Primer

Unconventional colour vision

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Colour vision in humans is 'middling' at best, both figuratively and literally in the animal visible spectrum of 300–750 nm. This comes as a surprise to many of us as we cannot imagine the need to see more colours than the millions we can manage. The fact is that many animals have colour vision that exceeds our red–green–blue (RGB)-based trichromacy. Birds and reptiles, along with several freshwater fish, have four colour receptors, for example, extending both ends of the human visible spectrum (400–700 nm), and may be termed tetrachromats. Horses, dogs, some primates and barracuda, on the other hand, have only two spectral classes of photoreceptors, and may be likened to red–green colour blind humans in performance; they are dichromats. Some animal groups, including insects, smaller fish, most birds and even mice make use of the ultraviolet (UV), a part of the spectrum we avoid, while others may see the spectrum that we have available to us but in more detail (Figure 1). The past two decades have also revealed animals with the potential for 'penta'-chromacy and beyond. Stomatopods (mantis shrimp) and butterflies possess up to twelve spectral sensitivities in their eyes and our mind boggles at the potential for 'dodeca'-chromatic colour space. How does a shrimp's brain decode a twelve-dimensional colour space, if indeed it does? Do butterflies require a higher level of colour vision to interpret the information of colours their wings seem to contain? Are we missing something?

In this Primer, we suggest simple answers to these questions, and point to some of the problems that arise when we assume that all animals conduct colour vision the same way. Stomatopods and butterflies, but also others including water fleas (*Daphnia*) and some fish, appear

to have too many colour channels, based on their lifestyle or ecology. These animals may be termed unconventional in the way they process the colour message, in their behavioural interactions with colours or in the ways their colour systems are subdivided across the retina to sample different areas of visual space (Figure 2). Humans and other primates with cone cells sampling in three different wavelength ranges earn the classification trichromat by combining this information such that the ratio of excitations from different cone types encode colour. Our receptors, frequently labelled S, M, and L for short, medium and long wavelength, are maximally sensitive around 435, 540 and 565 nm (Figure 1). The broad-band shape of these sensitivities indicates the lack of secondary filtering, to tune or change where the visual pigment responsible for each sensitivity absorbs maximally. Our lens in fact does filter out some light below around 400 nm, removing the UV that other animals may find useful. Filtering and sharpening of spectral sensitivities by photostable pigments or photoreceptor tiering is often critical to the more unconventional colour vision types as we discuss below (Figure 1).

The spacing of the three human spectral sensitivities is also noteworthy, with the M and L channels overlapping more than would seem sensible if one were designing a system from scratch. The detailed possible reasons for this are outside the scope of this Primer, but receptor sensitivity spacing is important for part of our discussion here. Compare, for example, humans and the also trichromatic, although short-wavelength-shifted, honey bee in Figure 1. Where spectral sensitivities are placed in the spectrum reflects the environmental and evolutionary history of the organism and provides important clues as to the type of colour vision it constructs. Links between environmental constraints and vision form part of the rich world of visual ecology, a discipline pioneered by John Lythgoe (1979) and recently reviewed by Cronin *et al.* (2014).

Ways of investigating colour vision
On discovering an animal that may have colour vision, three areas of

knowledge are useful in determining if its colour sense is something like ours or done differently: first, the number, shape and chromatic spacing of the spectral sensitivities (Figure 1); second, behind the retina, how interneuronal channels encode a chromatic message to the brain; and third, the behaviour of the animal relative to spectra (light and reflectance) in its environment.

Behavioural interrogation is the most complete way of discovering the limits and type of colour sense as it involves both the full system and reveals how colour vision may be implemented by the animal in question (Figure 3). The anatomy and cellular component of the retina is relatively easy to determine, while the colour channel combination is the hardest to demonstrate. It is almost certainly this that has led to some of the inaccurate interpretations of other animal colour vision, as we tend to assume, for example, that any species with three spectral sensitivities must be trichromatic. Dichromacy, trichromacy and tetrachromacy are often used rather loosely once we know only the retinal complement of spectral channels, and in fact to correctly label a system, we need to know how and if the channels combine information. Humans are true trichromats, as we use all three S, M and L receptors, in combinations such as S–(L+M) or (M–L) to encode relative ratios of excitation, as the cones view colour in the outside world. It is the different cone response triplets that encode the many colours we identify while (M+L) is used for luminance or intensity tasks.

One advantage of colour vision is to increase contrast between objects; a red apple against a green tree, for example. The trichromatic primates may have specifically added a third (L) photoreceptor in order to resolve red/green differences to identify objects such as ripening fruit, young forest leaves or female hind-quarters in oestrus. What about tetrachromats, possible 'penta'-chromats, 'octo'-chromats and 'dodeca'-chromats? Are they attempting to combine colour channel outputs in a way that adds dimensionality to their colour space and enables them to examine colour differences in ever increasing

exquisite detail? The answer beyond tetrachromacy is almost certainly no and part of the reasoning here requires a short aside into physics and the nature of colour.

Why is trichromacy common?

In the early 1980s, Horace Barlow and others noted that the colour vision status quo for many species was trichromacy, with some venturing as far as tetrachromacy, and this was for a good reason. He used the physical information available in colours, their spectral reflectance curve shape and positioning, to demonstrate that distinguishing almost all colours on earth can be achieved with three spectral channels of a typical visual pigment breadth and shape, over the 400–700 nm human visible spectrum. To expand interest into UV, four spectral sensitivities are needed. Beyond this number, the returns in information are diminishingly small. Tetrachromatic birds and reptiles narrow the response curves of visual pigment alone with photostable filters, coloured oil droplets in their eyes. This makes a better fit of their evenly spaced four sensitivity classes in the spectrum with less redundancy or overlap and, as Misha Vorobyev (2003) has pointed out, increases the discriminatory power of their colour vision close to perfection. Once animals do go beyond four, rather than assuming some sort of discriminatory superiority in 'penta'-chromacy, we must start looking for non-conventional colour vision explanations.

Animals with simple colour vision lifestyles may have four spectral channels, but not use them for complex colour tasks. Comparing the tetrachromatic bird (Figure 1E), with the apparently (but not really) tetrachromatic water flea (Figure 1B; see also Smith and Macagno, 1990), and assuming both have the same level of colour sense would be a mistake. Even within the limits of three or four channels, it may be that unconventional colour vision kicks in and this brings us back to behaviour.

Behaviour and ecology

Before further discussion of 'penta'-chromacy and beyond, it is worth asking why all animals are not tetrachromats. Phylogenetic background may play a role but,
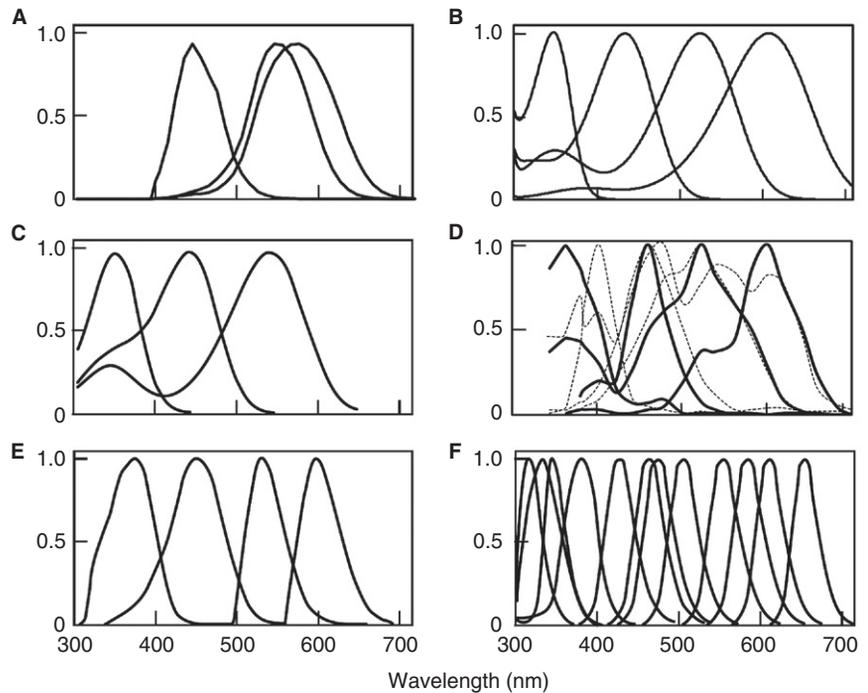

Figure 1. Comparative spectral sensitivities.
(A) Human; (B) the water flea, *Daphnia* (plotted as visual pigment templates but based on electrophysiological measurements of Smith and Macagno, 1990); (C) honeybee; (D) butterfly, the Japanese yellow swallowtail *Papilio xuthus*; (E) bird, the blue-tit *Cyanistes caeruleus*; (F) stomatopod, *Neogonodactylus oerstedii*.

as Dan Nilsson and others have noted, vision evolves rapidly to needs and adaptational reasons, including behavioural and environmental constraints, are likely to be more important. Expanding on environmental factors first, John Lythgoe (1979) drove the idea that marine fish often have only two colour channels because they inhabit spectrally limited water depths or types, and may view objects through long distances underwater. In either circumstance, the selective absorbance of wavelengths at either end of the spectrum limits effective colour vision to the blue–green. In other words, there is not sufficient colour contrast information to need more than two spectral channels. Dichromatic dogs and horses, on the other hand, inhabit a terrestrial world, but have evolved to take advantage of only those spectral regions important to them. Just because there is a 300–750 nm spectrum available does not mean it has to be used. This behavioural limit is analogous to species-specific frequency ranges in hearing, which again may depend on livelihood or task. This may seem a bit off-subject, but the selective use of spectral regions is important for understanding the way polychromatic eyes or parts of eyes may look into different spectral zones for different jobs.

Staying with four colour channels for a moment longer, behavioural considerations also allow us to suggest why the waterflea *Daphnia* is unlikely to be tetrachromatic, like a bird. Although this diminutive crustacean displays four relatively well spread spectral channels at 348 nm, 434 nm, 525 nm and 608 nm (Figure 1B), its low resolution compound eye (containing only 22 ommatidia, the visual units of the compound eye) and simple lifestyle certainly put it in what we are calling the unconventional colour vision category.

Almut Kelber and Daniel Osorio (2010) identified four grades of colour vision on a behavioural scale: first, colour taxes or light environment seeking; second, wavelength-specific behaviours directed towards objects; third, colour learning through neural representative of colour; and fourth, colour appearance including colour categorisation.

Figure 2. Regionalisation of spectral sensitivities.
(A) The apposition compound eye of the stomatopod *Odontodactylus japonicus,* shown on the right, with mid-band region of enlarged ommatidial facets (photographs: Roy Caldwell). This zone contains twelve spectral sensitivities (Figure 1F). (B) UV-induced auto-fluorescence in *Papilio xuthus* (left) and tapetal reflection under white light epi-illumination in *Pieris rapae* (right). In addition to the regionalization, these pictures indicate that the butterfly eyes are locally a random mesh of spectrally heterogeneous ommatidia.

This is useful, as long as we also accept that some animals may cross category boundaries or possess multiple grades. The colour world of *Daphnia* seems largely confined to the first of these categories. It displays charmingly labelled 'colour dances' in response to different wavelengths of illumination rich in UV or middle wavelengths: minute migrations that enable the animals to position themselves in the water column to both find food (mid-spectrum green algae) and avoid damaging UV irradiation close to the surface or shore. It is likely these behaviours are hard-wired to one colour receptor or simple colour receptor combinations and complex comparisons between the four channels are not made.

Insects that climb the spectrum
Adriana Briscoe and Lars Chittka (2001) have detailed how many insects, such as the bee (Figure 1C), have an evolutionarily ancient, short-wavelength-biased trichromacy. The basic set of UV, blue, green channels apparently pre-dates the emergence of flowering plants (angiosperms), Chittka noting that fossil bee's nests predate flowers is evidence for this interesting order of events. Flowers

are important in the lives of many insects, and it is possible that flower colours evolved to suit or at least became co-adapted with an existing colour sense in insects. Although there are many orange and red flowers, the long wavelength end of the spectrum seems largely untapped by many insects, but the old dogma that red flowers were for the birds has been overturned.

In fact, the more we look at insects the more exceptions we find. Behavioural evidence that the leafhopper, *Nephotettix cinticeps*, responds to very long wavelengths, beyond 700 nm, requires the use of the green photoreceptors that remain sensitive at these wavelengths. The Mediterranean beetle *Pygopleurus israelitus* moves its long wavelength photoreceptor to 628 nm, possibly in an effort to interact more effectively with the predominantly red flowers of its habitat. Some butterfly species also shuffle their three colour channels towards longer wavelengths, while others take full advantage of this untapped spectral real-estate by adding extra spectral sensitivities up the spectrum beyond green and also in-filling with broad-band or multi-peaked sensitivities (Figures 1D and 3B).

Extending the range of a trichromatic system, behaviourally or physiologically, comes within conventional colour vision, as might adding a fourth sensitivity for tetrachromatic discriminations. As Figure 3B demonstrates, butterflies are tremendously variable, both within and between family, and even between genders. They certainly venture into the realms of unconventional colour vision, with well established sets of receptors from three up to at least nine spectrally distinct photoreceptor classes (Arikawa and Stavenga, 2014). Cutting several long but fascinating stories short, some are just 'normal' trichromats, others retain trichromacy but add spectral channels beyond this, some appear to elevate to tetrachromacy with four sensitivities, while at the extreme, butterflies such as the swallowtail *Papilio xuthus* extend to tetrachromacy but also add further channels on top of this (Figure 1D).

Not all tasks are necessarily colour-related, with luminance and movement detection being driven often by a subset of green receptors (Figure 3B). Butterflies are perhaps more colour-obsessed than other animals, using it for navigation, feeding in and around flowers, looking at each other (mate choice and territoriality) and choice of egg-laying site, which brings with it a real effort to get the right green leaf. Some of these behaviours clearly require the complex colour judgement that trichromacy or tetrachromacy allows (the third category in the scheme above); however, others are managed more simply. Put another way, a butterfly such as *P. xuthus* with eight spectral channels does not have two different tetrachromacies for any reason.

Behaviourally, butterflies have been known for many decades to respond innately to specific wavelength ranges and undertake stereotyped behaviours. *Pieris rapae* with its six types of spectral receptors (Figure 3B) shows an open space escape reaction to UV/violet, a proboscis extension feeding behaviour to blue and an egg laying reaction to green. These are wavelength-specific behaviours in the second category of the scheme above, and are innate responses

to specific stimuli, not involving any wavelength discrimination or learning. They differ from simple taxes in that they usually involve interaction with small objects, flowers, leaves or other butterflies, rather than extended light sources. These behaviours are also most likely hard-wired, driven by simple neuronal connection rather than complex comparisons and may even be run by a single spectral class of receptor — we don't know yet. Intuitively, it is simple to see how once a new task is required for survival, another receptor sensitivity is added; another tool in the toolbox of spectral solutions.

An unusual example of red sensitivity addition was found in the family Pieridae. A sulphur butterfly, *Colias erate*, creates red channels through selective filtering of a green-absorbing rhodopsin by the reddish screening pigments around the rhabdom, not by adding new visual pigment opsins. Because of the sexual dimophism in the pigments, females have three more channels in the red wavelength region, while males add only one (Figure 3B). The behavioural need for the common 660 nm receptors is probably to detect red flowers for foraging, but the array of three receptors in the red wavelength region in females may be for finding high quality leaves on which they lay eggs. Filtering with photostable pigments in or around the rhabdoms (photoreceptors) is a feature of butterflies and is what results in the emissions seen from their eyes when we shine light in (Figure 2B). This, along with selective colour reflections from tracheal tapeta generally tune and shift existing spectral sensitivities in butterflies and, like the oil droplets in bird eyes, may optimise the chromatic space examined, enhancing colour distinctions. The even spacing of papilionid tetrachromacy (Figure 1D) is evidence of such selective filtering, not to increase number as in *C. erate*, but to tune within a spectral area.

Eye-shine in butterflies results from the filter colour and photoreceptor type and is both beautiful and useful (Figure 2B). It has been used to map and categorise the multiple photoreceptor types a single species may exhibit and demonstrates
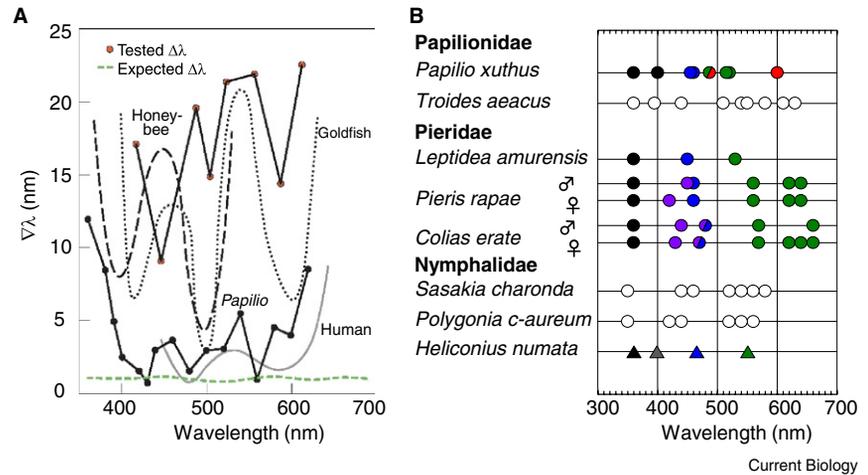

Figure 3. Comparative wavelength discrimination in different animals and spectral sensitivity variability in butterflies.
(A) Wavelength discrimination functions (Δλ) determined through behavioural testing with the animal choosing between narrow-band stimuli. Coloured data points (red and green) are for the stomatopod *Haptosquilla trispinosa*; other discrimination functions are labelled according to species (based on Koshitaka *et al.* 2008 and Thoen *et al.* 2014). The very different observed and expected result and apparently 'poor' discrimination in stomatopod indicate a form of colour signal processing different to other species, including the butterfly. (B) Butterflies whose spectral receptors are identified. Positions of circles indicate spectral sensitivity peak wavelengths determined by electrophysiology coupled with cell marking. Positions of triangles are absorption peaks of visual pigments predicted from spectral sensitivity of the entire retina. Symbols that are coloured indicate absorption spectra of visual pigments whose gene expression has been shown in respective photoreceptors. Black and gray correspond to ultraviolet. Note that some photoreceptors co-express two visual pigments with different absorption spectra.

that many species subdivide the eye into areas containing different photoreceptor sub-classes (Figure 2). This also helps in understanding the sometimes bewildering photoreceptor variety in a single eye, as different regions are assigned different behavioural tasks. Dorsal rim areas of compound eyes are found in many insects and contain a majority of UV receptors associated with navigation through polarisation from a UV-rich sky. Butterflies and other insects, including bees, further subdivide the eyes into zones that may be used for foraging; the downward looking areas, mate detection; the forward looking zones and other specified dorsal regions sometimes for either mate detection or prey detection against the sky. Each area is populated by the receptors and spectral sensitivities required for each job. Subdivision such as this (Figure 2) explains the apparent overlap or redundancy seen if we plot all butterfly spectral sensitivity on a single graph (Figure 1). The only confusion here is our way of plotting an otherwise beautifully evolved set of sub-tasks.

Stomatopods are not butterflies
As crustaceans, stomatopods are closer to cladocerans (*Daphnia*) than butterflies, yet as the work of Tom Cronin and one of us (J.N.M.) has shown, they also have multiple spectral sensitivities for colour vision: they also subdivide their compound eyes, use photostable filters and specific photoreceptor arrangements to tune receptor spectral sensitivities and have multiple receptor classes. Twenty receptor types have been defined: twelve for colour, six for polarisation and two with overlapping function for luminance tasks. The colour sensitivities reside in the top four rows of ommatidia of the so-called mid-band eye region, clearly delineated by the flanking peripheral regions by increased ommatidial size externally and internally by several modifications including sets of colour filter and receptor tiers (Figure 2A). This places them firmly in the unconventional colour vision camp.

A glance at the sharply tuned sensitivities, extending far into the UV (see Bok *et al.* 2014), suggests some form of order, more so perhaps than the overlapping and differently shaped sensitivities in butterflies (compare

Figure 1D,F). This same even spacing and receptor overlap around the 50% absorption level can be seen in bird and butterfly tetrachromats and insect trichromats. All are solutions to sample colour space evenly and comprehensively. From Horace Barlow (1982), we already know that a dodecahedral space is ridiculous. So, are they combining four trichromatic or three tetrachromatic systems, each responsible for a different spectral sub-zone? As with all animals discussed here, our major area of ignorance lies in the neuronal interconnectivity beyond the eye, but early indications in fact did suggest a set of six dichromatic colour analysers, each with a spectral region within which chromatic differences would fall out in great detail. The need for colour constancy in the diverse illumination habitats of the aquatic world also supported this solution; however, new evidence suggests an even more unconventional setup and a way of achieving colour analysis so far totally unique.

To cut to the chase, instead of comparing receptor outputs in what is called opponent processing and is the basis of even the lowliest dichromacy, stomatopods may identify colours as a series of excitation patterns. Several lines of evidence suggest this unconventional solution, the most compelling being the recent demonstration by Thoen *et al*. (2014) that they are in fact very poor at colour discrimination. Their wavelength discrimination ability ($\Delta\lambda$) is far worse than those of butterflies, bees, humans, birds and goldfish (Figure 3A). In such tests, animals must distinguish two stimuli of increasingly similar, relatively monochromatic, spectral distribution and the confusion point is where threshold lies for that spectral region. Two or three optima fall close to the overlapping regions of adjacent spectral sensitivities (see, for example, tetrachromatic goldfish and trichromatic human or bee in Figure 3A). Wavelength discrimination at best is around 2 nm in humans and better than that in places for the tetrachromatic butterfly *P. xuthus*. Theoretically stomatopods should be better than this across most of the spectrum (see green curve in Figure 3A) but in fact they are the worst so far tested with this behavioural task (red data points in Figure 3A).

As with butterflies, the details of this unconventional way of constructing a colour sense is still under investigation; however, the current working hypothesis of binning excitations into the twelve available colour sensitivities would yield a coarse but fast colour detection mechanism. Stomatopods may possess a memory library or 'look-up-table' of colours and respond determined by innate and/or learned neural mechanisms associated with these. While hypothetical still, another line of evidence supporting the idea is the way stomatopod eyes sample the world. The three black so called pseudopupils in Figure 2A indicate where the eye looks and indicates that 70% of the whole eye, including all the colour receptors from the mid-band, look into a 10° strip of space. The result of this unusual optical arrangement is that the animal must scan the strip over objects to take in their detail and the resulting constant scanning eye movements are a feature stomatopods are also famous for. A coloured object is therefore not discriminated best by spectral sensitivities looking at the same spot in the world, but by a sequence of colour receptors scanned over the object whose outputs are binned and the binning pattern registered as a colour pattern rather than a colour ratio.

Summary

Butterflies and stomatopods are certainly outliers in their unconventional colour sense and despite some similarities at first glance, in fact sample the world of colour very differently. In one way, butterflies are relatively conventional, possessing either tri-or tetrachromatic colour vision, then just adding one or several task-specific sub-mechanisms onto this. It is the stomatopods so far that have really pushed the boat out into a different colour vision mechanism. Over 400 million years of independent evolution they have arrived at a solution with more in common with the way a satellite sensor examines the colours of the earth than other animals.

Remember, however, that unconventional colour vision is not just the realm of the serially polychromatic. Apparently waterfleas with four classes of spectral receptors living in ponds operate a task-specific spectral sense with no need, or indeed neural processing power, to construct a complex discriminatory mechanism. It seems they have the butterfly added-extra set without the more complex comparative chromatic mechanisms, although in truth, conclusive behavioural proof is lacking. Behavioural observation of colour vision in the ecological context of each animal is vital before making the distinction between conventional and unconventional. Just counting spectral sensitivities is never enough.

Further reading

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