Behavioural evidence for polarisation vision in stomatopods reveals a potential channel for communication

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Polarisation sensitivity (PS) – the ability to detect the orientation of polarised light – occurs in a wide variety of invertebrates [1,2] and vertebrates [3–5], many of which are marine species [1]. Of these, the crustacea are particularly well documented in terms of their structural [6] and neural [7,8] adaptations for PS. The few behavioural studies conducted on crustaceans demonstrate orientation to, or local navigation with, polarised sky patterns [9]. Aside from this, the function of PS in crustaceans, and indeed in most animals, remains obscure. Where PS can be shown to allow perception of polarised light as a 'special sensory quality' [1], separate from intensity or colour, it has been termed polarisation vision (PV). Here, within the remarkable visual system of the stomatopod crustaceans (mantis shrimps) [10], we provide the first demonstration of PV in the crustacea and the first convincing evidence for learning the orientation of polarised light in any animal. Using new polarimetric [11] and photographic methods to examine stomatopods, we found striking patterns of polarisation on their antennae and telson, suggesting that one function of PV in stomatopods may be communication [12]. PV may also be used for tasks such as navigation [5,9,13], location of reflective water surfaces [14] and contrast enhancement [1,15–18]. It is possible that the stomatopod PV system also contributes to some of these functions.

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Results and discussion

Polarised light production and detection

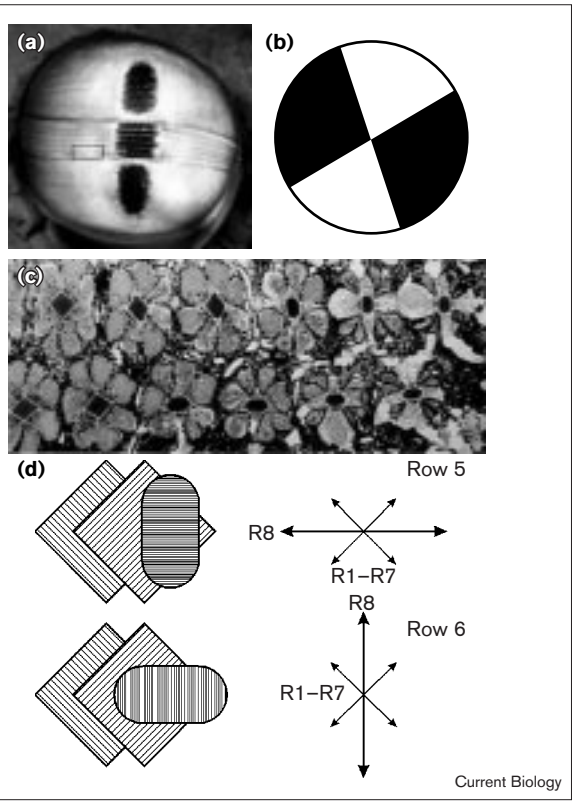
Polarised light exists in the ocean to a depth of several hundred meters [19], generated by the scattering effect of small particles or by reflection and refraction from the water surface. In still conditions, there may also be a direct transfer of the polarised pattern of light from the sky, but this is rapidly degraded over depth [19]. More locally, polarised light can be produced by reflective surfaces such as fish scales [16] or arthropod cuticle [20].

Arthropod and cephalopod retinae are inherently most sensitive to light with an E-vector (the principal vibrational axis [1]) parallel to the microvillar tubes of membrane from which their rhabdomeric photoreceptors are constructed (Figure 1) [18]. PV requires photoreceptors having two, or preferably three, microvillar directions [21] in order to distinguish E-vector orientation. Crustaceans commonly possess two populations of receptor cells with orthogonally arranged microvilli [2,6], and in some cases there are indications that opponency between these cell groups exists at the neural level [7,8]. This 'static' method of PV relies on the differential input from cells whose microvilli are at different angles to each other [1,21]. Theoretically, 'serial' PV may also exist using unidirectional dichroic detectors, rotated about their angle of maximum sensitivity to register a time-varying differential signal to polarised light. Serial PV has never been demonstrated in animals but is possible in mantis shrimps as their eyes show frequent rotational movements [22]. This movement about the long axis of the receptors (Figure 1b) results in the angular positions of the dichroic microvillar arrays within the eye changing — relative to the outside world — over time, providing the basis for serial PV.

Stomatopods and PS

The apposition compound eye of stomatopods is structurally complex (Figure 1) [6]. Through subdivision of the eye into distinct regions, each with a specific function, a single eye can analyse spatial detail, disparity information, colour and polarised light [10]. Six rows of enlarged ommatidia form the mid-band, and two of these (designated rows 5 and 6) are structurally well adapted for PV (Figure 1). The microvillar directions in rows 5 and 6 suggest two possibilities for static PV. Firstly, three directions of sensitivity exist (three-dimensional PV), fulfilling the requirements for completely unambiguous PV [1,10,21]. Second, we know that the spectral sensitivities of R8 and R1–R7 cells (nomenclature explained in Figure 1) in rows 5 and 6 of the mid-band are maximal near 350 and 500 nm [23]. Therefore, two spectrally distinct static PV systems may coexist, one with input from R8 cells, which are sensitive to ultraviolet (UV) light, and one with input from R1–R7 cells, which are sensitive to blue/green light. This is a theoretically ideal PV system [21,24], consisting of two two-dimensional PV channels operating in parallel,

Figure 1



The anatomical basis for complex PS in stomatopod eyes. **(a)** The right compound eye of *Odontodactylus scyllarus* (anterior view). The mid-band is composed of six rows of ommatidia running around the equator of the eye, the bottom two rows of which (rows 5 and 6) contain ommatidia capable of PV (magnification ×20). **(b)** Diagram of the rotational angles that the mid-band of the eye in (a) is capable of occupying. The range over which rotational movements occur is shown by the filled sectors (taken from [22]). **(c)** Transverse, light micrograph through the mid-band row 5 and 6 photoreceptors (rhabdoms) contained within the boxed portion in (a) at the level where the R8 cells join R1–R7. Row 5 and 6 rhabdoms are constructed, like those of many crustaceans, with a two-tiered configuration: a small single cell designated R8, overlying a rhabdom of interdigitating microvillar layers composed of seven cells, R1–R7. The curvature of the eye allows both to be seen in planar section [6]. The distally placed R8 cell rhabdoms have an oval-shaped cross section, are sensitive to UV light [27] and contain unidirectional microvilli [6]. Rhabdoms of R1–R7 cells are diamond-shaped in transverse section, sensitive to blue/green light and constructed from orthogonal interdigitating microvilli [6] (magnification ×500). **(d)** Diagrammatic representation of the microvilli (left) and resultant E-vector maximum sensitivity directions (right) in R8 and R1–R7. Each row is potentially capable of three-dimensional PV. Alternatively, R8 cells may be part of a two-dimensional UV-sensitive PV system and R1–R7 cells of a separate two-dimensional blue/green-sensitive system ([7]; see also [1,21] for a further discussion of dimensionality in PV systems).

each with its own spectral region. Combined with the potential for serial analysis, stomatopods clearly possess the potential for formidable PV capability. In colour vision, however, the possession of several types of spectral channel does not mean the animal is capable of true colour vision [21]. This is equally so for PV, and behavioural proof is required to demonstrate how the system is used in nature.

Behavioural experiments to show true PV

In behavioural tests, two stomatopod species *Gonodactylus chiragra* and *Odontodactylus scyllarus* were trained by operant conditioning to feed from white Plexiglass cubic or cylindrical containers, on one side of which a polarising filter was cemented. The experimental design was similar to that used by Marshall *et al.* [25] to demonstrate colour vision in stomatopods. It takes advantage of the mantis shrimp's curiosity and desire to break into foreign objects using their 'smashing' raptorial appendages [12]. When presented with a choice of three randomly arranged containers, none of which contained food and only one of which presented the E-vector orientation or pattern to which animals had been trained, stomatopods chose the container to which they had been trained, at levels significantly above chance (Figure 2). The experiments fell into three sets demonstrating that stomatopods can learn to choose a polarisation contrast in preference to a brightness contrast (series A); that they cannot be trained to brightness alone (series B); and that they can be trained to a single E-vector direction (series C).

In series A, *O. scyllarus* was trained using a simple polarising contrast pattern of two adjacent triangles of Polaroid with 90° E-vector orientations. This pattern was backed by the white 'perspex' square face of a cube (Figure 2). In tests, shrimps were given a choice of the polarising cube or two different neutral density (ND) cubes randomly chosen from five densities that had an ND value spanning that of the Polaroid material and were also made of abutting triangles of filter (see Figure 2 legend for experimental details).

The results of these experiments, while suggestive, do not conclusively demonstrate PV, as the Polaroid cube may have appeared as a brightness pattern to the PV system, or subtle differences between Polaroid and ND may have been visible to the animals. Ideally, one would like to vary the intensity of the Polaroid independently of E-vector orientation. We found this methodologically impossible, however, as combination Polaroid sheets and different ND filters or backgrounds created lamination patterns and colours distinguishable by the stomatopod colour vision system [25]. No combinations could be found that remained neutral or achromatic in the 300–400 and 675–700 nm ranges, for which stomatopods are known to possess multiple colour photoreceptor classes [23]. Instead, a second (series B) and third (series C) set of experiments were conducted.

In series B, using ND containers of six different densities, we tested whether *O. scyllarus* and *G. chiragra* could discriminate between feeding containers on the basis of intensity alone (see Figure 2). All attempts at this failed, suggesting that choice of the Polaroid containers in series A was mediated only through PV. This still leaves

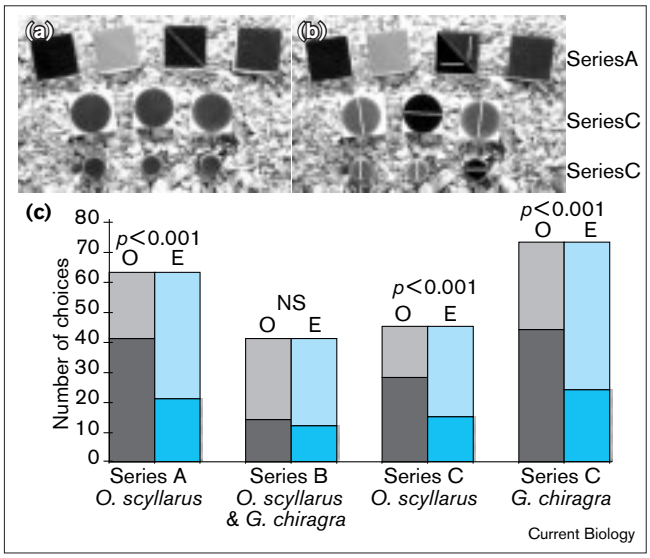


Figure 2

Methods and results of behavioural tests. **(a)** The feeding containers used in the different experiments arranged approximately as seen by stomatopods and photographed together for convenience. Top row (series A), ND and Polaroid cubes. In trials, only two randomly chosen ND cubes were used from a set of five (ND = 0.3, 0.6, 0.9, 1.2 and 1.8). Three of these ND cubes are shown for comparison with the Polaroid cube. Middle row (series C), Polaroid cubes used for *O. scyllarus*. Bottom row (series C), Polaroid tubes used or *G. chiragra*. **(b)** As in (a), but photographed through a vertical polarising filter. Food containers with horizontal Polaroid therefore appear darker compared with (a). All containers have Polaroid filter (Polaroid HN38S) glued to them, except in the top row where only the third cube from the right is covered by two triangles of Polaroid; the other three containers in the top row are each covered with two triangles of ND filter. E-vector orientation has been drawn on Polaroid cubes for clarity. The Polaroid filter was glued to feeding containers using double-sided tape and examined with a polarisation analyser to reveal any gluing patterns and the E-vector orientation. The food was placed inside the tube or cube and the open end(s) sealed with a circular or square glass coverslip using Vaseline. Stomatopods could break through this glass to get at the food within. Containers were arranged behind an opaque removable screen prior to trials. Their position was randomised. Those used for training, which contained food (mussel, shrimp or fish), were never used in trials, where no food was present. The arena was lit from behind the containers, relative to the stomatopod, providing diffuse illumination to minimise reflections that might alter the polarisation signal. Algae was allowed to grow on the sides of the aquaria for the same reason. Further experimental details are available in [25]. **(c)** Results of the three test series conducted. In all cases (except where only ND cubes were used), stomatopods chose E-vectors to which they had been trained at levels significantly above chance (χ^2 *p* values given). Bars show the total number of choices; the dark and light division in each bar represent the 'correct' and 'incorrect' choices, respectively. O, observed results; E, expected results if choices were random; NS, not significant. Series A, four *O. scyllarus* were trained to a Polaroid pattern and given a choice similar to the top row of cubes in (b), but with only two ND and one Polaroid cube. Cubes were 15 mm^3. The ND of the Polaroid averaged 0.7. Series B, three *O. scyllarus* and three *G. chiragra* were trained to single ND values in the series 0.0 (equivalent to white perspex), 0.3, 0.6, 0.9, 1.2 and 1.8 and tested against two other randomly chosen NDs from the same range. Series C, three *O. scyllarus* were trained to containers, similar to the middle row in (b), each having a single circular Polaroid glued to it, and eight *G. chiragra* were trained to the tube-shaped containers (which are easier to manipulate by these smaller shrimps) similar to those shown in the bottom row of (b).

the possibility that, although detected by the polarisation-sensitive elements in the eye, the triangular pattern of the Polaroid cube was perceived as a brightness pattern, so a final set of experiments was performed.

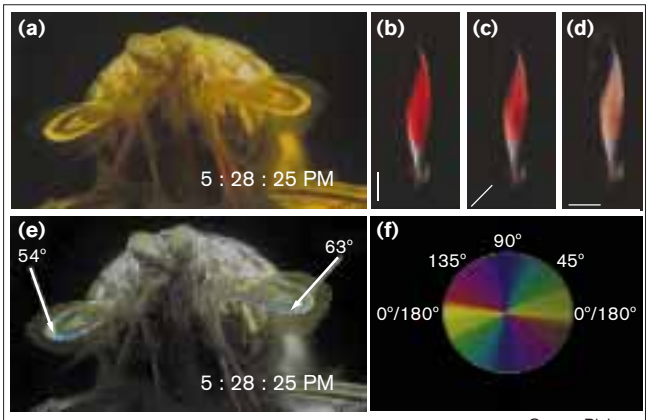
In series C, *O. scyllarus* and *G. chiragra* were trained to feed from a cube or cylinder, on one end of which a single round piece of Polaroid was cemented. This provided no pattern, forcing the stomatopod to discriminate differing E-vector orientation alone. Individual stomatopods were trained to horizontal or vertical E-vector orientations (relative to the bottom of the aquarium; see Figure 2 legend for precautions regarding reflections). In tests, they were given a choice of three cubes or cylinders, one with the E-vector to which they were trained, the other two with E-vectors orthogonal to this (Figure 2a). Both species chose the E-vector to which they had been trained, significantly above chance levels. Series B experiments showed that brightness was not being used and indeed, any of the three targets used in series C will vary in brightness level to any one dichroic detector because of rotations of the eye [22] (Figure 1). The correct choice must therefore be the result of E-vector learning, independent of brightness.

This is the first demonstration that an animal can be trained to choose one particular E-vector orientation over others. All other training paradigms in arthropods have examined other areas of PS (see [1] for a review). Similar experiments have only ever been performed with cephalopods, but in every reported case either brightness cues were not controlled or the choices were between patterns constructed of Polaroid (as in series A), again leaving open the possibility of pattern discrimination rather than E-vector discrimination.

Polarised signals and communication

The ability to recognise polarised light reflected from a discrete object strongly indicates that polarisation patterns may be useful in nature. We therefore examined *O. scyllarus* and *G. chiragra* carapaces, using imaging polarimetry [11] and Polaroid filters, for signs of patterns of polarised light. In both species, areas on the telson and, more notably, the antennal scales showed strong polarisation activity (Figure 3). The antennal scales, which reflect different E-vectors depending on the angle at which they are held (Figure 3), are often presented, for example, in the 'meral spread' [12], a commonly used display, and in other front-on encounters (Figure 3a). The telson may also be presented in a curled defensive posture [12]. Intriguingly, the antennal scales also show a change in colour from yellow to red depending on the angle of the analysing Polaroid filter (Figure 3b–d). Such a colour change may be biologically relevant. The stomatopod's unique colour-vision system, which is also situated in the mid-band region of the eye, is

Figure 3



E-vector optical activity of stomatopod antennal scales. **(a,e)** Frontal views of *G. chiragra* from video images. (a) True colour image. (e) False colour image generated with a portable imaging polarimeter [11]; coloured areas indicate polarised regions. The hue of the coloured areas reflects the E-vector angle shown in **(f)**, which was generated using an imaging polarimeter to view a polarisation axis finder (Oriel radial polarisation filter 25328) whose E-vector varies with radius angle [11]. The E-vector pattern varies with the position of the antennal scale. Note also the polarisation of light from the antennal scale setae. **(b–d)** A single antennal scale of *O. scyllarus* photographed through a plane polarising filter, the angle of which is represented by the lines at the bottom left of each panel. Note how the true colour, shown here, varies with the analyser angle. There are two coloured layers in the antennal scale, a red layer underlying a yellow one. Only the yellow layer is optically active and its reflected E-vector is approximately perpendicular to the long axis of the antennal scale. Its contribution to the colour is reduced when the analysing Polaroid is perpendicular to yellow's maximum E-vector (that is, parallel to the antennal scale). The possible function of this complex colour–polarisation interaction is unknown, but it may enhance the contrast of this signalling structure.

capable of registering such a change [10,23]. The mid-band in stomatopods may therefore be involved in communication in both colour and polarisation space.

Communication using polarised signals has been suggested for other arthropods on the basis of similar evidence [20,26,27]. Within the cephalopods, octopuses are known to recognise simple polarisation cues [18] and patterns [28]; squid use PV to capture prey [29]; and, during operant conditioning, *Sepia officinalis* has recently shown to reflect stereotyped polarised patterns on the frontally directed surfaces of its arms [17]. This latter example provides a remarkable parallel to the use of polarised light in stomatopods. The interesting problem now, both in cephalopods and stomatopods, is to decode the messages communicated in the polarised light domain.

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