



Color Vision in Honey Bees: A Historical Perspective

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ABSTRACT

Research in the honeybee has laid the foundations for our understanding of insect colour vision. The trichromatic colour vision of honeybees shares fundamental properties with primate and human colour perception, such as colour constancy, colour opponency, segregation of colour and brightness coding.

KEYWORDS:-COLOR, INTENSITY, BEHAVIOUR, CHROMATIC, ADVANTAGE

AN INTRODUCTION

Colour vision is mainly used by animals for phototaxis and in object detection. Wyszecki and Stiles (1982), define colour vision as 'that aspect of visual perception by which an observer may distinguish differences between two structure-free fields of view of the same size and shape, such as may be caused by differences in the spectral composition of the radiant energy'. This definition of colour is based on the measurement of the physical property of light i.e. the spectral composition or wavelength information and detaches itself from the human sensation from which most basic concepts of colour vision are derived (Kelber et al., 2003). Thus, colour vision can be described as the ability of a visual system to extract and process information contained in the spectral composition of light. It enables an organism to reliably distinguish objects based on the wavelengths of light they reflect, emit, or transmit. Colour is a multiple-signal stimulus and individual aspects of colour such as intensity, wavelength, spectral purity, and colour contrast can be used by animals in different behavioural contexts (Lunau and Maier, 1995). Humans can distinguish chromatic (hue and saturation) and achromatic (brightness) properties of a colour, while colour vision is assumed to be independent of brightness or intensity in other animals i.e. they do not use brightness to detect and distinguish colour. Based on this assumption, an animal is said to have colour vision if it can distinguish two spectrally different stimuli regardless of their intensities (e.g. Menzel, 1979; Neumeyer, 1991; Goldsmith, 1991). One of the advantages of colour vision is the ability to detect and discriminate objects under varying illuminations such as under shadows or in shallow water, where intensity differences from the object cause large variations in receptor signals. In these scenarios it is advantageous to depend on the ratio of signals from different photoreceptors rather than depending on intensity (Rubin and Richards, 1982; Mollon, 1989; Maximov, 2000). The pervasiveness of colour traits and the visual systems that process these signals itself is the proof of how important a stimulus colour is in interpreting and analyzing visual environment (Jacobs, 2009). Thus, it has understandably been a central theme for investigation in the visual ecology of several animal taxa. A prerequisite for any visual system capable of colour vision is the presence of at least two photoreceptor types with different spectral sensitivities for the comparison of signals and neural mechanisms to facilitate comparing signals arising from these photoreceptors and known as opponent mechanism (Backhaus, 1991; Vorobyev et al., 1998). If an animal has n photoreceptors it will possess an achromatic channel in which signals from all photoreceptors are summed together, and $n-1$ chromatic channels where the signal inputs will be subtracted between the photoreceptors. The number of the photoreceptors and thus the number of chromatic channels vary substantially across animal kingdom from two in most mammals (dichromacy), three in bees, new world monkeys and humans (trichromacy), four in birds and some butterflies (tetrachromacy) and up to sixteen classes of photoreceptors in some stomatopods (Marshall et al., 1991). Photoreceptors with different sensitivities have evolved independently in arthropods and chordates (Porter et al., 2011). Colour vision is the most studied sensory modality and plants and animals have evolved colouration to be detected or to avoid detection, as well as to facilitate learning and memory of salient (both desirable and undesirable) stimuli in the environment (Guilford and Dawkins, 1991; Bradbury and Verhencamp, 1998; Stevens, 2013). Excellent examples are fruit colouration and primate trichromacy and floral colours and the bee visual system. Primate trichromacy is well adapted to detect ripe fruits from the green foliage background (Mollon, 1989; Regan et al., 2001; Surridge, et al., 2003) and angiosperm floral colours are clustered in the wavelength regions where hymenopteran are most sensitive to changes in wavelengths (Chittka et al., 1993; Menzel and Schmida, 1993; Vorobyev and Menzel, 1999). These examples demonstrate how important colour is in shaping populations and communities (Menzel and Schmida, 1993; Schemske and Bradshaw, 1999; Binkenstein et al., 2013).

A historical perspective of colour vision in bees

The existence of colour vision in non-human animals has been a subject of fascination among scientists curious about understanding how the world appears to animals. The lion's share of what we understand about non-human colour vision comes from hymenopteran insects, mainly the Western

honeybee *Apis mellifera*. More than a century ago, Lubbock (1882) demonstrated that honeybee foragers repeatedly visited colour cards that they were trained to associate with sucrose reward and a few years later he discovered that ants were sensitive to ultraviolet light (Lubbock, 1888), a sensory capacity that the humans lacked. This suggested that non-human animals see the world very differently and therefore posed an intriguing problem for subsequent research. It was Karl von Frisch's (1914) seminal observations which proved that honeybees possess true colour vision. Von Frisch trained honeybee foragers to feed on sugar water in a dish placed on a coloured card. He later placed the coloured card together with grey cards of different intensities, reasoning that if the bees relied only on intensity cues, but not on colour cues then one of the grey cards which matched the intensity of colour card would also be visited by the bees, which was not the case. Thus, the honeybee became the first non-human animal in which colour vision was established. This landmark finding propelled studies using honey bees as a model to investigate the mechanisms involved in colour vision. Later, Kuhn (1924) demonstrated that honeybees also detect ultraviolet and that the bees' colour discrimination abilities are robust when colours are from different spectral regions separated by 80-100 nm. Further investigations demonstrated effects similar to simultaneous colour contrasts and complementary colour pairs in bees (Kuhn, 1927). Daumer (1956) obtained the first behavioural proof of trichromatic vision in bees, a first in an animal, by employing colour mixing experiments. The first electrophysiological recordings from photoreceptors were recorded from honeybees (Autrum and Zwehl, 1964; Menzel, 1975; Menzel and Blakers, 1976) and confirmed trichromacy in bees. Honeybees have a fully functional trichromatic vision with UV, Blue and Green receptor types which are maximally sensitive at wavelengths of 344nm, 436nm, and 544nm respectively

Honeybee eyes

Honeybees have apposition compound eyes; an eye design which best suits their diurnal lifestyle. *A. mellifera* workers have approximately 5500 ommatidia (Jander and Jander, 2002; Streinzer et al., 2013), while workers of the Eastern honeybee *A. cerana* have approximately 5000 ommatidia (Somanathan et al., 2009; Streinzer et al., 2013). Nine photoreceptors, comprising eight elongated large receptor cells and one small cell are present in each ommatidium which protrudes to the optic lobe in the brain (Figure 2). Each photoreceptor is made up of distinct visual pigments comprising two units - a chromophore (mostly retinal) and an opsin protein with about 370 amino acids. Opsins are formed of seven transmembrane helices integrated into the microvilli of the rhabdomeres forming a pocket where the chromophore is housed (Deeb and Motulsky, 1996). Opsins undergo conformational changes on absorbing quanta of light leading to the phototransduction cascade. Initially, the eight large receptors present in honeybee were thought to be comprised of four L (long-wavelength sensitive, $\lambda_{max} = 544$ nm) receptors, two M (middle-wavelength sensitive, $\lambda_{max} = 436$ nm) receptors and two S (middle-wavelength sensitive, $\lambda_{max} = 344$ nm) receptors and the ninth small receptor, presumably a UV receptor (Menzel and Backhaus, 1991). However, recent molecular studies have identified three different ommatidial types, each with different sets of spectral receptors (Wakakuwa et al., 2005), and each type unevenly distributed in the compound eye. Type I ommatidia have all receptor types and are Figure 2 Frontal view of bee head (scanning electron micrograph) - showing essential features of color processing in the brain. Information from the UV, blue, and green receptors is relayed from the first optic ganglion, the lamina, to the second optic ganglion, the medulla, by monopolar cells (LMCs); cell bodies are symbolized by filled circles. These cells feed into color opponent cells (drawn in red and black) found both in the medulla and lobula, either directly or via interneurons. Chromatic opponent cells receive antagonistic input from the different color channels, and project to the protocerebrum (Used with permission from Chittka and Brockmann, 2005). made up of six L-receptors, one M-receptor and one S-receptor, Type II ommatidia have six L-receptors and two S-receptors, while Type III ommatidia contain six L-receptors and two M-receptors (Menzel and Blakers, 1976; Wakakuwa et al., 2005). The relative composition of the Type I, II and III classes of ommatidia in the compound eye are 44%, 46% and 10% respectively (Wakakuwa et al., 2005). The spectral sensitivity of the ninth photoreceptor and its significance in colour vision is still ambiguous.

Neural wiring and mechanisms of colour vision in honeybees

The visual information processing takes place in the optic lobe of the bee brain, which is comprised of three distinct ganglia namely the lamina, medulla and lobula (Figure 2). Of the nine receptors present in each ommatidium, six form short visual fibers and protrude to the lamina (Ribi and Scheel, 1981). These six receptors are L-receptors and the axons of three other photoreceptors project to the medulla through the lamina. Out of these three, two can be either S and M- receptors or one each based on the ommatidial type (Menzel and Blakers, 1976; Friedrich et al., 2011). The third long visual fiber is the projection of the ninth small photoreceptor (Ribi, 1975). Intracellular recordings have demonstrated the presence of spectrally opponent interneurons which respond differentially to the spectral lights in the lamina, medulla and the lobula of the optic lobe (Menzel, 1974; Kien and Menzel, 1977; Hertel, 1980; Hertel and Maronde, 1987; Riehle, 1981; Maronde, 1991; Yang et al., 2004). The presence of spectrally opponent interneurons sensitive to colour which act as the neural substrate for coding colour (chromatic) and brightness (achromatic) cues in the honeybee has been well established. However, the predominance of L-receptors which mediates movement related tasks in the ommatidia imparts the bees with high sensitivity and better resolution in achromatic vision than in colour vision (Menzel, 1981; Srinivasan and Lehrer, 1984, 1988; Giurfa et al., 1996; Hempel de Ibarra et al., 2001). Coloured objects which are larger than 15° in angular size i.e. subtending an angle of 15° or more on the bees' eye will be detected and discriminated through the chromatic channel and targets subtending visual angles from 15° to 5° are detected by achromatic visual pathway mediated by the L-receptors (Giurfa et al., 1996, 1997; Giurfa and Vorobyev, 1998). Trichromacy at the retinal level and opponency at neural level forms the basis of colour vision in honeybees (Menzel and Backhaus 1989; Brandt and Vorobyev 1997). Colour discrimination is achieved through four stages. In the first stage, light is absorbed as quanta by the photoreceptors. In the second post-receptor or neural stage, receptor signals are processed and subtracted, facilitated by successive morphological and physiological layers in the brain. In the third stage, outputs from post-receptor mechanisms are compared between stimuli and an estimate of similarity between them is drawn. In the fourth and final stage these estimates of similarity are translated to appropriate behavioural responses.

Honeybee colour vision models

Evidence for trichromacy from behavioural data (Daumer, 1956), wavelength discrimination function (von Helverson, 1972), electrophysiological readings from intracellular recordings (Autrum and Zwehl, 1964; Menzel, 1975; Menzel and Blakers, 1976) and the demonstration of colour constancy i.e. the ability to perceive a colour as the same under different illumination conditions (Neumeyer 1981; Werner et al., 1988), made it possible to develop several models for colour discrimination (Table 1). These models have been explicitly used to address questions pertaining to the visual ecology of the honeybee (e.g. Lunau, 1990; Chittka and Menzel, 1992; Menzel and Shmida, 1993; Kevan et al., 1996; Vorobyev and Brandt, 1997; Waser and Chittka, 1998; Vorobyev and Menzel, 1999; Shreshtha et al., 2013). All models are based on the basic concept of classical metric theory of human color discrimination (Helmholtz, 1896; Schrodinger, 1920) and assume that achromatic vision is not used for detecting colour stimuli and that two independent opponent channels formed by the combination of signals from three types of photoreceptors code for chromatic aspects of the colour stimuli. The colour opponent mechanism results in a receptor-based colour space (Backhaus, 1991; Brandt and Vorobyev, 1997). The distance between the chromatic loci of two colour stimuli in the colour space modeled can be used to predict whether those stimuli will be perceived as different stimuli by the bees. The detectability increases as the distance between the stimuli in the colour space increases. Models for colour discrimination can be used to calculate the distance between the colour loci of the stimuli. However, it does not account for or describe perceptual differences. The distance between the loci in the colour space can be used to describe the perceptual differences between colours since it is positively correlated to discriminability of the stimuli. The distance and, accordingly, the detectability increase with increasing signal- to noise ratio of chromatic mechanisms, which is generally a function of the intensity of the light stimulus. Models can further be classified according to their dependence of the signal- noise ratio on the light intensity. The Maxwell triangle and the RNC models postulate that signal-to-noise ratio, and thus detectability, is independent of light intensity while, the GCO and the RNQ models postulate that the signal-to-noise ratio increases with increasing light intensity, which means that bright stimuli are easier to detect. The COC and hexagon models postulate that signal-to-noise ratio decreases with increasing light intensity, thus making bright stimuli difficult to detect. All physiological models assume at least four distinct stages of colour processing (Figure 3). They are as follows: 1) Number of quanta of light absorbed by the photoreceptors per unit time 2) Coding for achromatic and chromatic signals 3) Comparing the signals 4) Resulting behavioural response Colour opponent coding (COC, Backhaus, 1991) model was the first model of honeybee colour vision postulated based on behavioural data (Backhaus, 1991). This model was derived from the multidimensional scaling of color similarity experiments (Backhaus et al., 1987). The two scales resulting from those experiments were interpreted as color opponent mechanisms. The predictions of this model are in good agreement with behavioural data. This model is explicit on the neural implementation of four stages of colour processing. The hexagon model (Chittka, 1992) is a generalist model which is not based on any behavioural data. This model employs a Euclidian matrix to calculate colour distance instead of the city-block matrix used in COC. This model is a modification of Maxwell triangle which was not calibrated to any behavioural data and as a result it fails to predict spectral sensitivity functions with sufficient accuracy (Vorobyev and Brandt, 1997). These two models assume non-linearity of quantum catches resulting in receptor signals. General colour-opponent coding model (GOC, Brandt and Vorobyev, 1997) and Receptor Noise Limited models (RNL, Vorobyev et al., 1998) assume that quantum catches are linear functions and use Riemannian matrix to calculate colour distances. This model does not make explicit assumptions about the factors limiting performance. On the other hand RNL model postulates that colour discrimination is limited only by the noise in the receptor signals. Thus, this model which assumes that color discrimination is limited only by the noise of photoreceptors constitutes the minimal model of color discrimination.

Bee colour vision and Angiosperm floral colours

Colour vision in each single species or taxonomic group is well adapted for one or a few vital tasks and for insect pollinators such as the honeybee it is fine tuned to detect and discriminate flowers which provide them with nectar and pollen. Angiosperm flowers act as 'sensory billboards' by providing a suite of signals, to advertise nutrient-rich rewards to animal pollinators (Kevan, 1985; Raguso and Willis, 2002; Raguso, 2004; Goyret, 2010). The two most important signals are aimed at the visual and olfactory sensory systems of pollinators and include colour, pattern, shape and size and floral scents. Out of these, floral colour signals are most addressed in context of floral trait evolution compared to any other trait. Colour is an important multi-dimensional signal cue with properties such as contrast, hue, saturation and pattern, and acts as an effective releaser of responses in flower visitors (Lunau and Maier, 1995). Colour is considered to be amongst the most important floral features that several pollinators rely on (Menzel and Backhaus, 1991; Menzel and Shmida, as it also triggers behavioural reactions like important cues such as olfactory cues (Kevan, 1978; Lunau, 1990, 1992; Chittka and Menzel, 1992). Advances in our understanding of colour vision and subsequent neural processing as well as the availability of colour vision models, make the honeybee a popular model organism to study the co-evolution of angiosperm floral colours and pollinator visual systems in general. Peitsch et al., (1992) compared spectral sensitivities of photoreceptors in more than 40 species of hymenopteran pollinators (bees and wasps) and demonstrated that they all share trichromacy and have similar spectral sensitivities with a few exceptions in which a long wavelength receptor is present (red sensitive) in some species. Hence, the honeybee visual system can be used to represent hymenopteran pollinators in general. Plant fitness is dependent on the perception and appropriate behaviours elicited by floral signals in pollinators, and hence strong selective pressure to improve the detection and attractiveness of these signals is expected. Pollinator-mediated selection is thought to be the driving force behind the diversification of angiosperms and evolution of colourful floral displays (Grant, 1949; Schemske et al., 1978; Bawa, 1990; Kay and Sargent, 2009; van der Niet and Johnson, 2012). It is hypothesized that flower colours evolved to facilitate the detection, discrimination and memorisation by pollinators and thereby ensuring pollinator constancy and efficient pollen transfer. Pollination systems have thus been compared to a market place where the pollen and nectar are advertised using floral traits such as colour to selective foragers such as bees (Heinrich, 1977; Menzel and Shmida, 1993; Chittka, 1997). Hence strong and most importantly distinct signals should be used to ensure flower constancy and efficient pollen transfer (Kevan, 1978; Waser, 1983; Chittka et al., 1999). Bees avoid switching between flower species with very distinct signals, demonstrating the importance of signal divergence in inducing flower constancy (Waser, 1983; Wilson and Stine, 1996;

Chittka et al., 1999). The trichromatic visual system of honeybees and other hymenopteran pollinators predates angiosperm evolution and was likely present in the Devonian ancestor of these insects (Briscoe and Chittka, 2001). Floral colours and pollinator visual system can be considered as an evolutionarily adapted signal- receiver system which facilitates the optimal discrimination of flower colours (Menzel and Backhaus, 1991). Interestingly, when optimal sets of photoreceptors which could efficiently discriminate flower colours (from Israeli flora) were theoretically modeled (Chittka and Menzel, 1992), the peak sensitivities were very similar to photoreceptor sensitivities of hymenopterans recorded by Peitsch et al. (1992). Later, Chittka et al. (1993) showed that flower colours are clustered into distinct regions of the bee colour space and the UV component of trichromatic vision in bees does not have much salience when compared to blue and green receptors. Further analysis of more than 1000 floral spectral reflectances revealed that flowers do not exhibit the full range of colours that bees can detect and discriminate (Vorobyev and Menzel, 1999); floral colours were not uniformly distributed but were clustered in the colour space in such a way the entire range of colours that the bees can potentially discriminate were not exploited (Chittka et al., 1994; Vorobyev and Menzel, 1999). The uneven distribution of floral colours in few perceptually significant colour categories in the bee colour space may be explained by the generic colour vision system of bees and other generalist pollinators which may have allowed plants to sufficiently diverge in floral colours within the limits of phylogenetic and developmental constraints (Rauscher, 2008). A co-evolutionary relationship between the clustering of floral colours and bee visual system is rather unlikely as bees have equally spaced photoreceptors and the moderate overlap in their sensitivities impart bees with a general purpose colour vision system which is not specifically evolved to detect and discriminate colours in any particular region of the spectrum (Hempel de Ibarra et al., 2014). The bee colour space is sufficiently large and the floral colours only forms distinct clusters within the colour space and does not cover gamut of colours that the bees can discriminate.

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