Chittka, L. & Kevan, P.G. (2005) Flower colour as advertisement. In Dafni, A., Kevan, P.G., Husband, B.C. (eds.) Practical Pollination Biology. Enviroquest Ltd., Cambridge, ON, Canada, pp. 157 – 196

4.3 Flower colour as advertisement - By Lars Chittka and Peter G. Kevan

4.3.1 Introduction

Many pollinators' spectral perception extends from ultraviolet (UV) through to the red part of the electromagnetic spectrum. For natural daylight, this range is, practically speaking, from 300 nm to about 700 nm. Most animals with colour vision have three (trichromatic) or four (tetrachromatic) systems involving perceptions in UV, blue, green, and red. Trichromats may use UV, blue, and green (as in many insects) or blue, green, and red (as familiar for human beings). Many mammals are blue-green dichromats (Jacobs 1993), whereas arthropod dichromats typically have UV and green receptors, or blue and green receptors (Chittka 1996a; Briscoe and Chittka 2001). Many insects have multiple (more than four) spectral receptor types (Briscoe and Chittka 2001). It is important to recognize that each of these primary wavebands constitutes only one of at least two, and perhaps mostly three or more involved in colour perception (Kevan *et al.* 2001). Thus, singling out UV reflections is improper in studies of pollination (Kevan *et al.* 2001), although that practice may have merit in narrower contexts (*e.g.* taxonomy, biochemistry, biophysics) (Eisner *et al.* 1973; Burr *et al.* 1995). Similarly, ignoring the nature of the colours of the backgrounds against which flowers are presented is improper (Kevan 1978; Chittka *et al.* 1994). Flowers and floral parts change as flowers age, proceeding through various reproductive phases and possibly offering different rewards to pollinators. Such changes may or may not be visible to the human eye. The signals may improve the corporate images of groups of flowers, yet guide pollinators only to sexually receptive ones in the group (Weiss 2001). Thus, floral colours should be considered ideally with regard to:

- The colour vision system of the receiving animal.
- The colour of the background against which the flower blooms.
- The colour contrast between flower and background.
- Other simultaneously presented cues (visual and olfactory).
- Presence and exposure of rewards.
- Other co-flowering species.

The methods for measuring or designating floral colours must be such that they are meaningful to other scientists (*i.e.* standard) and replicable. *Table 4.3* presents the commonly used ways in which flower colours are described or measured or both.

For most methods of measuring floral colours, the flowers must be removed from the plants, and placed in a setup that fulfils the

conditions stipulated for each method. This is not a problem, however: most flowers do not usually change their colours within hours or a day, so they can be carried to the laboratory without problems. They can even be sent long distance with courier and rapid postal services. For transport, flowers should be packed in conditions which prevent desiccation. They should be placed in plastic bags or sealed containers, with some moisture, for example by spraying a few drops of water into the bag before closing. The containers should not be exposed to heat. If they are kept in containers for longer periods of time, it can also help to keep them in cooler bags, possibly with freezer gel packs.

4.3.2 Human visual evaluation

Floral colours can be typified by reference to human abilities to distinguish and match colours. Although this method does not take into account the ultraviolet component of floral colours, it can be useful. We advocate the use of standard arrays of colours to identify floral colours in a way that can be understood by other people. One of the internationally recognized standards for identifying colours is by the Munsell Book of Colour (www.munsell.com), but horticultural colour charts are smaller and specifically aimed at the colours of flowers and vegetation (1996 Horticultural Colour Chart issued by The British Colour Council in collaboration with The Royal Horticultural Society, www.rhs.org.uk). Horticultural colour charts provide numerical designations, standardized names in several languages, and examples from specific flowers. A similar approach can be through colorimetrically standardized commercially produced materials. The Pantone ® Colour Formula Guide (1997; www.pantone.com) is especially good in that both flat (for most flowers) and glossy (for some like buttercups) colour samples are provided with numerical designations. The Pantone colours are not diverse from white to pale grey, though. Patterns of floral colouration can be typified by use of these charts and drawings.

The use of colour photographs also serves well, but one should be aware that films vary in how true they are to colour, and may fade differentially with age. Digital photography is also highly useful. However, colour imagery is expensive to reproduce with the level of accuracy needed to transfer information on colouration. Approaching floral colours through human appreciation has been useful in making generalizations about changes in floral colours as flowers age, with respect to colour polymorphisms, seasonal changes and biogeographical differences in the frequency of occurrence of floral colours (Kevan 1983). Nevertheless, caution must be used in making inferences about pollination because human appreciations of colour are not analogous to the capacities of other animals to distinguish colours.

Table 4.3 Methods of colour determination and measurement.

Method	Advantages	Challenges and Disadvantages
Human visual evaluation	A rapid method for large samples, data can be assembled from existing floras and records. Standard colour charts can be used.	UV patterns can not be discerned. Various authors use different criteria for colour naming. Different colour charts provide different designations. Standard colour charts are expensive.
Spectrophotometry	Gives accurate measurements of reflectance (spectral reflectance curve) over the entire spectrum. Data can be stored automatically on computer spread-sheets. Colour loci can be calculated and presented on standard diagrams. Rapid calculations allow for colour contrasts, *etc.* to be presented. Modern technology makes this approach relatively easy to use.	Equipment is somewhat expensive and requires computer interface. Fine details of colour patterns, floral guides, *etc.* can not be readily assessed with accuracy. Requires careful calibration for incident light, white and black standards, as data are being collected.
Reflectance spectrophotometry (double beam scanning spectrophotometry with integrating sphere for reflectance measurements).	Gives accurate measurements of reflectance (spectral reflectance curve) over the entire spectrum. Data can be stored automatically on computer spread-sheets. Colour loci can be calculated and presented on standard diagrams. Rapid calculations allow for colour contrasts, *etc.* to be presented. Allows calibration simultaneous with measurements.	Equipment is somewhat expensive and requires computer interface. Fine details of colour patterns, floral guides, *etc.* cannot be readily assessed with accuracy. Laboratory instrument with internal standard light source. Spectral reflectances of coloured samples placed on one of the integrating sphere's ports are compared with a white standard placed on the other.

159

Method	Advantages	Challenges and Disadvantages
Photography with wide-band mono-chromatic filters and UV reflecting grey scale.	Allows for capturing images of fine details in colour patterns. Provides approximate spectral reflectance curves based on matching reflectance of study objects with that of the grey scale. Simpler and cheaper, but less accurate, adaptations can be used. Film is inexpensive.	Requires care. Quartz lenses to allow for best images in UV to be captured are expensive. Glass lenses attenuate UV (use with care), and haze-coated glass lenses block it (do not use for UV images: artefacts result). Care must be taken with focus adjustments, especially at shorter wavelengths. Artefacts result from overexposures, especially in UV.
Black and white video-viewing	Handy to use in the field. Can be used with wide-band monochromatic filters and grey scale as in photography.	Use with UV-passing filter will also capture Infrared images, so these need to be separated. No bee-subjective colours can be calculated.
Colour video viewing	Can be used to produce analogous colour images (UV becomes blue, blue becomes green, and green become red).	Technologically highly specialized and beyond the scope of this manual. Trichromacy only. Hardly used. No bee-subjective colours can be calculated.
Digital photography	The inexpensive digital cameras are not sensitive, or not uniformly sensitive in ultraviolet.	Untested, but probably prone to difficulties in interpreting the images. No bee-subjective colours can be calculated.
Hyperspectral imaging; computerised images taken through a series of interference filters (Chiao *et al.* 2000).	Images contain information on spectral content as well as spatial arrangement of colours; a promising method for evaluating floral colour in conjunction with pattern.	Equipment is expensive; evaluation of data is complex and beyond the scope of this chapter.

160

4.3.3 Photography with respect to pollinator vision by using broad-band monochromatic filters and grey scale

4.3.3.1 Background and theory

Flower photography is an important way of recording floral form and colours, and offers a way of visualising patterns that are invisible to human beings. Because many pollinators, including insects and birds, see in the ultraviolet part of the spectrum, there has been great interest in recording ultraviolet reflections from flowers (Kevan *et al.* 2001). Flower photography to examine the optical features of flowers that potentially are useful as cues to flower visitors must consider all salient wavebands of reflection. This means that photography needs to consider the three primary colours in trichromats, such as most bees (ultraviolet, blue, and green) and human beings (blue, green, and red) and the four (and possibly more) primary colours for tetrachromats or polychromats such as some birds and butterflies.

Some components of floral colour and colour patterns can be inferred within the confines of human colour vision (as noted above) but ultraviolet is one waveband of reflection that must be captured by instrumentation (photography or reflectance spectrophotometry or both). The same instrumentation can also be used to capture reflections in the other primary wavebands, so, for the sake of rigour and completeness, why not use it for that as well? The principle of photography to examine floral colours as they relate to pollinator colour vision is to take a series of photographs that capture reflections in each of the primary colours (monochromatic reflections) of the pollinators. Although not entirely necessary, we advocate that photographs should be taken in intermediate wavebands as well. This process can allow for quite closely estimated colour measurement based on a photographically derived approximate spectral reflectance curve (see reflectance spectrophotometry). Modern instrumentation allows much more accuracy than was possible when this approach was first used (Kevan 1972; Kevan 1983). We describe how to make photographs across the pollinator visual spectrum, what instrumentation and materials is needed, and how to interpret the results.

Photography relies on light, camera, lens, and film. Separating the pollinator visual spectrum for photography into broadband monochromatic relies on the use of coloured filters. To capture the images on film requires adjustments of the camera, lens, and film for proper exposure and focus. Lighting is highly critical.

We advocate placing the flower to be photographed against a black background (photographers' black flock paper) on a stand in diffuse (shade) facing north (in the northern hemisphere). The black paper provides for control for exposure: it should be black, or nearly so, on the eventuating photograph. The diffuse light eliminates

shadows and mirror like reflections that can make the photographs difficult to interpret. Light from the north is slightly richer in ultraviolet that light from the south. Absolutely critical to this sort of photography is control for exposure of the film. Inclusion of a grey-scale that reflects more or less uniformly from ultraviolet to red in the photographs is critical. The white part of the scale should be white and the black part and background black. The intermediate grey parts of the scale, even if only one is used, should be grey in the eventuating photograph. It is through the comparison of the greyness (reflections) of the various floral parts with the greyness of the scale that a spectral reflectance curve can be generated. How to make and calibrate a grey scale is described below.

Various cameras can be used, but the most satisfactory are those with detachable lenses. These allow for bellows (or extension rings) to be used for close up photography, and use of lenses that allow passage of ultraviolet light. Thus, 35 mm single-lens-reflex cameras are useful as is larger format cameras such as Hasselblad CM. The lens to be used is absolutely critical! There are, in leading journals, published "ultraviolet" images which were captured through lenses especially coated to block ultraviolet (to reduce the effects of "haze"). These photographs are from lengthy exposures that captured reflections in the narrow band of the highly reduced amount of light passed by the lens at its shortest wavelengths of transmission and the filter at its longest wavelengths. They are artefacts.

What lenses should be used? Glass attenuates ultraviolet light, but does transmit some of the longer waveband of ultraviolet in daylight. Thus, simple, non-coated, glass lenses can be used (*e.g.* Tessar lenses). It is advised that if plans are for use of a glass lens that the transmission curve be determined from the manufacturer or by transmission spectrophotometry. As a rule of thumb, the thinner the glass of the lens, the more ultraviolet will be transmitted. Also, the adhesives used to make complex lenses are ultraviolet absorbing. The best lenses to use are made of quartz or fused silica. Although such lenses themselves are not expensive, making them into lenses for photography requires an understanding of optics. The lenses specifically made for cameras are expensive and often have to be especially ordered.

Focus is a problem. Glass and quartz lenses refract different wavebands of light differently. This is what creates the rainbow of light as it emerges through a glass or quartz prism. The shorter the wavelength, the more the light is refracted. Thus, light from ultraviolet to blue on passing through a camera lens are refracted so that the image in these colours does not fall on the same focal plane as most of the visible light. It falls in front of the main visible image. Thus, when taking close up photographs in the ultraviolet, violet, and even blue (with wide apertures), focus must be adjusted by decreasing the distance from the lens to the film. The distances involved depend on

the lens and the filter (waveband) and need to be determined in preparation for photography (*Figure 4.1*). The Hasselblad system's Carl Zeiss UV Sonnar (1:4.3 f = 105 mm) is chromatically adjusted from ultraviolet to red so that no focus adjustment from what is seen through the lens in the human visible part of the UV-Vis is needed.
We suggest using highly sensitive (fast) black and white panchromatic film. All these sorts of films are sensitive to exposure from ultraviolet to the visible red. Their sensitivity drops rapidly in the near infrared. The most satisfactory films have ISO (ASA) ratings at between 300 and 400. The reason for using a fast film is that photographs in natural daylight and outside are possible, using bellows extensions to allow for close-up photographs through filters. The bellows and the filters both require exposure adjustment because the amount of light reaching the film is reduced by closeness and the filter. The film can be "forced" to even higher ISO, allowing for reduced exposures (duration or aperture). It that is done, developing of the film must be adjusted accordingly in the dark room. Remember that faster films are grainier, and forcing them makes them even grainier. Thus, fine detail, probably unimportant to the scientific interpretation of the photograph, but aesthetically pleasing in print, can be lost.

Figure 4.1 The photographic equipment in use, showing the vertical black backdrop against which the flowers, vegetation and grey scale are photographed, and the tripod-mounted camera with cable shutter-release, lens, filter holder, and filter in place.

Exposure is adjusted through shutter speed and aperture (f-stop). Through-the-lens light meters are the most practical because the meter is measuring the light coming into the camera from the image that will be captured by the photograph. Experienced photographers may prefer to use hand-held light meters to obtain exposure measurements directly from the object (flowers and backgrounds) and then adjust for the effects of close-up and filter. The combination of shutter speed and aperture that suits the situation is a judgement call on the part of the photographer. Fast shutter speeds are useful if one is outside and the subjects move with breezy gusts and such, but then the aperture must be increased so that depth of field is lost. Depth of field, how deep the focus is, is important for examining details, especially for tubular or complex flowers. Generally speaking,

small apertures and longer shutter speeds are desirable. It is often difficult to read through-the-lens exposure meters with a black background and filters in place. The former problem can be overcome by providing a pale slip of background where the meter readings are displayed, the latter is best overcome by obtaining a meter reading without no filter and then making photographs with double and quadruple exposures (filter factors).

The filters to be used should accord with the UV-Vis. We advocate a series of broadband (*ca.* 100 nm) monochromatic filters that serially overlap each others' transmissions by about 50 nm. *Table 4.4* provides a list, with suggested filter factors, and *Figure 4.2* provides data on their transmission spectra. The filters have to be held in front of the camera's lens so a filter holder is required. This simple item may have to be custom-made so that it clips on the front of the lens and has a slotted part that can accommodate each filter serially and in turn.

With camera, light-meter, film, close-up bellows or tubes, lens, filter-holder, filters, stand, and tripod at hand, set-up can start. The tripod should be extended to allow the photographer to work comfortably with the camera, close-up attachments, and lens mounted on it. The assembled equipment should be directed at and focused on the stand that displays the flowers and associated objects. The stand, with its black cover as backdrop, may be a sheet of cardboard, styrofoam, *etc.* that will allow the subject to be mounted. It is often easier in the field if the stand provides a vertical surface, but that is a matter of personal preference. The photographic system is now ready for use.

On the black backdrop, the flower(s), vegetation, grey-scale, and labels should be affixed. More than one flower can be placed on the backdrop for comparisons between species, ages, aspects, *etc.* For speed in the field, we advocate mounting the flowers *etc.* on the backdrop with insect pins. The need for the grey scale is explained above, a sample of vegetation should be included to represent the natural backdrop of the flowers. Labels are important and should carry the filter designation as a double check against carefully taken notes. The completed series of photographs should resemble *Figure 4.3.*

Table 4.4 Suggested array of broad-band monochromatic filters for photographic analyses of colours of objects across the animal visual spectrum from ultraviolet to red. Each filter has an approximate band width of 100 nm, overlapping by about 50 nm. These filters are chosen because of their fairly uniform and high transmittances. Filter factors in multiples of light meter reading at 6 cm, per cent reflectance of paper labels with respect to magnesium oxide and magnesium carbonate for each filter, and reflectance value used for calculation of flower colour.

Filters make and number	Trans-mission range (nm)	Filter factor	Reflectance of paper (%)	Reflectance value used (%)**
Kodak 18 A	300 – 400	6	< 30 – 85	60
Kodak 35	350 – 450	2	45 – 90	85
Kodak 98	400 – 500	3	80 – 90	85
Kodak 65	450 – 550	6	80 – 90	85
Kodak 61	500 – 600	4	80 – 90	85
Ilford 626	550 – 650	8	80 – 90	85
Kodak 25	600 -	3	80 – 90	85
Ilford 608	650 -	48	80 – 90	85
Kodak 87	750 -	- *	--	--

* - Film loses sensitivity at about 650 nm. ** - Reflectance values used to calculate flower colours represent reflectance of paper at peak of transmission of the filters.

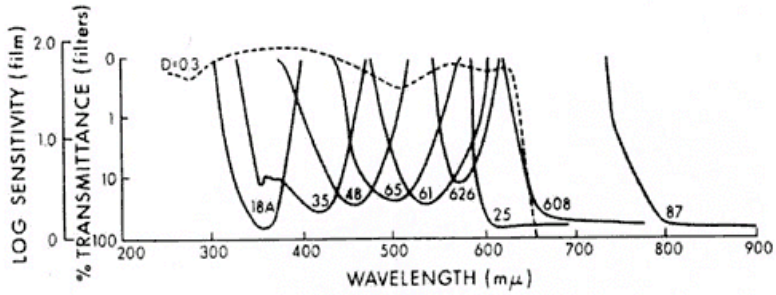

Figure 4.2 Percent transmission of the filters used for analysis of corolla colour (data from Kodak publication B-3 (1966) and Ilford Technical Information Sheet F 20-1); and film sensitivity (log reciprocal of exposure in ergs/cm^2 required to produce specific density of base plus fog, D = 0.3) of Kodak Tri-X pan film (Technical sheet 24-320 supplied from Kodak on request) as a function of wavelength in nanometers. The filter factor for each filter is noted near the peak transmittance of each filter.

Figure 4.3 A series of photographs of the yellow flower of *Opuntia humifusa* taken through an ultraviolet filter (upper left; Kodak 18A); a blue filter (upper right; Kodak 98); a green filter (middle left; Kodak 65); a yellow filter (middle right; Kodak 61); a red filter (lower left; Kodak 25); and no filter (lower right).

Because the process of UV-Vis photography involves numerous and repetitive steps, careful note-taking is required and assistance helpful. Once the objects are composed on the backdrop, the light meter reading should be taken and recorded. The first photograph(s) should be without any filter and serve as a reference. Then UV-Vis photography starts in earnest, with one two or three photographs taken through each filter in order in turn. As each filter is placed in the filter holder and the photographs ready to be taken, the label in the picture should be checked to make sure it is correct, focus adjustments made according to what has be predetermined as needed for that particular filter, and exposures adjusted according to the filter factors. The data on exposure should be recorded for each and every photograph. We advocate bracket exposures, *i.e.* one at the exposure determined by light meter and adjustments, and then another with 2-fold exposure (another f stop or double the time). Depending on the number of frames (exposures) on the film in use, extra photographs can be taken to bracket further for 0.5x exposure, and so on. In general, we find that 2 pictures without filter, then 2 or 3 with each filter for the 4 primary colours, and 1 or 2 for each intermediate filter is sufficient and allows for some final ultraviolet photographs at closer ranges for especially interesting parts of flowers, and room for correcting errors with extra photographs on a roll of 24 frames.

We advocate special attention be paid to photographs capturing ultraviolet and green images. It is suggested that images from the green part of the spectrum may be more important that hitherto realized because of the role of the green receptor in the insect ommatidia in shape, size, and pattern recognition outside the realm of colour *per se* (Kevan *et al.* 2001; Spaethe *et al.* 2001).

The exposed film should be removed from the camera and stored for developing. Depending on the photographer's requirements (was the film forced or not?), the film can be developed, fixed and the negatives examined. Prints can be made from those negatives with the best and most information.

The negatives can be used for compiling approximate spectral reflection curves by use of densitometry. With the white, black and grey standard in each photograph, it is possible to compare the greyness (optical density) of each object and its part with the standard. The densitometric measurements from the standards correspond to percent reflection, as predetermined, so that an estimate can be obtained of the percent of light in each waveband from the flowers, their parts, and the vegetation. The graphical representation becomes an approximate spectral reflection curve and that can be used to place the colour represented by that curve in a colour space as described for reflectance spectrophotometry.

Protocol 4.2 Preparation and use of the grey-scale for measuring reflectance

Materials

- MgO and carbon powders
- liquid collodion propylene oxide
- filter paper (Watman #1)

Method

1. Mix the powders (MgO and C) by volume to get the necessary mixture (*Table 4.5*). The percentage of reflectance for each mixture is given in *Table 4.3.*
2. Dissolve 100 mL collodion in 100-150 mL propylene oxide.
3. Add 100 ml powder mixture, add mix to 100-150 mL. solvent and stir, till it is thin enough to paint.
4. Paint the filter paper and tape it down on a hard flat surface to prevent buckling and cracking during drying.
5. Cut small chips (1-2 cm) of all the mixtures, and stick them in ascending order on a strips of tape to get the full (1 to 14, *Table 4.5*) grey-scale.

4.3.3.2 Spectral photography in a "nutshell"

Spectral reflectance photography involving the capture of UV images is conceptually simple. It involves using a standard camera with **film** that is sensitive from UV through to red. Black and white panchromatic films serve well, and films with ISO of around 400 can be used outside and without artificial lighting (see below). The **lens** on the camera must pass UV light sufficiently well for it to expose the film. Quartz lenses are best, glass lenses serve but do attenuate UV, and haze-coated glass lenses block UV and should never be used. The reflected light from flowers and their background is captured on the film, making familiar snap-shots. To determine the reflections (how much and where from the flower and its background) in the various wavebands representing the primary colours (UV, blue, green, and red) a series of broad-band monochromatic **filters** is placed in front of the lens and the photograph taken. However, to be sure that the photograph is not an artefact (published artefacts are numerous), one must be sure that the film is **properly exposed** and not overexposed (the main source of artefact). Thus, some standard should be placed alongside the object being photographed. A **grey scale** is best, but these require hand-building. Standard papers can

also be used to assure the investigator that their photographs are exposed more or less properly. In any event, the photographer needs to have reference to objects that are visibly and UV-black and visibly and UV-bright in the resulting pictures. The minimum requirement (two-step scale on black and white) is normally a black backdrop to the photograph and a piece of white paper that is known to be highly reflective in UV alongside the composition of flower and other objects of interest (*e.g.* vegetation). The source of **light** is also important. Daylight in shade is recommended because that avoids shadows and specular (mirror-like) reflections that result in direct sunlight and sometimes by flash.

The set-up consists of a black backdrop onto which the flower and other objects (including the grey scale or white standard paper) are placed, with camera (best on a tripod and with cable shutter release) with appropriate lens focused and ready for filters, film, and light meter (*Figure 4.1*). The **light meter reading** (through the lens or otherwise) sets the standard for the exposures, but the filters themselves attenuate light by their very function, so **exposures** are greater (longer or with larger apertures) than read from the light meter. A filter factor (double, quadruple the light meter reading) is then applied and is the standard for that filter. It is usual to "bracket expose" several pictures (3) with each filter, one at the adjusted light meter reading, and one at half that exposure and one at double that exposure. Black and white film is not expensive.

Protocol 4.3 Outdoor UV photography of flowers

Materials

- camera with cable shutter release, extension rings or bellows, and UV transmitting lens (see text for suggestions)
- tripod
- black background on which to mount the flower for photography
- film (*e.g.* Kodak Tri-X panchromatic; colour slide film may be used, but UV images are produced as blue)
- broad-band monochromatic filters, or UV filter (e.g. Kodak 18A) alone
- Pre-calibrated UV-Vis reflecting grey scale (see *Protocol 4.2*) or other standard (see text)
- Labels indicating the filter in use for each photograph to include in photographs

Method

A. *Photography method*

1. In the shade, and preferably with north sky light (in the Northern hemisphere) set up camera with film and with accessories, on tripod. Leave the UV filter off until ready to shoot. The image is not visible through the UV filter.
2. Arrange black background at suitable distance from camera and lens to allow photographs of flowers to fill as much of the frame as possible.
3. Mount flower(s), with an example of vegetation if needed, on the black background.
4. Mount grey scale, or other standard, next to flower(s) so that it appears in the frame.
5. Mount label next to flower(s) so that it appears in the frame.
6. Focus object in the camera. If using a fully chromatically adjusted lens, the no further adjustment is needed. If the lens is not chromatically adjusted from UV to visible, the focus adjustments must be made with accessories provided for focusing UV images, of focus adjustment calibration curves must be made (e.g. Kevan 1972). It is necessary to increase the object to lens distance, i.e. the lens to focal plane distance at shorter wavelengths (*e.g.* UV (18A) and violet (35)).
7. From this point, be sure to record everything in a notebook as work progresses: frame number, exposure used (f-stop and shutter speed), filter in place and notes.
8. Take a light meter reading corresponding to the speed of the film in use, and intended speed for development of the film (if "forcing").
9. Take three photographs without filter (control) and bracket expose either side of the light meter's reading. No label is required.
10. Add filters in sequence and take three photographs through each, bracketing on either side of the exposures as increased according to the filter factor multiplications. Be sure to add in the filter labels for each change of filter. It is easy to forget to add the label. Record all activities in a notebook. It is easier to work with a partner; one person operates the equipment and the other records the activity.
11. Develop film as appropriate and examine negatives. Make prints (positives) of the best pictures from each filter.

B. *Finding the suitable aperture*

1. Shoot an entire role of film at various f stops (shutter speed of 1/60 or 1/30 sec), with electronic flash as illumination source and the 18A filter in place.
2. Develop the film and print it in the usual manner. Choose the best quality photograph and indicate the appropriate L value.
3. Calculate the flash guide number (F) as follows (43) F = subject distance (SD) x lens aperture setting (L).
4. Use the F value to determine future exposure with the same camera, film, and flash attachment. When changing lens-subject distance, the new lens aperture is as follows: L = (F)/(SD).

Equipment

Film: Any panchromatic black and white film can be used. Because of the use of close-up rings or bellows and the filter factors needed, fast film is recommended. ISO of 300 and above is useful (*e.g.* Kodak Tri-X Pan (ISO 320)).

Cameras: The kind of film camera is not critical. Standard 35 mm single-lens-reflex cameras and larger format cameras (Hasselblad, Rolleiflex) can be used. Pinhole cameras can be used, too, but exposure times are long.

Lenses: There are several brands of quartz or fused silicon lenses available. All are expensive (Zeiss UV-Sonnar 105 mm lens (bayonet mount for Hasselblad or Roleiflex); Hammatsu A4968 50mm UV lens (C-mount); Nikon 105 mm UV lens; Pentax 85 mm UV lens). Technical information on camera systems and lenses should be obtained from the manufacturers or their agents. Focus adjustments are required for some of the lenses, but the Zeiss UV-Sonnar is chromtically adjusted for focus across the pollinator visual spectrum and beyond. Individual lenses can also be obtained (Edmund Scientific) for creating optics for photography, but this is beyond the scope of this book. Glass lenses may be used if they are non-coated and as thin as possible (*e.g.* Tessar lenses), but many are "haze coated" and have ultraviolet absorbing adhesives used in their assembly. These can not be used.

Filters: The series of filters that provides the greatest insights into colour patterns on flowers comprises a set of more or less 100 nm broad-band monochromatic filters. The 18A (also *e.g.* Schott UG -11, Hoya U 360, and others) is a special glass filter that passes UV light from about 300 to 400 nm, with peak transmission at about 350 nm which is in concert with the peak sensitivity of the UV receptor of the eyes of most insects so far studies. The next filters in the series pass violet, blue, blue-green, green, yellow-green, yellow, orange, and red

light, again monochromatically at peak transmissions at 400, 450, 500, 550, 600, 650,700, and 750 nm, respectively (*Figure 4.2*).

Grey scales can be made in various ways. Mixtures of MgO or $BaSO_4$ and carbon black can be made and painted on surfaces that can be cut and arranged into a grey scale. MgO or $BaSO_4$ white reflectace coatings can be mixed with carbon black to achieve the same end (*Table 4.5*). Whatman No. 1 or other filter paper can be dipped in water containing carbon black to obtain various levels of grey. Papers with computer generated greys laser-printed can also be made. In all cases, the scale needs to be calibrated by reliable reflectance spectrophotmetry across the pollinator visual spectrum so that it can be used with confidence, especially for photography in the ultraviolet. Daumer (1958) used a transmitting grey scale built into the camera, but this requires special equipment and expertise to accomplish. Failure to expose photographs properly causes artefacts (see *Figure 4.4* for pseudo-UV-reflection).

Other equipment required is a tripod with mount that accords with the camera system in use, a bellows attachment to allow the lens to be used close-up so that floral subjects more or less fill the photographs, a filter holder that allows the filters to be easily mounted and demounted in front of the lens (filter frames that are used on photographic enlargers work well, but may need adapting to attach to the lens), a cable release is desirable to eliminate camera shake for long exposures (more than 1/30 sec), and the backdrop (black flock paper over a surface that allows floral subject matter to be pinned on to it is the simplest). Flash-photography should be used with caution because of the likelihood of shadows and specular (mirror-like) reflections being recorded and confusing the images.

4.3.4 Measuring spectral reflectance by single beam spectrophotometry

Spectral reflectance measurements from flowers are necessary when one wants to predict *quantitatively* how colour appears to non-human animal. Such measurements must be taken under defined illumination conditions, with a fixed distance and angle between probe and flower, and with flowers arranged as a flat surface. This implies that such measurements cannot be taken from intact flowers in the field (for transport methods, see above). In recent years, spectrometers have become comparatively affordable and more easy to use. *However*, do not assume that one can just buy a spectrometer and data will start flowing. J. Marshall (University of Queensland) warned that using a spectrometer is about as difficult as learning to drive a car. This means, anybody can do it, but it takes practice, and several traps need to be avoided.

The authors (and several colleagues) have used the S2000 by Ocean Optics http://www.oceanoptics.com/homepage.asp (Dunedin,

FL, USA;) and we detail its use below. Although the system is relatively inexpensive, it requires care in its operation. The spectroradiaometer LI 1800 from Li-cor (Lincoln, Nebraska, USA; http://www.licor.com) is more expensive but less problematic to use. It is presently being tested and seems to work well for spectroradiometry in general and for transmittance (see Morandin *et al.* 2002) and reflectance (Kevan unpublished). Other devices not tested by the authors are made by Oriel (Stratford, CT, USA; http://www.oriel.com), Zeiss (Jena, Germany, http://www.zeiss.com) and by Soliton (Gilching, Germany; http://www.soliton-gmbh.de). Ocean Optics has branches in several countries, but it is worth comparing prices: they may not be cheapest in any given country. In some European countries, the Ocean Optics products are sold by Avantes (Eerbeek, Netherlands; http://www.avantes.com).

Table 4.5 How to make grey scales that reflect more or less uniformly across the spectrum from ultraviolet to red, MgO, and carbon black mixtures.

Mixture No.	% by volume MgO	% by volume C
1	10	90
2	40	60
3	60	40
4	70	30
5	80	20
6	85	15
7	95	10
8	93	7
9	95	5
10	96	4
11	97	3
12	98	2
13	99	1
14	100	0

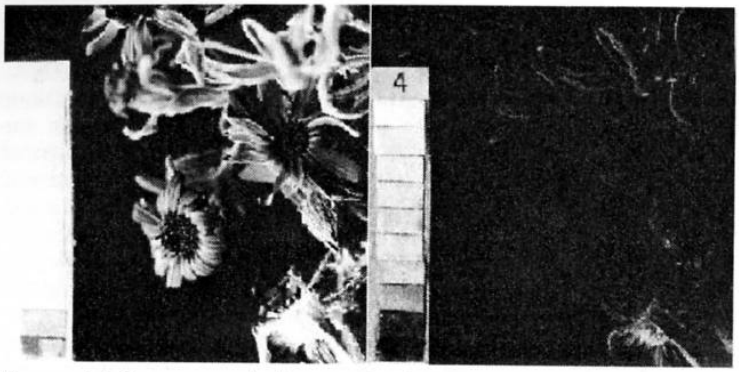

Figure 4.4 Over-exposed photograph of *Haplopappus lyallii* in UV (left). Illusion of bright UV reflectance is given, but reflectance of ligulate florets corresponds to dark end of grey-scale (gradient lost by overexposure compared with correctly exposed photographs on the right) at about 5% only. Hirsute vegetation on right is barely visible in the UV light. Both photographs were taken on the same occasion on the same role of film. Printing was identical for both.

The Ocean Optics S2000 is very handy (at 14x10.5x4 cm, it has approximately the size of a walkman). The S2000 must be mounted by the provider with a diffraction grating, an optical element that separates incident polychromatic radiation into its constituent wavelengths. Thus when ordering the S2000, make sure the supplier fits it with diffraction grating No.2, which admits light between 200 and 850 nm. If one buys from Ocean Optics directly, the preconfigured S2000 UV-Vis Spectrometer, which already has the correct diffraction grating inbuilt, can be ordered Other components that we had integrated into the S2000 by the supplier are a detector collection lens (L2), a UV-detector upgrade (UV2), a special detector coating (OFLV-200-850) and an entrance slit of 50µm (all by Ocean Optics). The S2000 is connected to a PC with an interface card (ISA-bus type A/D converter, ADC 1000, Ocean Optics). Special interface cards (DAQ-700 PCMCIA by National Instruments, with cable CBL-2-NI, Ocean Optics) are available for laptops, so that the S2000 can be easily moved around. It is very important to insert and remove the card only as described in the manual: doing otherwise can corrupt the card - the authors found out the hard way. Connections through USB ports are also possible. This is done using an external interface card (ADC1000-USB, Ocean Optics) and has the advantage that the same card can be used for both desktop and laptop PCs. The S2000 is powered through the computer, so no special power source is necessary. Some spectrometers have serious low sensitivity problems in the range below 340 or 350 nm, and this is often not even known to the company representatives. A good way to test this

is to save the white standard (see below), then measure the white standard again and then divide the second measurement by the first. If reflectance measurements shows serious deviations from the 100% line below 350 nm, it may suffer from low sensitivity problems. There is no way to correct this problem, except to buy a different spectrophotometer, or a light source with higher UV output.

Another essential component is a light source with high UV output. Whether intensity in the UV is sufficient can be tested using the method above. We have used the Deuterium/Halogen light source DH 2000 by Ocean Optics. This light source is compact but not portable; it needs to be connected to a regular wall outlet. The lamp requires some time to stabilise its output, so one should leave it switched on for at least one hour before starting actual measurements. It is also possible to use daylight as illumination source. Because daylight changes over time, sometimes rapidly, it is absolutely essential to use conditions under which such changes are minimal (avoid conditions when the sun is frequently obscured by clouds) and to calibrate measurements (*i.e.* measure white standard) before every single measurement. Direct sunlight should be avoided: the setup should be placed in the shade. It is also possible to collect data when the sky is evenly clouded.

Other necessary hardware includes a fibre optic cable that guides the light (UV and Vis) reflected from the object to the spectrometer, and a second fibre optic cable between the light source and the object (Lucas *et al.* 2001), or a coaxial reflectance probe. We have good experience using a reflectance probe with a bifurcate fibre optic cable (Ocean Optics R200-7, 2 m length). This is basically a Y-shaped cable, where the upper two ends connect to the light source and the spectrometer, and the lower tip is the probe pointed at the object to be measured. This probe contains a central *read* fibre, and 6 other ones arranged in a ring around that central fibre, which illuminate the object.

Measurement geometry is critical. It is important that all objects (and the white standard) are measured under rigourously controlled and identical geometry (distances and angles). It is not possible to just direct a probe at an intact flower and take a measurement in the field. Geometry should be arranged to minimise the contribution of specular reflectance of glossy objects (Galsterer *et al.* 1999). We are interested in diffuse reflection only. If using a probe, it should not be pointed at the object at a perpendicular angle! Use a 45° angle instead. If using two fibre cables, avoid anything close to a mirror geometry, where incidence angle and reflectance angle are equal. One classic arrangement in optical measurements is the 0/45 set-up, where the probe is placed at 0° and the illuminant at 45°. Another possible arrangement is shown in Lucas *et al.* (2001).

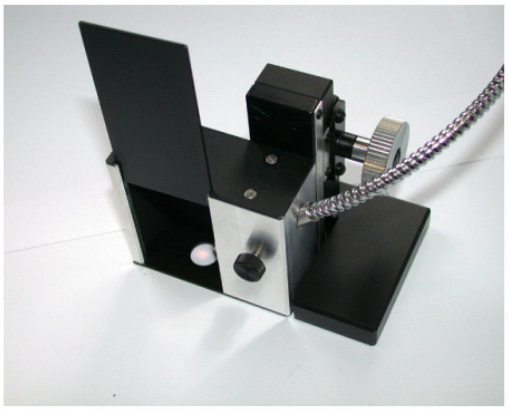

Figure 4.5 Set-up for measuring floral spectral reflectance with an Ocean Optics probe. The object is placed beneath a hole in the floor of a black-lined chamber. The chamber's height can be fine-controlled with a microscope drive. A sliding door in the front of the chamber allows to view the area of the flower to be measured. The probe is inserted through a hole at an angle of 45° relative to the object surface.

The measurement geometry needs to fulfil several additional requirements. First, one needs to be able to fine-control the area of the object that is being measured. Flower patterns can be rather fine-grained, so it is essential that the precise area of which the measurement is being taken. Can be seen Some commercially available probe holders, such as the RPH-1 by Ocean Optics, do not fulfil this prerequisite. Second, one must be able to control the distance between object and the end of the probe. Different objects, and the white standard, need to be measured at exactly the same distance. This can be tricky because different objects can have different heights, so placing the objects on the same surface while leaving the light guides in a fixed position won't work. One good solution is a small box painted black on the inside, with a small entrance hole to place on top of the object. The black box is useful to exclude influences from external light sources. The light beam and the axis of the measuring light guide must be carefully aligned on the same point in the centre of the entrance hole (if a coaxial probe is used, only that must be centred on the entrance hole; *Figure 4.5*). If natural daylight is used as the illuminant, an even simpler setup can be used (*Figure 4.6*). To assess what is the best geometry for the gear at hand (especially the distance between object and probe), it is also important to consider the acceptance angle of the measuring light guide. This can me measured by back-illuminating the light guide, but the information should also be available in the manual.

The software supplied by Ocean Optics to process the information from the S2000 is called *OOIBase32 Spectrometer Operating Software*. The authors have no personal experience with this software. They used *SpectraWin* by Avantes / Top Sensor Systems, which is quite easy to use and allows export of data to ASCII or

Excel® file formats, which can then be processed further by other software. Using this program, the hardware must first be configured as specified by the manual.

Figure 4.6 Measurement set-up for spectral reflectance with daylight as the illuminant, placed on a white standard supplied by Ocean Optics. This is a simplified version of the setup in *Figure 4.5.*

Reflectance is always given relative to a known standard, so the standard must be measured before reflectance measurements can commence. The white standard offered by Ocean Optics (diffuse reflectance standard WS-2) is not cheap, but it is also possible to use a pellet of freshly pressed Barium sulphate powder (Lucas *et al.* 2001; Wyszecki and Stiles 1982). It is important to adjust two things in combination while measuring the white standard because this is the brightest object to be measured. These are the distance between measuring guide and object, and the integration time. The further away the white standard (or other objects) are from the probe, the worse is the signal-to-noise ratio and the larger is the measured area. Thus, the object should not be too far away. If, on the other hand, the object is too close, some of the peaks from the light source may be in saturation, so that the spectrometer does not capture the full intensity range of the reflected spectrum. In this case, some of the peaks of the reflectance curve will appear to be "chopped off". If this happens, the distance should be enlarged a little, so that the full dynamic range of the spectrophotometer is exploited. Likewise, it is possible to adjust the integration time to avoid having either too low signal or saturation.

It is also necessary to measure dark data before measurements can commence, by switching of the light source, or completely blocking the measuring light guide. This is done to exclude the

equipment's own dark noise. Reflectance, R, (in percent of the white standard) at wavelength λ is the determined by:

$$R_\lambda = 100 * (sample_\lambda - dark_\lambda) / (reference_\lambda - dark_\lambda) \qquad (4\text{-}1)$$

SpectraWin for Ocean Optics gear performs this calculation automatically once set to reflectance mode. It is essential to do frequent calibrations (measure white standard and dark data). This is especially so when using natural daylight, for two reasons. One, the illumination spectrum may change rapidly depending on whether conditions. Two, spectrometer sensitivity tends to drift over time, particularly on hot days or in conditions with pronounced temperature changes. On hot days, spectrometers can be cooled using freezer gel packs. Frequent calibrations can be necessary under more controlled laboratory conditions, although once every 15 minutes should be sufficient for the S2000. Artificial light sources are also subject to spectrum or intensity changes.

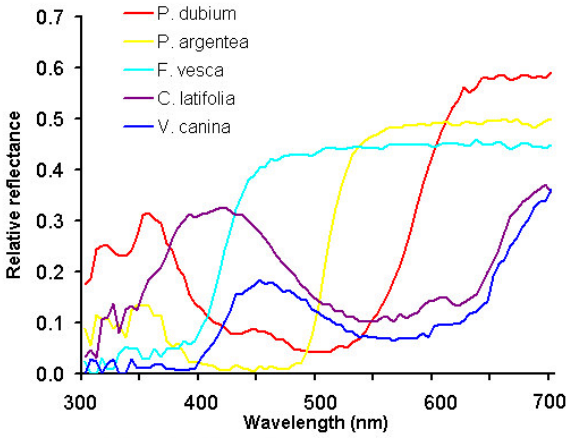

Figure 4.7 A set of typical floral spectral reflectance functions. The flowers measured are: yellow *Potentilla argentea* (with UV reflectance); red *Papaver dubium* (with UV reflectance); blue *Viola canina* (without UV reflectance); violet *Campanula latifolia* (with UV reflectance) and white *Fragaria vesca* (without UV reflectance).

Floral reflectance can be analysed by gluing samples on a small square block (*e.g.* 25x25x3 mm). This is done by wrapping the block with black electric insulation tape, but with the sticky side out. It is important that the sticky side of the tape is black, so that no light is reflected back from under the petals. Floral petals are then carefully pressed onto the sticky tape, so that the surface is as flat as possible. The minimum possible area can be determined by knowing the

acceptance angle of the probe (or light guide to the spectrometer) and the distance to the object. If the structure to be measured is smaller than this area (as might be the case for small floral nectar guides), a possibility is to glue equally coloured parts onto the tape like fish scales. Completed measurements should look as in *Figure 4.7*.

Protocol 4.4 Spectral reflectance measurements with S2000 by Ocean Optics

Materials

- S2000 Spectrometer (Ocean Optics)
- deuterium-halogen light source (DH 2000, Ocean Optics)
- computer and software to evaluate spectral reflectance measurements
- fibre optic cables between light source and object, and between object and spectrophotometer
- white standard
- probe holder
- black sticky tape (electrical tape)
- small block (wood, metal or plastic; *e.g.* 25 × 25 × 3 mm) to glue flowers on

Method

1. Connect the spectrometer with computer as explained in the manual.
2. Set up software and configure hardware as explained in manual.
3. Connect fibre optic cables to probe holders, spectrometer, and light source.
4. Switch light source on about 1h before measurements, to allow time for stabilization.
5. Wind black sticky tape around a small block, with the sticky side out.
6. Glue floral parts to be measured onto the sticky tape. Make sure the floral surface is even, and floral parts of same colour fill the entire measurement area.
7. Perform calibration.
   a. Measure the white standard. Adjust the distance between probe and white standard as explained in the text (not too

close, not too far). Save measurement to disk.

b. Perform a dark measurement. Disconnect light guide from light source.

c. Cover the probe with dark cloth. Save measurement to disk.

8. Take measurements of flowers. Save to disk.

9. Recalibrate about every 15 minutes.

10. Once measurements are finished, convert measurements to ASCII or Excel® format for further processing.

4.3.5 Measuring spectral reflectance by double beam scanning spectrophotometry

Spectrophotometers are commonly used in laboratories for measuring the transmittance or absorbance (one is the inverse of the other) of liquids placed in standard cuvettes or of transparent solids. The resulting data indicate the amount of various materials present in the liquids or solids, and the waveband of absorbance can be used to identify the material, and its amount present. Double beam reflectance spectrophotometers shine a beam of a standard light through the sample, and a second identical beam through an adjacent standard, and measure the amount that is passed through. The scanning instruments produce light of known and systematically changing wavelengths as it passes through the sample and the standard. The wavelength of the light is systematically changed by a pivoting prism so that the scan ranges according to the investigator's settings. Extracts of floral pigments can be analysed this way so that a spectral absorbance or transmittance curve from UV through to human visible red can be obtained. Such data are of little use in measuring floral colours because the pigments and cellular structures of floral parts interact to produce the colour of the flower. Nevertheless, insights from such data can be gained, and such studies are valuable in taxonomy, physiology and biochemistry.

The same spectrophotometers are available with attachments for measuring reflected light from the surfaces of objects, such as flowers. The attachments, referred to as integrating spheres, use the same double beam scanning principles, but the beams of light enter a spheroid chamber that is coated with fully spectrally reflective white (UV to near IR) on the inside and that has two ports. Over one port is placed the study objects (*e.g.* petals or leaves) while over the other is placed a white standard. The light enters the spheroid, is reflected and diffused by the white inside, the white standard, and the object of interest. The light that exits from the spheroid has become altered by the object, and is measured for its spectral qualities. Thus, the data collected by the instrument are of its spectral reflectance.

4.3.6 From spectral reflectance to photoreceptor excitation

To predict what signals a pollinator's colour receptors will generate when viewing a particular object, such as a flower, requires not only knowledge of the reflection spectrum of that object. In addition, we need the spectral sensitivity curves of the colour receptors. These can differ greatly between different types of pollinators, but are quite similar for most species of bees (Peitsch *et al.* 1992). Bees most commonly are trichromats, with receptors most sensitive near 345 nm (UV-receptors), 440 nm (blue receptors) and 535 nm (green receptors; Briscoe and Chittka 2001). Therefore the honeybee's (*Apis mellifera*) spectral sensitivity curves can be seen as reasonably representative approximations for other species of bees (*Figure.4.8, Table 4.6*).

The relative sensitivities of the photoreceptors types must also be known. Receptors *adapt*, which means that their relative sensitivity *increases* when they are poorly stimulated (such as in low light conditions, or when light is poor in that receptor's spectral domain). Relative sensitivity *decreases* when the receptor is, on average, strongly stimulated. This means that the sensitivity depends both on the spectrum of the illuminating daylight, and the reflectance spectrum of the predominant background. The sensitivity factor R is determined by:

$$R = 1 / \int_{300}^{700} I_B(\lambda) \cdot S(\lambda) \cdot D(\lambda) \cdot d\lambda \qquad (4\text{-}2)$$

$I_B(\lambda)$ is the spectral reflectance function of the stimulus; $S(\lambda)$ is the spectral sensitivity function of the receptor, and $D(\lambda)$ is the illuminant, $d\lambda$ is the wavelength step size. For most purposes, it is sufficient to use a standard function (D65; (Wyszecki and Stiles 1982), which we display in *Figure 4.9*; *Table 4.6.* Note that the illumination spectrum must be quantum based – energy based spectra are irrelevant for vision (Endler 1990). In most conditions under which bees view flowers, the background will be green foliage. We present a reflectance spectrum of a typical green leaf in *Figure 4.9* and *Table 4.6.*

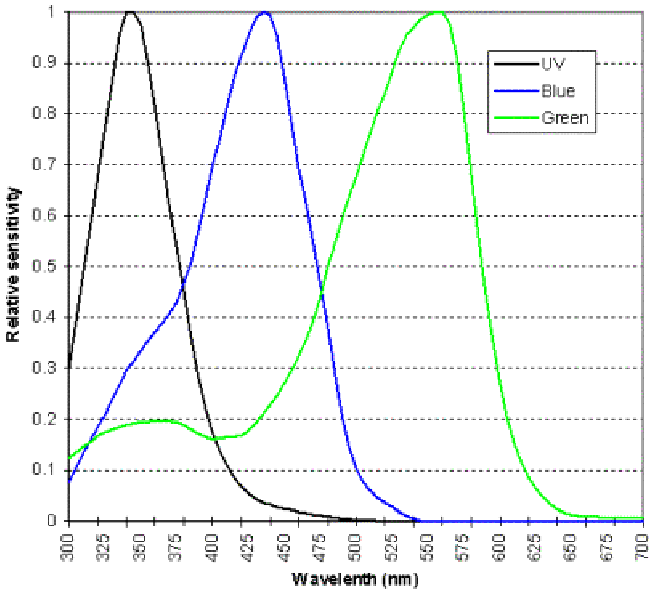

Figure 4.8 The spectral sensitivities functions of the honeybee (*Apis mellifera*) UV, blue, and green receptors, normalised to a maximum of unity. The honey bee functions are representative for a large number of Apoidea (Peitsch *et al.* 1992).

In non-technical terms, R is calculated by multiplying, for each wavelength value, the reflectance value of the background with the sensitivity of the photoreceptor at that wavelength, and with the strength of the illumination at that wavelength. The sum of all products, then, is the integral. *Table 4.6* can be used to exercise this procedure. For example, to calculate R for the UV receptor, use Excel® or a similar spreadsheet program to multiply the column for the UV receptor's spectral sensitivity curve with the column for D65 and with the column for the adaptation background. Calculate the sum of all these products and divide 1 through this sum to obtain R. Repeat the same procedure for the blue and green receptors' R values. Using the values in *Table 4.6*, the following R's should result:

$R_U = 5.23$; $R_B = 1.01$; $R_G = 0.33$

Table 4.6 Spectral sensitivity functions of the honeybee's (*Apis mellifera*) UV, blue and green receptors (Peitsch *et al.* 1992); standard daylight irradiance spectrum D65 (Wyszecki and Stiles 1982); a reflectance function of a typical green leaf (Chittka *et al.* 1994); reflectance function of a typical yellow, UV-absorbing flower, *Lotus corniculatus* (Chittka 1996b).

Wavelength (nm)	UV	Blue	Green	D65	Leaf	Flower
300	0.3	0.077	0.125	0.001	0.059	0.009
305	0.394	0.105	0.135	0.009	0.046	0.006
310	0.487	0.132	0.146	0.026	0.041	0.023
315	0.581	0.159	0.155	0.065	0.046	0.004
320	0.675	0.185	0.167	0.113	0.046	0.006
325	0.769	0.208	0.174	0.164	0.048	0.009
330	0.862	0.239	0.18	0.206	0.054	0.027
335	0.956	0.267	0.185	0.226	0.045	0.023
340	1	0.297	0.189	0.237	0.046	0.017
345	0.998	0.314	0.192	0.256	0.045	0.003
350	0.975	0.335	0.194	0.273	0.05	0.005
355	0.9	0.354	0.195	0.284	0.048	0.043
360	0.812	0.372	0.197	0.293	0.045	0.011
365	0.71	0.389	0.198	0.315	0.054	0.014
370	0.618	0.407	0.196	0.332	0.049	0.018
375	0.537	0.43	0.194	0.335	0.053	0.011
380	0.449	0.47	0.188	0.332	0.051	0.022
385	0.362	0.507	0.18	0.352	0.051	0.011
390	0.284	0.57	0.172	0.389	0.053	0.025
395	0.221	0.633	0.165	0.476	0.055	0.008
400	0.173	0.7	0.161	0.579	0.055	0.013
405	0.137	0.751	0.163	0.617	0.057	0.005
410	0.109	0.816	0.165	0.651	0.063	0.007
415	0.086	0.871	0.166	0.671	0.064	0.014
420	0.068	0.914	0.168	0.686	0.067	0.02
425	0.054	0.954	0.178	0.669	0.071	0.005
430	0.043	0.987	0.192	0.671	0.069	0.01
435	0.036	1	0.21	0.729	0.076	0.008
440	0.032	0.993	0.229	0.807	0.081	0.016
445	0.027	0.951	0.248	0.864	0.083	0.006
450	0.025	0.87	0.271	0.912	0.086	0.009
455	0.022	0.779	0.297	0.934	0.088	0.012
460	0.018	0.692	0.325	0.947	0.088	0.007
465	0.015	0.626	0.355	0.946	0.086	0.004
470	0.013	0.544	0.394	0.947	0.084	0.01
475	0.011	0.466	0.439	0.958	0.083	0.005
480	0.009	0.383	0.5	0.973	0.088	0.009
485	0.007	0.292	0.545	0.952	0.087	0.008
490	0.005	0.208	0.593	0.939	0.09	0.013
495	0.004	0.144	0.639	0.944	0.09	0.02
500	0.002	0.102	0.677	0.956	0.098	0.035
505	0.002	0.076	0.72	0.959	0.102	0.055
510	0.002	0.058	0.766	0.96	0.109	0.093

Wavelength (nm)	UV	Blue	Green	D65	Leaf	Flower
515	0.001	0.046	0.805	0.957	0.119	0.125
520	0.001	0.036	0.841	0.953	0.132	0.167
525	0.001	0.028	0.885	0.975	0.147	0.206
530	0	0.018	0.925	0.992	0.159	0.241
535	0	0.01	0.954	0.992	0.165	0.267
540	0	0.004	0.97	0.986	0.166	0.284
545	0	0.001	0.986	0.993	0.17	0.299
550	0	0	0.994	0.996	0.175	0.309
555	0	0	0.999	1	0.177	0.322
560	0	0	1	0.979	0.172	0.333
565	0	0	0.977	0.969	0.163	0.339
570	0	0	0.911	0.963	0.155	0.346
575	0	0	0.806	0.966	0.148	0.346
580	0	0	0.681	0.971	0.144	0.351
585	0	0	0.559	0.943	0.14	0.347
590	0	0	0.45	0.923	0.134	0.354
595	0	0	0.355	0.929	0.13	0.364
600	0	0	0.271	0.944	0.128	0.357
605	0	0	0.204	0.95	0.125	0.366
610	0	0	0.152	0.954	0.124	0.369
615	0	0	0.114	0.953	0.12	0.367
620	0	0	0.086	0.951	0.116	0.359
625	0	0	0.064	0.934	0.114	0.355
630	0	0	0.047	0.923	0.116	0.365
635	0	0	0.034	0.927	0.114	0.359
640	0	0	0.023	0.937	0.112	0.36
645	0	0	0.016	0.923	0.108	0.351
650	0	0	0.012	0.913	0.101	0.339
655	0	0	0.01	0.917	0.098	0.342
660	0	0	0.01	0.925	0.099	0.337
665	0	0	0.009	0.945	0.097	0.326
670	0	0	0.008	0.957	0.095	0.306
675	0	0	0.007	0.947	0.095	0.303
680	0	0	0.006	0.931	0.099	0.311
685	0	0	0.006	0.886	0.103	0.298
690	0	0	0.006	0.853	0.106	0.334
695	0	0	0.007	0.859	0.128	0.361
700	0	0	0.006	0.862	0.143	0.367

Thus, the UV receptor is 16 times more sensitive than the green receptor, and the blue receptor is 3 times more sensitive than the green receptor. These theoretically determined values are almost identical to those measured experimentally in the honeybee (Helversen 1992). The relative amount of light (quantum catch) absorbed by each spectral receptor type is:

$$P = R \int_{300}^{700} I_S(\lambda) \cdot S(\lambda) \cdot D(\lambda) \cdot d\lambda \qquad (4\text{-}3)$$

$I_S(\lambda)$ is the spectral reflectance function of the stimulus. Calculations can be made using *Table 4.6* and a spreadsheet program. To calculate the UV receptor's quantum catch, multiply the column for the UV receptor's spectral sensitivity curve by the column for D65 and by the column for the *Lotus corniculatus* flower. Calculate the sum of all those products, and multiply it by R to obtain P_U. The flower spectrum in *Table 4.6* should result in the following:

$P_U = 0.29$; $P_B = 0.17$; $P_G = 1.53$

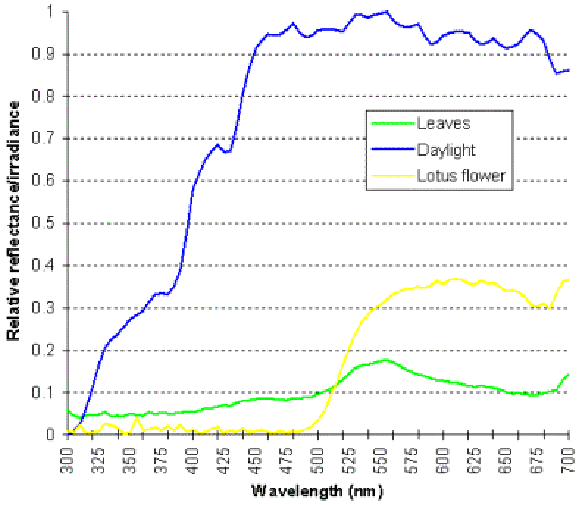

Figure 4.9 Spectral reflectance functions of a yellow, UV-absorbing flower of *Lotus corniculatus*, and a green leaf (Chittka *et al.* 1994). Daylight normfunction D65 is given on a biologically relevant quantal (not energy) basis.

For control, try the same procedure with the leaf background. It should yield the following values:

$P_U = 1$; $P_B = 1$; $P_G = 1$

The quantum catch in the photoreceptors P (*Equation 4-3*) is the input to the photoreceptors, not the input to the insect brain. On a

neural level, the brain performs "calculations" with graded potentials generated by receptor cells. These signals are not linearly related to the logarithm of the quantum flux that forms the input to the receptor (Naka and Rushton 1966). When the maximum excitation of the photoreceptors is set to 1, the nonlinear phototransduction process is well described by:

$$E = P / (P + 1) \qquad (4\text{-}4)$$

where P is the stimulus strength, in units such that for P = 1, E = 0.5 (*i.e.* half the maximum potential (Naka and Rushton 1966); for details see (Chittka 1992). Therefore, for the *Lotus corniculatus* flower:

$$E_U = 0.23; \qquad E_B = 0.15; \qquad E_G = 0.6$$

With our model, it is assumed that the photoreceptors display half their maximal response when stimulated by the light reflected from the adaptation background. Thus, for the adaptation background, E equals 0.5 in each photoreceptor. Precise predictions of both electrophysiological (Lipetz 1971; Wasserman 1978; Laughlin 1989) and behavioural (Valberg *et al.* 1986; Backhaus 1992) data are possible with these formulae, even when stimulus intensity is varied over several log units. Although the mathematical description of the non-linear transduction process in photoreceptors has been known for several decades, some workers still attempt to model bee colour discrimination with linear models (*e.g.* Brandt and Vorobyev 1997; Hempel de Ibarra *et al.* 2000). Even though such models may predict behavioural data reasonably well in some cases, they are fundamentally flawed because they are based on inappropriate input variables. They will therefore not be discussed in detail in this chapter.

For a calculation of colour spectral purity, and colour contrast between two coloured stimuli, see subsequent section on colour spaces. The brightness of colour stimuli is traditionally calculated as the sum of the three photoreceptor excitations E, but bees do not appear to use such a brightness signal when foraging among flowers (Giurfa and Lehrer 2001; Spaethe *et al.* 2001). Bees use a different intensity signal in a number of contexts: this is the signal of the long wavelength, or green receptor (see introduction). Because the background, by definition, yields a signal of 0.5 in each photoreceptor, green contrast between a flower and the background is the degree to which any given stimulus generates an excitation value different from 0.5 in the green receptor. Because excitation can range from 0 to 1, the maximum green contrast between flower and background is 0.5.

4.3.7 Plotting flowers in bee colour space

A colour space, or chromaticity diagram, is a representation of an animal's colour perception, designed so that distances between points generated by two colours are related to the animal's predicted ability to distinguish those colours (Wyszecki and Stiles 1982; Chittka and Waser 1997). Much ink has been wasted in recent years over the question of what is the most appropriate colour space for bees (*e.g.* (Hempel de Ibarra *et al.* 2000). Before we take sides on this issue, a historical perspective on the search for the appropriate colour space in another animal species, *Homo sapiens*, is in order. The immense difficulties that were encountered in that search should warn us that the construction of an equidistant colour space for any animal will be a laborious, if not an impossible, enterprise.

For many years, colour scientists have been searching for a colour space for human observers (or a corresponding colour difference formula) that would describe colour discrimination equally well in different spectral domains, different directions from a given point in colour space, at different intensities, *etc.* (Jameson and Hurvich 1968; Werner and Wooten 1979). Scientists acting at the forefront of this search came to somewhat pessimistic conclusions:

"*The hoped-for chromaticity diagram with such properties came to be called "uniform". The search for it extended over 50 years and seems no nearer to its goal than at the beginning. Much of the accumulated evidence indicates that the goal is unattainable...*" - D.L. MacAdam (1986)

"*None of the many colour-difference formulae that have been proposed over the last several decades is considered a sufficiently adequate solution to the problem.*" - G. Wyszecki and W.S. Stiles (1982)

Psychologists have resigned themselves to the fact that a colour space may be nothing more than a rough guidance map. Because of the complexity of information processing involved at different neuronal stages (*e.g.* retina and cortex), it might be impossible to design a colour difference formula that functions independently of viewing conditions, adaptation states, stimulus size and duration, *etc.* This is despite the fact that colour discrimination (or colour matching) data in humans are extremely easy to collect, compared with animals which ca not verbally express their subjective colour experience. MacAdam's (1986) conclusions were based on many thousands of data points in which human subjects were tested for just noticeable differences in different areas of colour space. Because colour discrimination data in animals are only obtainable through associative conditioning procedures, they are much more noisy than those in humans. Performance in discrimination tasks can be confounded by generalisation, or it can depend on the amount of training, previous experience outside the experimental paradigm, motivation, and other factors. This means that the search for a uniform colour space will

certainly be more difficult than in humans. Nevertheless, Campenhausen (1993, p.160) announced that what had not been possible for humans in many decades, had been accomplished in the honeybee: the development of a comprehensive theory (and a corresponding colour space) that explains all phenomena and processes of bee colour vision. In what follows, we evaluate some of the colour spaces proposed for bees, and their respective strengths and weaknesses. We conclude that the evidence is far too slim to support Campenhausen's claim. But we do have some useful models which predict the behaviour of bees in colour-related tasks reasonably well.

4.3.7.1 Colour triangle

This chromaticity diagram (*Figure 4.10*) has the advantage of being easily intelligible by non-specialists. It is based on the quantum absorptions in each photoreceptor divided by the total quantum catch, so that the *relative* quantum absorptions u, b and g (in the UV, blue, and green receptors) are:

$$u = P_U / (P_U + P_B + P_G) \qquad (4\text{-}5a)$$

$$b = P_B / (P_U + P_B + P_G) \qquad (4\text{-}5b)$$

$$g = P_G / (P_U + P_B + P_G) \qquad (4\text{-}5c)$$

Technically, the colour triangle is a plane through a three-dimensional vector space. In this space, the three axes correspond to the quantum catch values in the three different photoreceptor types. The co-ordinates of a coloured object in the colour triangle are determined by:

$$x = 0.8667(g - u) \quad \text{and} \quad y = b - 0.5(g + u) \qquad (4\text{-}6a \text{ and } b)$$

The co-ordinates of the colour triangle corners are as follows: lower left corner: x=-0.8667; y=-0.5; tip: x=0; y=1; lower right corner: x=0.8667; y=-0.5. The spectrum locus connects the loci of monochromatic lights, *i.e.* those which contain only a single wavelength. Because monochromatic lights are the purest colours theoretically possible, the spectrum locus delimits an area of colour space outside which no colour loci can occur. The spectrum locus is calculated as follows. For each photoreceptor type, calculate R as in *Equation 4-2*. Then, calculate adapted photoreceptor sensitivity curves by multiplying the receptors' sensitivity values for each wavelength value with R for that photoreceptor. Finally, calculate u, b and g values by dividing each receptor's sensitivity value at that wavelength by the sum of all sensitivity values of the three receptor colour types:

$$u = S(\lambda)_U / (S(\lambda)_U + S(\lambda)_B + S(\lambda)_G) \quad (4\text{-}7a)$$

$$b = S(\lambda)_B / (S(\lambda)_U + S(\lambda)_B + S(\lambda)_G) \quad (4\text{-}7b)$$

$$g = S(\lambda)_G / (S(\lambda)_U + S(\lambda)_B + S(\lambda)_G) \quad (4\text{-}7c)$$

Now, determine triangle co-ordinates using *Equation 4-6a and b*. The mixture line between the short and the long wavelength end of the spectrum (the UV-green or "bee purple" line) is a straight line between the locus for the short wavelength end (*e.g.* 300 nm) and the long wavelength end (*e.g.* 550 nm; at wavelengths above 550 nm, the loci in the colour triangle do not change anymore).

Each point in the colour triangle corresponds to a specific *ratio* of receptor quantum catches for a given coloured object. As in other colour spaces, the angle of a colour as measured from the centre corresponds to hue. The dominant wavelength of a stimulus is derived by drawing a straight line from the centre of colour space through the colour locus, and determining the wavelength at which this lines intersects the spectrum locus. Distance from the centre corresponds to spectral purity. Mixtures between two colour loci lie on a straight line that links these two colour loci.

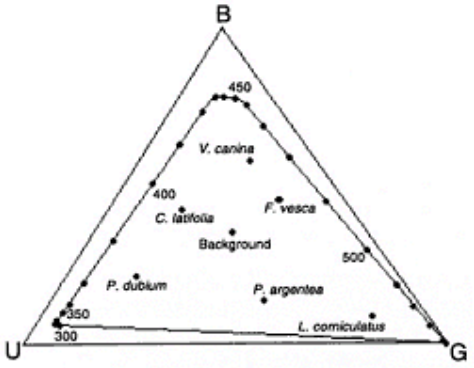

Figure 4.10 The colour triangle for trichromatic bees. The adaptation background, by definition, lies in the achromatic centre of the colour space. Reflectance from the flower of *Lotus corniculatus* stimulates predominantly the bees' green receptor, and therefore lies in the green corner of the triangle. The spectrum locus (which connects the loci of monochromatic lights, (*i.e.* those containing only one waveband) is given in 10 nm steps. Mixtures of two colours lie on a straight line between their two colour loci, as illustrated by the UV-green mixture line.

Unfortunately, the colour triangle is poorly suited for predicting the degree to which two colour stimuli are distinguishable (Chittka 1992; Chittka and Waser 1997). That is because the variables on which it is based, photoreceptor quantum catches, are not those with which the brain operates. Any realistic model of how colour is perceived must take into account the non-linear transduction function of the receptors (*Equation 4-4*).

4.3.7.2 The colour opponent coding (COC) space for honeybees

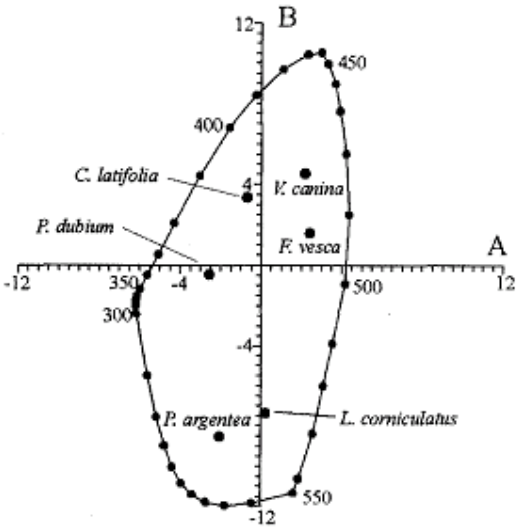

Figure 4.11 The colour opponent coding (COC) space for honeybees *(Apis mellifera)*. The axes correspond to two colour opponent mechanisms which combine the photoreceptor excitations with the following simple arithmetic: $A = -9.86\ E_U + 7.7\ E_B + 2.16\ E_G$; $B = -5.17\ E_U + 20.25\ E_B + 15.08\ E_G$. The continuous curve denotes the spectrum locus, in 10 nm steps from 300 to 550 nm. The bottom segment of the spectrum locus connects the loci of 300 and 550 nm in nine mixtures of the two lights in ratios of 0.9:01; 0.8:0.2, and so forth. Colour loci of monochromatic lights, adjusted for equal brightness according to Abney's law (sum of relative photoreceptor voltage signals $\sum E_i = 1.5$). Colour loci for flowers (whose reflectance is presented in *Figures 4.7* and *4.9*) are also given.

To create an appropriate colour space we need to know not only the physical properties of the colours, but also what signals these

colours cause the animal's photoreceptors to send to the brain, and how the brain integrates the signals. This approach was taken by Menzel and Backhaus (1989) and Backhaus (1991). They calculated photoreceptor excitations using *Equations* 4-2 to 4-4, and postulated that the photoreceptor signals are integrated by means of two types of colour opponent processes, one of which is UV – bluegreen antagonistic, and another which is blue – UV-green antagonistic. The excitation values of the two colour opponent mechanisms A and B are determined by

$$A = -9.86\ E_U + 7.7\ E_B + 2.16\ E_G \qquad (4\text{-}8a)$$

$$B = -5.17\ E_U + 20.25\ E_B + 15.08\ E_G \qquad (4\text{-}8b)$$

Backhaus (1991) claims these equations are borne out both by neurophysiological and behavioural evidence (see below for caveats). The two values A and B can be plotted in an orthogonal X-Y co-ordinate system, so that the axes correspond to the excitation values of the neurons A and B (*Figure 4.11*). The co-ordinates of each coloured stimulus are simply determined by calculating photoreceptor excitation values according to *Equations 4-2* to *4-4*, then inserting the resulting values into *Equations 4-8a* and *b*. According to Backhaus (1991), the distance (colour contrast) between two colours in this colour opponent space is not Euclidian, so that it cannot be measured simply with a ruler. Rather, distances are determined using a city-block metric, also called Manhattan metric. This means that to calculate the distance between two points, the distances along axis A and axes B are simply added up – so distances are determined very much like in a modern city with a rectangular layout (hence the terms city-block, or Manhattan metric; *Figure 4.11*). Thus, the colour difference formula for two colour stimuli with co-ordinates A_1, B_1 and A_2, B_2, is:

$$D = |\ A_1 - A_2\ | + |\ B_1 - B_2|$$

This formula can be used to calculate the colour contrast an object makes with its backdrop, or to determine the bee-subjective colour differences between the flowers of two different species. The spectrum locus cannot be determined as easily as for the colour triangle. This is because the loci of colours (including those of monochromatic lights) in the COC space change with intensity. For this reason, it is useful to introduce a convention to normalise the intensity of monochromatic lights to the same value. Backhaus (1991) and Chittka (1992) have normalised the monochromatic lights of the spectrum locus to adaptation light intensity. It follows from *Equations 4-2* and *4-3* that for the adaptation background, each photoreceptor contributes a relative quantum catch of 1. If intensity is calculated as the sum of the 3 photoreceptor quantum catches, then intensity is 3 for the background. Thus, for calculating the photoreceptor excitations for each monochromatic light, we adjust the intensity of each spectral light so that it produces a sum of photoreceptor quantum catches of 3. This is done following formulae *4-9a* to *c*. These receptor quantum catch values need to be converted into relative photoreceptor voltage signals using *Equation 4-4*. The co-ordinates for each wavelength of the spectrum locus are calculated using *Equations 8a* and *b*.

$$P_U = 3 * S(\lambda)_U / (S(\lambda)_U + S(\lambda)_B + S(\lambda)_G) \quad (4\text{-}9a)$$

$$P_B = 3 * S(\lambda)_B / (S(\lambda)_U + S(\lambda)_B + S(\lambda)_G) \quad (4\text{-}9b)$$

$$P_G = 3 * S(\lambda)_G / (S(\lambda)_U + S(\lambda)_B + S(\lambda)_G) \quad (4\text{-}9c)$$

where $P_{U,B,G}$ are the quantum fluxes in the UV, blue and green receptors, and $S(\lambda)$ is the adapted spectral sensitivity of the photoreceptor at the wavelength in question. The adapted spectral sensitivity curve is calculated by multiplying the spectral sensivity curve with the range sensitivity factor as determined by *Equation 4-2*.

The UV-green mixture line is determined by calculating mixtures of 300nm and 550nm in several discrete (for example, 9) steps. The quantum catches for the UV, blue and green receptors for each mixed light are calculated as follows: to predict the UV receptor's quantum catch for a mixture of one 10ths of 300nm and nine 10ths of 600nm, simply add one 10th of its quantum catch for 300nm, and nine 10ths of the quantum catch for 600nm. Do the same for the blue and green receptors, then calculate the co-ordinates of the mixture light in colour space according to *Equation 4-6a* and *b*). Then, procede to the next mixture ratio. Calculate receptor signals using *Equation 4-4*, and colour loci according to *Equation 4-8a* and *b*.

The COC model is simple and therefore attractive. It is an ambitious attempt to link behavioural and neurobiological data to form a comprehensive model of colour vision in an insect. It is this model that Campenhausen, (1993) heralded as being more comprehensive than any model that had been designed for humans (see above). However, the derivation of the COC model still involves a number of open questions. First, the behavioural colour discrimination data used to obtain the model were obtained from only a very small section of colour space in the blue-green area (Backhaus et al., 1987). Therefore, it is inappropriate to conclude that the same colour difference formula might apply in any other area of colour space. Second, the evidence that the two types of colour opponent neurons (and only these two types) demanded by the model actually exist in the honeybee brain has been overstated. Kien and Menzel, (1977) found only one type of colour opponent neuron frequently. This type was excited by UV light, and inhibited by blue and green light (UV+ B-G-). These cells differ widely in the strengths of inputs from the blue and green receptors, and so Backhaus' assumption of a single set of weighting factors is a simplification. The mirror image type (UV- B+ G+) was also found, so there is evidence that the assumption of a UV vs. blue-green mechanism in the COC model might have a physiological correlate. However, the other type of neuron postulated by the model, a tonic neuron with excitatory input from blue receptors, and inhibitory input from UV and green receptors (UV- B+ G-), or its mirror image, UV+ B- G+, were never described in

any original article. Kien and Menzel (1977) report a *single* recording from a neuron excited by blue light, but they point out that evidence for inhibition by UV and green light was weak. Such neurons, or their mirror image type, UV+ B- G+, are noted in reviews after Backhaus postulated their existence from modelling, first in Menzel and Backhaus (1989). They cite Kien and Menzel (1977) and Hertel (1980) as the original papers where this neuron was found, but it is not reported in either paper. Thus, the origin of the recording of a tonic UV+ B- G+ neuron, and the question of how commonly it is found, is unclear. Finally, it is not clear why only tonic neurons (which react to light with a sustained response) should contribute to colour coding. Phasic neurons (which react only at the onset of light) were found also with a third type of spectral antagonism: UV- B- G+ (Hertel and Maronde 1987), along with a single neuron of the UV+ B- G+ type (Hertel 1980).

Third, multidimensional scaling was used to determine the number of dimensions of the COC colour space, and its metric (Backhaus *et al.* 1987). Multidimensional scaling has long been known to produce artefacts when applied to colour vision (Torgerson 1958). It is especially inappropriate to decide between a Euclidian and a City-block organisation of colour space (Wuerger *et al.* 1995, and references therein). In fact, Chittka (1990) explicitly showed that the colour discrimination data used by Backhaus cannot be used to distinguish statistically between the two types of metric. This has implications that go beyond how colour distance is determined within colour space. If, in fact, the metric might be of the Euclidian type, the axes of colour space can be freely rotated. Imagine two points in a simple x-y coordinate system. The Euclidian distance between these two points will be unaffected if the x and y axes are both rotated by 45, 60, or 180 degrees (the same does not apply in a City-block metric: if the grid of streets and Avenues in Manhattan were tilted by 60 degrees, distances one would have to walk between two fixed points would change greatly). *If* the axes can be freely rotated, this means that an infinity of pairs of colour opponent mechanism can be used to generate the same set of colour distances. Chittka *et al.* (1992) found an entirely different set of colour opponent processes to best explain the same behavioural data. But this pair of colour opponent processes produced almost identical distance proportions in colour space as the COC model. Thus, given the limitations of the behavioural and neurobiological data currently at hand, we can not be sure just which set of colour opponent processes underlie colour coding in honeybees. The COC model may turn out ultimately to be largely accurate for predicting colour difference, but we need much more data to substantiate this conclusion. And its application to species other than the honeybee, as attempted by *e.g.* Gumbert and Kunze (2001), is certainly inappropriate.

4.3.7.3 The colour hexagon

Our analysis of the COC model shows that at this point we cannot be sure which precise set of colour opponent mechanisms might be responsible for colour coding in bees. On the other hand, we have good evidence from a number of Hymenoptera that colour coding is done using *some* combination of two nearly orthogonal colour opponent mechanisms (Chittka *et al.* 1992). This background was the rationale for designing a more general colour opponent space, the colour hexagon, where different colour opponent directions are weighted equally, and which is less dependent on only particular mechanisms of colour opponency (*Figure 4.11a*). The colour hexagon is a projection of the three dimensional photoreceptor signal space, whose axes x, y, and z correspond to photoreceptor signals E_U, E_B, and E_G (Chittka 1996b). The co-ordinates of the six corners of the hexagon, clockwise from the lower right corner, are: x_1= -0.8667, y_1=-0.5; x_2=-0.866, y_2=0.5; x_3=0, y_3=1; x_4=0.8667, y_4=0.5; x_5=0.8667, y_5=-0.5; x_6=0, y_6=-1. The co-ordinates of any colour are defined by the receptor signals derived according to *Equation 4-4*, inserted into the following equations:

$$x = 0.8667(E_G - E_U) \qquad (4\text{-}10a)$$

$$y = E_B - 0.5(E_G + E_U) \qquad (4\text{-}10b)$$

The spectrum locus is calculated as for the COC space (*Equations 9a-c*), except that *Equations 4-10a* and *b* are used instead of *4-8a* and *b* to calculate the co-ordinates in colour space. The colour hexagon shares with the colour triangle the virtue of simplicity: for non-specialist readers, it is easy to interpret the location of a colour locus to obtain its bee-subjective hue and spectral purity. Points within the hexagonal colour space are defined by constant difference between receptor signals, which is just the sort of algorithm performed by the colour-opponent system in the bee's brain. It can be shown by simple geometry that the excitation value for any opponent mechanism with known weighting factors can be derived from the hexagon coordinates x and y, given a few realistic assumptions about the nature of colour opponent mechanisms (Chittka 1996b).

The colour hexagon is also useful for categorising colours. This procedure makes no assumptions of categorical colour processing in the bee brain. It is simply a convention that allows scientists to communicate about bee colours. Chittka *et al.* (1994) introduced the terms UV, UV-blue, blue, blue-green, green, UV-green and uncoloured. Which colour category a particular colour locus falls into, can be derived from a graphical representation, or mathematically using the following relationships (*Figure 4.11b*). If the distance from

the centre of the colour hexagon is <0.1, the object is categorised as uncoloured. Otherwise:

If expression is true then the colour is:		
$(E_B + E_G + 3 \times \lvert E_B - E_G \rvert) / 2$	$< E_U$	UV
$(E_U + E_B - 3 \times \lvert E_U - E_B \rvert) / 2$	$\geq E_G$	UV-blue
$(E_U + E_G + 3 \times \lvert E_U - E_G \rvert) / 2$	$< E_B$	blue
$(E_B + E_G - 3 \times \lvert E_B - E_G \rvert) / 2$	$\geq E_U$	blue-green
$(E_U + E_B + 3 \times \lvert E_U - E_B \rvert) / 2$	$< E_G$	green
$(E_U + E_G - 3 \times \lvert E_U - E_G \rvert) / 2$	$\geq E_U$	UV-green

Colour distance in the colour hexagon are determined using a Euclidian metric, so that for two colour stimuli with co-ordinates x_1, y_1 and x_2, y_2, the colour distance D is:

$$D = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$$

Distances between colour loci in the colour hexagon correlate significantly with behavioural colour discrimination in many studies and several species of trichromatic insects (Chittka *et al.* 1992; Chittka and Waser 1997; Chittka *et al.* 2001; Spaethe *et al.* 2001). This is despite the fact that the distance proportion of the colour hexagon are not adjusted to any behavioural data, as opposed to, for example, the COC space. In one recent study, Brandt and Vorobyev (1997) found that a different model predicted data from a spectral threshold experiment in bees (Helversen 1992) better than the colour hexagon. However, this finding was unsurprising: the authors adjusted their model to precisely the same data they then tested it on. This is clearly an invalid procedure. In terms of the trade-off between parsimony of assumptions that go into the hexagon model, and the precision for predicting behavioural colour discrimination data, this is still the best model at hand.

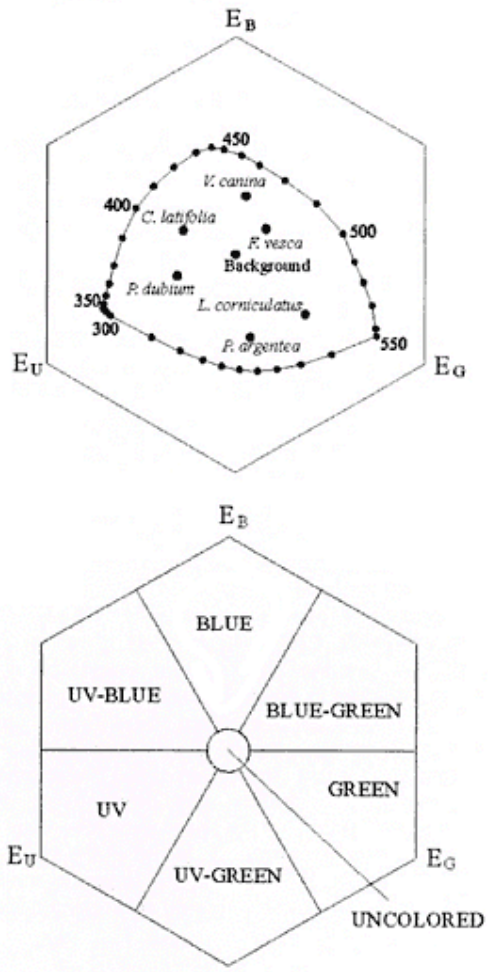

Figure 4.12 a) The colour hexagon is a general colour opponent space which, unlike the COC space, makes no specific assumptions about underlying colour opponent mechanisms. For calculation of spectrum locus see *Figure 4.11.* **b)** Colour terms in the colour hexagon. Bee colours can be easily categorised in the colour hexagon, simply by evaluating which sector a colour falls into. This convention facilitates communication about bee colours.

Literature

BACKHAUS, W. 1991. Color opponent coding in the visual system of the honeybee. *Vision Research.* 31:1381-1397.

BACKHAUS, W. 1992. The Bezold-Brücke effect in the color vision system of the honeybee. *Vision Research* 32:1425-1431.

BACKHAUS, W., R. MENZEL, AND S. KREISSL. 1987. Multidimensional scaling of color similarity in bees. *Biological Cybernetics.* 56:293-304.

BRANDT, R., AND M. VOROBYEV. 1997. Metric analysis of treshold spectral sensitivity in the honeybee. *Vision Research* 37:425-439.

BRISCOE, A.D., AND L. CHITTKA. 2001. The evolution of color vision in insects. *Annual Review of Entomology* 46:471-510.

BURR, B., D. ROSEN, AND W. BARTHLOTT. 1995. Untersuchung zur Ultraviolettreflexion von Angiospermenblüten III. Dilleniidae und Asteridae s.l. Tropische und subtropische Pflanzenwelt. *Akademie der Wissenschaften und der Literatur Mainz*. 93:1-93.

CHIAO, C.-C., T.W. CRONIN, AND D. OSORIO. 2000. Color signals in natural scenes: characteristics of reflectance spectra and effects of natural illuminants. *Journal of the Optical Society of America A* 17:218-224.

CHITTKA, L. 1990. Von den Photorezeptorsignalen zum Wahlverhalten der Honigbiene im Farbunterscheidungstest: Statistische Analyseverfahren bei der Erschliessung der Informationsverrechnungsmechanismen. *Diplomarbeit FU Berlin*.

CHITTKA, L. 1992. The color hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *Journal of Comparative Physiology A* 170:533-543.

CHITTKA, L. 1996a. Does bee colour vision predate the evolution of flower colour? *Naturwissenschaften* 83:136-138.

CHITTKA, L. 1996b. Optimal sets of colour receptors and opponent processes for coding of natural objects in insect vision. *Journal of Theoretical Biology* 181:179-196.

CHITTKA, L., W. BEIER, H. HERTEL, E. STEINMANN, AND R. MENZEL. 1992. Opponent colour coding is a universal strategy to evaluate the photoreceptor inputs in hymentoptera. *Journal of Comparative Physiology A* 170:545-563.

CHITTKA, L., A. SHMIDA, N. TROJE, AND R. MENZEL. 1994. Ultraviolet as a component of flower reflections, and the colour perception of hymenoptera. *Vision Research* 34:1489-1508.

CHITTKA, L., J. SPAETHE, A. SCHMIDT, AND A. HICKELSBERGER. 2001. Adaptation, constraint, and chance in the evolution of flower color and pollinator color vision. *In* Cognitive Ecology of Pollination. L. Chittka and J.D. Thomson, editors. University Press, Cambridge. 106-126.

CHITTKA, L., AND N.M. WASER. 1997. Why red flowers are not invisible for bees. *Israel Journal of Plant Sciences* 45:169-183.

DAFNI, A. 1992. Pollination Biology – a Practical Approach. University Press, Oxford.

DAUMER, K. 1958. Blumenfarben, wie sie die Bienen sehen. *Zeitschrift Vergleichende Physiologie* 41:49-110.

EISNER, T., M. EISNER, P.A. HYYPIO, D. ANESHANSLEY, AND R.E. SILBERGLIED. 1973. Plant taxonomy: ultraviolet patterns of flowers visible as fluorescent patterns in pressed herbarium specimens. *Science* 179:486-487.

ENDLER, J.A. 1990. On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society* 41:315-352.

GALSTERER, S., M. MUSSO, A. ASENBAUM, AND D. FÜRNKRANZ. 1999. Reflectance measurements of glossy petals of *Ranunculus lingua* (Ranunculaceae) and of non-glossy petals of *Heliopsis helianthoides* (Asteraceae). *Plant Biology* 1:670-678.

GIURFA, M., AND M. LEHRER. 2001. Honeybee vision and floral displays: from detection to close-up recognition. *In* Cognitive Ecology of Pollination. L. Chittka and J.D. Thomson, editors. University Press, Cambridge. 61-82.

GUMBERT, A., AND J. KUNZE. 2001. Colour similarity to rewarding model plants affects pollination in a food deceptive orchid, *Orchis boryi*. *Biological Journal of the Linnean Society* 72:419-433.

HELVERSEN, O.V. 1972. Zur spektralen Unterschiedsempfindlichkeit der Honigbiene. *Journal of Comparative Physiology* 80:439-472.

HEMPEL DE IBARRA, N., M. VOROBYEV, R. BRANDT, AND M. GIURFA. 2000. Detection of bright and dim colours by honeybees. *Journal of Experimental Biology* 203:3289-3298.

HERTEL, H. 1980. Chromatic properties of identified interneurons in the optic lobes of the bee. *Journal of Comparative Physiology* 137:215-231.

HERTEL, H., AND U. MARONDE. 1987. The physiology and morphology of centrally projecting visual interneurones in the honeybee brain. *Journal of Experimental Biology* 133:301-315.

JACOBS, G.H. 1993. The distribution and nature of colour vision among the mammals. *Biological Reviews* 68:413-471.

JAMESON, D., AND L.M. HURVICH. 1968. Opponent-Response Functions Related to Measured Cone Photopigments. *Nature* 58:429-430.

KEVAN, P.G. 1972. Floral colors in the high arctic with reference to insect-flower relations and pollination. *Canadian Journal of Botany* 50:2289-2316.

KEVAN, P.G. 1978. Floral coloration, its colorimetric and significance in anthecology. *In* The pollination of flowers by Insects. Vol. 6. A.J. Richards, editor. Linnean Society Symposium Series. 51-78.

KEVAN, P.G. 1983. Floral colors through the insect eye: what they are and what they mean. *In* Handbook of experimental pollination biology. C.E. Jones and R.J. Little, editors. Van Nostrand Reinhold, New York. 3-30.

KEVAN, P.G., AND W.G.K. BACKHAUS. 1998. Color vision: ecology and evolution in making the best of the photic environment. *In* Color vision - perspectives from different disciplines. Backhaus, W., editor. Walter de Gruyter & Co., Berlin. 163-183.

KEVAN, P.G., L. CHITTKA, AND A.G. DYER. 2001. Limits to the salience of ultraviolet: lessons from colour vision in bees and birds. *Journal of Experimental Biology* 204:2571-2580.

KIEN, J., AND R. MENZEL. 1977. Chromatic properties of interneurons in the optic lobes of the bee. II. Narrow band and colour opponent neurons. *Journal of Comparative Physiology A*. 113:35-53.

LAUGHLIN, S.B. 1989. The role of sensory adaptation in the retina. *Journal of Experimental Biology* 146:39-62.

LIPETZ, L.E. 1971. The Relation of Physiological and Psychological Aspects of Sensory Intensity. *In* Handbook of Sensory Physiology. W.R. Loewenstein, editor. Springer, Berlin. 192-225.

LUCAS, P.W., T. BETA, B.W. DARVELL, N.J. DOMINY, H.C. ESSACKJEE, P.K.D. LEE, D. OSORIO, L. RAMSDEN, N. YAMASHITA, AND T.D.B. YUEN. 2001. Field kit to characterize physical, chemical and spatial aspects of potential primate foods. *Folia Primatologica* 72:11-25.

MACADAM, D.L. 1986. Color measurement. Theme and variations. Springer-Verlag, Berlin. 228 pp.

MENZEL, R., AND W. BACKHAUS. 1989. Color vision honey bees: Phenomena and physiological mechanisms. *In* Facets of vision. D. Stavenga and R. Hardie, editors. Springer, Berlin. 281-297.

MORANDIN, LA, TM LAVERTY, RJ GEGEAR, AND PG KEVAN. 2002. Effect of greenhouse polyethylene covering on activity level and photo-response of bumble bees. *Canadian Entomologist* 134: 539-549

NAKA, K.I., AND W.A.H. RUSHTON. 1966 a. S-potentials from colour units in the retina of fish (Cyprinidae). *Journal of Physiology* 185:536-555.

PEITSCH, D., A. FIETZ, H. HERTEL, J. DE SOUZA, D.F. VENTURA, AND R. MENZEL. 1992. The spectral input systems of hymenopteran insects and their receptor-based colour vision. *Journal of Comparative Physiology A* 170:23-40.

RAGUSO, R.A. 2001. Floral scent, olfaction, and scent-driven foraging behavior. *In* Cognitive Ecology of Pollination. L. Chittka and J.D. Thomson, editors. University Press, Cambridge. 83-105.

SCOGIN, R. 1983. Visible floral pigments and pollinators. *In* Handbook of experimental pollination biology. C.E. Jones and R.J. Little, editors. Scientific and Academic Editions, New York. 160-172.

SPAETHE, J., J. TAUTZ, AND L. CHITTKA. 2001. Visual constraints in foraging bumblebees: Flower size and color affect search time and flight behavior. *Proceedings of the National Academy of Science (USA)* 98:3898-3903.

TORGERSON, W.S. 1958. Theory and methods of scaling. Wiley, New York.

VALBERG, A., T. SEIM, B.B. LEE, AND J. TRYTI. 1986. Reconstruction of equidistant color space from responses of visual neurones of macaques. *Journal of the Optical Society of America A* 3:1726-1734.

VON CAMPENHAUSEN, C. 1993. Die Sinne des Menschen. Georg Thieme Verlag, Stuttgart. 254 pp.

WASSERMAN, G.S. 1978. Color vision: an historical introduction. John Wiley & Sons, New York.

WEISS, M.R. 2001. Vision and learning in some neglected pollinators: beetles, flies, moths, and butterflies. *In* Cognitive Ecology of Pollination. L. Chittka and J.D. Thomson, editors. University Press, Cambridge. 171-190.

WERNER, J.S., AND B.R. WOOTEN. 1979. Opponent chromatic mechanisms: relations to photopigments and hue naming. *Journal of the Optical Society of America* 69:422-434.

WUERGER, S.M., L.T. MALONEY, AND J. KRAUSKOPF. 1995. Proximity judgments in color space: tests of a Euclidian Geometry. *Vision Research* 35:827-835.

WYSZECKI, G., AND W.S. STILES. 1982. Color science: Concepts and methods, quantitative data and formulae. Wiley, New York.