The Journal of Experimental Biology 208, 1803-1815
Published by The Company of Biologists 2005
doi:10.1242/jeb.01610

The ecology of visual pigment tuning in an Australian marsupial: the honey possum *Tarsipes rostratus*

Petroc Sumner[1,*], Catherine A. Arrese[2] and Julian C. Partridge[3]

[1]*Department of Visual Neuroscience, Division of Neuroscience, Faculty of Medicine, Imperial College London, St Dunstan's Road, London W6 8RP, UK*, [2]*School of Animal Biology, University of Western Australia, Crawley, WA 6009, Australia* and [3]*School of Biological Sciences, University of Bristol, Bristol, BS8 1UG, UK*

*Author for correspondence (e-mail: p.sumner@imperial.ac.uk)

Accepted 16 March 2005

Summary

While most mammals have no more than two types of cone photoreceptor, four species of Australian marsupial have recently been shown to possess three types, and thus have the potential for trichromatic colour vision. Interestingly, the long-wave cones of the honey possum *Tarsipes rostratus* are tuned to longer wavelengths than those of the other species measured to date. We tested whether the honey possum's long-wave tuning is adaptive for visual tasks associated with its almost unique diet of nectar and pollen. We modelled three tasks: (1) detecting food-rich 'target' flowers against their natural background of foliage or other vegetation; (2) discriminating target flowers from flowers of non-target species; (3) discerning the maturity of the most important target flowers. Initial comparisons of trichromacy *vs* dichromacy generally favoured the former, but interestingly dichromacy was no disadvantage in some cases. For tuning, we found that overall the honey possum's long-wave tuning is more adaptive than that of the other marsupial species. Nevertheless, the optimal tuning for tasks 1 and 2 would be at longer wavelengths still, implying that a different pressure or constraint operates against a further long-wave shift of the honey possum's L cone tuning. Our data show that a possible ecological pressure may be provided by the third task – the difficult and potentially critical discrimination of the maturity of the animal's major food supply, the flowers of *Banksia attenuata*.

Key words: colour vision, ecology of vision, trichromacy, dichromacy, mammal, long wave cone, middle wave cone, optimisation.

Introduction

Background to mammalian colour vision

Until recently it was thought that the colour vision of all mammals except primates was very limited. In order to have colour vision, an animal must possess at least two types of photoreceptor that differ in their absorption spectra; that is, in their sensitivity to different wavelengths of light. This is because retinal photoreceptors signal only the total number of photons they absorb per unit time, and thus they cannot intrinsically distinguish spectrally different signals. For a single class of receptors any light source can be made to match any other by a suitable adjustment of luminance, regardless of spectral composition (Rushton, 1972). If an animal has two types of cone photoreceptor the photon catch of these cones can be mapped into two dimensions and its colour vision is said to be dichromatic. Such an animal will have the potential to discriminate one dimension of chromaticity as well as lightness and darkness. Most placental mammals have dichromatic colour vision, as do some 2% of humans, but some mammals are thought to have no colour vision at all, having only one class of cone photoreceptor (e.g. Jacobs, 1993; Peichl et al., 2001).

In contrast to the relatively poor colour vision of most studied mammals, many birds, reptiles and fish have four classes of daylight-functioning photopigment sequestered in at least as many types of retinal cone. Such retinal complexity gives these species the potential for tetrachromatic colour vision, which has been confirmed in some species by psychophysical experiments (e.g. Neumeyer, 1992). It is believed that four classes of cone photosensitive protein (opsin) existed well before the mammals diverged from the reptiles (e.g. Collin et al., 2003; Yokoyama, 2000), and that the placental mammals retained only the longest and shortest wavelength-sensitive classes. Some primates, including an ancestor in the human lineage, then re-evolved a third cone opsin by creating two subtypes of the long-wave class (e.g. Bowmaker et al., 1991; Jacobs, 1993). It was thought that these primates were the only mammals to have more than two cone visual pigments until Arrese et al. (2002b) discovered three cone pigments in two species of Australian marsupial, the fat-tailed dunnart *Sminthopsis crassicaudata* and the honey possum *Tarsipes rostratus*. It is currently unknown how many other marsupial species may have such retinal complexity and

behavioural experiments have yet to demonstrate trichromacy in any – microspectrophotometric (MSP) measurements suggest that the quokka *Setonix brachyurus* and quenda (Southern brown bandicoot, *Isoodon obesulus*) have three cone pigments (Arrese et al., 2005), while electroretinogram, molecular and behavioural evidence suggests that the tamar wallaby *Macropus eugenii* is dichromatic (Deeb et al., 2003; Hemmi, 1999; Hemmi et al., 2000). It is also unknown whether the middle-wave cone pigments discovered in marsupials are related to the middle-wave cone pigments of other vertebrates such as fish, reptiles and birds and thus likely to have been retained from ancestral mammals, or whether they are related to marsupial long-wave pigments and thus mid-wavelength sensitivity is likely to have been 're-evolved' by these marsupials in a manner analogous to the opsin duplication seen in primates.

Presence of marsupial M cones

Middle-wave cones (M cones) seem to be absent in some marsupial species and present in others (Arrese et al., 2002b, 2005; Deeb et al., 2003; Hemmi, 1999; Hemmi et al., 2000). Whether the marsupial M cones have been inherited or re-invented, they are very unlikely to have been maintained if they offered no selective advantage. Even if there is no extra energetic cost to having more types of cone in the retina, colour processing takes up neural resources, and more importantly, there are visual performance costs associated with multiple receptor types. For example, spatial acuity must be reduced, because, if only one class of receptor is used to provide spatial information, the density of this class must be less if there are more photoreceptor types present; but if more than one class is used, the difference in their signals adds noise to spatial information and there is the additional problem of chromatic aberration (different wavelengths cannot concurrently be in focus on the retina) (Osorio et al., 1998; Regan et al., 2001). Even in the absence of costs, genetic mutations would act in the direction of losing visual pigments if there were no selective pressure to keep them. There are numerous examples of species that have reduced their colour vision capabilities. For example, in addition to the general reduction to two cone classes in placental mammals, many species are being discovered to have retained only one cone class. These include all whales and seals tested (Peichl et al., 2001), two nocturnal primates (Jacobs et al., 1993, 1996; Wikler and Rakic, 1990), racoons (Jacobs and Deegan, 1992; Peichl and Pohl, 2000) and some rodents (summarised in Ahnelt and Kolb, 2000). It is thus pertinent to attempt to discover potential advantages of trichromacy in marsupials, and thereby attempt to identify the selective pressures acting on the evolution of their visual system.

Tuning of marsupial L cones

Trichromacy may offer a number of advantages over dichromacy. As such, simple comparison of trichromacy with dichromacy might not identify the crucial tasks important to each species. Interestingly, while the marsupial M cones so far discovered are all very similar to each other, different tunings for the long-wave cones (L cones) have been found in even the small sample of Australian marsupials measured to date. The L cone wavelengths of peak sensitivity (λ_{max}) are in the range 530–538 nm for four of the six measured species – tamar wallaby *Macropus eugenii*, quokka *Setonix brachyurus*, fat-tailed dunnart *Sminthopsis crassicaudata* and stripe-faced dunnart *Sminthopsis macroura* (Arrese et al., 2002b, 2005; Deeb et al., 2003; Hemmi et al., 2000; Strachan et al., 2004). However, the quenda (Southern brown bandicoot, *Isoodon obesulus*) has a λ_{max} of 551 nm, and the honey possum *Tarsipes rostratus* has the longest wave tuned L cone, with a λ_{max} at 557 nm (Arrese et al., 2002b). The honey possum is a unique marsupial: it is in the diprotodont order, but it is the only species in its family/superfamily (Strahan, 1995). The other measured diprotodont species are the tamar wallaby and quokka, which have L cone λ_{max} in the range 530–538 nm. Arrese et al. (2002b) suggested that the longer wavelength sensitivity of the honey possum may be correlated with the specific requirements of its visual ecology. The honey possum has a diet virtually unique amongst marsupials, feeding almost exclusively on nectar and pollen, and it has numerous morphological adaptations, particularly to its tongue, skull and gut, for this diet (Richardson et al., 1986). Arrese et al. (2002b) suggested that the honey possum's L cones may be well tuned to enhance the '*detection of yellow and red nectar-producing flowers, particularly in a crepuscular light environment*', and/or to '*assess the maturity of flowers, which are yellow or red when ripe but green when unripe*'. We set out to test these suggestions *via* specific hypotheses.

We divided the honey possum's need to find food into three tasks: (1) detecting food-rich 'target' flowers amongst their natural background (foliage or other vegetation); (2) distinguishing target flowers from flowers of non-target species; (3) discerning the maturity (correlated with food value) of flowers of the most important target species (see Fig. 1). For each of these tasks we assessed first whether the honey possum's trichromacy offered an advantage over the dichromacy apparent in most mammals. More interestingly, we asked whether the tuning of the honey possum's L cones provided advantages compared with the tuning of the other marsupial's L cones, and whether any other hypothetical tuning would be better still. To this end, we developed the methodology of Vorobyev and Osorio (1998) by combining it with the analysis used by Regan et al. (1998) and Sumner and Mollon (2000a). In this way we hoped to identify the critical visual tasks that may have provided selective pressure for the evolution of cone spectral sensitivities in the honey possum. Our study differs from previous studies of this kind on other animals in that all radiance spectra were measured directly in the field, rather than estimated from multiplying spectral reflectance with a measure of spectral irradiance of daylight (the latter method does not, for example, take into account light transmitted through leaves or petals; see for example, Sumner and Mollon, 2000a). Since honey possums can be active at various times of day from afternoon through dusk and evening,

and then again before dawn until mid morning (Arrese and Runham, 2002; Vose, 1973), we made measurements under different light conditions (sun, cloud, shade, dusk), and kept these separate in the analysis.

Materials and methods

Teleradiometry

A calibrated teleradiometer was constructed with which spectral radiance (photons s^{-1} m^{-2} sr^{-1} nm^{-1}) could be measured from areas of particular visual targets such as flowers, leaves etc. in the natural environment of honey possums. The teleradiometer consisted of a Nikon Micro UV Nikkor 105mm (f/4.5) lens mounted on a modified Nikon FM2 camera body. A fibre optic (Ocean Optics P200-5-UV/VIS, Dulven, The Netherlands; 5 m long, 200 μm diameter) was coupled to the back of the camera such that its entrance was in the film plane of the camera. Light was thus focused by the camera lens from a small area of the field of view (marked in the view finder of the camera for alignment during measurements) into the fibre optic. The field of view was a circle of ca. 9.3 mm diameter at 5 m distance. The fibre optic led to a spectroradiometer (Ocean Optics USB2000) sensitive to the wavelength range 250–850 nm. Light measurements were recorded using Ocean Optics OOIBase32 software, using a Toshiba notebook PC powered by an external 12 V battery, which allowed all-day operation.

Calibrating the teleradiometer involved measuring a NIST-traceable light source (Ocean Optics DH-2000-CAL) of known radiant output. The teleradiometer calibration was found to vary with both focus distance and f-number of the lens, but knowledge of these variables allowed the data recorded in the field to be corrected to absolute spectral radiance for use in subsequent analyses.

Field measurements

Field work was conducted at two sites in Western Australia, both of which are known habitats for honey possums: Mount Lesueur National Park near Jurien, and Whiteman Park near Perth. The former is an eroded laterite landscape of some 27 kha, vegetated with scrub comprising a diverse flora of more than 800 species, many of which are shrubs less than 2 m high but ranging from ground plants to trees. The latter is a metropolitan area parkland that includes some 700 ha of mixed bushland, from which the general public is largely excluded. Spectral radiance measurements were recorded with the teleradiometer, which was mounted on a short tripod (ca. 0.25 m high) providing a 'possum's eye view' of the surrounding vegetation. In total, some 2300 spectral radiance measurements were made, including both general vegetation and the leaves and flowers of over 15 identified species of plants. Measurements were made from dawn to post-dusk and in a variety of weather conditions ranging from full cloud to full sunlight. Time of day, weather and orientation of the teleradiometer relative to the sun were recorded with the spectral radiance data for inclusion in analyses. Spectral radiance data were recorded in the field as ASCII text files that were managed and merged by category using Microsoft Excel 2000 before being further processed using Matlab (The Mathworks Ltd.) version 6.5.

Fig. 1. *Banksia attenuata* (3 m high) showing inflorescences ranging from immature (green) though mature (yellow) to senescent (brown/grey). Also shown is shrub of *Eremaea beaufortioides* (orange flowers).

Quantum catches

For each stimulus radiance spectrum, quantum catches were calculated for each class of cone photoreceptors by multiplying the stimulus radiance spectrum by the cone's spectral sensitivity (i.e. corrected spectral absorptance) over the full range of wavelengths to which the cone would have any sensitivity. Q_i, the quantum catch for receptor i, is thus given by:

$$Q_i = \int_{300}^{800} S(\lambda) R_i(\lambda) d\lambda , \quad (1)$$

where λ denotes wavelength, $S(\lambda)$ is the stimulus radiance spectrum and $R_i(\lambda)$ is the receptor's spectral sensitivity. Each receptor sensitivity (effective absorptance) function was generated using the rhodopsin visual pigment template of Govardovskii et al. (2000), adjusted for self-screening (assuming a cone outersegment length of 20 μm and a specific absorbance at the λ_{max} of 0.0065 μm^{-1}; Arrese et al., 2002b) and the spectral transmission of the lens (we used data for the placental mouse, *Mus musculus*, lens; R. H. Douglas, personal communication). The λ_{max} values for honey possum cones were taken from Arrese et al. (2002b). For most aspects of the analysis only the relative, not the absolute, quantum catch needs to be known. However, absolute quantum catches are needed for the estimate of quantum noise, explained below. To estimate absolute quantum catches we assumed a 0.0001 sr solid angle for the stimulus (corresponding to a 1 cm^2 circular stimulus at a distance of 1 m), a pupil diameter of 3 mm and a

100 ms integration time (Hood and Finkelstein, 1986). For trichromatic honey possums we estimated that L cones covered 10% of the retinal area (Arrese et al., 2002a), and the ratio of L:M:S cones was 10:5:1 (Arrese et al., 2002b), whereas for a hypothetical dichromatic animal lacking M cones, the L:S ratio and the total retinal area covered by cones remained approximately the same, so the L cone area increased to 15% and the S cone area to 1.5%. We also made minor adjustments for light reflected back from the cornea (5%; Hecht et al., 1942), the quantum efficiency of rhodopsins (0.67; Knowles and Dartnall, 1977) and the fact that the effective sampling aperture of cones is probably smaller than their diameter (0.8; Geisler, 1989; although how the marsupial cone oil droplets affect this is unknown). While these chosen values are only estimates and therefore their product may vary up to an order of magnitude, the effect of varying them is exactly equivalent to varying intensity in the illuminant, which changes over several orders of magnitude during the daytimes at which honey possums are active. We have tested several illuminant conditions and have also artificially varied the level of the illuminant in our analyses to check that this does not affect the results and conclusions given in this paper.

Chromatic distances

From the cone quantum catches, chromatic distances between stimuli were calculated following the technique of Vorobyev and Osorio (Vorobyev et al., 2001; Vorobyev and Osorio, 1998). This technique has the advantage that the post-receptor neural 'wiring' of an animal's visual system does not need to be known, because the model assumes that receptor noise is the main limitation in near threshold chromatic discriminations. The quantum catches give each stimulus a position in a colour space whose axes are the signals from each class of cone. For a dichromat the colour space is thus two-dimensional. Each stimulus is in fact described not by a point but by an ellipse that represents the probability spread of the stimulus' position given that there is noise (i.e. error) in the receptor signals. The centre of the ellipse is given by the calculated quantum catches of the two cone classes and the width of the ellipse in each direction is given by an estimate of the signalling noise for each cone class (see below). The noise-scaled chromatic distance between two stimuli is then calculated as the distance between the centres of each ellipse in the chromatic direction, scaled by the width of the ellipses in that direction (the achromatic signal is not used). This calculation for a dichromat reduces to:

$$(\Delta S)^2 = \frac{(\Delta f_L - \Delta f_S)^2}{e_L{}^2 + e_S{}^2}\ , \qquad (2)$$

where ΔS is the noise-scaled chromatic distance between two stimuli; Δf_L is the difference between the L cone signals for the two stimuli, this receptor signal being the log of the cone quantum catch, $f_i = \ln Q_i$ (Osorio et al., 2004); Δf_S is similarly the difference between the S cone signals for the two stimuli; e_L and e_S are the noise in the L and S cone signals, respectively (see below).

For a trichromat the colour space is three dimensional, and each stimulus is therefore represented as an ellipsoid whose centre is given by the calculated S, M and L cone signals, and whose extent represents the estimated signalling noise for each of these cone classes. The ellipsoid can be collapsed onto a two-dimensional chromatic plane, and the noise-scaled chromatic distance between two stimuli is then calculated as the distance between the centres of each stimulus' ellipse scaled by the width of the ellipses in that direction (see fig. 2 in Osorio et al., 2004). This calculation for a trichromat reduces to Eqn 3 below (for the formal derivation, see Vorobyev and Osorio, 1998):

$$(\Delta S)^2 = \frac{e_S{}^2(\Delta f_L - \Delta f_M)^2 + e_M{}^2(\Delta f_L - \Delta f_S)^2 + e_L{}^2(\Delta f_S - \Delta f_M)^2}{(e_L e_M)^2 + (e_L e_S)^2 + (e_M e_S)^2}\ . \qquad (3)$$

Signalling noise

The estimate of signalling noise for each cone class had two components: quantum noise and neural noise. Quantum noise represents the probabilistic way in which photons are absorbed by photopigment, whereas neural noise represents the variation in synaptic neurotransmitter release per photon absorbed. In very low light levels when quantum catches are low, quantum noise dominates. In higher light levels quantum noise becomes unimportant and neural noise dominates. Quantum noise is estimated as the square root of the calculated quantum catch, $\sqrt{Q_i}$, because the quantum catch follows a probabilistic Poisson distribution with a variance equal to the mean (i.e. the calculated quantum catch). Receptor neural noise is estimated as $\omega_i Q_i$, where ω_i is the 'Weber fraction' of each cone class. The increment in intensity needed to detect a target on a background is generally a constant proportion of the background intensity, and this proportion is known as the Weber fraction (e.g. Wyszecki and Stiles, 1982). The expression used for the standard deviation in signal due to the combined effects of quantum noise and neural noise is thus:

$$\delta Q_i = \sqrt{Q_i + (\omega_i Q_i)^2}\ , \qquad (4)$$

and the relative signalling noise in each receptor class for each stimulus:

$$e_i = \delta Q_i / Q_i = \sqrt{1/Q_i + \omega_i{}^2}\ . \qquad (5)$$

The combined signalling noise for stimuli A and B, whose chromatic distance we wished to calculate, is thus:

$$e_i = \sqrt{[1/Q_i + \omega_i{}^2]_A + [1/Q_i + \omega_i{}^2]_B} = \sqrt{1/Q_{iA} + 1/Q_{iB} + 2\omega_i{}^2}\ . \qquad (6)$$

We estimated the relative Weber fractions for the three honey possum cones classes as 0.05, 0.071 and 0.16 for the L, M and S cones, respectively. The ratio of these values is given by the square roots of the relative numbers of each cone in the retina. The absolute values have to be estimated from human values (e.g. Wyszecki and Stiles, 1982) and the few

measurements made for other species (e.g. Vorobyev et al., 2001). Given the small size of the eyes and the density of cones in the retina (Arrese et al., 2002a), we chose the value of 0.05 for the L cones as a reasonable estimate (see Vorobyev et al., 2001), being more than twice the value (half the sensitivity) of the human L cone system (e.g. Wyszecki and Stiles, 1982). For a hypothetical dichromatic animal lacking M cones, in order that the L:S ratio and the total retinal area covered by cones remained the same as for trichromats, the absolute numbers of L and S cones in the model increased, giving reduced Weber fractions of 0.04 and 0.13. Since these Weber fraction estimates are only approximate, we tested the effects of varying these values beyond the plausible range to check that the results and conclusions presented here were not altered in any important way.

The chromatic distances between stimuli calculated using Eqns 2 or 3 for dichromatic or trichromatic animals, respectively, were then used to assess the three foraging tasks of the honey possum: detecting target flowers in their natural background; discriminating flowers of target species from non-target species; and discerning the maturity of important target flowers. We explain these analyses with the simplest first, which is in fact the reverse order as listed above.

Discerning flower maturity

Each measured inflorescence of *Banksia attenuata* (see Fig. 1) was classified as immature if the flowers were not yet open, mature if they were open and scented, and senescent if open but there was no scent detectable to us. Following the procedure that Osorio et al. (2004) applied to ripe and unripe fruit, we found for each mature flower measurement the chromatic distance to the most similar non-mature flower (immature or senescent), by calculating chromatic distances to every non-mature flower measured in the same conditions and finding the minimum. This discrimination between mature and nearly mature or just senescent flowers represents the most difficult colour discrimination the honey possum has to make, and one which cannot be made on the basis of size and shape. We then simply calculated the mean and median of these chromatic distances for all mature flower samples. First we compared the outcome for a trichromatic honey possum with cone visual pigment λ_{max} values of 350, 505, 557 nm with the outcome for a hypothetical dichromatic honey possum with λ_{max} of 350 and 557 nm (lacking M cones). Then, to find the optimal tuning of the L cone, we calculated the outcome for hypothetical trichromatic honey possums with S and M cone λ_{max} of 350, 505 nm and an L cone λ_{max} varying from 510 to 650 nm. These hypothetical L cone spectral sensitivity functions were generated in the same way as the standard honey possum cone sensitivities, using the rhodopsin visual pigment template of Govardovskii et al. (2000), adjusted for self-screening and the spectral transmission of the lens. All of the above was repeated separately for measurements made in five lighting conditions: sun, cloud, early dusk (60 min to 15 min pre-sunset), sunset and post-sunset.

Discriminating flower species

Each measured species of flower was classified as a target species or non-target species according to the known diet of honey possums (Turner, 1984; Wooller et al., 1984, 1983). Unlike the case above, in which we could be sure that we measured the complete range of *Banksia attenuata* flower maturities, we could not measure the complete range of target and non-target flowers species that honey possums may come across, because some species were not in flower during the limited time of our study. In addition, the discrimination between a target and its chromatically nearest non-target may not be the most difficult discrimination, because sometimes two flowers had very similar colours but were very different sizes and grew on very different plants. Therefore, we considered that it was not appropriate to base this analysis purely on the chromatically nearest non-target to every target, and instead we calculated for each target the median chromatic distance to all the non-targets. All other aspects of the analysis were the same as for discerning flower maturity above. We did not have measurements for the brief periods of sunset and post-sunset, nor enough measurements of non-target species in cloud for a meaningful analysis. We did, however, have many measurements in shade for species other than *Banksia attenuata* (which being a tree among mostly shrubs is rarely in shade, see Fig. 1).

Detecting flowers in their natural environment

For each set of measurements of target species we also took measurements of the natural background, which was either foliage of the same species or the vegetation of surrounding plants. The ease of detecting a target depends not only on the difference between target and background but also on the homogeneity of the background (e.g. Desimone and Duncan, 1995). To capture this, Regan et al. (1998, 2001) and Sumner and Mollon (2000a) assessed the visibility of fruit in foliage using signal-to-noise ratios where the ‘noise’ in this case was the chromatic spread of the background foliage. Here we combine this approach with that of calculating chromatic distances using the model of Vorobyev and Osorio (1998) explained above (Regan et al. and Sumner and Mollon did not use this model, relying instead on knowledge of the post receptor chromatic channels in primates and calculating chromaticities analogously to MacLeod and Boynton, 1979). For each target the median chromatic distance to the background measurements was divided by a measure of the chromatic spread in the background, which was the standard deviation of the distances from that target to each background measurement. We also included a term for receptor noise that maintained a minimum level of overall noise in the signal-to-noise ratio (and as mentioned above we tested the effect of varying the estimates that contribute to this noise, and found that the conclusions presented below were not affected). Thus signal-to-noise ratios, where the noise was a combination of receptor noise and chromatic noise in the immediate visual environment, were calculated for each target within a species and lighting condition, and the mean over the targets was

taken. Each target species measured was analysed separately and our measurements also allowed analysis for five lighting conditions (sun, shade, cloud, dusk, dawn). As above, first we simply compared trichromacy to dichromacy, and then we varied the L cone tuning.

Results

Detecting flowers in their natural environment

Presence of M cones

Signal-to-noise ratios for detecting flowers amongst their natural backgrounds were calculated as explained above for trichromatic honey possums and for hypothetical dichromats lacking M cones. Table 1 shows the proportion of signal-to-noise available to the dichromat relative to the trichromat. The first analysis used all our measurements of general vegetation 'bush background' and all the target flowers that would naturally appear amongst this background (not including the flowers that grow on trees: i.e. *Banksia* sp.). It is clear that the dichromat would be at a substantial disadvantage relative to the trichromat in detecting these targets in this environment. Additionally, we tested individually the five main flower species in the honey possum's diet in our study environment against measurements of foliage taken immediately around the measured flowers. In all cases but one the dichromat would be at a clear disadvantage, but it is intriguing that the dichromat would be equally well able to detect *Banksia attenuata* flowers, the most important food source for the honey possums in our study environment. This is because the chromaticities of *Banksia attenuata* flowers are separated from the chromaticities of *Banksia attenuata* foliage mainly in the L–S direction in colour space. Analyses were performed separately for the different lighting conditions under which we were able to measure each species, and the dichromat/trichromat proportions given in Table 1 are averages across the conditions, but the pattern also holds true for each condition individually. The results given for *Banksia attenuata* used only mature flowers as targets, because the presence of immature or senescent flowers on a tree most often did not signal the presence of mature flowers. For all other species we used all measured flowers as targets because detecting immature or senescent flowers might act as a cue to the presence of mature flowers as there were always some mature flowers on every plant. Nevertheless, the pattern of results was the same whether all flowers or just mature flowers were used as targets. The advantage of trichromacy over dichromacy was also virtually unaltered if the hypothetical dichromat's L cone had a λ_{max} anywhere in the range 530–557 nm (the range of known marsupial L cones).

Table 1. *Proportion of trichromatic signal-to-noise ratio available to dichromats, for the task of detecting flowers in their natural background*

Targets	Background	Dichromat/Trichromat S/N ratio
All shrub flowers	General vegetation	0.53
Banksia attenuata	Immediate foliage	0.99
Banksia ilicifolia		0.83
Calothamnus quadrifidus		0.38
Eremaea beaufortioides		0.28
Verticordia grandis		0.37

S/N, signal-to-noise ratio.

L cone tuning

Fig. 2 shows the effect of altering the L cone tuning in a trichromat. Plotted separately are the results for detecting flowers amongst general bush background and for detecting each species against its immediate foliage surroundings (analyses were performed separately for each species for each lighting condition for which we had measurements, and we averaged conditions within each species and then took the average over species). It is clear that the L cone tuning of the honey possum offers higher signal-to-noise ratios than the shorter wave L cone tunings of other marsupials. However, longer wave tunings would be better still: the optimal L cone would have a λ_{max} of about 615 nm. This pattern of results holds true for most individual species against local foliage or subsets of bush background in different lighting conditions, not just the overall results as plotted.

Discriminating flower species

Presence of M cones

Chromatic distances for discriminating target flowers from

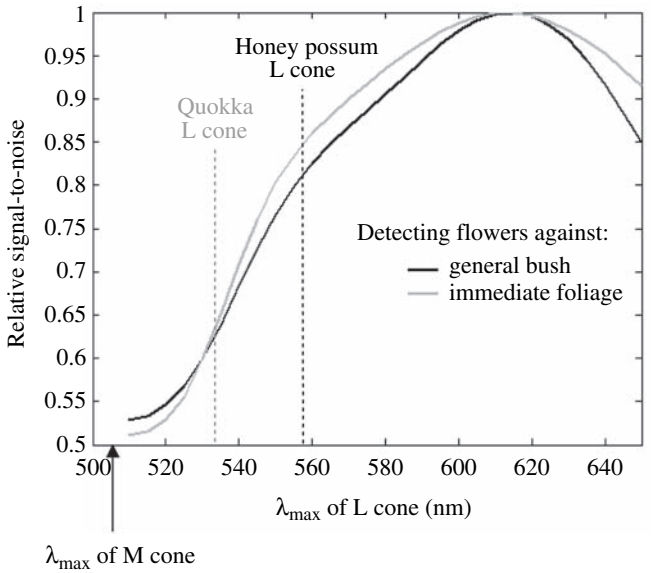

Fig. 2. Calculated signal-to-noise ratios achieved by different hypothetical L cone tuning (see Materials and methods) for the visual task of detecting flowers amongst their visual backgrounds (largely leaves and bark of vegetation). For both immediate and more general backgrounds the L cone tuning of the honey possum offers higher signal-to-noise ratios than the L cone tunings of other marsupials (e.g. quokka), but the best L cone would have a λ_{max} more long-wave than that found in any marsupials.

non-target flowers were calculated as explained in the Materials and methods for trichromatic honey possums and for hypothetical dichromats lacking M cones. For the measurements made in sun and shade, the mean chromatic distances for a dichromat were 94% and 77% of those for a trichromat. However, at dusk (for which we had very few measurements for non-targets) the mean chromatic distance for a dichromat was 114% that for a trichromat. This surprising result that chromatic distances were larger for a dichromat is possible because distances are calculated relative to receptor noise, which becomes smaller for a cone class when its density increases – in the modelling the M cones were not simply deleted, they were replaced by L and S cones, reducing the quantum noise and neural noise in these classes of cones. This reflects the fact that having an extra dimension of colour vision is not without cost to other aspects of vision, including any other dimension of colour vision. The pattern of results was the same if the dichromat's L cone had λ_{max} anywhere in the range 530–557 nm. Thus there was no clear and consistent disadvantage in being dichromatic for discriminating between the target and non-target flower species in the honey possum's environment. This unexpected result was produced because many of the discriminations required lie in the yellow-white-lilac colour direction, which is still available to a mammalian dichromat.

L cone tuning

Fig. 3 shows the effect of changing the L cone tuning for a trichromat. Discriminability between target and non-target flowers increases as the L cone sensitivity moves to longer wavelengths, meaning that the tuning of the honey possum's L cone is not optimal, but it is better than the tuning of other marsupial L cones. This pattern of results held true whether we used just the mature flowers or all measurements of each species, and also for subsets of the target species (e.g. leaving out *Banksia* species, which are more easily discriminable by their shape than other target flowers).

Discerning flower maturity – Banksia attenuata

Presence of M cones

Chromatic distances between mature *Banksia attenuata* flowers and the most similar immature or senescent flowers were calculated for trichromatic honey possums and for hypothetical dichromats lacking M cones (see Materials and methods). The means of these chromatic separations for a dichromat, as a proportion of the trichromatic signal, were 0.31, 0.94, 0.34, 0.49 and 0.34 for the measurements made in conditions of sun, cloud, early-dusk, sunset and post-sunset, respectively. Thus for the task of discerning the maturity of this crucial food resource, a dichromatic honey possum would be at a disadvantage compared to a trichromat (the pattern of results was the same if the dichromat's L cone had λ_{max} anywhere in the range 530–557 nm).

L cone tuning

Fig. 4 shows the effect of changing the L cone tuning for a

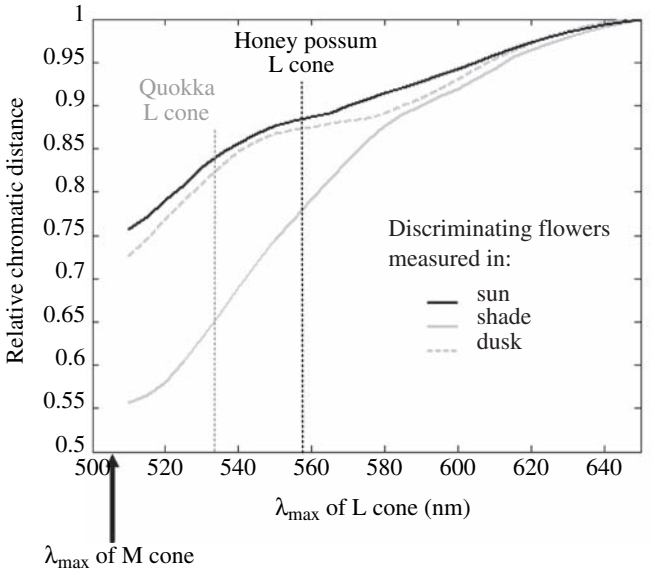

Fig. 3. Calculated noise-scaled chromatic distances achieved by different hypothetical L cone tuning (see Materials and methods) for the visual task of discriminating target flowers from non-target flowers. In all illumination conditions, the L cone tuning of the honey possum is more advantageous than the L cone tunings of other marsupials (e.g. quokka), but better still would be L cone tunings more long-wave than those found in any other marsupials.

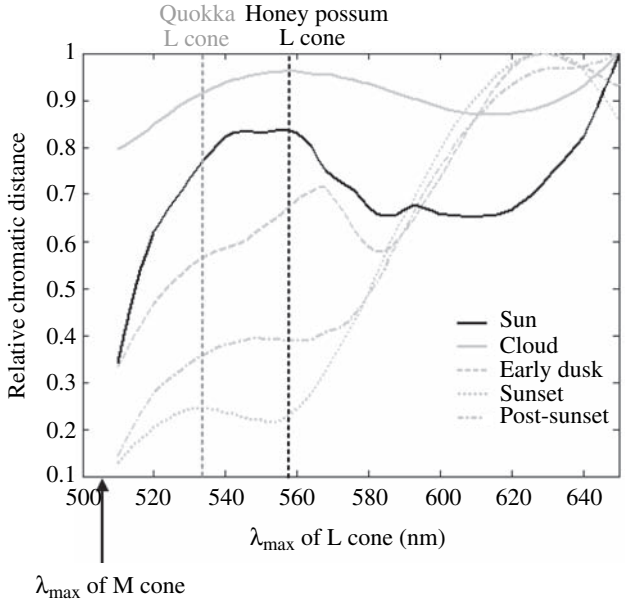

Fig. 4. Calculated noise-scaled chromatic distances achieved by different hypothetical L cone tuning (see Materials and methods) for the visual task of discriminating food rich (mature) from other (immature and senescent) inflorescences of *Banksia attenuata*, the most important measured food resource for honey possums. In this case, incrementally shifting the honey possum's L cone tuning to longer wavelengths would offer no clear advantage. For the conditions of sun and cloud, which represent larger portions of time than the dusk conditions, and for which we have many more measurements, a local optimum in L cone λ_{max} is evident close to the value found in the honey possum.

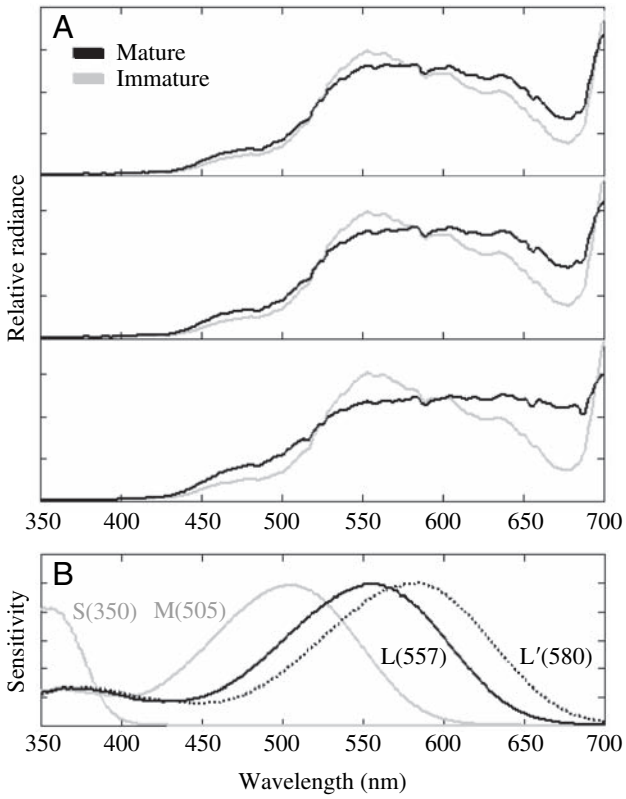

Fig. 5. Radiance spectra of *Banksia attenuata*, compared to spectral sensitivity of honey possum cones. (A) Three examples of mature, food bearing, inflorescences alongside the chromatically nearest immature inflorescence (see Materials and methods). The maturity of the mature samples increases from top to bottom panels. The spectra are normalised for luminance (i.e. the absorption of honey possum L cones is equated). (B) Relative sensitivity of honey possum S cone (grey), M cone (grey), L cone (solid black line), and a hypothetical L cone (L′) with λ_{max} value at 580 nm (broken black line). The sensitivity curves have been adjusted for lens absorbance and self screening (see Materials and methods). The tuning of the honey possum L cone is approximately aligned with the local peak of the immature radiance spectra – characteristic of chlorophyll. If the L cone λ_{max} was incrementally shifted from 557 nm to longer wavelengths, the contrast between the mature and immature spectra would reduce at first, and then increase again for much longer wavelength λ_{max} values. Note that with L and M cone λ_{max} values at 557 nm and 505 nm, the L:M absorption ratio is higher for immature than mature inflorences – the opposite relationship to that for human colour vision with λ_{max} values at 565 nm and 535 nm.

trichromat engaged in the task of discerning the maturity of *Banksia attenuata* flowers. Like the tasks tested above, the tuning of honey possum L cones is better than the tuning of L cones from other marsupials (except for the brief period just before sunset, when the light is changing very rapidly and for which we have fewest measurements). However, unlike the tasks tested above, there is no clear advantage to be gained by incrementally shifting the honey possum's L cone sensitivity to longer wavelengths, except in the early dusk and sunset conditions. For the sun and cloud conditions the honey possum's spectral tuning even seems to be locally optimal. *Post-hoc* analysis was used to investigate why these results were so different from those for the previous tasks, despite the overlap between spectral measurements and analysis. First, the pattern of results was found not to be influenced by including or omitting from the analysis the senescent flowers or the very immature flowers. Second, the results *were* altered by omitting the immature flowers, or by replacing them with *Banksia attenuata* leaves or other flower species. Thus it is the comparison of mature and nearly mature *Banksia attenuata* flowers that is crucial for the pattern of results obtained. Fig. 5 plots three examples of mature flower radiance spectra for comparison with chromatically near immature spectra. It can be seen that the honey possum's L cone tuning is approximately aligned with the local maxima of the immature spectra, at around 550–560 nm – a spectral peak associated with reflectance or transmission by chlorophyll-containing material. It can be seen that incrementally shifting the L cone tuning to longer wavelengths would reduce the contrast between immature and mature spectra, before increasing it again for much longer wave pigments.

Lastly the exact nature of the analysis plays a role. We followed the method of Osorio et al. (2004), which finds for each target the smallest chromatic distance to any non-target (i.e. the hardest discrimination). If the median chromatic distance to *all* non-targets is used instead (which we consider less appropriate for this type of task), this destroys the striking local optimum of tuning. This is because, as shown in Fig. 6, each mature sample often differs from its chromatically nearest immature sample in L:M cone absorption ratio, and thus it is helpful to have the L cone positioned in the spectrum where the relative radiance of the mature and immature samples is reversed compared to the region of the spectrum where the M cone is more sensitive. However, since both immature and mature samples are spread out in their ratio of S cone absorption to L and M cone absorption (i.e. how flat the spectra are; how desaturated the colour appears), then the median chromatic distance between each mature sample and all immature samples will indicate to a higher degree the differences in shortwave to longwave ratio. This contrast will not be helped by positioning the L cone where the relative radiance of the mature and immature samples is reversed. Instead it would be best to tune the L cone to where the sum of the L and M absorption is maximally different between samples. For the same reason, the above tasks of detecting flowers and discriminating flower species did not produce clear local maxima of L cone tuning around 560 nm because, even if the analysis used the nearest distractor as used in this section, the chromatic displacement to the nearest distractor was as often in the S cone direction as the L–M direction.

In sum, the results have shown that for the task of discriminating between mature and nearly mature *Banksia attenuata* flowers, the most important food resource in our study environment, shifting the L cone tuning to longer wavelengths would not generally be advantageous. This is because this task requires detection of changes in the L:M cone

absorption ratio (yellow-green to yellow in human terms), rather than more general differences in the ratio of short to long wavelengths. It is worth noting that for a honey possum, the mature flowers produce a lower L:M ratio than the immature flowers, which is opposite to the relationship for human colour vision, emphasising the importance of not relying on human vision for judgments of what other animals see.

Discussion

In this study we have tested the proposal that the colour vision of honey possums may be adapted to the specific requirements of its visual ecology. We defined three tasks related to its diet of nectar and pollen: (1) detecting target flowers in their environment; (2) discriminating between target and non-target flowers; (3) discerning the maturity of *Banksia attenuata* flowers, the most important food resource in our study environments. In summary, we found that the possession of M cones, and thus the potential for trichromacy, offered clear advantages over typical mammalian dichromacy for detecting most target flower species (task 1) and for discerning *Banksia attenuata* maturity (task 3). Surprisingly, however, there was no advantage for discriminating between flower species (task 2) or for detecting *Banksia attenuata* flowers (subset of task 1), demonstrating that trichromacy is not always advantageous over dichromacy, even for colour discrimination tasks where we (using human vision) might have expected it to be. Further, given that honey possums do have three cone types, we considered the tuning of the L cone, which differs from that in the other measured marsupial species. We found that for detecting target flowers (task 1) and for discriminating them from non-targets (task 2), the tuning of honey possum L cones is more advantageous than the tuning of other marsupial L cones. However, longer wavelength sensitivity would be better still, implying that there must be another factor acting to limit such longwave shifts. In contrast, for the task of discerning *Banksia attenuata* maturity (task 3), there would be no clear advantage in longer wavelength sensitivity, and the tuning of the honey possum L cones may be locally optimal.

Our data provide possible answers to three questions. Why do honey possums possess M cones as well as the S cones and L cones found in most other mammals? Why are honey possum L cones tuned to longer wavelengths than other marsupial L cones? Why are honey possum L cones not tuned to longer wavelengths still? Before we consider these questions in turn, it is important to note that an adaptationist approach can never definitively answer such questions about evolutionary causes. There will always be other possible pressures or constraints than the ones tested, or it may be that the trait in question has not itself been selected at all, but is a ‘spandrel’ (i.e. a byproduct of selection for another trait; Gould, 1997; Gould and Lewontin, 1979). Furthermore, the properties of sensory systems may be a compromise between many pressures, which may be one reason why the critical sensory tasks that affect the fitness of an individual animal are difficult to identify, despite sensory systems generally being considered to be subject to strong selection pressure. It is also important to distinguish between pressures that may operate on a trait now and the pressures that may have contributed to its selection in the past, and even a clear demonstration of increased reproductive fitness would identify only the former. However, an adaptationist approach can rule out possible causes of selection if it shows that an existing trait would offer no advantage over the possible alternatives. Thus by identifying tasks for which honey possum vision appears adapted, and tasks for which it is sub-optimal, we can identify or rule out candidate pressures that may have shaped it.

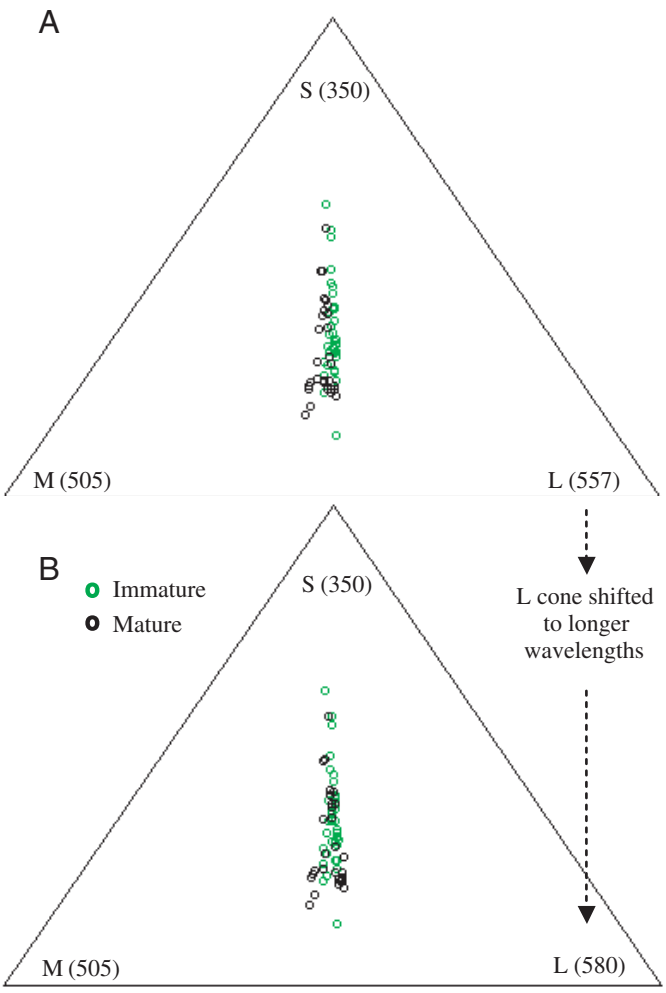

Fig. 6. Chromaticity diagrams depicting mature and immature *Banksia attenuata* flowers. Chromaticity contrast (relative to mean immature chromaticity) is plotted in Maxwellian coordinates (see Kelber et al., 2003). (A) Chromaticity for extant honey possums; (B) chromaticity for hypothetical honey possums with L cone λ_{max} at 580 nm (λ_{max} values beyond this being implausible for rhodopsins). It can be seen that this long-wave shift makes the immature and mature chromaticities less distinct from each other. Note that the mature samples lie on the left (M cone side) of the immature samples, whereas for human vision, they would lie on the right (L cone side).

Presence of M cones

It is not yet known whether the honey possum M cone pigment has been evolved relatively recently or whether it is related to M cone pigments in other vertebrates. If the latter is the case, it could be argued that the existing cones in marsupials have simply been inherited from shared ancestors with reptiles, and are no longer under any selective pressure. However, since retinal space is limited, reducing the number of different types of cone in a retina can produce performance benefits for tasks that rely on the remaining receptor classes. Spatial vision will be improved either by increasing the density of the receptors that contribute to the relevant pathway, or by making all contributing receptors identical (see e.g. Osorio et al., 1998; Regan et al., 2001). Furthermore, certain colour discriminations can also be improved by reducing trichromacy to dichromacy and thus increasing the number of cones in the remaining classes. This was demonstrated in the results for discriminating target flowers from non-targets (task 2). Additionally, in the absence of any pressure to maintain a particular photopigment, genetic mutation would act in the direction of making it non-operational. The presence of three cone types in honey possums stands in contrast to the general trend of colour vision reduction in placental mammals, where the ancestral M cones were lost from the whole group, followed by many separate examples of S cone loss (e.g. Ahnelt and Kolb, 2000; Collin et al., 2003; Jacobs and Deegan, 1992; Jacobs et al., 1993, 1996; Peichl et al., 2001; Peichl and Pohl, 2000; Wikler and Rakic, 1990; Yokoyama, 2000). Thus it is likely that the compliment of cone pigments present in honey possums has been maintained by some selective pressure. Our data show that this pressure is unlikely to have come from the ability to discriminate between target and non-target flower species (task 2), because, perhaps surprisingly, M cone loss would not cause much disadvantage for this task. However, the other tasks we tested do offer clear candidates for the selective pressure. If honey possums became dichromatic like other mammals and tamar wallabies, they would be disadvantaged in detecting many food-bearing flowers in their natural environment (task 1), although interestingly they would remain able to detect the most important food resource, *Banksia attenuata*. However, they would be disadvantaged in discerning the maturity of this important resource (task 3). Thus, although a dichromatic honey possum could detect *Banksia attenuata* flowers, it would have difficulty visually ascertaining which flowers were mature and food-bearing. The potential cost of this difficulty is discussed below.

L cone tuning

Why are honey possum L cones tuned to longer wavelengths than other marsupial L cones?

Vertebrates have a diversity of visual pigments, ranging in λ_{max} from ca. 350 nm to ca. 630 nm, and the inter- and intra-specific variation in visual pigment tuning has been subject to much study. Many correlations have been noted between spectral tuning of visual pigments and spectral irradiance of the light environment, particularly in the case of fishes (Bowmaker et al., 1994; Cummings and Partridge, 2001; Lythgoe et al., 1994). Other studies have focussed on critical visual tasks, emphasising spectral radiance as the raw signal used by visual systems (e.g. Osorio and Vorobyev, 1996; Partridge and Cummings, 1999; Regan et al., 1998; Sumner and Mollon, 2000a), and this is the approach we have taken. We have identified two tasks for which the longer wave tuning of honey possum L cones is more advantageous than the tuning of other marsupials: detecting flowers in their natural environment (task 1), and discriminating between target and non-target flowers (task 2; there was a smaller and less consistent difference between the honey possum and other marsupial tunings for the third task of discerning the maturity of *Banksia attenuata*). Thus these two tasks are therefore candidate pressures for the longer wave tuning of honey possum L cones. The ancestral λ_{max} values of mammalian groups remain obscure, so it is impossible to say whether the honey possum tuning or the shorter wave tuning of other marsupials is closer to the ancestral position of marsupial L cones. It is noteworthy though, that of the marsupial L cones so far measured, most have λ_{max} values in the range 530–540 nm, including the two other diprotodont species besides the honey possum (Arrese et al., 2002b, 2005; Deeb et al., 2003; Hemmi et al., 2000; Strachan et al., 2004). In addition, the L cones in most mammals are tuned to shorter wavelengths than in honey possums, with λ_{max} values down to around 500 nm (e.g. Ahnelt and Kolb, 2000; Jacobs, 1993). It is unknown whether the shorter wave tunings of other marsupials and other mammals are to be explained by ecological pressures or by a tendency for relaxed selection to produce drift to middle wave tuning. However, given that the known L cones of other marsupials and most mammals are tuned to shorter wavelengths, it seems likely that the honey possum L cone λ_{max} has been under selective pressure either to move to, or stay at, longer wavelengths. Our data supply two candidates for this pressure: detecting target flowers amongst leaves or general bush, and discriminating between target and non-target flowers.

Why are honey possum L cones not tuned to longer wavelengths still?

These two tasks, which can explain why longer wave tuning is found in honey possums than in other marsupials and most other mammals, cannot explain why honey possum L cones are not tuned to longer wavelengths still. The optimal λ_{max} for detecting flowers (task 1) is around 615 nm, and the benefit for discriminating flowers (task 2) continues to rise for λ_{max} values up to 640 nm (c.f. the optimal tuning of primate L and M cones for detecting fruit; Regan et al., 1998, 2001; Sumner and Mollon, 2000a; and for discerning fruit ripeness, Sumner and Mollon, 2000b). One reason that the L cone λ_{max} value of honey possums has not moved from 557 nm might simply be that as long as an animal's colour vision system is 'good enough' visual pigment tuning tends to be conservative. For example, most birds seems to have very similar sets of visual

pigments despite varied habitats and lifestyles (e.g. Hart, 2001). However, against this argument lies the fact that the L cone tuning of all marsupials is not the same, so it must have changed in some species since their common ancestor, and among mammals, L cone λ_{max} values vary from around 500 to 565 nm (e.g. Jacobs, 1993).

There are three basic ways in which the L cone λ_{max} value in honey possums could be shifted to longer wavelengths. First, exchanging rhodopsin for porphyropsin, in which the same opsin protein binds a different light absorbing molecule, causes a longwave shift (e.g. Knowles and Dartnall, 1977). However, although many fish and some reptiles employ porphyropsins, they are unknown in mammals. Moreover, even if this type of pigment was available to marsupials, porphyropsins are known to have higher dark noise than rhodopsins (Ala-Laurila et al., 2003; Donner et al., 1990), which might entail considerable cost for animals that forage in dim conditions as well as daylight. The second method for producing a long-wave shift would be to employ long-pass filters, such as the red oil droplets found in birds (e.g. Hart, 2001). Oil droplets are in fact present in honey possum retinae, but they are all transparent (Arrese et al., 2002a), presumably because filters must cut out some light, which would entail a cost at low light levels. Thus the fact that honey possums are active in dim light as well as daylight would act as a selective pressure against achieving longer wavelength sensitivity by employing porphyropsins or by using coloured oil droplets. Alternatively, it may be that the genetic steps needed to achieve porphyropsins or to recolour the oil droplets are highly improbable and simply never have occurred since honey possums diverged from other marsupial groups (who also have transparent oil droplets). The third method of achieving a long-wave shift in tuning would be to alter the amino acid sequence of the L cone opsin, and here it is possible that there are molecular constraints. However, while rhodopsins with λ_{max} values near 600 nm are unknown, they do exist at longer wavelengths than the honey possum's 557 nm: e.g. 565 nm in primates; 575 nm in guppies (a freshwater teleost); 565–575 nm in many birds (e.g. Archer et al., 1987; Hart, 2001; Jacobs, 1993). Therefore λ_{max} values for unfiltered rhodopsins may be ruled out beyond 575 nm by unknown molecular constraints, but this would not explain why honey possum L cones are limited to a λ_{max} value of 557 nm. We may speculate that honey possum L cone opsins could not achieve the long-wave shift to 565 or 575 nm because of some unknown molecular incompatibility that is not present in primates, guppies or birds.

However, in the absence of specific evidence for molecular limits, we turn to potential ecological pressures that might act against a long-wave shift of the honey possum L cone tuning. Such a selective pressure is suggested by our results for discerning the maturity of *Banksia attenuata* flowers: a task for which the honey possum L cone tuning may even be close to (locally) optimal. Is it feasible that such a specific task could override conflicting pressures from more general tasks? In fact, because the task essentially seems to be one of detecting the removal of chlorophyll from the flowers, it is probably applicable much more widely than to *Banksia attenuata*. Any flower whose crucial change in maturity (i.e. the availability of nectar and pollen) is correlated with removal of chlorophyll and a change in colour, in humans terms, from yellow-green to yellow, is likely to produce similar results. Such flowers include, for example, several other *Banksia* species not present at our field sites, but known to be important to honey possums (Turner, 1984; Wooller et al., 1984, 1983). In the habitats in which we worked, *Banksia attenuata* itself is certainly the most important source of food, being both a common plant and the one with largest inflorescences. In addition, this visual task is likely to be the most difficult that is required for a honey possum, since mature and nearly mature inflorescences cannot be distinguished by shape, size or position. Furthermore, unlike most other plant species that we measured, the presence of immature flowers does not signal the presence of mature flowers (the example in Fig. 1 was chosen because it contains mature, immature and senescent inflorescences in close proximity, but this was rarely the case). Given that *Banksia attenuata* are large plants with widely separated and exposed inflorescences, confusing inflorescences that offer food with those that do not entails a clear cost for a small terrestrial animal in terms of time, the energetic cost of climbing and exposure to predators. It is difficult to quantify the latter, because predation events are rarely observed and in any case avian predator populations may have changed in recent history. We can simply estimate the time cost, however, which is likely to be more important than the energetic cost of climbing for a small warm-blooded mammal that requires a lot of energy simply to stay alive. Immature inflorescences outnumbered mature ones in our study environments by at least 5 to 1, and the large, mature-sized, but still immature inflorescences were about twice as common as mature food-bearing inflorescences. If a honey possum makes a mistake of visiting just one immature inflorescence for every mature one, foraging efficiency may be reduced by half. A good sense of smell will go some way to reducing this cost, but smell does not have the same ability to localise targets from a distance as vision, even for an animal with relatively poor spatial acuity. It is difficult to know how important *Banksia attenuata* may have been to honey possums in the past, especially since the ranges of both plant and animal are likely to have decreased in recent history. However, since there are several other species of *Banksia* with similar inflorescences and with similar properties to those described above, it is not unfeasible that discerning the maturity of *Banksia* flowers is the most important single selective pressure maintaining in honey possums both the existence of M cones and the exact tuning of the L cones.

Conclusions

Our strongest finding is that the L cone tuning of honey possums is better adapted than the L cones of other marsupials for the tasks of detecting food-bearing flowers in their natural environment, and discriminating target flowers from distractor flowers. However, since further advantage in these two tasks

would be gained by even longer wave tuning, there must be a different factor limiting long-wave tuning shifts. This factor may be molecular, since it is rare for unfiltered rhodopsins to have longer wave λ_{max} values than 560 nm. Alternatively, an ecological pressure is suggested by our results for the task of discerning the maturity of *Banksia* flowers. Finally, it is of note that while the presence of M cones is generally advantageous for these tasks, there are specific examples where it is not, emphasising the difficulty in predicting the properties of animals' visual systems from a human visual perspective, without appropriate modelling.

The authors would like to thank Dr Julia Shand (University of Western Australia) for making the research possible, the staff at Whiteman Park staff for their assistance with access to the field site, Dr Elizabeth White (University of Bristol, UK) for help with field work and data formatting, and Dr Misha Vorobyev (University of Queensland, Australia) for comments. The authors would like to thank the Royal Society (P.S.), the UWA Distinguished Visitor Fund (J.C.P.) and the Australian Research Council (C.A.A.) for financial support.

References

Ahnelt, P. K. and Kolb, H. (2000). The mammalian photoreceptor mosaic-adaptive design. *Prog. Ret. Eye Res.* **19**, 711-777.

Ala-Laurila, P., Albert, R. J., Saarinen, P., Koskelainen, A. and Donner, K. (2003). The thermal contribution to photoactivation in A2 visual pigments studied by temperature effects on spectral properties. *Vis. Neurosci.* **20**, 411-419.

Archer, S. N., Endler, J. A., Lythgoe, J. N. and Partridge, J. C. (1987). Visual pigment polymorphism in the guppy *Poecilia reticulata*. *Vis. Res.* **27**, 1243-1252.

Arrese, C., Archer, M. and Beazley, L. D. (2002a). Visual capabilities in a crepuscular marsupial, the honey possum (*Tarsipes rostratus*): a visual approach to ecology. *J. Zool. Lond.* **256**, 151-158.

Arrese, C., Hart, N. S., Thomas, N., Beazley, L. D. and Shand, J. (2002b). Trichromacy in Australian marsupials. *Curr. Biol.* **12**, 657-660.

Arrese, C. A., Oddy, A. Y., Runham, P. B., Hart, N. S., Shand, J. and Beazley, L. D. (2005). Cone topography and spectral sensitivity in two potentially trichromatic marsupials, the quokka (*Setonix brachyurus*) and quenda (*Isoodon obesulus*). *Proc. R. Soc. Lond. B* doi:10.1098/rspb.2004.3009.

Arrese, C. and Runham, P. (2002). Redefining the activity patterns of the honey possum (*Tarsipes rostratus*). *Aust. Mammal.* **23**, 169-72.

Bowmaker, J. K., Astell, S., Hunt, D. M. and Mollon, J. D. (1991). Photosensitive and photostable pigments in the retinae of Old World monkeys. *J. Exp. Biol.* **156**, 1-19.

Bowmaker, J. K., Govardovskii, V. I., Shukolyukov, S. A., Zueva, L. V., Hunt, D. M., Sideleva, V. G. and Smirnova, O. G. (1994). Visual pigments and the photic environment – the cottoid fish of Lake Baikal. *Vis. Res.* **34**, 591-605.

Collin, S. P., Knight, M. A., Davies, W. L., Potter, I. C., Hunt, D. M. and Trezise, A. E. O. (2003). Ancient colour vision: multiple opsin genes in the ancestral vertebrates. *Curr. Biol.* **13**, R864-R865.

Cummings, M. E. and Partridge, J. C. (2001). Visual pigments and optical habitats of surfperch (Embiotocidae) in the California kelp forest. *J. Comp. Physiol. A* **187**, 875-889.

Deeb, S. S., Wakefield, M. J., Tada, T., Marotte, L., Yokoyama, S. and Graves, J. A. M. (2003). The cone visual pigments of an Australian marsupial, the tammar wallaby (*Macropus eugenii*): Sequence, spectral tuning, and evolution. *Mol. Biol. Evol.* **20**, 1642-1649.

Desimone, R. and Duncan, J. (1995). Neural mechanisms of selective visual-attention. *Annu. Rev. Neurosci.* **18**, 193-222.

Donner, K., Firsov, M. L. and Govardovskii, V. I. (1990). The frequency of isomerization-like dark events in rhodopsin and porphyropsin rods of the bullfrog retina. *J. Physiol. Lond.* **428**, 673-692.

Geisler, W. S. (1989). Sequential ideal-observer analysis of visual discriminations. *Psychol. Rev.* **96**, 267-314.

Gould, S. J. (1997). The exaptive excellence of spandrels as a term and prototype. *Proc. Natl. Acad. Sci. USA* **94**, 10750-10755.

Gould, S. J. and Lewontin, R. C. (1979). The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *Proc. R. Soc. Lond. B* **205**, 581-598.

Govardovskii, V. I., Fyhrquist, N., Reuter, T. and Kuzmin, D. G. (2000). In search of the visual pigment template. *Vis. Neurosci.* **17**, 509-528.

Hart, N. S. (2001). The visual ecology of avian photoreceptors. *Prog. Ret. Eye Res.* **20**, 675-703.

Hecht, S., Shlaer, S. and Pirenne, M. H. (1942). Energy, quanta and vision. *J. Gen. Physiol.* **25**, 819-840.

Hemmi, J. M. (1999). Dichromatic colour vision in an Australian marsupial, the tammar wallaby. *J. Comp. Physiol. A* **185**, 509-515.

Hemmi, J. M., Maddess, T. and Mark, R. F. (2000). Spectral sensitivity of photoreceptors in an Australian marsupial, the tammar wallaby (*Macropus eugenii*). *Vis. Res.* **40**, 591-599.

Hood, D. C. and Finkelstein, M. A. (1986). Sensitivity to light. In *Handbook of Perception and Human Performance,* volume 1: *Sensory Processes and Perception* (ed. K. R. Boff L. Kaufman and J. P. Thomas), pp. 1-66. New York: Wiley.

Jacobs, G. H. (1993). The distribution and nature of colour vision among the mammals. *Biol. Rev.* **68**, 413-471.

Jacobs, G. H. and Deegan, J. F. (1992). Cone photopigments in nocturnal and diurnal procyonids. *J. Comp. Physiol. A* **171**, 351-358.

Jacobs, G. H., Deegan, J. F., Neitz, J., Crognale, M. A. and Neitz, M. (1993). Photopigments and color vision in the nocturnal monkey, *Aotus*. *Vis. Res.* **33**, 1773-1783.

Jacobs, G. H., Neitz, M. and Neitz, J. (1996). Mutations in the S-cone pigment genes and the absence of colour vision in two species of nocturnal primate. *Proc. R. Soc. Lond. B* **263**, 705-710.

Kelber, A., Vorobyev, M. and Osorio, D. (2003). Animal colour vision – behavioural tests and physiological concepts. *Biol. Rev.* **78**, 81-118.

Knowles, A. and Dartnall, H. J. A. (1977). *The Photobiology of Vision*. New York: Academic Press.

Lythgoe, J. N., Muntz, W. R. A., Partridge, J. C., Shand, J. and Williams, D. M. (1994). The ecology of the visual pigments of snappers (Lutjanidae) on the Great-Barrier-Reef. *J. Comp. Physiol. A* **174**, 461-467.

MacLeod, D. I. A. and Boynton, R. M. (1979). Chromaticity diagram showing cone excitation by stimuli of equal luminance. *J. Opt. Soc. Amer.* **69**, 1183-1186.

Neumeyer, C. (1992). Tetrachromatic color-vision in goldfish – evidence from color mixture experiments. *J. Comp. Physiol. A* **171**, 639-649.

Osorio, D., Ruderman, D. L. and Cronin, T. W. (1998). Estimation of error in luminance signals encoded by primate retina resulting from sampling of natural images with red and green cones. *J. Opt. Soc. Amer. A* **15**, 16-22.

Osorio, D., Smith, A. C., Vorobyev, M. and Buchanan-Smith, H. M. (2004). Detection of fruit and the selection of primate visual pigments for color vision. *Am. Nat.* **164**, 696-708.

Osorio, D. and Vorobyev, M. (1996). Colour vision as an adaptation to frugivory in primates. *Proc. R. Soc. Lond. B* **263**, 593-599.

Partridge, J. C. and Cummings, M. E. (1999). Adaptations of visual pigments to the aquatic environment. In *Adaptive Mechanisms in the Ecology of Vision* (ed. S. N. Archer, M. Djamgoz, E. Loew, J. C. Partridge and S. Valerga), pp. 251-284. London, UK: Kluwer.

Peichl, L., Behrmann, G. and Kroger, R. H. H. (2001). For whales and seals the ocean is not blue: a visual pigment loss in marine mammals. *Eur. J. Neurosci.* **13**, 1520-1528.

Peichl, L. and Pohl, B. (2000). Cone types and cone/rod ratios in the crab-eating raccoon and coati (Procyonidae). *Invest. Ophthalmol. Vis. Sci.* **41**, 2630B876.

Regan, B. C., Julliot, C., Simmen, B., Viénot, F., Charles-Dominique, P. and Mollon, J. D. (1998). Frugivory and colour vision in *Alouatta seniculus*, a trichromatic platyrrhine monkey. *Vis. Res.* **38**, 3321-3327.

Regan, B. C., Julliot, C., Simmen, B., Viénot, F., Charles-Dominique, P. and Mollon, J. D. (2001). Fruits, foliage and the evolution of the primate colour-sense. *Phil. Trans. R. Soc. Lond. B* **356**, 229-283.

Richardson, K. C., Wooler, R. D. and Collins, B. G. (1986). Adaptations to

a diet of nectar and pollen in the marsupial *Tarsipes rostratus* (Marsupialia: *Tarsipedidae*). *J. Zool. Lond. A* **208**, 285-297.

Rushton, W. A. H. (1972). Pigments and signals in colour vision. *J. Physiol.* **220**, 1-31P.

Strachan, J., Chang, L. Y., Wakefield, M. J., Graves, J. A. and Deeb, S. S. (2004). Cone visual pigments of the Australian marsupials, the stripe-faced and fat-tailed dunnarts: sequence and inferred spectral properties. *Vis. Neurosci.* **21**, 223-229.

Strahan, R. (1995). *The Mammals of Australia*. Chatswood: Reed New Holland.

Sumner, P. and Mollon, J. D. (2000a). Catarrhine photopigments are optimised for detecting targets against a foliage background. *J. Exp. Biol.* **203**, 1963-1986.

Sumner, P. and Mollon, J. D. (2000b). Chromaticity as a signal of ripeness in fruits taken by primates. *J. Exp. Biol.* **203**, 1987-2000.

Turner, V. (1984). *Banksia* pollen as a source of protein in the diet of two Australian marsupials. *Oikos* **43**, 53-61.

Vorobyev, M., Brandt, R., Peitsch, D., Laughlin, S. B. and Menzel, R. (2001). Colour thresholds and receptor noise: behaviour and physiology compared. *Vis. Res.* **41**, 639-653.

Vorobyev, M. and Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Proc. R. Soc. Lond. B* **265**, 351-358.

Vose, H. M. (1973). Feeding habits of the western Australian honey possum, *Tarsipes spenserae*. *J. Mammal.* **54**, 245-247.

Wikler, K. C. and Rakic, P. (1990). Distribution of photoreceptor subtypes in the retina of diurnal and nocturnal primates. *J. Neurosci.* **10**, 3390-3401.

Wooller, R. D., Russell, E. M. and Renfree, M. B. (1984). Honey possums and their food plants. In *Possums and Gliders* (ed. A. P. Smith and I. D. Hume), pp. 439-443. Sydney: Surrey Beatty & Sons with Australian Mammal Society.

Wooller, R. D., Russell, E. M., Renfree, M. B. and Towers, A. (1983). A comparison of seasonal changes in the pollen loads of nectarivorous marsupials and birds. *Aust. Wildlife Res.* **10**, 311-317.

Wyszecki, G. and Stiles, W. S. (1982). *Color Science*. New York: Wiley.

Yokoyama, S. (2000). Molecular evolution of vertebrate visual pigments. *Prog. Ret. Eye Res.* **19**, 385-419.