Interaction of visual and odour cues in the mushroom body of the hawkmoth *Manduca sexta*

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Abstract

The responses to bimodal stimuli consisting of odour and colour were recorded using calcium-sensitive optical imaging in the mushroom bodies of the hawkmoth *Manduca sexta*. The results show that the activity in the mushroom bodies is influenced by both olfaction and vision. The interaction between the two modalities depends on the odour. The visual stimulus suppressed the response to a general flower scent (phenylacetaldehyde). In contrast, the response to a green leaf scent (1-octanol) was enhanced by the presence of the visual stimulus. The bimodal enhancement can thus not be explained simply by summation, but is a sign of true interaction.

Spectral sensitivities of a mimetic spider

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Misumena vatia (Araneae, Thomisidae) (Clerck 1857) is a mimetic spider living on flowers that can reversibly change its body colour from white to yellow in 10-15 days to be concealed from visiting pollinator preys by decreasing the chromatic and/or achromatic contrasts (Chittka, 2001). The degree of matching has been studied only according to the prey visual system, mainly due to the wide knowledge on bees vision (Backhaus, 1990). Indeed, whereas bees view of spider mimicry has been well studied (Théry and Casas, 2003; Théry and al., 2005; Chittka, 2001), nothing is known about *M. vatia* vision. Moreover, background matching has never been studied with respect to mimic visual system.

Thus, electroretinograms (ERGs) combined with selective adaptation were carried out to determine the number and nature of spectral classes of photoreceptors in the retina of each pair of eyes of *M. Vatia*. Moreover, visual fields of each eye were determinated. Our results strongly suggest that the spectral sensitivities of the fours pairs differ. Indeed, records of Posterior and Anterior Median Eyes (PME and AME) reveal three peaks in UV (around 340nm) and green regions (around 520 and 540nm; see figure 1 for PME) that suggest the presence of three classes of photoreceptors. Records of Posterior and Anterior Lateral Eyes (PLE and ALE) suggest four peaks in UV (around 340nm), blue-green (around 490nm) and green regions (around 520 and 540nm). Thus, PLE and ALE might be tetrachromatics.

Finally, we discuss the visual implications of this photoreceptor arrangement along the spectrum and its relevance for matching flowers.

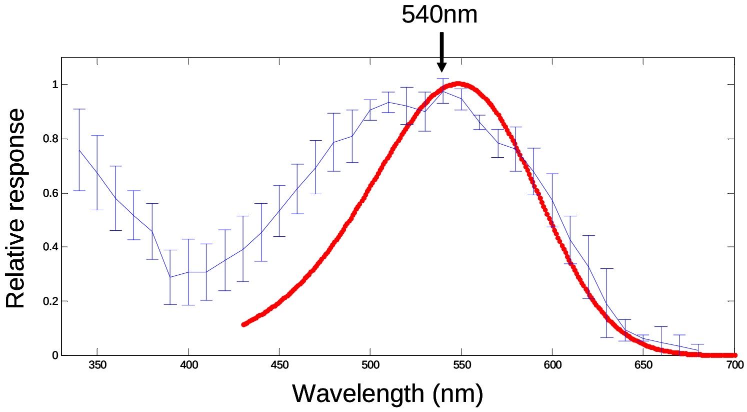

Figure 1: Spectral response curve for the posterior median dark-adapted eye (PME). The dashe line represents the predicted absorption curve of a template with a peak absorption at 545nm (Stavenga and al, 1993).

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Théry M., Debut M., Gomez D., Casas J. 2005. Specific color sensitivities of prey and predator explain camouflage in different visual systems. *Behavioral Ecology*. 16 : 25-29.
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Red leaf colouration and aphid colour choice in autumn

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Although the visual senses are of great importance for host finding in herbivorous insects, only little is known about their colour vision. In order to elucidate colour choice behaviour of migrating aphids in autumn, water traps (Ø 14cm) of 140 different colours were set out in the field in October 2007. Hues were varied in the range of blue, green yellow and red, and additional variation was introduced by adding varying amounts of black or white. Trap colours were measured with a spectrophotometer. The catch N of each trap was normalised to the maximum number of aphids N_{max} caught in a trap; the number $y=N/N_{max}$ was used as an indicator for the 'attractivity' of trap colours. Using information from previous electrophysiological work, the 'attractivity' was modelled with two input variables, the photon catch G that a given trap spectrum produced in a green receptor (with a peak sensitivity at λ_{max}=530 nm), and B the respective photon catch for a blue receptor (λ_{max} =460nm). This model was then applied to 2409 leaf spectra from 113 tree species to predict their relative attractivity for aphids. For the same leaf spectra we also ran a human colour naming model, in order to objectively determine which leaves could be called green, red, yellow, or brown. The colour of leaves that appear red to humans were indicated to be on average 68% less attractive to aphids than green leaves; yellow leaves were much more attractive than green ones. However, colour variation within the human name categories "red" or "green" resulted in overlapping attractivity ranges. Based on our results, the informed selection of leaf colors, using existing color variation within crop species, could serve as tool for aphid control.

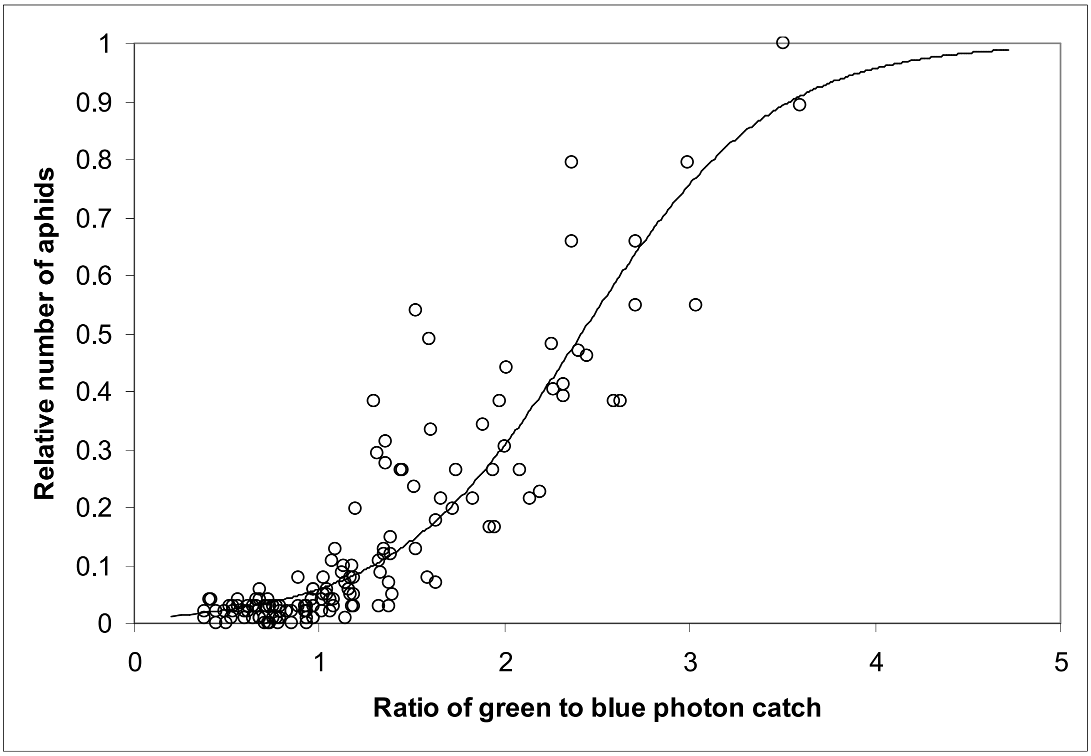

Fig 1. Relative aphid catch in coloured traps plotted against the x=G/B, the ratio of photon catches elicited in a green photoreceptor vs. a blue photoreceptor. Model: ln[y/(1-y)]=ax+b, with a=1.95±0.10, and b=–4.71±0.18 (mean ± s.e., r^2=0.81, n=140).

Beta-carotene deficient larval diet influences the use of visual and olfactory stimuli by foraging adults of Manduca sexta

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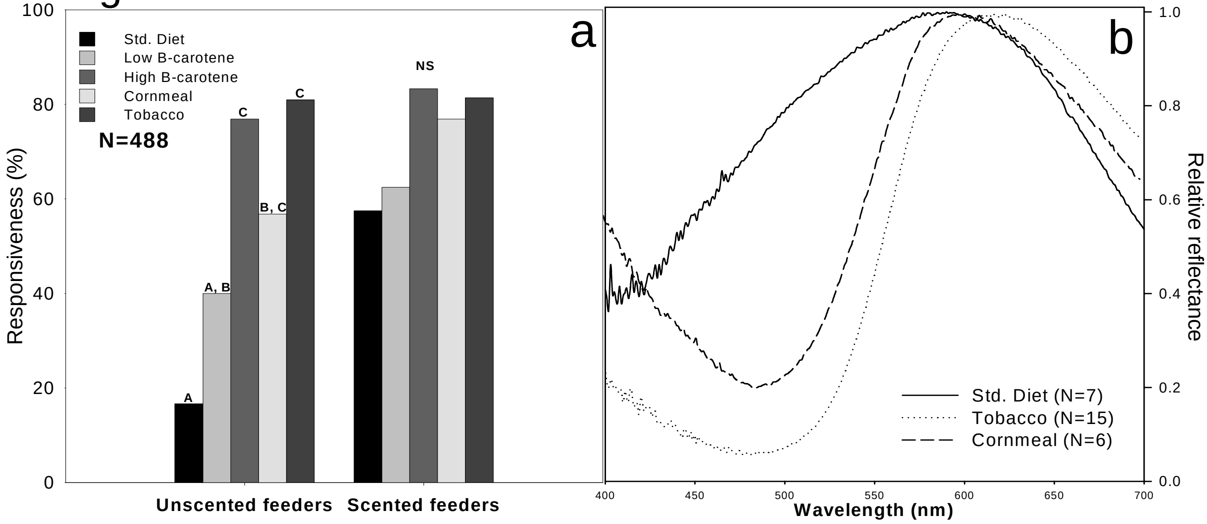
Mobile, foraging insects mediate plant reproduction by moving pollen between individual flowers. To do so, insects must first find and recognize flowers. Initially, they accomplish this task through innate responses to various sensory stimuli, especially conspicuous visual floral display and distinct fragrances. Past work on laboratory-reared *Manduca sexta*, a nocturnal hawkmoth native to the Americas, showed that both olfactory and visual cues are necessary and sufficient to elicit feeding responses. This contrasts with related hawkmoth species, like the nocturnal *Deilephila elpenor* and the diurnal *Macroglossum stellatarum*, which probe readily at concealed odor sources and unscented visual targets, respectively. Is the apparent requirement for multi-modal floral stimuli an artifact of laboratory diet? It is known that *M. sexta* larvae fed on diets deficient in β-carotenes have lower visual sensitivity and aberrant morphology of photoreceptor membranes. Thus, we investigated whether the amount of β -carotene content in larval diet can influence the nectar feeding responses of adult, naïve *M. sexta* to olfactory and visual cues. Moths reared on the standard wheat germ-based artificial diet almost exclusively fed when surrogate flowers were scented. In the presence of scent, there was no diet effect on the feeding behavior of moths (Fig. 1a). Moths spent more time searching when flowers were unscented, but searching time was independent of diet treatment. However, moths' feeding responses to unscented flowers increased with increasing β-carotene content of their larval diets, culminating in the positive control (their host plant, *Nicotiana tabacum*). Indirect addition of β-carotenes to the artificial diet (via cornmeal) appears to reconstitute the natural reflectance spectrum of the eye of *M. sexta* adults (Fig. 1b). Thus, the concentration of photopigment precursors in larval diet directly impacts the visual system of adult *M. sexta*, in ways that are compensated by the use of olfactory channels. We discuss this inherent sensory flexibility in the context of the foraging behavior by M. sexta and other hawkmoths.

Figure 1



Spectral tuning and evolutionary adaptation to different light environments in mysid shrimp.

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Questions of spectral tuning, the relation of spectral and thermal properties of visual pigments, and evolutionary adaptation to different light environments were addressed using a group of small crustaceans of the genus *Mysis* as a model. The general purpose was to clarify to what extent and on what time scale adaptive evolution has driven the functional properties of (mysid) visual pigments towards optimal performance in different light environments. An ultimate goal was to find the molecular mechanisms underlying the spectral tuning and to understand the balance between evolutionary adaptation and molecular constraints.

The light sensitivity of the visual pigment depends on interactions between the chromophore and the opsin. In the light of current data available on visual pigment physiology and molecular structure, spectral tuning of the visual pigment can basically occur only by two different means; (*i*) by changing of the chromphore or (*ii*) by altering the charge environment of the chromophore in the chrompohore pocket.

Visual pigment absorbance spectra were recorded from ten different populations of the *Mysis relicta* species group, six *M. relicta* (two from Finnish freshwater lakes, two from Swedish freshwater lakes and two from the Baltic Sea) and two *M. salemaai* (from the Baltic Sea) populations. In all populations, a single visual pigment was found, and judged by the shape of the absorbance spectra with pure A2 chromophore. The "Sea" populations were 20-35 nm blue-shifted within *M. relicta* as well as between *M. relicta* and *M. salemaai*, qualitatively consistent with the differences in their respective light environments

A comparison of amino acid substitutions between *M. relicta* and *M. salemaai* indicated that mysid shrimps have a small number of readily available tuning sites to shift between a "shorter"- and a "longer"-wavelength opsin. The totally consistent segregation of absorption maxima (_$_{max}$) into (shorter-wavelength) marine and (longer-wavelength) freshwater populations suggests that truly adaptive evolution is involved in tuning the visual pigment for optimal performance, driven by selection for high absolute visual sensitivity. On the other hand, the similarity in _$_{max}$ and opsin sequence between several populations of freshwater *M. relicta* in spectrally different lakes highlights the limits to adaptation set by evolutionary history and time.

Adaptation of the visual system to different spectral light qualities in terrestrial habitats

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Animals live in diverse habitats that vary in their light spectral quality. In terrestrial habitats the most extreme differences in spectral light quality occur between low and high altitudes, and between open habitats like grassland and dense forest (Endler 1993). However, most studies so far fail to show correlations between the spectral sensitivity of photoreceptors and the photic environment of the specific habitat, in spite of a distinct variation of spectral light quality among habitats of the species under comparison. Therefore, the possible mechanisms responsible for adaptation of terrestrial animals to their photic environment are still under debate.

Bumblebees (*Bombus*) may provide an ideal system to address these questions since they inhabit almost every terrestrial habitat and for many taxa the spectral sensitivity of their visual system is well characterized (Briscoe & Chittka 2001). Here we investigate whether bumblebees adapt to different photic environments by varying the relative level of visual pigments (opsins) in order to change the sensitivity of the respective photoreceptor types. The complex eye of bumblebees is composed of three different receptor types sensitive in the UV, blue and green part of the light spectrum (Briscoe & Chittka 2001). It has already been shown that the mRNA expression level of the long-wavelength (LWRh) opsin of *Apis mellifera* varies significantly over a 24 h cycle (Sasagawa et al 2003). We hypothesize that opsin expression levels correlate with the photic environments, e.g. bumblebees in alpine habitats, where UV radiation is high, express lower levels of UV opsin mRNA than bumblebees in lowland habitats or forests, where light spectrum is strongly shifted to longer wavelengths.

Using real-time PCR, we first characterised the expression level of three opsin mRNAs (UV, blue and green) of *Bombus terrestris* individuals kept under controlled 12:12 hours L:D light regime over a 24 h period. We then entrained two experimental groups to short- and long-wavelength shifted illumination, respectively, using color filters, and measured possible changes in the mRNA levels of the three opsins. Finally, we compared opsin expression levels of individuals of different *Bombus* species caught in the field from alpine habitats at altitudes > 2500 meters asl and from low land habitats.

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- Endler, J. (1993) The color of light in forests and its implications. *Ecol. Monographs* 63:1-27.
- Sasagawa, H., R. Narita, Y. Kitagawa and T. Kadowaki (2003) The expression of genes encoding visual components is regulated by a circadian clock, light environment and age in the honeybee (*Apis mellifera*). *Euro. J. Neurosci.*17: 963-970.

Eye Regionalisation in the Pale Clouded Yellow, *Colias erate*

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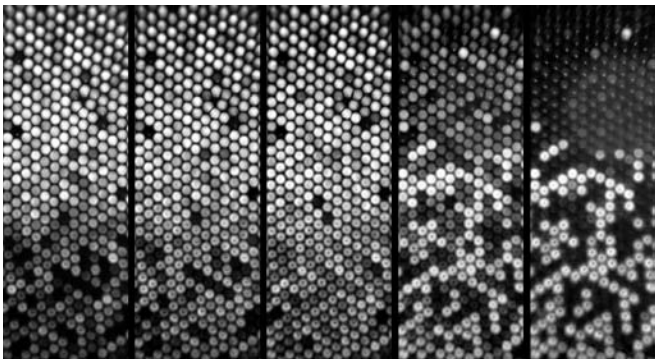
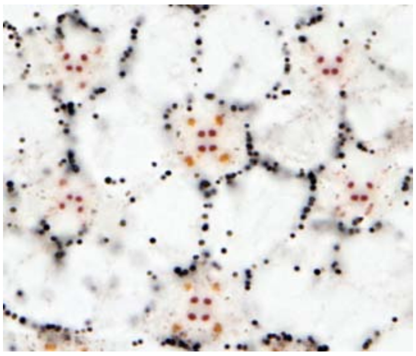
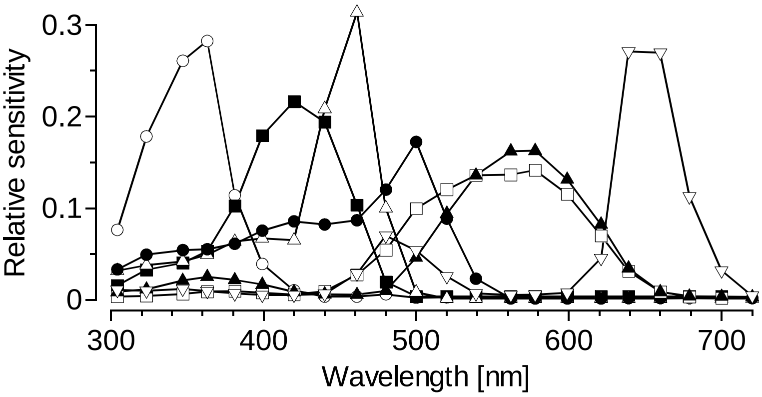
The eyes of butterflies are regionalised and contain several types of ommatidia with sets of photoreceptors expressing different rhodopsins. Some ommatidial types contain screening pigments that modify the spectral sensitivity of the photoreceptors from those of pure rhodopsin absorption spectra.



The distribution of ommatidial types in the eye of ***Colias erate*** was inferred from measurements of the eye shine, a phenomenon caused by a reflecting tapetum multilayer. The dorsal part of the eye has a rather uniform bright red eye shine. In the ventral part of the eye, we observed two types of ommatidia, red with fluorescence (Rfl, peak reflectance at 670 nm, fluorescence excitation <420 nm), and deep red (dR, peak reflectance at 720 nm). Histological sections demonstrated that the two ommatidial types of the ventral eye part have different longitudinal and transverse distributions of red pigment granules in the distal tier of cells R5-8. In Rfl ommatidia, pigment clusters are in a trapezoidal constellation while in dR ommatidia, clusters are in a rectangular constellation.



We measured the spectral and polarisation (PS) sensitivity of photoreceptors by intracellular recordings. We identified four short wavelength receptor types, ultraviolet (UV), violet (V), narrow blue (nB) and broad blue (bB), and three long wavelength types, narrow green (nG), broad green (bG) and red (R). V, nB and bB receptors have strong vertical PS, corresponding to the R1/R2 positions in the distal tier. The UV receptor has low PS. The nG receptor has low PS and probably represents the R3/R4 photoreceptors of the distal tier. Its sensitivity in the UV is substantially lower than what would be expected from the rhodopsin template. The bG receptor has no UV sensitivity, a broader bandwidth than the nG receptor and has strong oblique PS, indicative of the R5-8 photoreceptors of the proximal tier. The R type has oblique PS and the main peak at about 650 nm, which makes it the farthest red receptor found in insects so far; it also has a second peak at 480 nm.



We hypothesize that, similarly as in *Pieris rapae*, the R receptors are within dR ommatidia and contain the same rhodopsin as bG and nG receptors. We suggest that bG receptors have a broadened sensitivity spectrum due to self-screening and that the reduction of UV sensitivity in nG and bG cells results from screening by the red and fluorescing pigments and from rhodopsin absorption in R1-4 cells.

Top Figure: The dorso-ventral gradient of the eye shine. At 650, 670, 690, 710 and 730 nm (left to right). **Middle:** two ommatidia with rectangular and four with trapezoidal arrangement of the red screening pigment, at about 230 μm below the cornea. The function of the surrounding four orange pigment clusters is unknown. **Bottom:** Spectral sensitivities, normalised to unit area.

Sequence & expression of opsins in fiddler crab, *Uca Pugilator*

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Fiddler crabs are members of the ocypodid family of brachyuran crabs, and inhabit intertidal sand and mudflats. They exhibit a rich behavioral repertoire using primarily visual and vibratory signals to communicate during their complex territorial and courtship interactions. The carapace of many fiddler crabs is extremely colorful, and creates a high visual contrast when viewed against the mud flat background. Beyond merely enhancing effective luminance contrast, species specific coloration and their ability to change color in minutes has for a long time suggested the possibility of color as a major communication signal, particularly during social interactions. Recently the role of color vision has been established during mate choice by an Australian species, but the sensory and neural systems that mediate and limit such behavior, such as the number of spectral classes of opsins and their expression pattern across the retina, remain unknown. This project aims to address this issue by identifying and sequencing all retinal opsins and their expression pattern across the photoreceptor array by *in situ* hybridization. Results so far have identified partial fragments of four unique opsins from *Uca pugilator*, a North American fiddler crab, indicating the requisite substrate for color vision. The expression pattern of these opsins will further illustrate how color is used to perceive/extract information from specific portions of the visual world and thereby to mediate social behavior.