour combination, and the slopes of the lines for the different sets are quite similar. The effect of changes in chromatic contrast appears to be a shift in the *y*-intercept of these lines, with little or no effect on the slope.

Since, within each set, there was a linear relationship between detection probability and brightness contrast, and between sets there was a linear relationship between chromatic contrast and detection probability, we concluded that we could derive a single multi-linear regression equation, with brightness contrast and chromatic contrast as the two independent variables, that would summarize all the results from experiment 1. For this equation, the brightness contrast is based on the empirically determined spectral sensitivity function for *A. cristatellus* (see Fig. 1B,C) from 450 to 700 nm. Chromatic contrast is based on the empirically determined

quantal spectral sensitivity for each class of cone (all four cones) in the anoline retina (Fig. 1A).

The resulting equation is:

$$p = 0.400C_b + 0.429C_c + 0.156, \quad (1)$$

where p is the probability of detection, C_b is brightness contrast from 450 to 700 nm and C_c is chromatic contrast. The regression has an r^2 value of 0.71 (for the eight sets of trials from experiment 1) and explains a highly significant portion of the variation in the data (F=68, P<0.0001). It should be noted that, even for zero values of brightness and chromatic contrast, there is a small positive detection probability (p=0.156). This reflects a small level of positive response in the absence of contrast, presumably due to random shifts of gaze or to a response to the small amount of sound produced by the moving stimulus.

The predicted values of this model for each stimulus/background set in experiment 1 are shown in Figs 6 and 7 by a solid black line. In all cases, the predicted values from the multi-linear regression fell within the 95% confidence intervals of the simple linear regression for each individual set. This was also true when the equation was used to predict the results from experiment 3 (Fig. 9B). Thus, a single equation gives good predictions (within the 95% confidence intervals) for the full range of experimental stimuli used in the present study (except for stimuli dominated by wavelengths shorter than 450 nm). This equation allows us to predict the relative visibility of any dewlap spectrum viewed under any light conditions by *A. cristatellus*.

Some implications of these results

On the basis of these results, we can predict how changes in dewlap colour (i.e. both spectral quality and intensity) should influence signalling efficiency in *Anolis cristatellus*. This is a widely distributed species, with discrete populations found in a variety of habitats. There is variation among these populations in dewlap colour and, on the basis of the results presented here, it should be possible to determine whether these changes are in a direction consistent with evolution towards increased visibility by measuring dewlap reflectance, natural illumination and natural background spectra. Our earlier studies of a number of different anoline species have shown that their visual systems are very similar in basic anatomy and physiology (Fleishman et al., 1993; Fleishman et al., 1995; Fleishman et al., 1997; E. R. Loew, personal communication) and that their responses to visual stimuli of the type described here are quite similar (Fleishman, 1992). Further experimentation will be required to confirm that the quantitative rules for visual detection probability established here are applicable to other anoline species but, if they are, we will have a powerful tool for testing the hypothesis that among-species diversity in dewlap colour patterns has evolved as a result of selection pressure for increased signal visibility under different habitat conditions. Moreover, there is sufficient similarity in the way that many vertebrates process chromatic and brightness contrast (e.g. see Albright, 1991; Lythgoe and Partridge, 1991) to believe that the approach used here may also prove useful for studying the role of colour in signal efficiency in other vertebrate groups.

Enhancing the likelihood that a signal will initially be detected is only one role for signal colour. Once a signal has been detected by an animal, colour can serve other functions such as providing information about the identity (species or individual) and/or quality of the signaller. These functions are a fundamentally different task for the visual system compared with the detection task we have examined in our experiments and tend to be limited by the ability of a visual system to make fine discriminations between different spectra. In several recent studies, visual-system-based models of the ability of animals to distinguish among relevant natural spectral patterns have been developed (e.g. Chittka, 1996; Vorobyev et al., 1998; Vorobyev and Osorio, 1998). The visual task explored in the present study is different: it is a test of the influence of colour differences, well above threshold, on signal detection probability and, therefore, a different model was required to estimate chromatic contrast.

The colour of a visual signal will usually serve more than one function. These different functions require different tasks of the visual system, and there will be different evolutionary constraints on signal colour for each task. However, before a signal can serve any function, it must be seen by its intended viewer. Selection for high signal visibility is therefore likely to be a powerful force in the evolution of signal design. Here, we have established a method for predicting how differences in dewlap colour will influence the efficiency of displays in attracting the attention of conspecifics. We believe this information will be extremely useful for testing hypotheses about the influence of habitat light and visual-system responses on the evolution of the design of visual signals. We have also shown that signal visibility depends heavily on both the signal itself and the lighting conditions under which it is viewed. Habitat light conditions depend on vegetation structure and can be changed by even modest alterations in habitat. This study demonstrates that such changes have the potential to alter substantially the effectiveness of the visual signals of a species. These results may be important for conservation biology because they provide a means of predicting the effects of changes in habitat light conditions on signal efficiency.

Appendix: calculation of chromatic contrast

Chromatic contrast, as it is used in this paper, is defined as the perceptual difference between a signal and the background against which it is viewed based on the difference in spectral shape, independent of intensity. Chromatic sensation is largely a function of the ratio of stimulation of the different classes of cones in the retina. This information is then further processed by the retina and brain. We have good estimates of the quantal spectral sensitivity of each cone class in the anoline retina, but we have no information on the subsequent processing. We therefore quantify chromatic contrast in terms of the difference

in relative stimulation of cones produced by different colours, recognizing that this is only a first-order approximation of the actual perceived difference between colours. Approaches similar to this have been used in a number of studies to predict colour discrimination thresholds (see, for example, Arnold and Neumeyer, 1987; Neumeyer, 1986; Neumeyer, 1992) which were then compared with experimentally determined values, and the predictions were quite good.

Our calculations are based on the cone absorption spectra (corrected for filtering by oil droplets) shown and described in Fig. 1A. We start with the assumption that, in response to a white stimulus (a stimulus with equal quanta at all wavelengths from 300 to 700 nm), the neural stimulation from each of the four cone classes is equal. To satisfy this assumption, a correction is made for the difference in area under the curves of the different cone classes.

To compare two different colours (i.e. that of the background and that of the stimulus flag), we start by determining how each colour alone stimulates each class of cone. Each spectrum is multiplied by the spectral sensitivity of each cone class. The resulting value for each cone is multiplied by the area correction factor for that cone to give the stimulation of each cone class, which we can refer to simply as UV, S, M and L. A relative stimulation, X_i, is then calculated for each of the cone classes as follows:

$$X_{UV} = UV/(UV + S + M + L), \quad (A1)$$

$$X_S = S/(UV + S + M + L), \quad (A2)$$

$$X_M = M/(UV + S + M + L), \quad (A3)$$

and

$$X_L = L/(UV + S + M + L). \quad (A4)$$

In this way, the stimulus spectrum is reduced to a relative score from 0 to 1 for each of the four photoreceptor classes. We can think of these stimulation values as a set of four coordinates in a four-dimensional space in which relative stimulation of each cone class (from 0 to 1) represents an axis. We then repeat the same calculation for the second colour and determine its coordinates in this space. The coordinates for colour 1 are X_{UV1}, X_{S1}, X_{M1} and X_{L1}, and those for colour 2 are X_{UV2}, X_{S2}, X_{M2} and X_{L2}. Since we have constrained our cone excitation values for each spectrum so that they always add to a value of 1.0, each point in colour space has only three degrees of freedom and it is also possible to plot each spectrum as a point in a three-dimensional space referred to as a colour tetrahedron (see, for example, Goldsmith, 1990; Neumeyer, 1992; Neumeyer, 1998).

We now have each of the two colours described as a point in four-dimensional space, and the location in this space is a measure of the relative degree of stimulation of each of the different cones. We now assume that the greater the difference in this relative degree of stimulation between two colours, the more different they will appear to the lizard viewing them. We thus define 'chromatic contrast' as the Euclidean distance between the two points represented by the two different colours in the four-dimensional space. To estimate this difference, we calculate the vector distance between the two points as follows:

$$C_c = \sqrt{(X_{UV1}-X_{UV2})^2 + (X_{S1}-X_{S2})^2 + (X_{M1}-X_{M2})^2 + (X_{L1}-X_{L2})^2}, \quad (A5)$$

where C_c is chromatic contrast.

In summary, our chromatic contrast score is a measure of the difference in the ratio of stimulation of the four different cone classes that two different colour stimuli produce. The same procedure can be applied to any number of cone classes, and in this paper we also calculate chromatic contrasts based on sets of three cone classes.

This model is fairly simple in that it assumes that the input strength of each cone class to the perception is equal for an ideal white stimulus, and it does not take into account differences in cone size or retinal area occupied by different cone classes. Other models exist that take some of these issues into account (e.g. Chittka, 1996; Vorobyev and Osorio, 1998). Both these models require information about signal processing that is not available for anoline lizards, and they are designed to test the ability to discriminate among fairly similar spectra, rather than to estimate how different two spectra which may be easily discriminated, appear.

The model we use does not attempt to take chromatic adaptation of photoreceptors into account. The stimulus and background for the experiments occupied a tiny proportion of the total visual field (only a small opening in one wall of the cage). The majority of the visual field consisted of a dimly lit broadband grey background that presumably caused very little adaptation of photoreceptors. We explored the possibility of using the average radiance of this wall to replace white (i.e. equal quanta at all wavelengths) in the formulation of the model above. However, this modification weakened the predictive power of our model considerably, suggesting that the radiance in the visual field was sufficiently low, relative to the experimental stimulus and background, for chromatic adaptation to have had little effect. In any case, the aim of this modelling was to fit the empirically determined data, and the model described above (without assuming chromatic adaptation) works best.

In Table 3, we list the relative cone excitation values for each stimulus and background spectrum used in this study (after correction to make the cone excitation equal for an ideal white stimulus), and the chromatic contrast for each stimulus against each background.

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