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Behavioural evidence for colour vision in stomatopod crustaceans

Abstract If an organism can be taught to respond in a particular way to a wavelength of light, irrespective of that light's intensity, then it must be able to perceive the colour of the stimulus. No marine invertebrate has yet been shown to have colour vision. Stomatopod crustaceans (mantis shrimps) are colourful animals and their eyes have many adaptations which indicate that they are capable of such spectral analysis. We adopted an associative learning paradigm to attempt to demonstrate colour vision. Stomatopods readily learnt to choose some colours from arrays of greys, even when the correct choice colours were darker than the ones they had been trained to. Possible mechanisms underlying colour vision in these animals, and their ecological significance are discussed. A simple model is presented which may help interpret the complex stomatopod colour vision system and explain some of the learning anomalies.

Key words Stomatopod • Colour vision • Crustacean behaviour

Abbreviations ND neutral density • OD optical density • R8 Retinular cell 8 • R1-7 Retinular cells 1-7 • e.g. RID Distally placed R1-7 retinular cells in mid-band row 1 • e.g. RIP Proximally placed R1-7 retinular cells in mid-band row 1 • D/P Estimate of chromatic signal ratio

Introduction

The mantis shrimp Odontodactylus scyllarus lives in the spectrally rich environment of the reef top which it views with large apposition compound eyes. Each eye is hemisected by six clearly visible rows of specialised ommatidia called the mid-band (Manning et al. 1984; Marshall et al. 1991a). Four of these rows show adaptations which are strongly suggestive of colour vision (Marshall 1988; Cronin and Marshall 1989a; Marshall et al. 1991b). These specialisations include more than eight different visual pigments (Fig. 2b), tiered receptor cells and densely coloured filter blocks (Marshall et al. 1991b; Cronin and Marshall 1989b; Cronin et al. 1994b). O. scyllarus is astonishingly colourful and in common with other species in its superfamily (Gonodactyloidea), it shows off coloured areas of cuticle during threat displays (Caldwell and Dingle 1976; Hazlett 1979). Based on these varied observations it is likely that O. scyllarus possesses some form of colour vision.

To demonstrate colour vision, a behavioural test is needed in which the animal recognises objects of differing spectral properties irrespective of intensity (Menzel 1979; Burkhardt 1983; Neumeyer 1991; Thompson et al. 1992). Such tests have been performed on insects and a wide variety of vertebrates but rarely on crustaceans (Menzel 1979; Menzel and Backhaus 1991; Jacobs 1981). Studies of crustaceans colour vision generally examine phototaxis in relation to colour and are difficult to interpret as they may reflect what are known as wavelength specific behaviours rather than 'true' colour vision (von Frisch and Kupelwieser 1913; Smith and Baylor 1953; Stearns 1975; Hyatt 1974, 1975). Wavelength specific behaviours are stereotyped motor patterns, underlying various behaviours, which are triggered by photoreceptors responding to specific wavelengths (Menzel 1979). They are described in a variety of insects and exist in other animals instead of or as well as 'true' colour vision (Scherer and Kolb 1987a, b; Menzel and Backhaus 1991). As a result some caution is needed in interpreting results involving colour stimuli.

Within the Crustacea there are also a number of studies describing the possibility of colour vision based...
solely on retinal anatomy and visual pigment diversity (Stowe 1980; Leggett 1979; Lall and Cronin 1987; Smith and Macagno 1990; Marshall 1988). Such claims for colour vision are necessarily speculative. However tests on animals designed to demonstrate colour vision where diverse photoreceptor types exist have almost always proved positive. With over eight visual pigments and colour filters (Marshall et al. 1991b; Cronin and Marshall 1989a), it would be odd if stomatopods did not possess some form of colour vision.

The task required of our shrimps exploits the prey capture and defence mechanism of the so called ‘smashing’ stomatopods like *Odontodactylus scyllarus*. Using enlarged, hardened second maxillipeds, these mantis shrimps crack open hard bodied prey and inflict lethal blows on rivals. Raptorial limbs use a storage-release muscle mechanism to generate enormous force for the rapid forward strike, one of the fastest animal movements known (Burrows 1969; Caldwell and Dingle 1976). Animals were given hollow plastic cubes containing food with the 5 open sides sealed by glass coverslips and the remaining solid side covered with coloured or grey plastic. They learned within seconds to pick up cubes and within minutes to crack them open, gaining entry usually through the replaceable glass sides (Fig. 1).

While some colours of plastic; green, red and yellow, were easily distinguished from various shades of grey by *O. scyllarus*, the blue plastic chosen was not. A simple model is presented which, based on the colour reflectance characteristics and retinal sensitivities, may help explain this problem. The suggested model system also holds one possible explanation for the tremendous complexity found in the stomatopod retina.

**Materials and methods**

*Odontodactylus scyllarus* of both sexes were purchased from tropical fish suppliers and housed individually in standard marine aquaria where half a flower pot acted as a burrow. (Aquastar) fluorescent tubes provided the best possible lighting for the aquaria, including the UV range (Fig. 2a).

Food cubes were constructed from white perspex blocks (21 mm$^3$) with an accessible cavity, drilled from five of the sides, into the centre (Fig. 1). Coloured plastic squares and a range of neutral density (ND) filters (ND-0 - white perspex, no covering filter, ND-A - optical density (OD) 0.3, ND-B - OD 0.6, ND-C - OD 0.9, ND-D - OD 1.2, ND-E - OD 1.8) were attached to the one intact face of individual cubes by using double sided sticky tape. The coloured cubes were primed with a piece of food (usually cockle, shrimp or mussel) for training runs, filled with water and the five open sides closed off with glass coverslips held on with petroleum jelly. In the test situations, all the cubes were empty and these cubes were kept separate from the training cubes to avoid the possibility of remaining odour confounding the visual discrimination task.

At the start of each experiment the animal was partitioned into its burrow with a sheet of black perspex. Three blocks, one coloured and two grey, were then arranged symmetrically in front of the burrow, with their solid faces toward the animal. The relative position of the coloured cube was varied systematically and the neutral density cubes, chosen from a series containing two of each ND type, were both selected at random. A choice was scored as the first cube the animal picked up. Each animal was tested twice a week after an initial four to six week period of training runs which were also conducted twice a week. This test of asking the shrimps to choose a colour from a range of greys follows the basic experimental design used many times in the past (e.g. von Frisch and Kuppelwieser 1913, Fukushi 1990).

Figure 2a illustrates the spectral reflectance of coloured cubes, two of the neutral density cubes used and the emission spectrum of the “Aquastar” fluorescent lamps which illuminated the arena. All reflectance and radiometric measurements were made with “Sub-Spec” a custom-built (Andor Technology/Oriel) spectroradiometer and a “Spectralon” white reflection standard. For the calculations detailed in Fig. 4, radiometric values of light (in photons/sec/cm$^2$/sr/nm) reflected from each cube were used. “Sub-Spec” was calibrated from 300–800 nm against a known emission standard prior to these measurements. Cube reflectances were measured in the experimental arena and therefore represent our best estimate of the spectrum that reached the shrimp’s eyes from each cube.

**Results**

Stomatopods could learn to discriminate what appeared to us as red, green or yellow, but not blue, from various shades of grey. These results are detailed in Fig. 3 which shows observed versus expected-by-chance results for each cube colour type. Choices of red, green and yellow cubes from greys are significantly higher than the 1:3 chance level. For blue however choice frequencies close to 1:3 were observed. It also proved impossible to train animals to choose a particular shade of grey (ND-A) from a selection of others, a task which would depend solely upon an ability to discriminate and remember intensity (Fig. 3a). In colour preference tests animals also showed no innate preference for any one of the cube colours. There was no preference for cube position.