

adhesion proteins implicated in integrin activation, including integrin-linked kinase and the filamin-binding protein, migfilin (Figure 1). However, whether these interactions are important for activation or for kindlin-mediated integrin signaling, focal adhesion formation, and cell spreading is unclear and elucidation of the mechanisms of kindlin function will likely require detailed knowledge of its interactions.

What about the kindlin FERM domain? Sequence analysis indicates that kindlins are composed solely of an atypical FERM domain. FERMs are widespread protein–protein interaction domains, typically composed of three subdomains (F1, F2 and F3) that assemble into a compact clover-shaped module. The kindlin FERM is most similar to that of talin, which is unusual in having an additional amino-terminal domain (F0), a large, flexible, membrane-binding loop in F1, and forming an extended structure rather than the compact one seen in all other FERMs to date (Figure 1). Like talin, kindlin contains an integrin-binding site within the F3 subdomain, but a unique and defining feature of kindlin FERMs is the insertion of a pleckstrin homology (PH) domain within an F2 subdomain loop, suggesting that phospholipid binding will be important for kindlin function. It is intriguing that kindlin and talin, two proteins intimately involved in integrin activation, both possess atypical integrin-binding FERMs. A deeper knowledge of the similarities and differences between talin and kindlin is likely to aid in understanding the molecular basis of kindlin activity and of integrin activation in general.

Where can I find out more?

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Primer

Polarisation vision

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Putting on a pair of polarised sunglasses is as close as most of us get to seeing polarised light. Photographers also use polarising filters and, in both cases, the reason for placing such filters in front of eye or camera is to reduce glare and increase contrast within the image or scene viewed. Animal visual systems also utilise polarised light for these purposes, along with navigation, sexual signalling and detecting water. They rarely, if ever, use optical filters to achieve polarisation sensitivity; instead it is an intrinsic property of their photoreceptors. Linear polarising sensitivity is common in the animal kingdom, particularly in invertebrates such as arthropods (insects, crustaceans and spiders) and cephalopods. Linear polarising sensitivity is also known in vertebrates, including fish, birds and a few amphibians and reptiles. In truth, this ability is probably more widespread than we think, and in the cephalopods and many crustaceans it may replace colour vision. While circular polarising photography — used for cancer detection in medical imaging and for (explosive) mine detection underwater — might be considered an obscure man-made optical trick, some animals also have circular polarising sensitivity. Before going on to describe how and why animals utilise polarised light, we briefly examine what polarised light is, why it is called linear or circular, where it comes from and where it is frequently found in natural environments (Figure 1).

Polarised light and where to find it Polarisation can be confusing. Remember that light can be thought of as a wave as it propagates through space. The wavelength of light, measured in nanometres, is used to describe the spectrum, and in colour vision, animals are sensitive to different parts of this spectrum from the ultraviolet to far red (near 300 nm to over 700 nm). Colour vision requires at least two populations of photoreceptors sensitive to different

parts of the spectrum plus a neural comparison of excitation between these to set up the sensation of colour. In polarisation, it is the electrical vector (e-vector) properties of the light waves that are significant. Polarising photoreceptors are often restricted in spectral sensitivity to around 380 nm on land and 500 nm underwater. Indeed, it is important that polarisation sensitivity does not confuse spectral variability with polarisation differences, and colour photoreceptors may deliberately destroy any intrinsic polarisation sensitivity that photoreceptors possess (Figure 2).

Light from the sun is unpolarised, producing e-vectors vibrating at all possible angles perpendicular to the direction of travel of the light beam (Figure 1). When this beam is scattered or passes through dichroic filters, in which some e-vectors are passed while others are absorbed, it becomes polarised. The end result is that most light now vibrates in a single plane, generally denoted by an angle relative to vertical. Dichroic filters wholly (100%) or partially polarise the light that passes through them, depending on the molecular nature of the filter material and the wavelength of the light. Thus, the angle of the e-vector and the degree of polarisation are important variables for polarisation sensitivity, the third being the intensity or brightness of the light beam.

Reflections are an abundant and sometimes confusing source of polarised light on land. Light reflected from shiny dielectric surfaces such as water, waxy leaves and some animal parts (Figure 2) has its e-vector parallel to that surface, while the others are refracted or absorbed. At a specific angle known as Brewster's angle (around 53° for water), the degree of polarisation reaches 100%. Reflection is in fact an example of coherent scatter. Incoherent particle scatter (Rayleigh scattering) is another widespread source of polarised light both in the sky, and underwater and at scatter angles of 90° in air, polarization may reach 100%, while underwater, due to multiple scattering events, the degree of polarisation rarely exceeds 50%.

These extended fields of polarised light, the celestial hemisphere and aquatic background space-light, can be viewed using sun glasses. At dawn or dusk, looking up into a clear blue

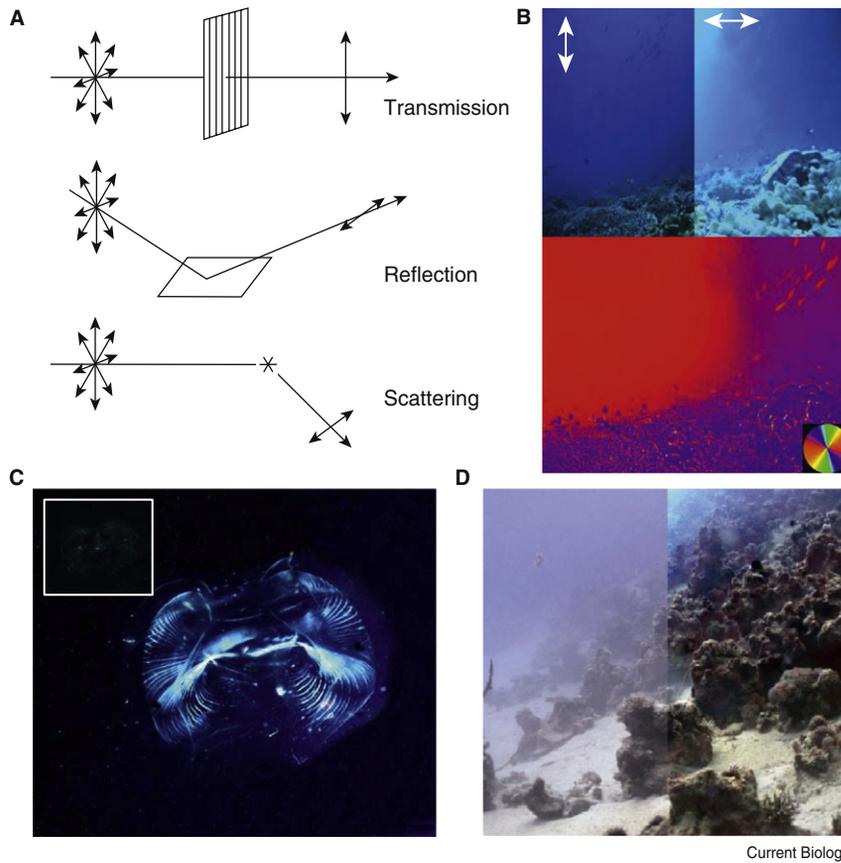


Figure 1. Natural sources and two potential uses of polarised light. (A) Three ways that linearly polarised light may be created: transmission, reflection and particle scatter. (B) Horizontally polarised light in an underwater scene at 20m off The Great Barrier Reef, Australia. The top photograph, split in two and re-combined, shows a reef scene at mid-day. The left top half of the photograph is taken through a vertically oriented polarising filter, and the right half through a horizontal polarising filter (as depicted by the double headed arrows). The same scene is shown below but here the angle of polarisation has been calculated from the two photos above (along with a third photograph with polariser at 45°), and encoded such that red is horizontal (see angle colour key inset bottom right). (C) Camouflage breaking, a possible use of single-axis polarisation sensitivity. Lobate ctenophores (comb jellies) use transparency for camouflage (inset). When placed between two polarising filters, the backing one horizontal and imitating light underwater, and the front filter vertical representing a possible polarisation sensitive photoreceptor, the muscle bands of the ctenophore become clearly visible as they are birefringent, that is they change the angle of polarised light as it passes through the tissue. (Photographs courtesy of Edith Widder.) (D) De-hazing. Polarisation vision may help animals see further underwater. Equipped with two orthogonal polarisation sensitivities and the ability to optimise these may allow aquatic animals to ‘remove’ scattered polarised light. The left side of the photo shows the best result using a single polariser, and the right half is a two-channel polarisation-based de-hazed image. (Figure courtesy of Yoav Schechner, reproduced with permission from Schechner and Karpel 2004.)

sky and rotating your head reveals a dark band of polarisation (90° from the sun on the horizon), splitting the sky into solar and anti-solar halves. Some insects actually make these odd head or body rotations to find direction (Figure 3). The celestial pattern can enter water, where it is visible at shallow depths through Snell’s window, the 46° cone of light into which the 180° hemisphere above water is refracted. Probably more relevant to aquatic animals is

the polarisation pattern beneath this window resulting from the horizontal scattering of sunlight in the direction of the observer. At mid-day this produces a broad horizontal e-vector light field, tilting up to around 45° when the sun is on the horizon (Figure 1).

There are few reflections in the aquatic realm, compared to terrestrial habitats, because of the smaller refractive index differences between objects and water. This makes the background light field, and some

specifically constructed animal signalling systems, the only strong sources of polarised light in water (Figure 2). Whatever its source, both underwater and on land, a polarisation pattern is present that changes with sun angle and direction and provides a reliable pattern for navigation.

So far we have described linear polarising phenomena, where the e-vector of the wave remains at a fixed angle. By convention this is often shown as a double-headed arrow, representing an end-on view of the wave travelling in our direction (Figures 1 and 3). In circular polarisation, instead of the arrow remaining in one plane relative to the light beam, it rotates either clockwise or anticlockwise (right- or left-handed), the helical path of the e-vector arrow-head looking like a right- or left-handed screw, respectively. A full explanation of this is provided in the further reading. It is astonishing enough here to realise that some animals, such as stomatopod crustaceans and possibly beetles, want to see circular polarised light at all.

How animals sense and interpret polarised light

Most animals lack optical polarising filters; instead, their individual photoreceptors are sensitive to polarised light. All visual pigments (more precisely, their chromophores) are dichroic. A vertical e-vector is preferentially absorbed by a visual pigment chromophore also oriented vertically. Two additional, higher-level structural features set up polarisation sensitivity within the eye: the anatomy of the photoreceptor membranes containing visual pigments and the orientation of the photoreceptors within the eye, relative to the outside world.

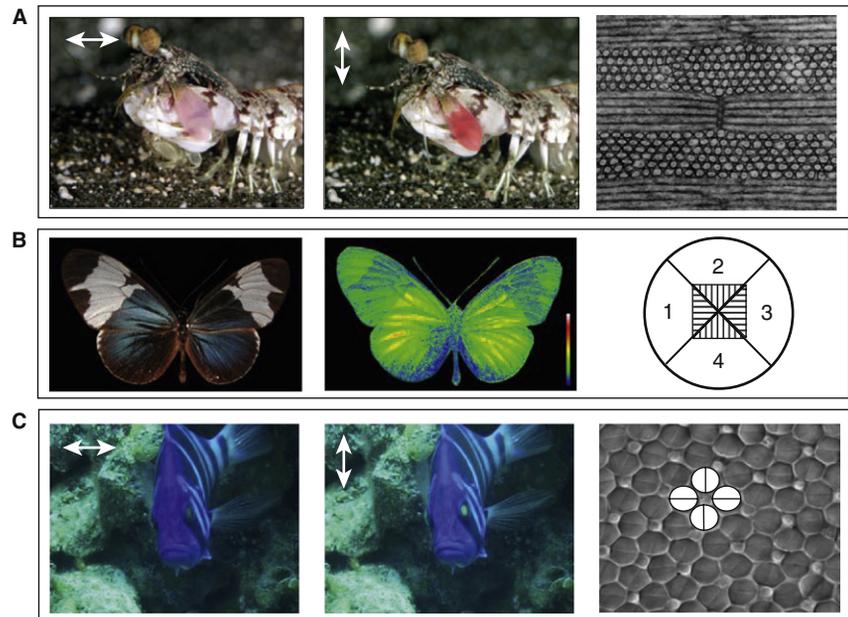
Each photoreceptor cell in many invertebrate compound or simple eyes constructs a *rhabdomere*, the part of the cell containing visual pigment, from tubules of rolled up membrane called microvilli (Figure 2). The geometry of the visual pigment molecules within this finger-like projection, and also in some cases alignment of the visual pigment molecules, give each microtubule an overall preferential e-vector sensitivity along the long axis of the tube. Rhabdomeres in polarisation-sensitive *ommatidia* of insects and crustaceans (there is one ommatidium per facet in a compound eye, each

containing several, commonly eight, rhabdomere-bearing cells), as well as adjacent receptors in cephalopod retinas, generally show orthogonal microtubule arrangement, indicating sensitivity to e-vectors at 90° to each other (Figure 2). While microvilli could be arranged at any angle, this 90° arrangement forms the basis of many polarisation-sensitive systems.

Although rarely demonstrated (Figure 3), opponency at higher neuronal levels is presumed to compare signals from orthogonal cell populations. For example, a single ommatidium viewing a lake surface would have cells with horizontal microvilli excited greatly and those with vertical microvilli excited much less. Opponent processing by interneurons contacting these cell populations carries the information necessary to identify the lake as a source of horizontal e-vectors. There are several species of insects that seek out water such as lakes and ponds in this way. Success in locating the lake requires that, relative to the outside world, the microvilli in receptor units are vertical and horizontal in the first place.

Knowing the arrangement of polarisation-sensitive photoreceptors within the eye and the eye's position relative to the outside world is critical for a clear understanding of how e-vector information is encoded. For example, insects that navigate using the polarised pattern of the sky have specific upward looking *dorsal rim* photoreceptors which are arranged in a fan of orthogonal e-vector sensors. Through rotational head or body movements, insects can match up to the celestial e-vector pattern, locking on to set their internal compass during the day. Each compound eye ommatidium has a specific polarisation sensitivity, determined easily from anatomical or physiological study. Simple eyes require more care as the projection of each photoreceptor out into the world, and specifically its e-vector sensitivity, must be accounted for. Spider and cephalopod simple eye photoreceptors possess microvilli, like those in insects and crustaceans, making this task easier, especially as their microvilli are also orthogonally arranged within local retinal areas or, in spiders, sometimes between eyes.

Understanding polarisation sensitivity in vertebrates is



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Figure 2. Polarised light reflections and e-vector sensitive photoreceptors.

(A) Left: Mantis shrimp (stomatopod) polarising reflections are revealed on the antennal scales, the paddle shaped appendages, of *Odontodactylus latirostris* by photography through vertical and horizontal polarising filters. Right: a transmission electron micrograph of a crustacean, photoreceptor (rhabdom) (photographs by Roy Caldwell). (B) Left: Nymphalid butterfly (*Heliconius sapho*) polarising reflections. Left photograph is a normal image and right is a false colour image where the degree of polarisation follows the inset key from 0–100%. Right: a schematic insect photoreceptor in transverse section with a central rhabdom made of microtubules constructed by four surrounding cells. These abutting rather than overlapping microvilli are arranged in two orthogonal directions. Cells 1 and 3 and then 2 and 4 would provide opponent input to interneurons interpreting the polarised signal. (Photographs courtesy of Jonathan Douglas.) (C) Fish may possess polarisation sensitivity based on the orthogonal arrangement of their double cones. A photoreceptor wholemount, right, shows double cone members (with a set of four double cone inner segments over-drawn for clarity) that may preferentially reflect polarised light. Left: the hamlet *Hypoplectrus indigo*, photographed through orthogonal polarising filters may possess a polarising filter in the cornea, as indicated by the different reflections from the eyes.

more difficult, as rod and cone photoreceptors are not constructed from microvilli, but rather have membrane stacks of flattened disks or plates. Light passing through these normal to their surface does not encounter preferentially oriented sets of visual pigments. As a result, the cone photoreceptors thought to be polarisation sensitive in vertebrates must have secondary adaptations to permit differential responses to polarised light. These modified photoreceptors are often *double cones*, two cones that are stuck closely together. Although double cones are present in the eyes of most vertebrates known to have polarisation sensitivity, fish provide the clearest examples. To start with, fish double cones may be arranged in an orthogonal manner in the retina, a strong indication of the presence of polarisation sensitivity (Figure 2).

Further modifications include: stacking the disks on their side so the visual pigment chromophore does become preferentially aligned (only known in one fish family, the anchovies), reflection of polarised light at the joining membrane or septum of double cones in fish and, again in fish, a small inherent dichroism resulting from a slight tilting of the membrane plates. These latter two solutions provide only weak polarisation sensitivity and, compared to invertebrates, the retinal substrate for sensing polarised light in vertebrates is less well understood.

So far we have considered orthogonal systems with two directions of polarisation sensitivity. A full analysis of polarisation requires three directions of sensitivity with e-vector reception at 60° separations, for example. This three-way input avoids null points, allowing the

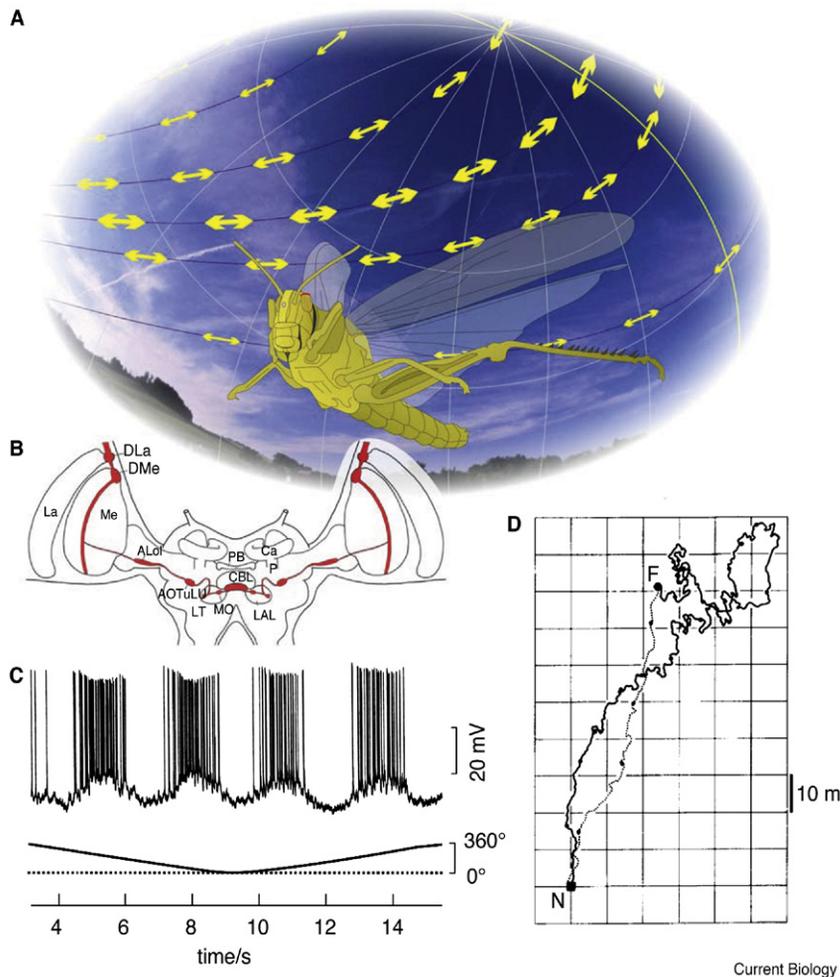


Figure 3. Insect navigation using the celestial pattern of polarisation. (A) A schematic locust flies under a sky panorama on which has been superimposed the celestial polarisation pattern, the e-vectors within which are represented by double-headed arrows of different thickness for different degrees of polarisation. (Figure courtesy of Stanley Heinze and Uwe Homberg.) (B) Polarisation vision neural pathways (red) in a schematic locust brain. These lead from photoreceptors in the specialised dorsal rim eye area (red in A) through the optic lobes and into the central complex of the brain. (Figure courtesy of Uwe Homberg, reproduced with permission from Träger *et al.* 2008.) (C) An intracellular recording from a POL interneuron in the cricket *Gryllus campestris* responding to an input stimulus of a rotating e-vector (0–360° shown below). Bursts of spikes recorded from POL interneurons encode polarisation opponency from two large populations of photoreceptors in the dorsal rim area of the eye. (Figure courtesy of Tom Labhart, reproduced with permission from Wehner and Labhart 2006.) (D) The sort of behaviour that this navigation system can conduct is direct home finding after long foraging runs, here seen in the desert ant *Cataglyphis*. The outward searching track is dark, the direct run to home having found food and light. (Figure courtesy of Rüdiger Wehner.)

determination of e-vector angle, degree of polarisation and intensity. It is the basis of the false colour images in Figures 1 and 2. Interestingly, all currently known polarisation systems are simpler than this (Figure 3), having receptors with only two orthogonal e-vector sensitivities. Polarisation photoreceptor arrays exist, notably in the dorsal rim of insect eyes, where the angle between adjacent orthogonal-pair photoreceptors changes gradually

(Figure 3). This spatial arrangement is used for navigation, but apparently does not disentangle all polarisation parameters. Electrophysiological recording in brains and optic neuropils (signal interpretation centres) provides no evidence for such complex deconstruction of polarised light, and this is an interesting difference with colour vision where many animals pass beyond dichromacy to tetrachromatic systems and beyond.

Neural integration of polarising signals is best understood in the relatively simple nervous systems of insects (Figure 3). So-called POL interneurons in insects, located in the optic lobes of, for example, crickets, ants and locusts, exhibit variable spiking activity to a rotating polarising stimulus, usually with 180° periodicity (Figure 3). E-vectors eliciting minimum and maximum activity are generally orthogonal. Opponent neurons are also found deep within the central complex of the insect brain. E-vector-sensitive areas exist in the crayfish central nervous system, but crustacean systems are less well characterised. Recordings from the brains of fish also reveal e-vector-correlated activity.

There are no cells known that exhibit intrinsic circular polarisation sensitivity. Instead, circularly polarised light is converted to linear by optical filters. In the eyes of stomatopods, these filters, in fact themselves sections of an overlying rhabdomere, are $\frac{1}{4}$ wave retarders (see further reading) which recover the 90° offset constituent vectors of the incoming light. They thus pass linearly polarised light to underlying linear receptors. Little is known about any polarisation opponency in stomatopods. We do know from direct electrophysiological recording that stomatopod photoreceptor sets are capable of multiple angles of linear polarisation sensitivity in two wavelength ranges, UV and blue/green, as well as both left- and right-handed circular polarisation sensitivity. The possible behavioural relevance and neural decoding of this astonishingly complex system remains a challenge for the future.

Uses of polarised light, known behaviours and assumed possibilities

Polarisation behaviours are sometimes divided into polarisation-sensitivity-mediated behaviours and those requiring true polarisation vision. Polarisation-sensitivity-mediated behaviour is stereotyped and linked directly to a polarising pattern or signal. Navigation falls into this category. True polarising vision requires higher levels of processing. Here animals must discriminate polarization properties (for example, the e-vector angle) and then perform associative learned responses accordingly. Cephalopods and stomatopods are probably the only animals for which polarisation vision

has been convincingly demonstrated, but, as in our title here, it is often used as a blanket term to cover both capabilities.

Navigation

Direct behavioural evidence exists for the navigational ability of insects. In all aspects, this is the best studied polarisation-sensitivity system we know. The desert ant *Cataglyphis* has a *dorsal rim* system and uses the celestial polarisation pattern to find direct routes to its home nest in a featureless environment (Figure 3). Direct paths are also preferred by dung beetles, this time away from the dung pat to avoid competition over their dung-balls. In both ants and beetles, predictive mistakes made when polarising filters are held above the animal provide a direct demonstration of how this system is used. Remarkably, dung beetles and possibly nocturnal bees can navigate using the celestial polarization pattern of a moonlit night sky.

Evidence of celestial pattern navigation also exists in fish (notably salmonids, presumed to view the celestial pattern through Snell's window) and birds. Local polarisation-based navigation is suggested in the water flea *Daphnia*, where the true underwater field of scattered polarised light orients the animal's vertical movements. Finally in navigation, the water-finding response of water beetles is certainly horizontal e-vector driven, making these animals crash into man-made sources of horizontal e-vector when they mistake a wet car or tarmac road for a lake.

Signals and contrast enhancement

Good behavioural evidence exists for linear polarisation sensitivity in cephalopods and stomatopods, and for circular polarisation sensitivity in stomatopods. In all cases, the animal was required to learn a polarising signal in a choice test with food as a reward. A potential circular polarisation phototaxis has also recently been suggested in the metallic coloured scarab beetles. In all three animal groups, their polarisation sense may function in polarisation signalling between individuals or sexes. Cephalopods such as squid and cuttlefish, as well as stomatopods and indeed butterflies, are known to reflect linearly polarised light from specific body areas (Figure 2). Some

stomatopod species and scarab beetles reflect circularly polarised light as well. In stomatopods and butterflies there are differences between the sexes so sexual selection and a desire for covert signalling may have driven the evolution of these signal pathways. For the marine animals, polarising signals may be more reliable than colour, as water selectively changes wavelength but not e-vector angle over depth. In none of these examples of potential polarised signalling have successful manipulations been performed to show the value or indeed real purpose of the signal.

Indirect evidence suggests that linear polarisation sensitivity could be used in camouflage breaking of transparent or silvery animals in the ocean, because such animals either reflect polarised light or are *birefringent* (see Figure 1 for explanation). Despite their appeal, recent evidence indicates that both these hypotheses are flawed (see further reading) or at least not as simple as we would like them to be. Camouflage breaking is a category of contrast enhancement using linear polarisation sensitivity; de-hazing, de-glinting and other suggested noise reduction mechanisms based on linear polarisation sensitivity also come under the umbrella of contrast increase. Underwater, in particular, haze from forward scatter prevents vision over much distance. Inspired by stomatopod vision, Yoav Schechner recently proposed a de-hazing algorithm for machine vision. Interestingly, it requires just two channels of orthogonal e-vector sensitivities and uses a simple subtractive process between resultant images, analogous in some ways to *opponent* processing (Figure 1). Again, while an attractive possibility for the orthogonal sensitivities of cephalopods, crustaceans and possibly fish, as de-hazing can at least double imaging distance, no direct proof exists.

Future directions in polarisation vision research

Polarisation vision is so poorly understood that a glib "all areas above" might do. Investigating the de-hazing system is a clear priority for underwater work, both in terms of behavioural measurements that show animals with orthogonal systems can see further than predicted and

in finding the neural integration that might encode the algorithm. We would like to progress in understanding the circular polarisation sensitivity systems and encourage colleagues to look for this sensory capability in other animals, as there are some obvious anatomical features to start with. In the vertebrate world, a clear and physiologically backed-up retinal substrate for linear polarisation sensitivity is a holy grail that has been polished with near misses over the years. In both vertebrates and invertebrates, it is surprising that dichroic filters in front of the receptors have not been described. Figure 2C shows a possible case of this that we are investigating in fish. Finally, the terrestrial world of linear polarisation sensitivity is dominated by navigation using the celestial large field pattern, while in the aquatic world, linear polarisation sensitivity focuses on signals and potential contrast enhancement mechanisms. It would be worthwhile to invert these emphases and look for more signals and contrast enhancement on land and navigation underwater using the predictable pattern of scattered polarised light.

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