FLOWERS THROUGH INSECT EYES: THE CONTRIBUTION OF POLLINATOR VISION TO THE EVOLUTION OF FLOWER COLOUR

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THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

QUEEN MARY, UNIVERSITY OF LONDON

2010

ABSTRACT

Flowers' colours are an essential element of their ability to attract visits from pollinators. However, the colours as they appear to human observers can differ substantially from their appearance to insect pollinators, and so it is essential to consider pollinator vision in any study of the ecology of flower colour.

In this thesis I describe how I have overseen the development of an online database to provide accurate information on floral spectral reflectance measured without human observational bias. This resource allows a more accurate consideration of flower colours in future studies, and permits investigations of flower colours within and across habitats. Using the records in this database, I analysed flowers from two European habitats for spatial or temporal changes, modelling the colours according to insect visual perception. I discovered that the insect-colour composition of the plant communities does not change either along an altitudinal gradient or throughout the year. These novel and ecologically-relevant analyses contradict previous observational studies, but support the theory of a pollination "market" in which flowers compete for pollinator visitation.

I then describe my experimental investigations into the visual capabilities of two pollinators and how this may relate to what colours of flowers they visit. Firstly I study the foraging behaviour of bees under spatially inconsistent illumination and how this impacts on their choice behaviour. I revealed patchy light can have measurable effects on bee foraging behaviour: they intentionally choose familiar over unfamiliar illumination, which may impact on the flowers they visit in complex natural environments. Secondly, I detail the new evidence for a red-sensitive photoreceptor in South African monkey beetles, a major pollinator in a habitat containing many long-wavelength-reflecting flowers, which are not classically "attractive" to bees. Throughout this thesis, I explore how pollinator vision has shaped the evolution of flower colours in different contexts.

This thesis is dedicated with love to a remarkable and generous woman who overcame her own fear of insects in order to teach me just how fascinating they truly are.

For my mother

Petula Arnold
(24th November 1954 – 19th October 2009)

ACKNOWLEDGEMENTS

It is a pleasure to thank, first and foremost, my supervisors, Professors Lars Chittka and Vincent Savolainen, for their support, advice and input throughout my thesis. Their enormous expertise and occasional tough love has been invaluable.

I am also deeply grateful to the members of the Chittka lab, past and present, who have always been available for advice and troubleshooting: Drs Nigel Raine, Tom Ings, Elli Leadbeater, Nehal Saleh, Mathieu Molet and Mathieu Lihoreau, and Oscar Ramos-Rodriguez, Ralph Stelzer, Helene Muller, Kate Hunt and Mu-Yun Wang. In addition, I thank the administrative and technical staff of Queen Mary who have supported me during my time here.

Several people's programming expertise laid the groundwork for the development of FReD; in particular, I thank Chris Ingram and Beth Hellen, and Samia Faruq for providing further coding when the FReD project continued under my direction. Additionally, I would like to thank the Positive Internet Company for their technical support when launching FReD online.

The electrophysiology work would not have been possible without the support, apparatus and guidance of Dr Peter Skorupski, and I am also endebted to Dr Thomas Döring for his suggestions and guidance. I also thank Dr Jonathan Colville and Dr John Manning for taking care of so much paperwork and organisation in order to provide me with monkey beetles for these experiments.

Several organisations and companies have supported my project in many different ways: the BBSRC and the Royal Botanic Gardens, Kew have provided funding (BBS/S/L/2005/12155A). Koppert Biological Systems and Syngenta Ltd. have supplied bees for the behavioural experiments.

I would also like to thank the editors and referees whose detailed and patient comments improved a number of manuscripts arising from my PhD work, including Dr Neal Williams and Dr Tamar Keasar, who provided very useful constructive criticism of my

early publications. Likewise, I thank the friends who offered to proofread chapters of this thesis: Caitlin, Matthew, Lyra, Helen C, Jenny, Helen S, Linda and Stuart.

Lastly, but certainly not least, I owe a huge debt of gratitude to my family and friends for their loyal support through the ups and downs inevitable in any PhD. In particular I thank Katie, Ulrica, my flatmates Elizabeth, Anu and Felicia, and my parents, Mike and Petula Arnold. Petula will unfortunately never see the conclusion of this thesis, but I hope she would have approved.

Sarah Arnold
March 2010

SUMMARY OF INPUT

I wrote the papers for Chapters III and IV. My contributions to the data chapters are as follows:

II. I finalised the development of FReD version 1.0 (initiated by E. Hellen), and supervised development of FReD version 2.0 by S. Faruq, who performed the programming and coding according to a specification which I developed in collaboration with L. Chittka and V. Savolainen. I tested the completed application and oversaw development of new features, bug fixes and fine-tuning. I screened the database manually for problems with records and updated many records according to revised nomenclature for plant species. I set up the current webhosting and oversaw implementation of the database as a live website.

III. The data for the alpine study was collected by L. Chittka in 1992. I conducted the data analysis and interpretation for the study.

IV. The data for the phenology study was collected by L. Chittka in 1993-4. In the phenology study, the randomisation analysis was performed by S.C. Le Comber according to a specification I developed in consultation with L. Chittka. I performed the other statistical analyses for that study.

V. I designed and conducted all behavioural experiments in parts 1 and 2 and performed all statistical analysis on the results. Some of the data collection in part 3 was performed by H.W.H. Mak under my supervision and according to a protocol I designed. I designed the experiment in part 4 in collaboration with H.W.H. Mak; he collected all the data, but I performed all the analysis.

VI. The flower readings were taken by V. Savolainen and M.P. Powell. The test animals were collected by J. Colville in collaboration with J. Manning. I conducted all the electrophysiological experiments with mentoring from P. Skorupski and performed all analysis and interpretation of results.

DECLARATION

I declare that that the work in this thesis is all my own, with the exception of the contributions from others mentioned in the "Summary of input" section above.

Sarah Arnold
March 2010

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Arnold S.E.J., Le Comber S.C. & Chittka L. (2009) Flower colour phenology in European grassland and woodland habitats, through the eyes of pollinators. *Israel Journal of Plant Sciences* 57:211-230.

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OTHER OUTPUTS

Arnold S.E.J. , Savolainen V. & Chittka L. (2008) FReD: The floral reflectance spectra database. *Nature Precedings.* doi:10.1038/npre.2008.1846.1

CHAPTER I

INTRODUCTION

The colours of flowers have fascinated people for thousands of years (Grant, 1950), yet the purpose of their colour, size and many aspects of their morphology is not to delight the human eye but as an advertisement of their presence – and the rewards they offer (or, in some cases, fail to offer) – to insect and vertebrate pollinators (Chittka and Kevan, 2005; Raguso, 2004). Their visual appeal and conspicuousness greatly enhances their chances of being visited by animals able to carry their pollen on to conspecifics and thus afford them reproductive success.

Angiosperms probably first evolved around 140 million years ago (Sun et al., 2002; Willis and McElwain, 2002), but the radiation of the group and the innovation of a prominent floral display with petals and colour is more recent, occurring perhaps only 90-100 million years ago (Busch and Zachgo, 2009; Crane et al., 2009). The evolution of the flower has enhanced the reproductive success of angiosperms and contributed to the enormous diversification into all the species of flowering plants seen today. However, there is also increasing evidence indicating that pollination predates the evolution of angiosperms – several species of modern cycads have quite specialised pollination systems (Schneider et al., 2002; Tang, 1987), and a recent study by Ren (2009) taking into account morphological evidence from fossilised scorpionflies, analysis of the food deposits in their probosces and consideration of the morphology of a now-extinct group of gymnosperms, the Czekanowskiaceae, concluded that it was highly likely that a specialised scorpionfly-Czekanowskiaceae pollination system existed long before angiosperms radiated.

FLOWER COLOUR DEVELOPMENT: PIGMENTS

How are flower colours produced? Most flower colours are a result of chemical pigments present in the cells of the flower petals. Particularly significant are the

carotenoids and the anthocyanins. Carotenoids are complex organic molecules whose presence results in a yellow-orange colour appearance to human eyes, both in flowers and in fruit (Jordan, 2004). They are typically divided into two types: the carotenes and xanthophylls (the latter contain oxygen; the former do not).

Anthocyanins are a subclass of flavonoids containing a sugar group. They are present in all tissues, but when they occur at higher concentrations in petals they are the cause of most blue-violet-red shades observed in flowers by humans (Gottsberger and Gottlieb, 1981). The exact colour appearance conferred by these pigments is determined by the pH of the intracellular environment (Stewart et al., 1975).

As understanding of animal behaviour progressed it became apparent that the purpose of flower colours is to entice pollinators to visit them, acting in concert with other features such as floral odour to become "sensory billboards" (Raguso, 2004). This is true both of the pigmentation and the structural contributions to a flower's colour appearance. Experiments involving colour knockouts and different colour morphs show that flowers lacking their typical pigments may receive fewer pollinator visits (Brown and Clegg, 1984; Comba et al., 2000; Dyer et al., 2007; Levin and Brack, 1995) (note, however, that many of these studies did not investigate the effect of colour changes on plant floral odour, which can have large effects on flower visitation (Kunze and Gumbert, 2001)).

FLOWER COLOUR DEVELOPMENT: STRUCTURAL COLOUR

Flower colour appearance can also be enhanced or altered by structural modifications of the petals. Some types of epidermal cells serve to concentrate photons and intensify pigmentation colours (Glover and Martin, 1998; Noda et al., 1994). These conical epidermal cells are present in around 70% of flower species studied, so one can surmise they serve an important purpose and their visual appeal may be a contributing factor to this (Dyer et al., 2007). Similarly, striations on the petal epidermis present can induce iridescent effects by refracting light of different wavelengths by different amounts (Whitney et al., 2009a), similar to the structural colour observed on butterfly wings (Vukusic, 2006; Vukusic et al., 2002), beetles (Seago et al., 2009) and some damselflies (Vukusic et al., 2004). The result is that viewing the petal from different angles

produces a different impression of colour; such iridescence can be detected and learned by insects (Whitney et al., 2009b). Such structural colours can produce a more intense saturation than can be achieved by pigments, but in general the majority of flower colours are nonetheless pigment-mediated.

ANCESTRAL FLOWER COLOURS

As a result of the biochemical and developmental pathways involved in pigment production, the colours a flower can assume are constrained by evolutionary history. In order for a brightly- coloured flower to emerge from a lineage that has previously only been white, the plant must evolve or co-opt from other biochemical pathways the enzymes necessary to produce coloured pigments. In contrast, a plant group with brightly coloured flowers may be able to give rise to a white-flowered lineage relatively easily by loss of a functional enzyme at some point in the pathway, such as when a chalcone synthase enzyme is disrupted in *Antirrhinum*, *Ipomoea, Dendranthema* or *Delphinium* (Coberly and Rausher, 2003; Yang et al., 2002). Strong evidence of this comes from a recent meta-analysis by Rausher (2008) in which he found many examples of pigments being lost from taxa at a greater rate than pigment-gain events, but only one taxon (*Costus*) in which gains of pigmentation appear to have happened more frequently.

The ancestral flower colour associated with the first flowers was therefore unlikely to have been highly pigmented and to have appeared, for example, blue, pink or purple. We are uncertain exactly what colour the first flowers were, but early flowers were likely to have resembled those of the extant plant groups considered “basal” – specifically the ANITA-grade plants (Thien et al., 2009).

In any case, in some lineages it seems that the evolution of differently-coloured pigments in flowers has in some way benefited them, as distinctive and striking colours such as red (in *Papaver*, *Mimulus*, *Penstemon* and *Romulea* just by way of illustration) and blue (in, to name but a few examples, *Iris*, *Delphinium*, *Ipomoea*) appear in many taxa of angiosperms convergently (Bernhardt, 2000; Chittka et al., 1994; Cronk and Ojeda, 2008).

White flowers present something of a conundrum – there are many flowers that appear white to humans. However, almost universally, these flowers are UV-absorbing (Kevan et al., 1996). Therefore, as will be explained later, to insect eyes they could be predicted to be as distinctly colourful as cyan or yellow appears to humans, since the flowers stimulate two out of three of most trichromatic insects' photoreceptors. By contrast, UV-reflecting white flowers are very uncommon (Chittka et al., 1994). This implies that white flowers are under selection by visual systems as much as those that appear colourful to human eyes.

FLOWER COLOUR IN THE CONTEXT OF POLLINATION

Why might a flower's colour affect the number of visits received by it? Most pollinators – insects or vertebrates – possess colour vision (Briscoe and Chittka, 2001; Frisch, 1914) (note, however, that bats appear to have rather limited colour vision (Winter et al., 2003), and rodents and small marsupial pollinators likewise tend to be rod-dominated dichromats (Ahnelt and Kolb, 2000; Strachan et al., 2005)), and therefore are able to detect the colour properties of a flower. Colour can have implications for salience; flowers that are highly visible against a background will be more easily detected and therefore more frequently visited than flowers that are less conspicuous (Dyer et al., 2007; Giurfa et al., 1996; Spaethe et al., 2001). This mechanism alone would favour the evolution of flowers with high contrast with their background, at least as detected by the relevant pollinators.

Additionally, however, pollinators may have innate preferences for particular flower colours (Giurfa et al., 1995; Lunau and Maier, 1995; Raine et al., 2006) and so may tend to choose to visit certain colours more frequently even with no prior flower-visiting experience. But the overall picture is not so simple: pollinators can overcome these preferences and learn to associate particular colours with a reward, even colours that are not considered "innately attractive" to them (Menzel, 1985b; Menzel and Erber, 1978). Rewards offered by flowers may include nectar (Baker and Baker, 1973), pollen (Betts, 1920; Jones, 1997), aromatic oils (Buchmann, 1987; Vogel and Machado, 1991) or heat (Dyer et al., 2006; Seymour et al., 2003). Thus, the flower colour serves as an

advertisement, both making the flower conspicuous to a pollinator and typically providing information about the reward contained within.

However, there are fundamental differences in the colour vision used by us as human observers to view flowers and that of the pollinators. As it is the perception of pollinators which will ultimately lead to reproductive success for flowers, any consideration of flower colour must take into account how those colours are perceived by the relevant pollinators. This is investigated further in chapters III and IV, in which the colour composition of floral communities is modelled in bee and fly colour space in order to look for spatial or temporal changes relating to their pollination ecology.

OVERVIEW OF THE COLOUR VISION OF INSECT POLLINATORS

I now present a summary of the colour vision capabilities of bees and other Hymenoptera, moths, butterflies, dipteran flies and beetles. Although other insect groups, e.g. crickets (Micheneau et al., 2010), can also contribute to pollination, these guilds are considered to be the major pollinators in most community studies (Johnson, 2004; Lázaro et al., 2008; McCall and Primack, 1992; Ollerton et al., 2009a). Some bird species (e.g. hummingbirds (Altshuler, 2003; Brown and Kodric-Brown, 1979; Castellanos et al., 2003), passerine birds (Ollerton et al., 2009b) and sunbirds (Pauw, 1998) can also serve as pollinators and indeed are the primary pollinators of many flower species (Bruneau, 1997; Dalsgaard et al., 2009); their physiology is substantially different from that of insects and their visual apparatus and capabilities are also very dissimilar (Goldsmith, 1980; Goldsmith et al., 1981).

Insects have compound eyes consisting of hundreds or thousands of ommatidia, discrete units containing the photoreceptor cells and support structures. An ommatidium comprises a set of photosensitive cells and the associated support structures. There are typically around eight elongate retinula cells, arranged around a central rod – the rhabdom, which serves as a light-guide to incoming light. The rhabdom contains the rhabdomeres, which contain the visual pigments (Goldsmith and Philpott, 1957) (Figure 1).

The compound eyes in insects may all appear to be superficially similar, but in fact there is a great diversity of structure, enabling them to trade off sensitivity and acuity in different ways. Firstly, insect eyes can be of either apposition or superposition type (Exner, 1891). The apposition eye, thought to be the ancestral form, contains optically isolated ommatidia, and the fractions of images provided by each ommatidium are assembled in the brain to form a coherent image. The superposition eye is typical of many nocturnal insects: the ommatidia are not optically isolated and several ommatidia are able to focus light on to each of the retinula cells, producing a single, erect image in the eye (Land et al., 1999; Schwab, 2006). Superposition eyes increase sensitivity by a factor of between 10 and 1000 compared to apposition eyes (Gaten, 1998).

Additionally, the rhabdoms can function in either an open or closed systems. In the open system, the individual rhabdomeres are separated from each other so that each photoreceptor cell functions semi-independently as a "picture point", giving better spatial resolution without an increase in number of ommatidia. In contrast, the closed system consists of fused rhabdomeres and results in only a single picture-point per ommatidium (Zelhof et al., 2006).

The photopigment genes in insects and their spectral tuning – and thus the spectral sensitivities of insects – are fairly well conserved for such a large taxon (Briscoe and Chittka, 2001), certainly predating the evolution of flowers and flower colour (Chittka, 1996a). However, different groups have their own fine-tuning adaptations, including some insect species which are putatively tetra- or even pentachromatic (Briscoe and Chittka, 2001). Even with the same receptor physiology, the way in which incoming colour information is processed can differ dramatically between different insect groups. Here, I outline what is known about the colour vision of the main insect groups involved in pollination.

Bees. The most important pollinator group worldwide, economically and ecologically, is the bees. There are over 16000 species of bee worldwide (Danforth et al., 2006), including both solitary species and social species. Based on studies of the honeybee *Apis mellifera* and various bumblebee species, the vision of bees is particularly well-characterised compared to other insects.

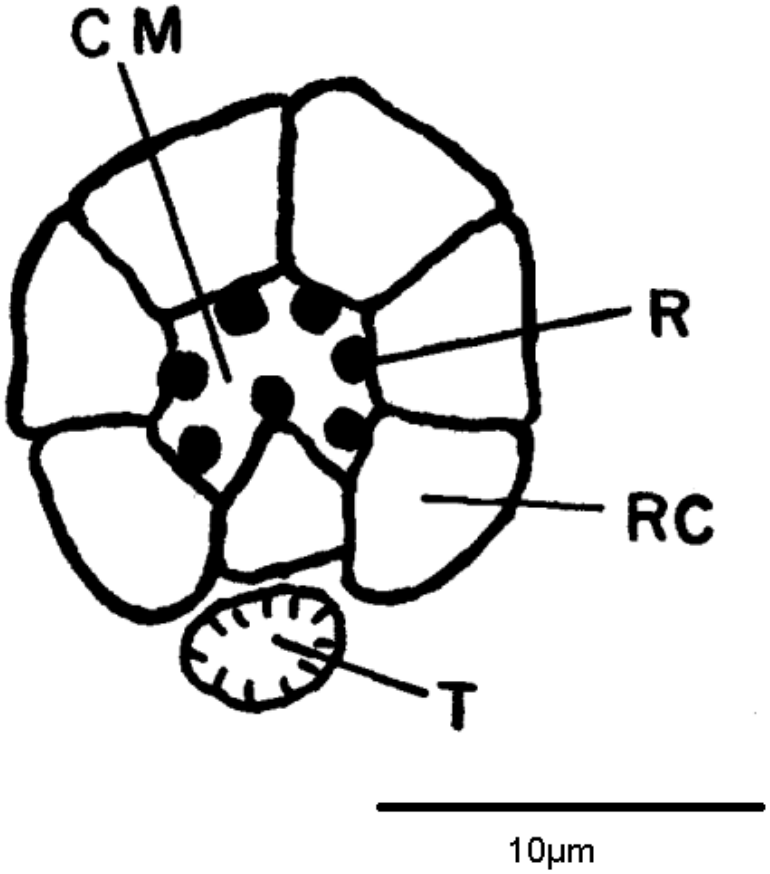


Figure 1. Transverse-sectional diagram of a single ommatidium from *Drosophila melanogaster*, as seen under a microscope - taken from Goldsmith and Philpott (1957). CM refers to the rhabdom's central matrix; R indicates the location of a rhabdomere, part of the overall rhabdom in the centre of the ommatidium, RC indicates one of the retinula cells and T is the trachea of the ommatidium. The seventh and eighth retinula cells are arranged with one vertically above the other so that only seven are visible in transverse section.

Bees do in fact have five eyes, two of which are large compound eyes and the other three being simple ocelli located on the back of the head that are unlikely to be image-forming but may have a role in controlling flight course and direction (Kastberger, 1990a; Kastberger, 1990b). Bees have apposition eyes, the rhabdoms of which are of the closed variety. Their eyes are typically relatively large and high resolution for their body size, as bees rely highly upon the visual medium for foraging success and locating flowers. The ommatidia all contain eight photosensitive cells, of which six always contain a green-sensitive opsin. The other two cells may be either both sensitive to UV, both sensitive to blue, or there may be one blue-sensitive and one UV-sensitive cell present; these three types of ommatidium are distributed rather randomly across the retina (Spaethe and Briscoe, 2005; Wakakuwa et al., 2005).

Almost all species of bee are diurnal (Kelber et al., 2006), though some are crepuscular or even nocturnal (Kelber et al., 2006; Warrant et al., 2004). Bees that forage in dim light typically have very large eyes for their body size (Warrant et al., 2004).

Colour vision in bees was first proven definitively by Karl von Frisch (1914), and with one known exception (a tetrachromatic solitary bee, *Callonychium petuniae* (Peitsch et al., 1992)) they are now known to be trichromatic (Daumer, 1956; Daumer, 1958), with receptors exhibiting peak sensitivity to UV, blue and green light (Peitsch et al., 1992; von Helversen, 1972). Both behaviour and physiological studies show that bees have good colour vision and use it extensively when foraging. Figure 2 shows the specific receptor sensitivities of some bee species as tested by Peitsch (1992), with further information on the receptor sensitivities of other Hymenoptera for comparison.

Several bee species have been demonstrated to have an innate preference for blue and violet flowers, as humans would perceive them, but they rapidly overcome this with training (Brandt et al., 1989; Giurfa et al., 1995; Gumbert, 2000). However, in some bee species and subspecies, a secondary unlearned preference for red flowers has been observed, though it is not as strong as that for blue/violet (Chittka et al., 2004). This is somewhat surprising given that their receptors are relatively insensitive at these wavelengths, but could be ecologically relevant in certain habitats in which there are many red flowers which, in the past, were pollinated principally by birds. If birds were once important pollinators in an ecosystem, the flowers may have developed

adaptations to this such as long wavelength reflectance; now, if pollinating bird species are no longer present, bees have access to a valuable source of energy (nectar) and adaptations to help them utilise these "bird flowers" such as a secondary red preference could benefit them (Chittka et al., 2004). Birds have long-wavelength receptors that enable detection of red light (Endler and Mielke, 2005) and therefore flowers appearing human red are often assumed to be adapted for bird pollination (Castellanos et al., 2003).

A bee with no specific training will tend to generalise perceptually similar flower colours to a large extent (Dyer and Chittka, 2004c), and therefore its ability to discriminate may appear poor. However, when a bee has received differential conditioning, in which some stimuli (such as flowers of a particular colour) are associated with a reward (such as nectar) and others are not, or are even associated with a threat or punishment (in the laboratory, bitter-tasting quinine hemisulphate solution (Chittka et al., 2003); in the wild, something such as a predator attack (Ings and Chittka, 2008)), the bees will slow down their decision-making process and demonstrate the ability to discriminate between very similar colours with great accuracy.

Nature presents a complex foraging environment in which the illumination is often neither constant nor consistent. Given that the spectral composition of illuminating light inevitably affects the spectral composition of the light reflected by an object and thus exciting the cells of an observer's retina, changing illumination has the potential to radically alter the colour appearance of flowers or other items. There is now a body of published literature demonstrating bees' abilities to compensate for changed illumination (Lotto and Wicklein, 2005; Neumeyer, 1981; Werner, 1987; Werner et al., 1988); in other words, bees possess colour constancy: the ability to discriminate colours correctly under spectrally different light conditions. It is likely that in most or all visual systems, the properties of photoreceptors to adapt to light intensity by up- and down-regulating receptor sensitivity will inevitably convey some level of colour constancy, and indeed when researchers have looked for colour constancy in animal groups they have generally found it (Balkenius and Kelber, 2004; Kinoshita and Arikawa, 2000; Neumeyer et al., 2002). Chapter V of this thesis will explore some aspects of bee foraging in environments containing complex and patchy illumination, which is in part

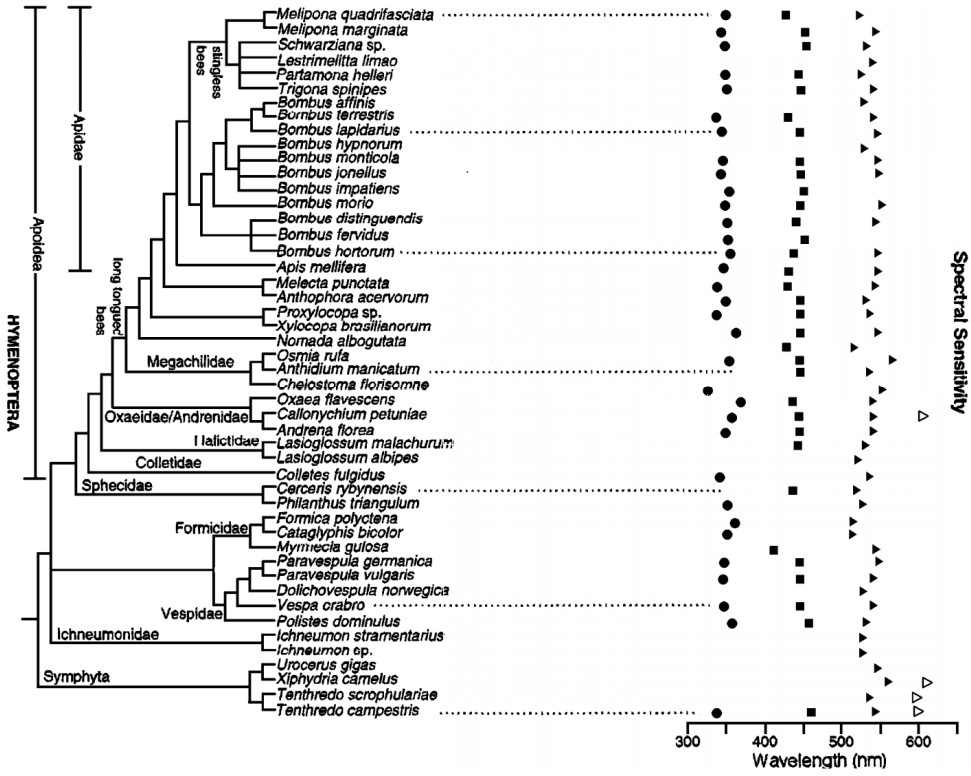


Figure 2. Receptor sensitivities for various Hymenoptera species, taken from Briscoe & Chittka (2001). The circles are the short-wavelength receptor (UV receptor), with the position corresponding to the peak sensitivity; the squares indicate the peak sensitivity for the medium-wavelength (blue) receptor and the closed triangles are the peaks for the long-wavelength (green) receptor. One bee species, *Callonychium petuniae*, also possesses a red receptor, as do many sawfly species.

determined by the limitations of bees' colour constancy and how accurately they can find coloured stimuli under changing light conditions.

Other Hymenoptera. Other Hymenoptera can also play a pollination role. Yellowjacket wasps such as *Vespula vulgaris* pollinate such plants as figwort and ivy; these have similar vision to that of bees in terms of colour discrimination and learning, as well as recognition and learning of shapes (Lehrer and Campan, 2004). There is little information on their innate colour preferences: as they possess a very similar set of colour receptors to bees (Figure 2), one might anticipate similar innate preferences based on the apparent homology of these other aspects of the visual system. However Real (1981) found that free-flying wasps with unknown previous experience preferred yellow artificial flowers to blue when the energetic rewards were equal, and Sharp and James (1979) reported a preference for yellow targets in an experiment performed with experienced foraging wasps of *Vespula squamosa*.

A special case of pollination by wasps is the case of fig-wasps (Agaonidae). This is discussed extensively by other authors (Janzen, 1979; Weiblen, 2002), but as it is believed to be principally mediated by olfactory cues rather than visual ones, fig-wasp pollination will not be discussed at length here.

Ants typically perform very limited pollination services as their thoracic metapleural glands secrete an antibiotic compound which inactivates pollen, and therefore pollen transfer by ants is normally unlikely to result in successful fertilisation (Beattie et al., 1984). However, myrmecophily has been observed in some plants (de Vega et al., 2009; Ramsey, 1995). Different studies have produced divergent results regarding the photoreceptor sensitivities of ants. Kretz (1979) claimed from behavioural experiments that *Cataglyphis bicolor* possesses at least three and possibly four photoreceptor types with its longest-wavelength receptor maximally sensitive to wavelengths corresponding to human-yellow, yet Mote and Wehner (1980) and Paul et al. (1986) find evidence of only two photoreceptor types in this species, and likewise *Formica polyctena* (Menzel and Knaut, 1973) and *Myrmecia gulosa* (Lieke, 1981) only seem to have two receptor types. It is likely that ants locate flowers predominantly by scent rather than colour cues, though there is new evidence that some species can perform accurate colour discrimination (Camlitepe and Aksoy, 2010).

Beetles. Some beetles do play an important role in pollination. The classic "beetle flower" of the pollination syndrome hypothesis is anticipated to be white or greenish (Faegri and van der Pijl, 1978), i.e. presenting poor green-contrast and relying weakly if at all upon colour for attraction.

However, one study by Dafni observes *Amphicoma* beetles visiting red, bowl-shaped flowers (Dafni, 1997). Equally, many flowers of Namaqualand and the Cape Floral Kingdom of South Africa are long-wavelength-reflecting (e.g. red/orange to humans) and pollinated by endemic monkey beetles (Scarabaeidae: Hopliini) (Colville et al., 2002; Johnson and Midgley, 2001). Many other flowers putatively pollinated by beetles are either bowl or urn-shaped (Van Kleunen et al., 2007), but traditional theories claim the flowers should also be strongly scented (Faegri and van der Pijl, 1978), a characteristic which is absent in both red flowers pollinated by *Amphicoma* and in the various large flowers of the South African Cape region pollinated by monkey beetles.

Beetles are thought to be highly significant in pollination of generalist flowers in Australian flora, as well as in other habitats with a Mediterranean climate and some tropical habitats (Bernhardt, 2000). In many such systems they are frequently associated with ANITA-grade (early-divergent) flowers, and are often considered to number among the most ancient pollinators (Bernhardt, 2000; Thien et al., 2009; Van Kleunen et al., 2007), despite that more recent evidence suggests specialised, non-beetle pollination systems predate the angiosperms and were far from primitive (Ren et al., 2009). However, some other beetle pollination systems, such as those described above, are highly derived.

Not much is known about the colour vision or preferences of flower-visiting beetles. Electrophysiological studies imply there are probably three or four photoreceptor types present in most species (Hasselmann, 1962; Lall et al., 1982). In chapter VI, I add to the knowledge in this area, focusing specifically on the monkey beetle (Hopliini) pollination system in South Africa's Cape region and using electrophysiological recordings to ascertain what types of photoreceptors they possess and how this may relate to their ecology and lifestyle.

Moths. Moths are important pollinators of the flowers of many plant species, especially during the night. Nocturnal and crepuscular moths might be expected to have poor colour vision, especially when one considers that many flowers known or believed to rely principally on moth pollination are white or very pale pink in colour (Faegri and van der Pijl, 1978). However, moths actually have excellent colour discrimination (Kelber, 1996; Langer et al., 1979) and can continue to differentiate coloured stimuli even at low light levels (Kelber et al., 2002; Schlecht, 1979), outperforming humans and bees substantially.

Physiological experiments show that many moths are trichromatic, with roughly similar photoreceptor sensitivities to bees (UV, blue and green) (Eguchi et al., 1982; Kelber, 1996). However, some may have an additional photoreceptor type and thus be tetrachromatic (Eguchi et al., 1982).

Moth colour vision capabilities extend beyond simple discrimination tasks under sun- and moonlight: the moth species *Macroglossum stellatum* has been demonstrated to possess colour constancy much as honeybees do (Balkenius and Kelber, 2004), and therefore it can adapt to changed illumination conditions and continue to perform colour learning and discrimination tasks accurately. This impressive capacity for colour vision implies that coloured flowers can appear very visually attractive even to nocturnal moths.

Indeed, behavioural experiments with the nocturnal hawkmoth *Manduca sexta* demonstrated that it possesses an unlearned preference for blue flowers (Goyret et al., 2008), and therefore one might conceive of a floral community in which moth-pollinated flowers may benefit from being blue. However, when foraging in nature, *M. sexta* most often visits white, UV-absorbing flowers – which may appear counterintuitive when taken in isolation, but is likely to be indicative of diverse selection pressures on moth-pollinated flowers in plant communities, perhaps including trade-offs of colour in order to reduce unwanted diurnal visitors.

Butterflies. Butterflies are important diurnal pollinators, especially of brightly coloured (e.g. human purple and pink) flowers with long corolla tubes. Their colour vision and innate preferences are highly variable: some species of butterfly are trichromatic, others

tetrachromatic and others are even potentially pentachromatic with five photopigments contributing to colour vision (Arikawa et al., 1987; Kelber and Pfaff, 1999).

It is thus impossible to generalise butterfly colour vision and flower preferences; instead, it is better to consider species individually (Neumayer and Spaethe, 2007). Colour preferences recorded in butterfly species in the field (i.e. experienced individuals) or in laboratory experiments (i.e. experimentally naïve individuals) include red for *Gonopteryx rhamni* (Kühn and Ilse, 1925), *Pieris rapae* and *Erebia nivalis* (Neumayer and Spaethe, 2007), yellow with UV reflectance for *Colia palaeno* (Neumayer and Spaethe, 2007), yellow with a secondary preference for blue and purple in *Battus philenor* (Weiss, 1997) and blue in *Papilio demeolus* (Ilse and Vaidya, 1956). Colour constancy has been demonstrated in one species of butterfly, the swallowtail *Papilio xuthus* (Kinoshita and Arikawa, 2000). Given that this is an ability it shares with bees and nocturnal moths, it is likely that this is a trait that is universal amongst the butterflies.

Flies. Flies can be hugely important pollinators, especially in extreme conditions such as high elevations (Kearns, 1992; Lázaro et al., 2008; Totland, 1993). Their visual systems vary immensely in the number of photopigments and it is still incompletely understood how the different photoreceptors interact to contribute to colour vision (though see Morante and Desplan (2008)). One study (Troje, 1993) has found that the blow fly (*Lucilia* sp.) possesses four photoreceptor types, but that it categorises colours differently from either bees or humans: rather than having a continuous scale of similarity between colours (e.g. a human finding leaf-green and lime-green to be distinct, but more similar to each other than either is to orange), it does not discriminate amongst colours within predefined discrete spectral ranges (Figure 3).

The most important pollinators among the Diptera are probably syrphids (hoverflies). These visit flowers to feed on pollen and nectar as adults and therefore have ample opportunity to move between conspecific flowers and potentially transfer pollen. They are often associated with yellow or white flowers (Faegri and van der Pijl, 1978), but behavioural experiments have demonstrated a diversity of preferences and also learning ability (Lunau and Maier, 1995; Sutherland et al., 1999). Indications are that most

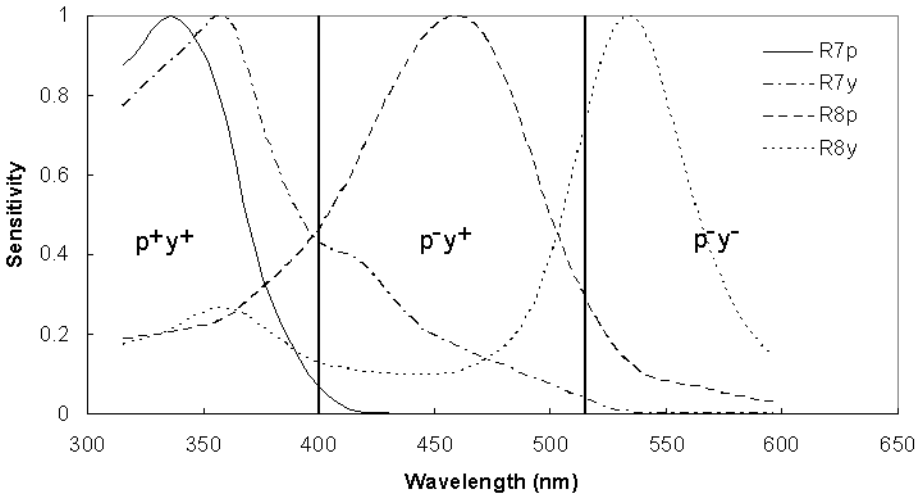


Figure 3. Receptor spectral sensitivity curves for a muscoid fly, *Lucilia* sp., after Hardie & Kirschfeld (1983). The fly possesses four photoreceptor types contributing to colour vision, two types of R7 receptor and two types of R8 receptor, the types being termed "yellow" and "pale" depending on the colour they appear when transmitting light (Troje, 1993). The vertical lines on the graph show the boundaries between fly colour categories as discovered by Troje (1993), within which all coloured stimuli are generalised: p^+y^+ indicates maximal stimulation of the R7p and R7y receptors and is typically termed "fly-UV"; p^-y^+ is "fly-blue"and p^-y^- "fly yellow". A fourth colour category, p^+y^-, is a "fly purple", stimulating both the extreme ends of its spectral range (via the R7p and R8y receptors).

species probably possess three or four photoreceptor types, with peaks broadly corresponding to those of bees (Horridge et al., 1975).

However, the extent to which flies use colour when foraging is unclear, but is likely to be less than that of bees. Notably, the visual apparatus flies dedicate to colour detection is quite different to that of bees – whilst bees have only one type of retinula cell in terms of its size, with green-, UV- and blue-sensitive cells all being largely the same size, in the flies so far studied there are two sizes. The R1-6 cells are all of the large type; however, these are not thought to have a colour-sensitive function as the opsin's sensitivity is too broad to allow colour discrimination. By contrast, the R7 and R8 cells are of the short variety, and are therefore much smaller than the broadband cells, but these are nonetheless the ones that are involved in colour discrimination (Borst, 2009); these are arranged vertically, one above the other (see Figure 1). There are two types of R7 cell and likewise, two types of R8 cell, differing in the colour of the pigments they contain ("yellow" or "pale") (Borst, 2009; Troje, 1993), thus providing the fly with four photoreceptor types thought to contribute to colour discrimination.

The physiology of the fly eye, with only the small R7 and R8 cells contributing to colour vision, combined with the colour-categorisation system discovered by Troje (1993) which seems to place low weight on fine colour discriminations, implies that flies probably do not use on colour for foraging behaviour as extensively as bees and Lepidoptera do, instead giving high precedence to odour information in locating food sources.

Another dipteran pollinator group of particular interest is the long-proboscid fly group in South Africa. These large-bodied flies from the Nemestrinidae can have a proboscis of up to 50mm long and specialise in pollinating plants with very long floral tubes such as *Lapeirousia* and some *Gladiolus* species (Goldblatt and Manning, 1999; Goldblatt and Manning, 2000; Johnson et al., 2002). These plants usually have purple to crimson flowers (Manning and Goldblatt, 1996) with very long (up to 70mm) floral tubes. The highly saturated and, to human eyes, intense colour of these flowers suggests that the long-proboscid fly species (of the genera *Moegistorhynchus*, *Stenobasipteron*, *Philoliche*, and *Prosoeca*) probably have good colour vision capabilities.

POLLINATION SYNDROMES

The pollination syndrome hypothesis is an idea put forward to attempt to explain floral characteristics in terms of their pollinators. It postulates a tight, specialised relationship ("lock-and-key") between various morphological and other traits of the flower, and the sensory, physical and learning capabilities of the insect assumed to pollinate it. For a long time, the pollination syndrome hypothesis was regarded as a reliable predictor of a plant's pollinator based on this suite of characteristics displayed by a flower, including colour, morphology, odour and nectar production (Faegri and van der Pijl, 1978; Grant, 1981; Vogel, 1954).

Indeed, used in the loosest sense, pollination syndromes can give a sweeping overview of which pollinators *might* be relevant to a plant, or equally what types of plant a pollinator might prefer to visit (Fenster et al., 2004; Reynolds et al., 2009). There are various species of plants whose flowers are indeed pollinated either by a single insect species or by a guild of insects that are related and of similar morphology (Johnson, 2005; Johnson et al., 2002; Johnson and Steiner, 2000). However, the pollination syndrome hypothesis is nonetheless an oversimplification for most habitats and insect-plant interactions, based on erroneous assumptions about the level of specialisation of most flowers (Arnold et al., 2009a; Arnold et al., 2009b; Hegland and Totland, 2005; Johnson and Steiner, 2000; Ollerton et al., 2009a; Waser et al., 1996); in actuality, most flowers are relatively generalist, and are pollinated by more than one group of insects. Likewise, relatively few insects visit just a single species or taxon of plants.

As previously mentioned, pollinators have impressive learning abilities and are often particularly adept at colour learning. Bees have been trained to virtually every colour they are physically able to detect and discriminate (Menzel, 1985b), and likely most other insects are similarly flexible in their learning capacities. This means that for flowers, being one particular colour is not necessarily any more advantageous than being another. However, there are some correlations between particular principal pollinators and particular colours – e.g. many putatively hummingbird-pollinated flowers are red, and this seems to have resulted in some cases of selection for the flowers to be less conspicuous to bees relative to hummingbirds, so the flower receives more avian visits and benefits from the birds' more efficient pollen transfer without

"wasting" nectar feeding less efficient bee pollinators (Castellanos et al., 2003; Schemske and Bradshaw Jr., 1999; Vickery, 1995).

However, there are inevitably some constraints on which pollinators are physically able to pollinate particular flowers. These, however, are typically constraints of morphology rather than colour. Large bees cannot enter very small flowers with long tubes and concealed nectar; equally, small bees lack the strength to open large *Antirrhinum majus* flowers.

MEASURING FLOWER COLOUR ACCURATELY

Human judgements of flower colour (in particular, when applying names to the colours) are intrinsically anthropocentric and, although they have a certain degree of consistency between individuals, fine colour judgements are often subjective (Roberson, 2005). They are affected by individual differences in human classifications of flower colour (for example, whether the flower is blue or purple) and only take into account the wavelengths visible to humans, which are essentially 400-700nm except in unusual cases such as extremely dark-adapted eyes.

These constraints mean that a scientist cannot formulate accurate studies and hypotheses about plant-pollinator interactions by observing flowers and recording their colours according to his or her own judgement. Some more accurate, unbiased assay is called for, one which encompasses wavelengths visible to animals beside humans: in particular, those animals whose pollination services determine a flower's fitness.

Fortunately, such a method is available. Spectrophotometers provide very accurate data on the precise colour of a flower, by measuring the proportion of incident light of all human and insect-visible wavelengths reflected off the surface of a flower. A typical spectrophotometer directs light – produced ideally by a light source containing all wavelengths from 300-700nm – on to the surface of the flower using optic fibres, then the reflected light is detected by the probe and compared to a reflectance standard (Chittka and Kevan, 2005).

This results in a reflectance spectrum such as those shown in Figure 4 for three European species, *Galium aparine* (white to humans, blue-green to bees), *Senecio vernalis* (yellow to humans; UV-green to bees) and *Salvia pratensis* (purple to humans, UV-blue to bees). In flowers, reflectance spectra typically change relatively smoothly and usually take one of a small number of forms corresponding to different colours (as perceived by a human or a bee) (Chittka et al., 1994). Step-functions are common in white, yellow and orange/red flowers, with the reflectance increasing from near-zero to perhaps as much as 50% of maximum over just a few tens of nanometres – as seen in *S. vernalis* and *G. aparine* in Figure 4. Peaks in reflectance are also typical of floral reflectances, and may occur around 350nm (UV – as in *S. vernalis*), 400-430nm (seen in blue and purple flowers – see *S. pratensis* – and also pink flowers that appear blue-green to bees), 550nm (associated with chlorophyll and therefore green pigments) or in the long wavelengths of 600nm and above (Kevan et al., 2001).

MODELLING COLOUR VISION SYSTEMS

Once some understanding of the neurological basis for colour vision in an animal has been obtained, it becomes possible to attempt to model this mathematically and graphically. No colour space is perfect – multiple colour spaces are in use for human colour vision and all have relative advantages and disadvantages (Chong et al., 2008; Jameson and D'Andrade, 1997; Wyszecki and Stiles, 1982) and the same is true of bee colour space models. All of them rely on certain assumptions about colour processing mechanisms and receptor sensitivities that may not always apply and indeed may not always be testable.

Building a colour space requires some knowledge of how sensitive the different photoreceptor types present in an insect retina are *relative* to one another, rather than just their sensitivities in isolation, since natural stimuli tend to excite more than one photoreceptor type and this can have key effects on colour appearance of an object. In addition, one must consider what other conditions have an effect on colour vision: the

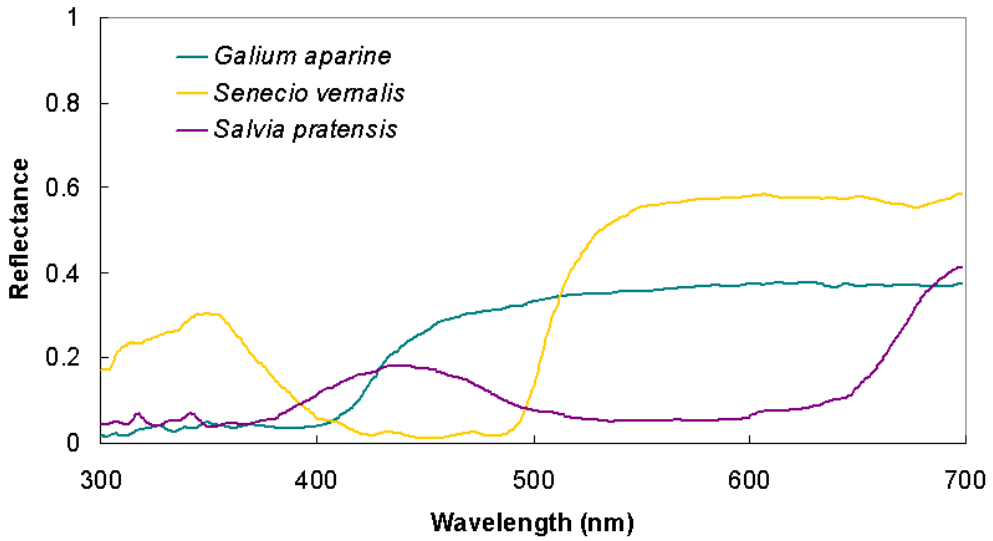


Figure 4. Sample spectral reflectances for three flower species from Central European grassland and woodland habitats (as studied in Gumbert et al. (1999) and with the reflectance data present in the Floral Reflectance Database). *Galium aparine* appears white to human eyes and blue-green to bees. *Senecio vernalis* appears yellow to human eyes and UV-green to bees. *Saliva pratensis* appears purple to human eyes and UV-blue to bees.

ambient light, the background colour, etc. One should also seek an understanding of how signals from the photoreceptor cells interact in the brain of the organism, e.g. inhibitory signalling and opponent coding (Backhaus, 1991). For a long time, bee vision research primarily used the triangular colour space model of bee vision initially based on research by Daumer (1956; 1958), consisting of three dimensions or axes corresponding to the relative quantum catch values of the three photoreceptor types (UV, blue and green). By excluding brightness from this model it is possible to visualise it as a two-dimensional graphic representation of bee colour perception, appearing as a triangular space with loci inside it corresponding to different colours of stimuli (Menzel, 1979; Menzel, 1985b).

However, this model did not take into account any measure of interactions and comparisons between the different photoreceptor signals. In addition, the colour triangle does not take into account the non-linear transduction process from quantum catch to receptor membrane potential change, which, among other things, means that receptors saturate at a certain light intensity and cannot increase their response above that level. To attempt to compensate for these shortcomings, Backhaus (1991) developed a model that incorporated the responses from two types of colour opponency neurons in the bee brain, $UV^{+}B^{-}G^{-}$ and $UV^{-}B^{+}G^{-}$, in which stimulation of one receptor type causes inhibition of the adjacent one(s). This gave rise to the straightforward Colour Opponent Coding (COC) model in which the two axes of the model corresponded to the relative $UV^{+}B^{-}G^{-}$ response and the relative $UV^{-}B^{+}G^{-}$ response. Since then, however, firm evidence has emerged for multiple types of colour opponency neuron (Yang et al., 2004), not just those used in Backhaus's equations, which limits the applicability of the COC model.

A more general colour vision model which does not weight any one colour opponency mechanism above any other has also been developed. This is the colour hexagon model (Chittka, 1992), which is now widely used. As in the other models, it is based on the relative excitations of the three photoreceptor types elicited by the stimulus and the opponency mechanisms in the bee brain. The calculations necessary to model coloured stimuli in this space are explained below.

The amount of light absorbed by one of the three photoreceptor types, i.e. photon capture, can be calculated using the following formula (from Chittka (1992), Spaethe (2001)):

$$P = R \int_{300}^{650} Si(\lambda)I(\lambda)D(\lambda)d\lambda \quad (1)$$

In this equation, P is the photon capture, Si is the spectral sensitivity function of the photoreceptor, I describes the spectral reflection of the object, D describes the spectral composition of the illuminant, and R expresses the adaptation to a green background stimulus:

$$R = 1/\int_{300}^{650} S(\lambda)I_B(\lambda)D(\lambda)d\lambda \quad (2)$$

Here, I_B is the spectral reflection of the background material to which the receptors have adapted. In a typical situation for a bee foraging in a natural environment one would expect the background to be green vegetation. The value of P for each photoreceptor can be transformed into a receptor excitation (E) by using the following equation:

$$E = P/(P+1) \quad (3)$$

These excitations can then be converted into a locus on a two-dimensional hexagonal diagram (Figure 5). The closer to one of the apices the locus is situated, the more the corresponding photoreceptor type is stimulated – for example, an object appearing blue to bees will stimulate their blue receptors most strongly and therefore the locus will be displaced towards the upper half of the hexagon (Figure 5). The conversion uses the following formulae:

$$x = (E_G - E_{UV})\sqrt{3}/2$$

$$y = E_B - 0.5(E_G + E_{UV}) \quad (4)$$

Here, the E values correspond to the calculated excitations for the three different photoreceptor types (UV, blue and green) present in the bee's retina. When the x and y

coordinates are plotted on a graph as described, the apices of the hexagon are located at (-0.866, 0.5), (-0.866, -0.5), corresponding to maximal stimulation of the UV receptor and zero stimulation of the other two, (0, 1), corresponding to maximal stimulation of the blue receptor and zero of the others, (0.866, 0.5), (0.866, -0.5), corresponding to maximal stimulation of the green receptor and zero of the others, and (0, -1).

The colour hexagon model is useful as the distance between two points on the diagram corresponds to the perceptual difference between the colours of the two stimuli to the bee. Therefore, the distance between two points (in colour hexagon units) can be used as a measure of colour dissimilarity according to bee colour perception.

Another colour vision model, for the blow fly (*Lucilia* sp.), was developed by Troje (1993). Based on behavioural experiments, he established that flies generalise colours within four discrete colour categories. So although a bee appears able to perceive two coloured stimuli as spectrally similar but discriminable, to a fly it seems that stimuli are either spectrally indistinguishable or completely different. This is mediated by the interaction between four photoreceptor types, R7y, R7p, R8y, R8p. This means that froma fly's point of view, all stimuli are either UV, "fly-blue", "fly-yellow" or "fly-purple" (Troje, 1993).

COLLECTING FLORAL REFLECTANCE SPECTRA

The necessity for full reflectance spectra for any stimulus modelled in colour space means that many researchers with an interest in pollination biology have taken such measurements of flowers (Chittka et al., 1994; Gumbert et al., 1999; Lunau, 2000; Menzel and Shmida, 1993). In order to be directly comparable, such spectra need to be measured with care and with known instrumentation (Chittka and Kevan, 2005). In particular, when it comes to flowers it is important to ensure that the light source contains sufficient ultraviolet light.

However, though many floral reflectance spectra exist, the measurements are dispersed throughout the world, in different file formats and physical locations. Were these measurements to be collected together in a single, central database accessible to any

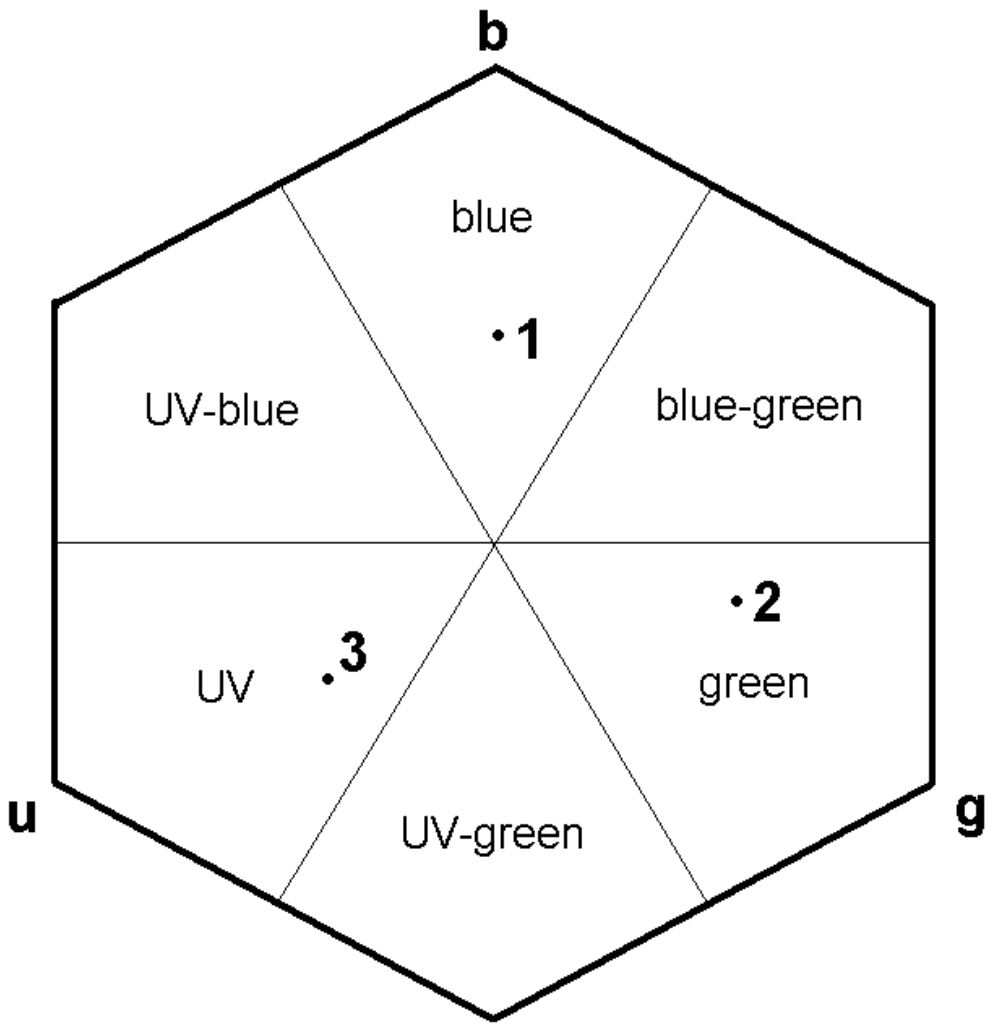


Figure 5. Honeybee colour hexagon, showing hypothetical loci (1, 2, 3) for three coloured objects in the colour space. The colour hexagon is a schematic representation of how a honeybee sees colours, showing coloured objects schematically as they appear in the bee's perception space; loci that are close together indicate perceptually similar colours. The three apices (b, g, u) indicate the three photoreceptor types a bee possesses – loci closer to one of the apices are those whose colours stimulate the corresponding photoreceptor most strongly, e.g. stimulus 1 stimulates the blue receptor mostly strongly and therefore is displaced towards the top of the hexagon. The hexagon can be divided into six segments which can be considered to be bee "colour categories", as shown on the diagram. Loci in each segment are assumed to be perceived as that colour by the bee.

researcher, larger-scale studies of flower colour would become possible. This would also facilitate more diverse uses of the colour information that can be extracted from reflectance spectra.

This was the motivation behind the development of the Floral Reflectance Database, a core part of this project introduced in chapter II. The creation of this database has also enabled other studies to take place, including analysis of whether the colour composition of flower communities changes along transects or over the course of the year, in addition to studies of how pollinator colour preferences and learning interact with the colours of flowers.

TRENDS IN FLOWER COLOUR, THROUGH POLLINATOR EYES

An immediate use of the Floral Reflectance Database is to make comparisons of flower colours within and between habitats, using not just human colour judgements, which are subjective and ecologically irrelevant, but the physical spectral properties of the flowers and insect models of colour vision.

It is possible, for example, to compare whether floral communities in arid or rainforest habitats differ significantly in different parts of the world. The most immediate way to analyse reflectance spectra is using a Principal Components Analysis irrespectively of any colour vision system. This reduces complex spectral information down to a smaller number of main dimensions of variation. The first two principal components might typically account for around 70% of the total variation in flower colour (Arnold et al., 2009b), and therefore although not exhaustive, are a reasonable assessment of the diversity of colours present in a given sample.

FLOWER COLOURS ACROSS THE WORLD

With the standard use of floral reflectance spectra and the development of the Floral Reflectance Database, it is possible to add a new dimension to studies of pollinator communities across the world. One can compare and contrast the colour composition of

whole plant communities from different habitats, latitudes or locations, or indeed as the database grows, focus on particular genera and families and examine whether there are trends or similarities and differences in the reflectance spectra of related flowers in different locations.

An example is shown in Figure 6, in which reflectance spectra from many species within the Orchidaceae (listed in Appendix I) are converted into bee colour hexagon loci and then grouped according to whether they grow in tropical, temperate or intermediate/ subtropical habitats. Although this is clearly not an exhaustive survey of the family, being based merely on the collected spectra available in the Floral Reflectance Database, it appears superficially based on where the loci fall in the hexagon that there are more bee UV-blue flowers in temperate regions and bee blue and blue-green flowers in the tropics. However, these differences are not significant (ANOVA, $F = 1.684$, $hdf = 4$, $edf = 58$, $p = 0.166$).

The pollination market hypothesis indeed predicts that a range of flower colours should be present in virtually all habitats, as evolution favours distinctiveness and conspicuousness in flowers (Friedman and Shmida, 1995; Gumbert et al., 1999). This might lead us to expect that all habitats would have very similar colour compositions, with approximately the same proportions of species of each colour present. An example of two datasets in which all the species growing in a particular study site have been sampled and spectral reflectance measurements taken are shown in Figure 7. As anticipated, a range of colours is present in both habitats.

However, one has to take into account other constraints: since distinctiveness is favoured, flowers will tend to be colours that are distinctive in the visual systems of the pollinating insects (i.e. if no insects in a particular habitat were to possess any capacity for colour discrimination in the red range, one might predict that habitat to be deficient in flowers with mostly long-wavelength reflectance). Therefore subtle variations in colour composition could occur according to the pollinating species playing a role in the habitat. Equally, pigments could be favoured for other pleiotropic reasons related to resistance to abiotic environmental factors (Ben-Tal and King, 1997; Chalker-Scott, 1999; Warren and Mackenzie, 2001). Evolutionary history may, as previously stated, play an impotant role: islands are a particularly unique example in which the plant and animal species may have passed through an evolutionary bottleneck; as the capacity to

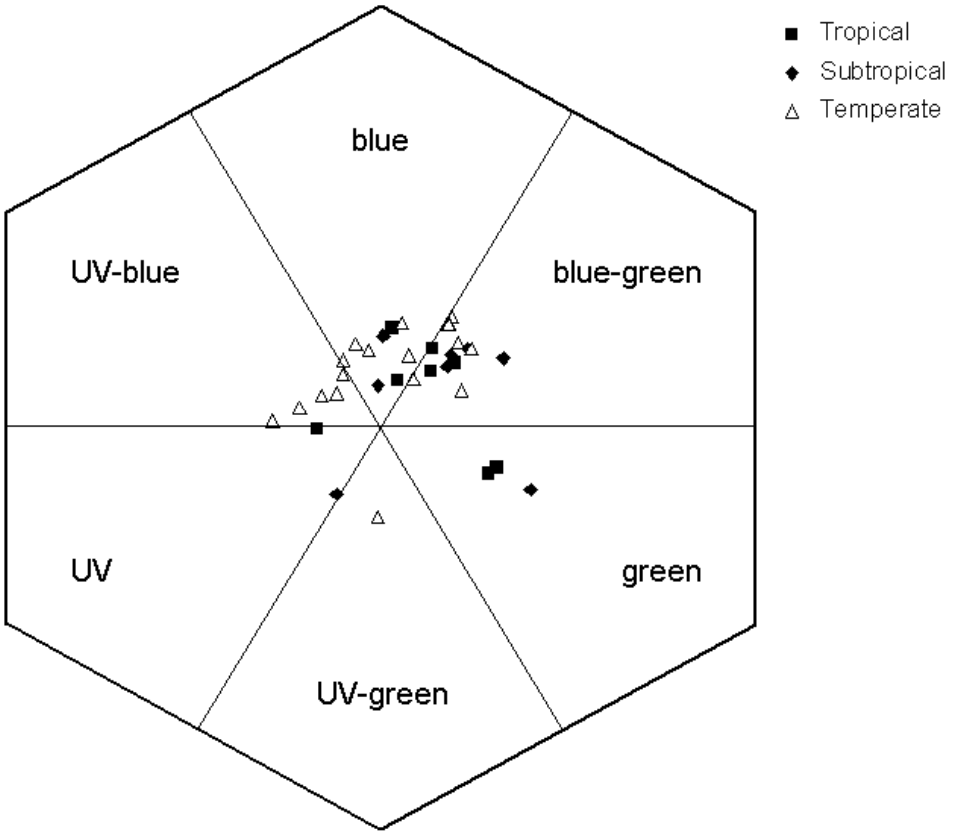
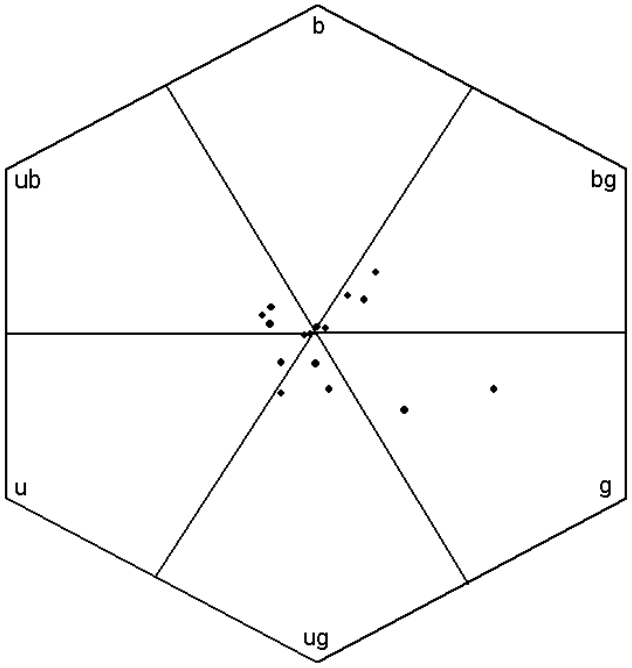


Figure 6. Honeybee colour hexagon plot of various species of Orchidaceae. The plant species here occupy diverse loci in the colour space and are divided up into those with tropical, subtropical and temperate native habitats. This is not an exhaustive survey of Orchidaceae by any means and therefore cannot be used to draw conclusions about global colour trends in this family, but serves as an example of how the colour hexagon can be used to visualise bee-colours of many different species for comparative studies.

a)



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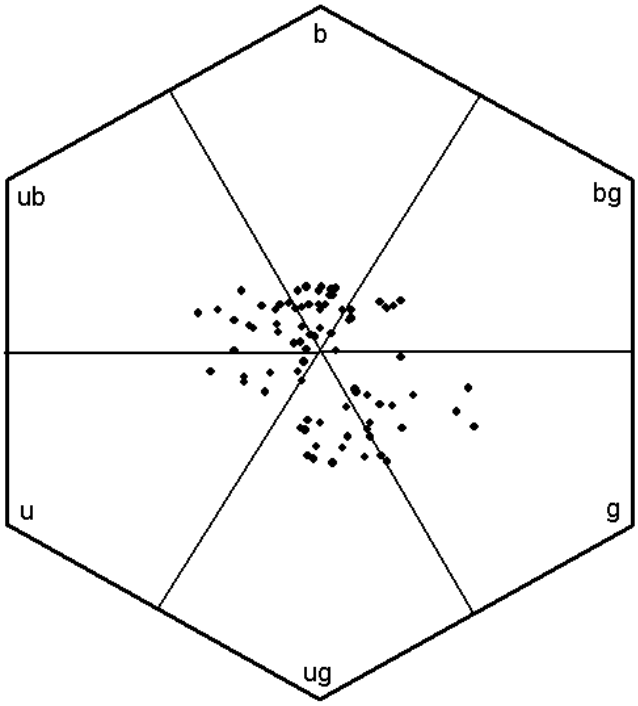


Figure 7. Colour hexagons showing the distribution of flower colours sampled from a site at a) Richtersveld, South Africa and b) Har Gilo in Israel. Both are relatively arid locations but Richtersveld is effectively semi-desert whilst Har Gilo is Mediterranean. In both cases, a wide range of flower colours are present in the habitats, suggesting selection favours distinctiveness of appearance rather than one particular colour being universally superior. The colour "category" segments are indicated by: b = blue, bg = blue-green, g = green, ug = UV-green, u = UV and ub = UV-blue.

synthesise certain pigments may be lost in the process, or genetic drift may be playing a larger role, it is possible that island plant communities may have unusual proportions of flower colours relative to mainland communities. Flowers in a community may therefore face a complex set of trade-offs, between being too similar to conspecifics and being too different, and also between pigments that would favour particular pollinators versus ones that protect against environmental challenges, all within the context of their evolutionary history (Arnold et al., 2009a; Arnold et al., 2009b; Gronquist et al., 2001; Whibley et al., 2006). Unravelling the pressures controlling flower colour in a particular plant species in a given community, therefore, is no simple task.

When flower colours are plotted on the bee colour hexagon, a pattern often emerges in which the flowers' colours tend to be located less frequently on boundaries between hexagon segments than towards the centres of these segments, with the clusters therefore appearing at regular intervals spaced 60° apart around the hexagon (Chittka, 1997). Figure 8 shows this phenomenon for species recorded by surveys carried out in four different countries (Brazil, Costa Rica, South Africa, Israel) (Chittka, 1997; Chittka et al., 1994; Menzel and Shmida, 1993). For these graphs, the vectors from the centre of the hexagon to each species' locus are calculated and the loci occurring at different angles from the centre – corresponding to different hues – can be plotted by frequency. This clustering phenomenon might perhaps be because insects find colours in the centre of categories easier to recognise or discriminate than colours that fall on category boundaries. However, evidence for how and indeed if bees categorise colours is still somewhat equivocal (Benard et al., 2006), and therefore it is difficult to say with any certainty whether the arbitrary segments of the colour hexagon correlate in any meaningful way with bee colour perception.

One can also perform Principal Components Analyses (PCA) on unprocessed floral reflectance spectra, to investigate differences in colour composition independently of any insect vision bias. Figure 9 shows the results of a PCA performed on the same Orchidaceae species as in the colour hexagon diagrams above. This time, however, the results are not weighted by spectral sensitivity of a viewing insect, but depend only on the physical properties of the flower reflectance functions. However, as might be anticipated, colours that appear broadly similar to humans or to bees still cluster together: note the cluster of points in the lower left-hand corner of the graph from

temperate locations, broadly corresponding to the UV-blue flower loci on the colour hexagon. In this analysis, the colours of orchids in tropical, temperate and subtropical habitats *do* differ significantly (ANOVA, $F = 3.565$, $hdf = 4$, $edf = 58$, $p = 0.011$). This indicates that the variation in spectral reflectances detected in this analysis may be in the longer wavelengths to which bees are less sensitive, and it therefore affects their loci in the space created by the PCA but not their colour hexagon loci. One cannot use a selective sample such as this to draw conclusions about the colours of all orchids worldwide, but it serves as an example of how spectral reflectance data from many species across multiple habitats can be analysed to provide information about global trends in flower colour and of how different ways of modelling and assessing flower colours may give rise to different results.

Figure 10 also investigates flower colours using a PCA. This shows the results of an analysis performed on the spectral reflectance data of the same four countries as in Figure 7. As might be expected according to theories of pollination markets and selection for distinctiveness, there is large degree of overlap in the clouds of points associated with different habitats, owing to the occurrence of a range of colours in all habitats. However, further analysis on a site-by-site basis may yield interesting differences in the proportions of species in each part of the distribution and in outlying points.

These analyses serve as very brief examples of the possibilities opened to us by assembling larger sets of floral reflectance data. By using accurate measurements of flower colour in spectral reflectance format, we can choose to take into account insect visual systems, or to rule out all visual systems including that of humans, when looking for evolutionary and ecological explanations for flower colour. Such datasets will facilitate future research into global trends in flower colour and pollinator behaviour.

THESIS AIMS

In this thesis I approach the question of how flower colour has evolved under selection by pollinators from a variety of angles. Overall, I seek to discover how different selection pressures can interact to affect the evolution of flower colour and how

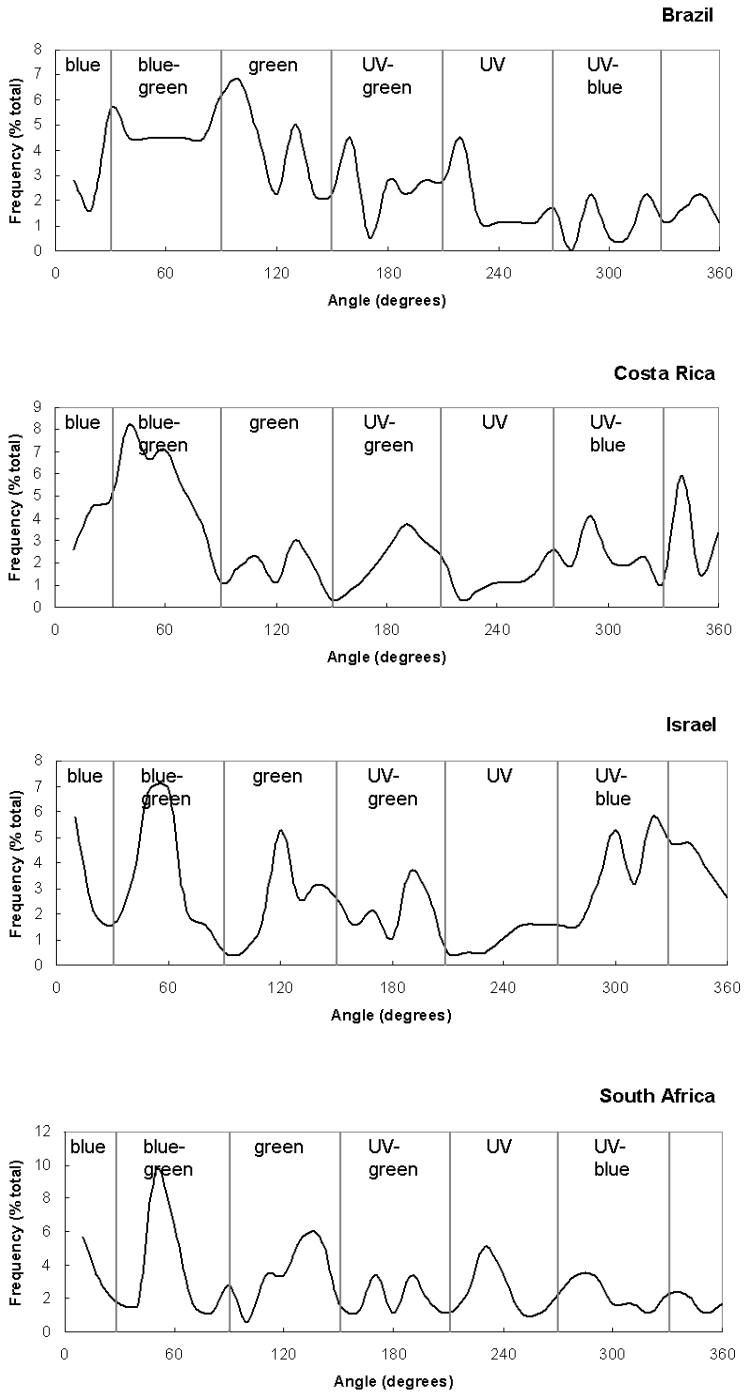


Figure 8. Angular vectors for the colour hexagon loci of the flower species from large collections of spectra, sampled from multiple sites in four countries: Brazil (Chittka, 1997; Chittka et al., 1994; Chittka et al., 1993), Costa Rica (M. P. Powell and V. Savolainen, unpublished), Israel (Menzel and Shmida, 1993) and South Africa (M. P. Powell and V. Savolainen, unpublished). 0° is the top apex of the hexagon, corresponding to the bee's blue receptor. There are similar trends for the flora of all four countries, with flower colours consistently clustered towards the centre of colour hexagon segments (which may be used arbitrarily as colour "categories"), and relatively few colour loci located on category boundaries.

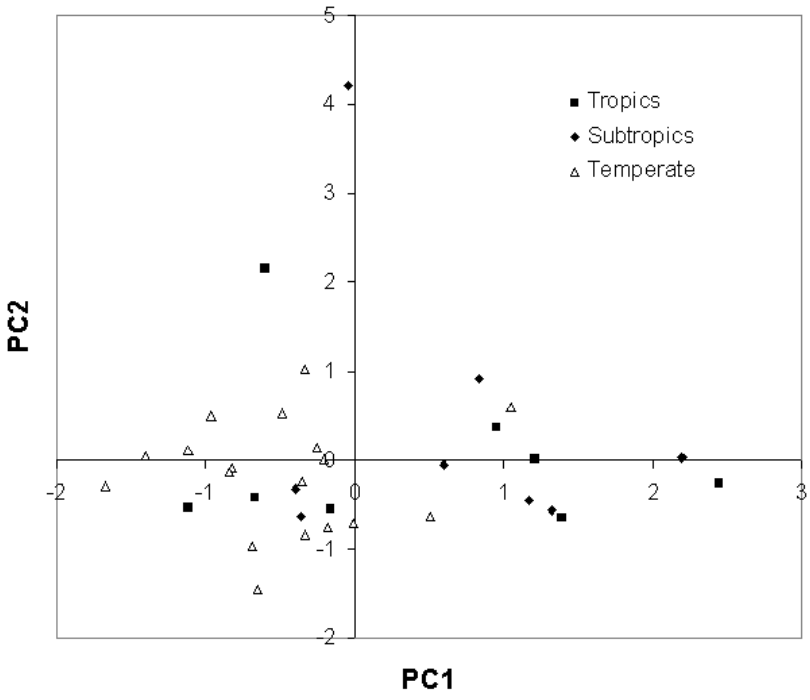


Figure 9. Principal Components Analysis performed on the same orchid species as in Figure 6. This analysis does not rely on any one visual system, but on the physical properties of the floral reflectances. Once again, the species show a diversity of colours, with clusters that loosely reflect similar bee and human colours. The tropical, subtropical and temperate orchids from the data set have significantly different colour ranges.

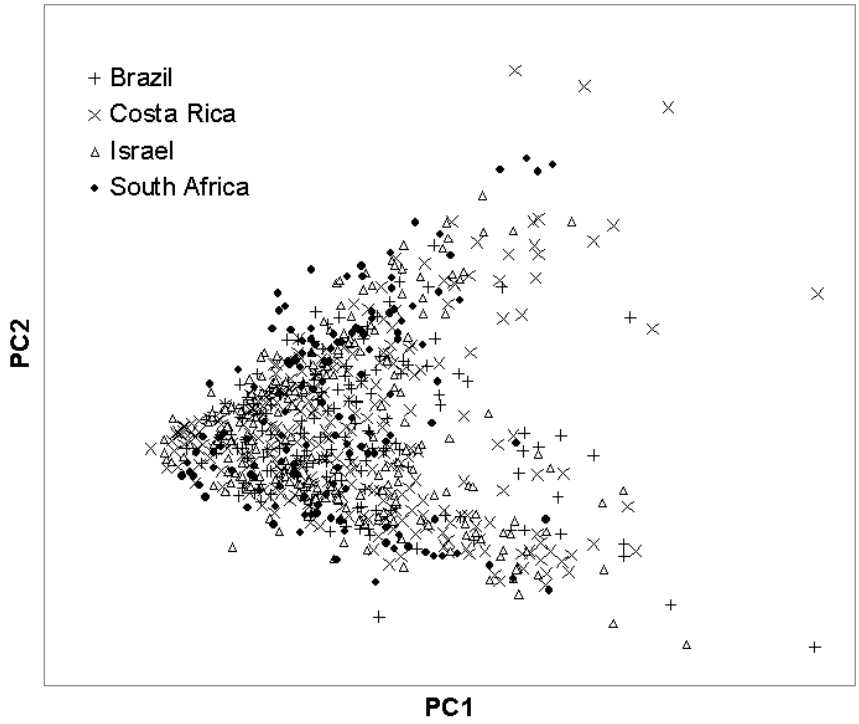


Figure 10. Principal Components Analysis performed on all the species used to compile Figure 7. It indicates that on a large scale the colour compositions – based on physical properties of spectra – of large regional floras tend to be roughly consistent.

particular flower colours can be favoured by a particular temporal, physical or biological context. By examining flower colour both at the habitat level and in terms of the behaviour and physiology of individual insect pollinator types I hope to provide more insight into the diverse pressures at work in pollinator-plant interactions and the ways in which flower colours are selected as a result.

Chapter II

I introduce in detail the Floral Reflectance Database (FReD), an online searchable collection of flowers' spectral reflectances, colour hexagon coordinates and other information related to their colour. This sets the background for later chapters, in which I examine some of the data in FReD in more detail, investigating whether the differing species compositions of pollinators active over spatial and temporal scales can cause different colour compositions of the flowers in two European plant communities.

Chapter III

I study an elevation gradient in Norway to see whether flower colour is influenced by altitude, and by the insects present at that altitude. This chapter aims to examine whether spatial changes in pollinator composition may give rise to changes in colour composition of floral communities, in accordance with the flower colours traditionally associated with particular guilds of insect pollinator.

Chapter IV

I focus on a set of German grassland and woodland habitats, investigating whether flower colours change throughout the year, possibly as a result of differing guilds of pollinators being active in different seasons.

Chapter V

The previous chapter considers that temperate woodlands especially are subject to changing light environments throughout the year, as a result of the leaf-cover in the

canopy during different seasons. In this chapter, I move to look more closely at insect behaviour under inconsistent light conditions and how this might influence flower choice. In particular I examine the behaviour of bumblebees foraging in spectrally inconsistent light over a spatial scale, a condition which mimics foraging in woodland edges and hedgerows.

Chapter VI

I focus on the unusual pollination systems in the South African Cape region (Namaqualand and the Cape Floral Kingdom). This region has a large number of brightly-coloured flowers which lack strong scents but possess long-wavelength reflection. I investigate whether the floral colour compsition of this region significantly differs from flower colour compositions in other global habitats. These long-wavelength reflecting flowers are pollinated primarily by beetles of the Hopliini tribe, but their colour vision has not previously been investigated. Using electrophysiology to study the colour vision capacity of three monkey beetle species, *Pachycnema crassipes*, *Lepisia braunsi* and *Clania macgregori*, I consider how these visual systems may exert selective pressure on floral appearance in the Cape Region.

CHAPTER II

FRED: THE FLORAL REFLECTANCE DATABASE - A WEB PORTAL FOR ANALYSES OF FLOWER COLOUR

INTRODUCTION

Flower colour and pigmentation are of interest to researchers in areas of both developmental biology and pollination ecology (Chittka, 1997; Dyer et al., 2007; Whibley et al., 2006). The colours of flowers are under selection by their pollinators (Kevan and Baker, 1983; Tastard et al., 2008; Waser, 1983a) and this has led to a large range of colours and colour patterns being present in the world's flora. However, flowers should not simply be categorised according to their colour appearance to a human observer, because pollinators have fundamentally different visual systems to humans, including sensitivity to different wavelength ranges. Most insects have at least three photoreceptors, normally responding to ultraviolet, blue and green light (Briscoe and Chittka, 2001; Peitsch et al., 1992); many insects have four or more spectral receptor types, whose sensitivity often extends into both long (>650nm) and very short (<300nm) wavelengths (Arikawa et al., 1987).

In spite of these differences, some studies investigating flower colours in plant communities have only considered these colours as humans perceive them (McCann, 1986; Warren and Billington, 2005; Weevers, 1952), an oversight that has been brought up repeatedly by past scholars (Daumer, 1956; Daumer, 1958; Kevan, 1972; Kevan, 1978; Kevan and Backhaus, 1998). Such neglect of insect vision is clearly inadequate, as two colours that look distinct to a human can look similar to a pollinator, and vice versa (Chittka et al., 1994; Kevan, 1983).

Spectral reflectance measurements constitute a significant improvement over merely considering flowers according to human-judged categories ("pink", "yellow", etc.). Not only are these subjective terms and not always used consistently between people, but

they do not reflect how the flowers appear to the organisms to which they should appeal, i.e. their pollinators.

We therefore developed the Floral Reflectance Database (FReD) to provide free, searchable access to reflectance spectra of a large number of flowers, thereby making available extensive information about flower colour that is not inherently human-biased and which can be used when considering the interactions between floral appearance and the visual systems of pollinators (Menzel, 1990; Menzel and Shmida, 1993). The information in FReD includes the ultraviolet reflectance of all the flowers measured, as well as their reflectance function in the spectrum visible to human observers. This allows us to build an accurate picture of how the flowers have evolved colours as an advertisement (Chittka and Kevan, 2005), and means that the colours of samples in the database can be modelled according to the visual capabilities of animals with colour vision systems different from humans, e.g. insects with an ultraviolet-sensitive receptor. Since the visual ecology of bees is so well understood, and they are also such important pollinators in a variety of habitats (Proctor et al., 1996), the Floral Reflectance Database has devoted particular attention to modelling and predicting flower colours as they appear to trichromatic bees to show how the flowers sampled would appear to bee eyes (Figure 11). However, it would be equally possible to analyse flower colours using another animal's visual system as the base.

The Floral Reflectance Database not only includes the reflectance spectra for the flower records contained within it, but the newest version also calculates the flowers' loci on the colour hexagon (Chittka 1992) and provides information on how the spectra excite each of the bee's three different photoreceptor types. It displays the information in the form of a spectral reflectance graph (Figure 12) and a diagrammatic form based on a pollinator vision model, the bee colour hexagon. The database records also contain information about where each sample was collected, as well as other floral parameters and the pollinators of the respective flower species, where known. We have brought together reflectance datasets from multiple studies for researchers to access and use freely.

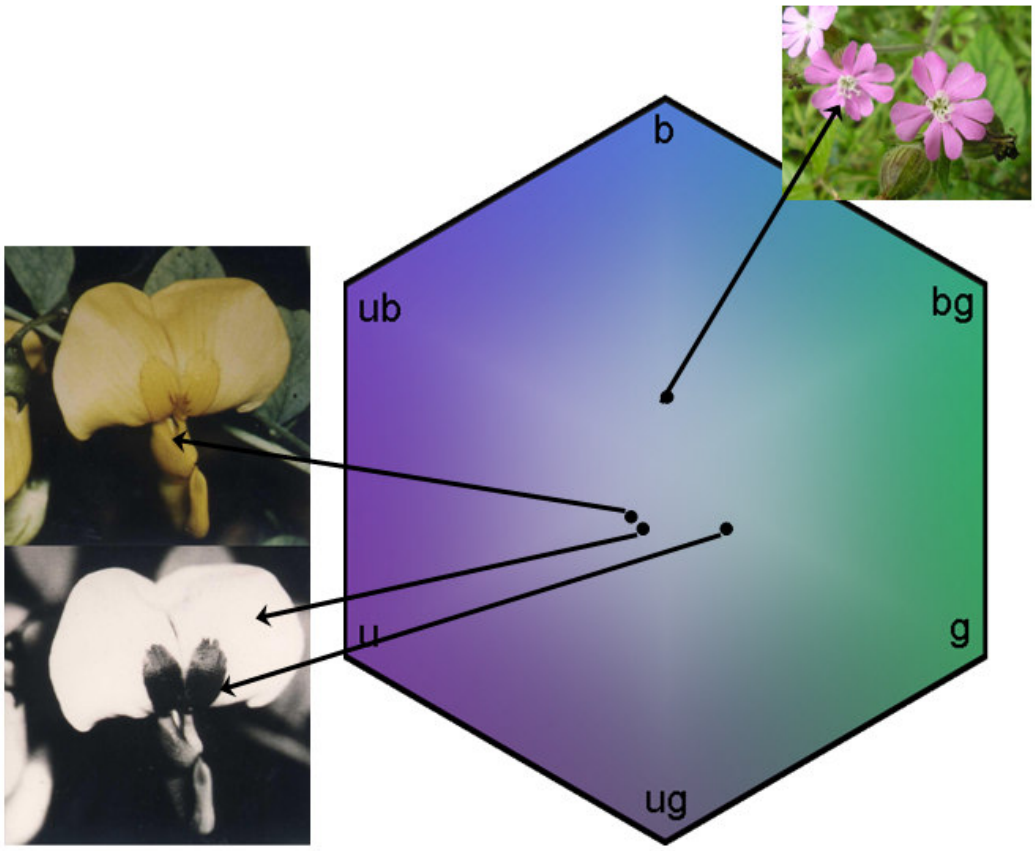


Figure 11. Bee colour hexagon diagram, showing the colour loci of three different parts of the yellow (to humans) flowers of *Colutea arborescens* (L.), including the UV-absorbing nectar guide, and also the locus of red campion, *Silene dioica* (L.). Photographs show *S. dioica* and also *C. arborescens* in both conventional and UV photography (upper picture: human vision; lower picture: UV photograph, showing markings not visible to humans but, in addition to longer wavelengths, visible to insects). Images and spectra for *C. arborescens* from Lunau (1993); *S. dioica* picture by S.E.J. Arnold.

DATA COLLECTION

The measurements in the database have been collected over the last 20 years from various sites around the world (Chittka, 1996b; Chittka, 1997; Chittka et al., 1994; Gumbert et al., 1999) and are included with permission from the collectors. Flower spectral reflectance functions were measured in the laboratory as soon as possible after collection, using a spectrophotometer (see references for model and technique – also see Chittka and Kevan (2005)). This involves directing a light (from a light source containing all wavelengths from 300 to 700nm) on to the surface of the flower and recording the proportion of incident light reflected at each wavelength using a spectrophotometer (e.g. as made by Ocean Optics (Dunedin, FL, USA) or Avantes (Eerbeek, Netherlands)) calibrated against a white standard such as one made of $BaSO_4$, which reflects light equally at all wavelengths. The readout was converted into a series of reflectance functions at wavelengths from 300 to 700nm, in increments of 1nm, a range that encompasses or exceeds the visible spectrum for most insects.

Outputs are standardised so all records in FReD are in the same format. Measurements are taken from several parts of a flower in some cases – where this has occurred, each record specifies what flower part was measured and whether this could be taken as the main colour of a flower (i.e. the dominant petal colour) or merely a secondary marking (e.g. nectar guides). Where possible, the spectral reflectance functions provided are an average for several inflorescences from multiple plants of the same species in that location, rather than simply based on a single sample.

FRED VERSION 1.0 – BETA VERSION OF DATABASE

The Floral Reflectance Database was developed originally as a beta (test) version, with basic search functionality and some other simple features.

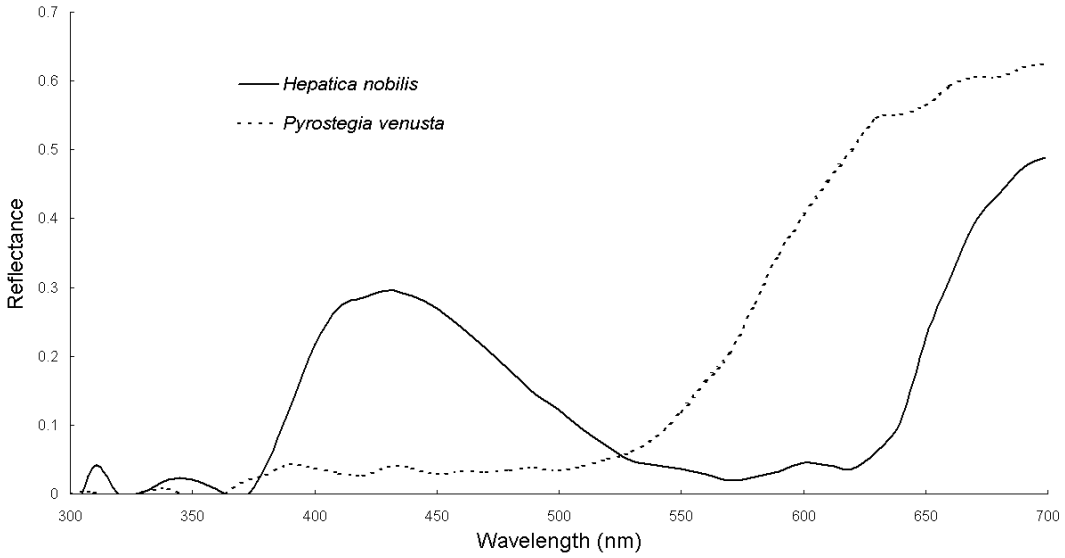


Figure 12. Reflectance spectra for *Hepatica nobilis* (Schreb.) (sampled in Germany (Gumbert et al., 1999)) and *Pyrostegia venusta* (Miers) (sampled in Brazil (Chittka et al., 1994; Chittka et al., 1993)). The reflectance is the total proportion of light at each wavelength reflected by the sample.

This version is still available at http://www.reflectance.co.uk and can be accessed by selecting the appropriate link on the front page of the site. It contains 2283 floral reflectance spectra, each spanning the wavelength range of 300 to 700nm in 1nm increments.

The beta version of the FReD website permits these records to be searched on the website either by keyword or by an advanced search form (allowing users to specify criteria such as genus, colour or location).

In addition to the reflectance files, each sample has associated information about the characteristics and collection location for the flower. These include the following fields:

- Location information: country, closest town, GPS data where available, elevation of collection site above sea level
- Taxonomy information: family, genus and species of the flower, and herbarium accession where available
- Plant characteristics: corolla diameter, floral tube length and plant height where available, principle pollinators where the information is available, information on colour to both human and bee eyes
- Collector information: name of collector and publication in which the reflectance data were first used (details of voucher specimens are given where available)

Using FReD v 1.0

The beta version of the database consists of a MySQL database with six tables (Figure 13), and a Java ServerPages (JSP) user interface, which provides an intuitive environment to allow the user to query the database. The user interface includes a basic keyword search (which will call all records containing a given word, for example "Asteraceae" or "green"), and also an advanced search, with a search form and options to select fields to display in the results. A user running either of these types of search will then be presented with a page of corresponding search results (Figure 14), with basic details for each record. Each record also includes a link to the full flower collection and referencing details, and also to an HTML file displaying the full spectral reflectance. Pollinator information is given where available, in a coded format – a pop-up window linked from the sidebar explains the codes used throughout the database.

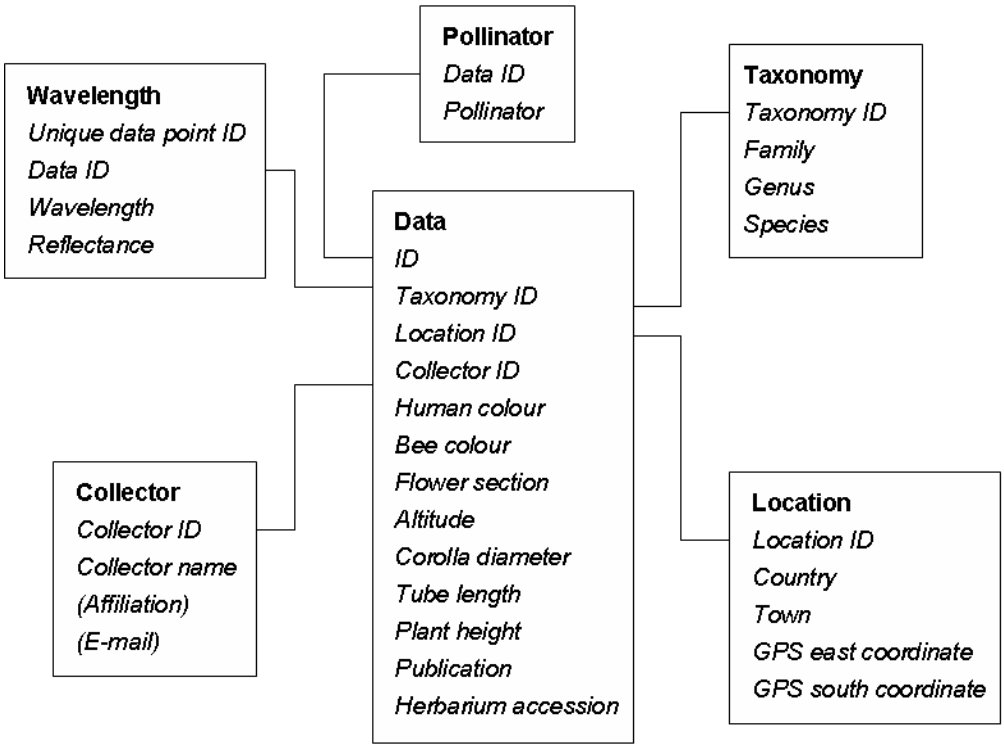
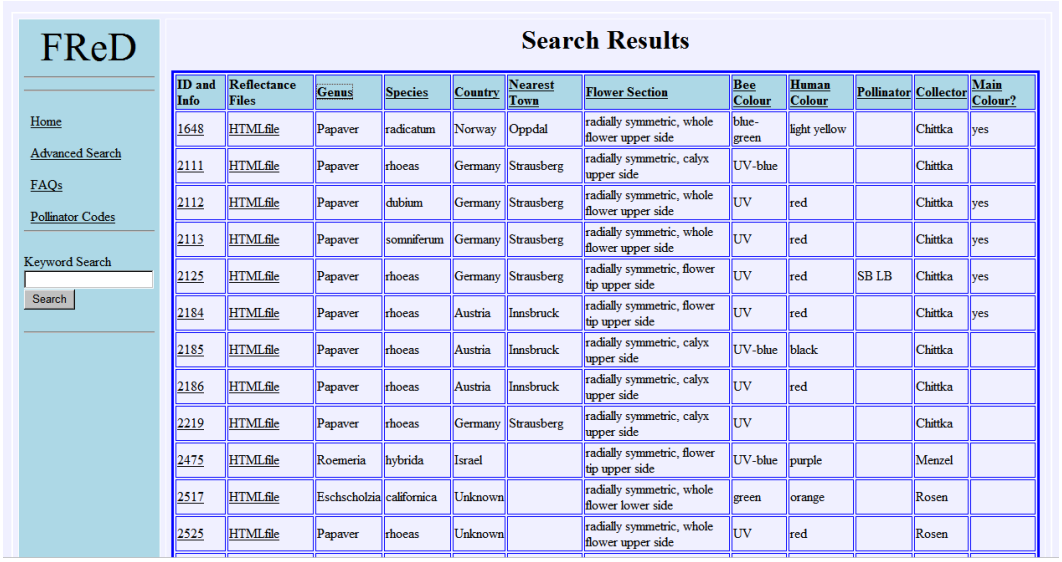


Figure 13. Database structure of FReD v1.0. Individual boxes indicate discrete data tables and the fields within each one. Lines linking boxes show data tables that are linked by identification codes (ID numbers); the linked fields are indicated here by a line from the originating table, mapping to the corresponding fields in subsidiary tables.



FReD

Home

Advanced Search

FAQs

Pollinator Codes

Keyword Search

Search

Search Results

ID and Info	Reflectance Files	Genus	Species	Country	Nearest Town	Flower Section	Bee Colour	Human Colour	Pollinator	Collector	Main Colour?
1648	HTMLfile	Papaver	radicatum	Norway	Oppdal	radially symmetric, whole flower upper side	blue-green	light yellow		Chittka	yes
2111	HTMLfile	Papaver	rhoeas	Germany	Strausberg	radially symmetric, calyx upper side	UV-blue			Chittka	
2112	HTMLfile	Papaver	dubium	Germany	Strausberg	radially symmetric, whole flower upper side	UV	red		Chittka	yes
2113	HTMLfile	Papaver	somniferum	Germany	Strausberg	radially symmetric, whole flower upper side	UV	red		Chittka	yes
2125	HTMLfile	Papaver	rhoeas	Germany	Strausberg	radially symmetric, flower tip upper side	UV	red	SB LB	Chittka	yes
2184	HTMLfile	Papaver	rhoeas	Austria	Innsbruck	radially symmetric, flower tip upper side	UV	red		Chittka	yes
2185	HTMLfile	Papaver	rhoeas	Austria	Innsbruck	radially symmetric, calyx upper side	UV-blue	black		Chittka	
2186	HTMLfile	Papaver	rhoeas	Austria	Innsbruck	radially symmetric, calyx upper side	UV	red		Chittka	
2219	HTMLfile	Papaver	rhoeas	Germany	Strausberg	radially symmetric, calyx upper side	UV			Chittka	
2475	HTMLfile	Roemeria	hybrida	Israel		radially symmetric, flower tip upper side	UV-blue	purple		Menzel	
2517	HTMLfile	Eschscholzia	californica	Unknown		radially symmetric, whole flower lower side	green	orange		Rosen	
2525	HTMLfile	Papaver	rhoeas	Unknown		radially symmetric, whole flower upper side	UV	red		Rosen	

Figure 14. Search results for FReD v1.0. When a user runs a basic or advanced search, the interface returns a table of results, with information on each of the samples matching the search criteria.

The list of search results can be sorted according to any of the fields returned (e.g. by genus) by clicking on the field label at the top of the column ("Genus").

FReD v 2.0: the finalised version

Following on from the beta version of FReD, a final version has now been developed. This has a new, more aesthetically appealing and more intuitive user interface, using more efficient code and a new coding language. The underlying database itself has also been rearranged for speed and efficiency so that in spite of containing largely the same data, the presentation formats are more useful to researchers visiting the site. Similarly, it incorporates large numbers of new features which may be of use to visitors to the website.

FReD version 2.0 contains a large number of spectral reflectance functions from flowers from all over the world, with the intention for more records to be added as they are collected and published. The most extensive information is available for flowers from Brazil and Germany.

The database remains a MySQL database but the new user interface is written in PHP. This is an open-source coding language which works in a similar way to JSP but is more widely supported and less processor-intensive. The online release of the database functions in all major browsers and is compatible with Windows, Linux and Mac operating systems; however, users of some less common browsers may experience problems with the HexSearch facility. The database is freely accessible for any user to search and view wavelength files.

The updated MySQL database now consists of 16 tables, dealing with information on the flower sample and characteristics, location, citation information, colour, collection and taxonomy information, and the wavelength measurements themselves (Figure 15).

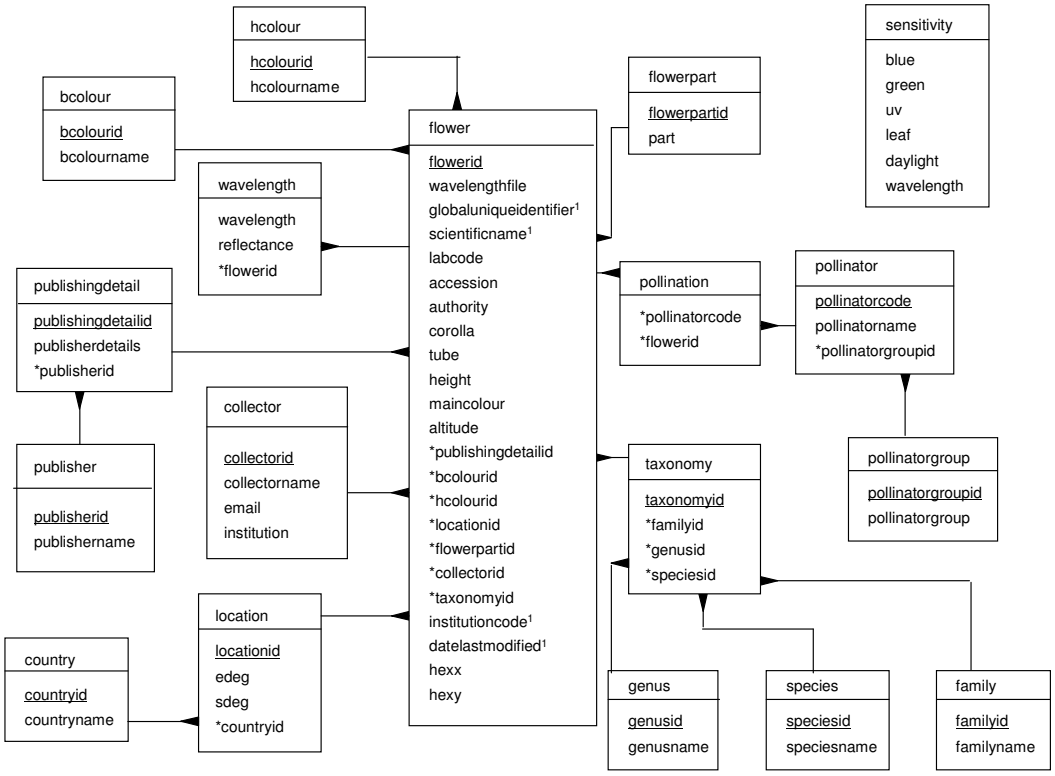


Figure 15. Table layout in FReD v2.0. The number of tables has been increased, but this has enabled the size of the database to be reduced to improve speed and efficiency of searches.

- The *Flower* table is the main table, containing important details of the sample taken, including altitude (m above sea level), plant height (cm), corolla diameter (mm) and tube length (mm) measurements, colour hexagon coordinates, and if the colour information represents the dominant colour of the flower. It also contains information on the herbarium accession number of the sample, if available.
- The *Taxonomy* set of tables provide details about the species and classification of the different flower samples. Where necessary, the colour morph or subspecies of flower can be specified in the "species" field to differentiate it from other samples of the same species.
- The *Location* set of tables provide details on where the flower sample was obtained, including GPS data where available.
- The *Flowerpart* table contains details of what flower section is being measured for each sample, e.g. calyx, tips of petals, upper lip of a zygomorphic flower, etc.
- The *Colour* tables give information on the flower colour, both as seen by a bee and a human (human colour judged by the collector in the field).
- The *Pollinator* set of tables contain the information pertaining to the pollinating species, where available.
- The *Collector* table provides information about the researcher who collected the samples.
- The *Publishing* tables give information about the published source and citation information for each sample listed in the database.
- The *Wavelength* table contains the reflectance measurements themselves.
- The *Sensitivity* table is not interlinked with the flower information, but contains information on honeybee photoreceptor sensitivity, spectral components of illumination and other measurements required to calculate colour hexagon coordinates.

The format of information contained on each flower sample in addition to the reflectance spectra is also summarised in Table 1.

Table 1 Summary of the searchable data fields in FReD and examples of the data format used in each

Field	Data type	Example
Family	varchar	Fabaceae
Genus	varchar	Trifolium
Species	varchar	repens
Authority	varchar	L.
ScientificName	varchar	*Trifolium repens* L.
Collector	varchar	Chittka
Bee colour	varchar	blue-green
Human colour	varchar	white
Main flower colour	varchar	Y
Flower section	varchar	radially symmetric, whole flower upper side
Country	varchar	Norway
Town/Area	varchar	Oppdal
GPS_East	float	[longitude coordinate, where available]
GPS_South	float	[latitude coordinate, where available]
Pollinator	varchar	bumblebees, large bees
Altitude	float	900
Height	float	15
Tube length	float	3
Corolla diameter	float	15
Publication	varchar	Chittka, L. 1996 J. Theor. Biol. 181:179-196
Herbarium accession	varchar	[herbarium accession details, where available]

Multiple samples of the same species

As previously mentioned, the database often contains multiple reflectance spectra for the same species. Different records may reflect different flower parts being sampled – e.g. the nectar guide versus the keel of the flower – in which case the part measured is specified in the "flowerpart" field. Alternatively, there may be records for different subspecies, cultivars or morphs; many species of plant have more than one floral colour morph (Whibley et al., 2006). In these cases, the "type" of plant sampled is also specified in the species field (e.g. "*Viola lutea* (w)" to indicate the white morph of *Viola lutea* (Huds.)). As the colour of the flower to human eyes is also recorded in the "human colour" field, it is additionally possible to infer the colour morph from this information.

Using the database – functions and features

The database web portal consists of several user-friendly features to facilitate access to the data and provide users with additional tools for analysis and consideration of flower colours. These include:

- Search facilities
- Colour hexagon display
- Reflectance graphs
- Raw reflectance data downloads
- HexSearch facility, to search for flowers with similar colour hexagon loci.

Search facilities

Visitors to the Floral Reflectance Database are able to use the search facilities to run basic or guided searches for flowers with specific characteristics, e.g. flowers from a particular location, of a particular species or colour, or a combination of these. The Advanced Search also allows the user to choose from which data fields he/she wishes to display results; a default selection is given, but they are free to edit this as they choose. As the basic search supports Boolean syntax (AND, OR, NOT, and use of quotes) (Frants and Shapiro, 1991), it resembles common search engines and thus is straightforward and intuitive to use.

Both types of search produce a table of results (Figure 16). The results can be ordered by field, by clicking on one of the column headings. A search summary is available at the top of the page, giving some descriptive statistics on the results returned (most common attributes of results, such as commonest colours, locations, etc.).

A user will then be able to view the reflectance spectra for all the search results. The use of AJAX (Asynchronous JavaScript And XML) technology keeps loading times as fast as possible by minimising the amount of unnecessary information displayed – a user is presented initially with abbreviated records, and can bring up a flower's full record in a pop-up window by clicking on an individual result. Equally, the search summary, containing a colour hexagon showing coordinates of all the results, is not displayed by default; however, it is available from a link at the top of the results page.

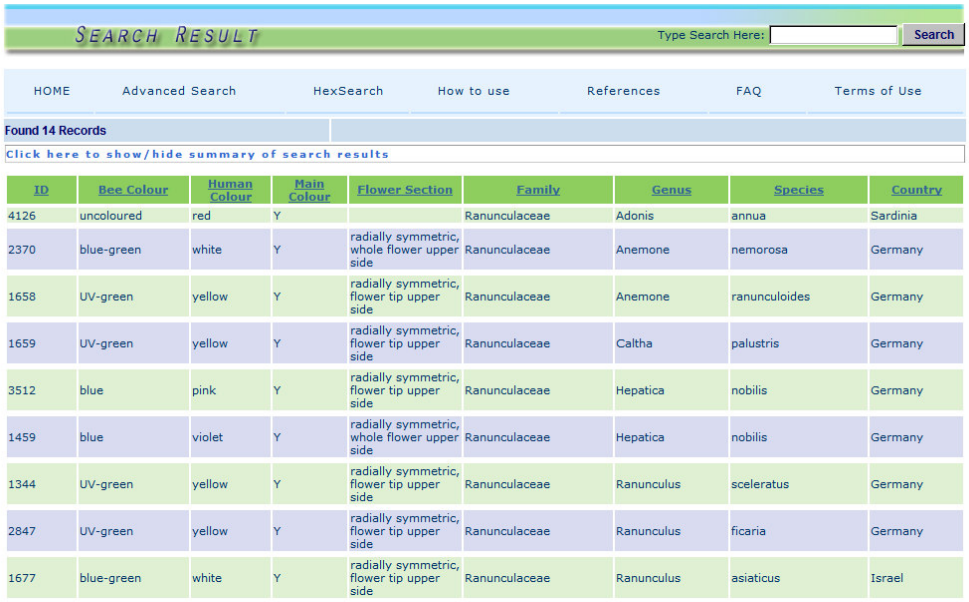
From the pop-up window for each flower record, there is a button to display the full reflectance data for the sample as a simple table of numeric values. From the page containing the table, it is possible to either return to the flower record, download the reflectance data in comma-separated values (.csv) format, or close the window and return to the table of search results.

Colour hexagon facility

The database also has the function to display the locus of each flower on a colour hexagon diagram. By making the colour loci available to users, they are able to obtain instant information about how the flower's colour might appear to a typical insect pollinator with a colour vision system similar to that of *Apis mellifera*.

The colour hexagon coordinates are calculated according to the methodology described in Gumbert, et al. (1999), taking into account the illuminating light, the reflectance of the background (assumed in the database to be leaves; an average leaf spectral reflectance is used as in Chittka (1997)) and honeybee spectral sensitivities over their visible wavelength range. Daylight spectral curves (D65) used in the calculations are taken from Wyszecki & Stiles (1982), leaf spectral reflectance data come from Chittka (1997), and honeybee spectral sensitivity curves are taken from Peitsch, et al. (1992).

a)



SEARCH RESULT

Type Search Here: Search

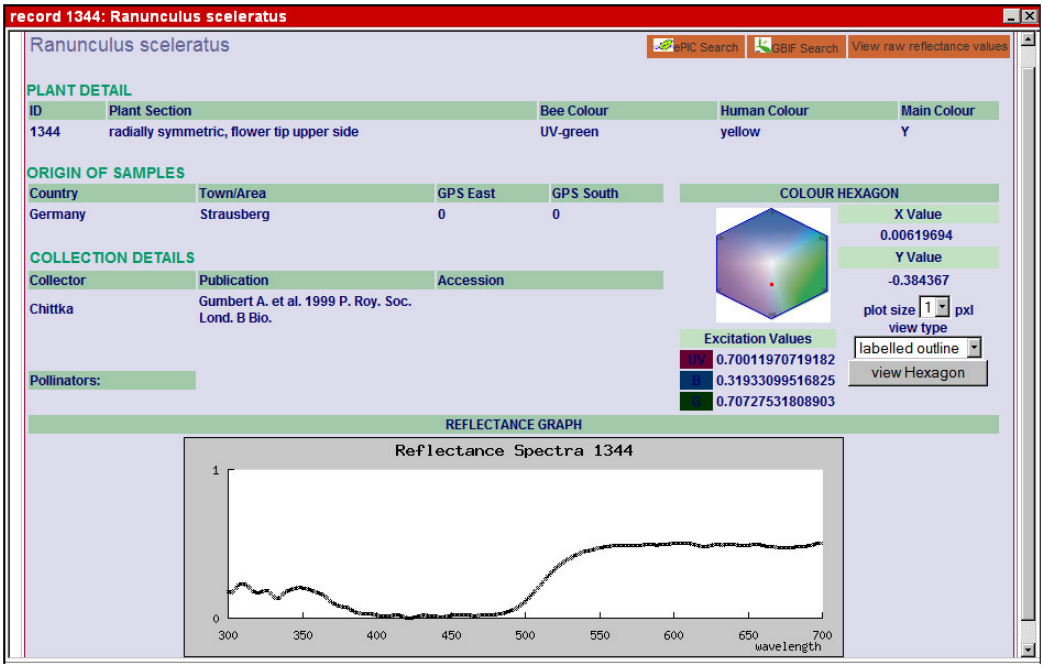
HOME | Advanced Search | HexSearch | How to use | References | FAQ | Terms of Use

Found 14 Records

Click here to show/hide summary of search results

ID	Bee Colour	Human Colour	Main Colour	Flower Section	Family	Genus	Species	Country
4126	uncoloured	red	Y		Ranunculaceae	Adonis	annua	Sardinia
2370	blue-green	white	Y	radially symmetric, whole flower upper side	Ranunculaceae	Anemone	nemorosa	Germany
1658	UV-green	yellow	Y	radially symmetric, flower tip upper side	Ranunculaceae	Anemone	ranunculoides	Germany
1659	UV-green	yellow	Y	radially symmetric, flower tip upper side	Ranunculaceae	Caltha	palustris	Germany
3512	blue	pink	Y	radially symmetric, flower tip upper side	Ranunculaceae	Hepatica	nobilis	Germany
1459	blue	violet	Y	radially symmetric, whole flower upper side	Ranunculaceae	Hepatica	nobilis	Germany
1344	UV-green	yellow	Y	radially symmetric, flower tip upper side	Ranunculaceae	Ranunculus	sceleratus	Germany
2847	UV-green	yellow	Y	radially symmetric, flower tip upper side	Ranunculaceae	Ranunculus	ficaria	Germany
1677	blue-green	white	Y	radially symmetric, flower tip upper side	Ranunculaceae	Ranunculus	asiaticus	Israel

b)



record 1344: Ranunculus sceleratus

Ranunculus sceleratus

ePIC Search | GBIF Search | View raw reflectance values

PLANT DETAIL

ID	Plant Section	Bee Colour	Human Colour	Main Colour
1344	radially symmetric, flower tip upper side	UV-green	yellow	Y

ORIGIN OF SAMPLES

Country	Town/Area	GPS East	GPS South
Germany	Strausberg	0	0

COLOUR HEXAGON

X Value 0.00619694

Y Value -0.384367

plot size 1 pxl

view type labelled outline

view Hexagon

COLLECTION DETAILS

Collector	Publication	Accession
Chittka	Gumbert A. et al. 1999 P. Roy. Soc. Lond. B Bio.	

Pollinators:

Excitation Values

UV 0.70011970719182

B 0.31933099516825

G 0.70727531808903

REFLECTANCE GRAPH

Figure 16. Sample search results from FReD v 2.0. a) First lines of the table of flower records returned by a search for "Ranunculaceae"; b) Sample flower record for one of the species in genus *Ranunculus*, showing the colour hexagon coordinates and spectral reflectance graph.

Using those data, the relative excitations of the bee's three photoreceptor types can be calculated, and these three vectors can be converted into coordinates in a two-dimensional colour space diagram (e.g. the colour hexagon), as shown in Figure 11.

The flower records present the colour hexagon coordinates for each sample on a schematic diagram, but also give the coordinates numerically, and the excitation values for the three bee photoreceptor types are provided for users who wish to use these values in alternative models of colour vision. The colour hexagon diagram for each record is provided as a Portable Network Graphics (PNG) file, and therefore can be downloaded by users if necessary.

Reflectance graph

Spectral reflectance functions for each record are displayed as a graph in the flower record, for users to assess what pattern of reflectance a flower possess, where the major reflectance peaks occur, etc. These are generated dynamically using the measurements in the Wavelength table, and displayed as a PNG file, so they can be displayed separately from the search results, and saved to a user's local hard drive if required.

HexSearch facility

This is an additional function which may be of particular use to researchers interested in mimicry or the effects of particular pigment compounds. It permits searches for flowers with similar bee colours rather than merely searching according to gross colour category.

The user can select up to ten loci of interest on the colour hexagon, which are searched simultaneously, and can specify their position on the hexagon by clicking in the relevant place on the map provided. The user then selects the radius of the search area (in colour hexagon units (hu)), and the function returns a page of results, comprising the flowers with colour hexagon coordinates within the area specified. Hex searches can either be general (e.g. specifying a 0.5 hu radius) or more specific (e.g. 0.05 hu). The centre point of the search can be moved as many times as required.

Downloading reflectance data and compatibility with other databases

The database was designed to be used by researchers, and thus we are aware that users may wish to download spectral reflectance curves for their own use. This option is available by selecting the option to "view raw data" and then "view CSV file". They can then download the reflectance measurements for each species as a .csv (comma-separated values) file, which can be imported into spreadsheets or into other databases.

In order to facilitate potential future integration of FReD into a larger meta-database, we have organised the database with a structure in line with the international DarwinCore standard. FReD is also linked from the website of the Royal Botanic Gardens, Kew, under their lists of data and publications. We have set up links from FReD's flower record pages to search results in the electronic Plant Information Centre (ePIC), the large plant database run by RBG Kew, and also to the Global Biodiversity Information Facility (GBIF). This means that a user searching FReD will also be provided with a link from each flower record to corresponding search results for that species in ePIC and GBIF, widening the information available to users of FReD about species in the database.

Applications for the database and future developments

We expect the Floral Reflectance Database to be a valuable tool to researchers wishing to make between-habitat or global comparisons of floral colour. With the large number of samples in the database already, it has applications in meta-analyses. We also anticipate its usefulness on a smaller scale, to provide detailed information on the exact colour of flowers of particular species.

By providing full reflectance spectra of all the samples, we are making available information which makes no *a priori* assumptions about the colour vision system viewing the flowers save that its sensitivity extends from 300-700nm. The database provides a selection of natural, ecologically-relevant stimuli that could be used in a variety of colour modelling studies (in the manner of Maloney (1986) and Chittka (1996b)). Additionally, as there are species from many plant families of differing ages, the data may, in conjunction with other information about species, have uses in studies

of flower colour evolution and investigations of how floral colour relates to other characteristics.

In the longer term, we intend to add more spectral reflectance readings, including data from South Africa and Costa Rica. We eventually hope to accept reflectance data from other users of the database provided that the measurements are of high quality and include the most important associated information about the sample being measured (i.e. at least species, flower section being sampled, relevant publications, location in which sample was collected). The database also has the potential to be extended to contain additional data fields of interest to pollination studies, such as details of flowering phenology.

We anticipate that as the database grows to encompass more species from diverse international locations, it will become an even more useful resource for many areas of research requiring an objective consideration of flower colours.

CHAPTER III

FLOWER COLOURS ALONG A NORWEGIAN ALPINE ALTITUDE GRADIENT: BEE AND FLY PERSPECTIVES, AND THE EFFECT OF PHYLOGENY ON ALPINE FLOWER COLOUR

INTRODUCTION

Plants growing in mountainous regions are faced with a range of challenges. As well as having to contend, potentially, with high winds, desiccation and extremes of cold, they also face increased ultraviolet exposure and pollinator limitation when the temperatures and winds grow too extreme for pollinating insects to fly (Totland et al., 2000). Many strategies employed for dealing with such habitats have already been investigated in depth (Totland et al., 2000), but what still warrants further investigation is how flowers at high altitude might respond in terms of their colour.

Why might some colours prove more beneficial for a plant over evolutionary time than others? There is some evidence that some colour morphs (particularly those containing anthocyanin pigments) have increased resistance to certain environmental conditions, e.g. desiccation (Warren and Mackenzie, 2001), cold (Ben-Tal and King, 1997; Chalker-Scott, 1999), and to other challenges such as herbivory (Johnson et al., 2008), all factors which are likely to differ in importance at different elevations. Similarly, the increase in ultraviolet at high elevations can be damaging to some plant cells, and it has been found that floral pigments such as anthocyanins may also confer protection against UV damage (Mori et al., 2005). The various protective effects of anthocyanin pigments would indicate that more strongly-coloured (blue, red, purple) flowers might be more abundant under more environmentally stressful conditions.

Additionally, flower colour is under selection by pollinators (Wertlen, 2006), as is suggested by the fact that insect colour vision by far predates flower colour, and yet bee photoreceptors, for example, are optimal for discriminating the colours of flowers (Chittka 1997). There are indeed several studies associating shifts in flower colours with

shifts in pollinator type (Altshuler, 2003; Bradshaw Jr. and Schemske, 2003). In alpine areas the numbers of pollinators present will decrease overall with increasing altitude, and will change in composition; some insect groups are less able to function at very high altitudes than others (Kearns, 1992; Totland, 1992). Therefore, different pollinator guilds dominate at different elevations and thus the selective forces on flower traits might be expected to differ. The pollination syndrome hypothesis, which has often been used as the basis for studies of pollination systems for many years (Faegri and van der Pijl, 1978), postulates a strong association between different pollinator guilds and particular suites of floral characteristics, in particular aspects of morphology and colour (e.g. the zygomorphic, closed and blue/purple "bee flowers"; large, white "moth flowers" with long corolla tubes). Based on this theory, a changing pollinator composition at different elevations may be expected to lead to different "optimum" colours being present at different altitudes depending on the dominant pollinator types and the colours that appeal to them.

For example, the ability of flies to forage at higher elevations than bees (Kearns, 1992; Lázaro et al., 2008; Totland, 1993) might perhaps lead us to expect that the flower colours traditionally thought to be associated with fly pollination (appearing white and yellow to humans) would be more abundant at high altitudes. Flowers appearing white (and also pink) to humans are mostly blue-green for bees and other trichromatic insects (Kevan et al., 1996), whereas yellow flowers can be either green or UV-green, depending on their UV reflectance, to such insects (Chittka et al., 1994). The recent study by Lázaro et al. (2008) also noted that butterflies in the Norwegian mountains seem to become more important pollinators as a group at higher elevations (constituting just 2% of the flower-visiting insects at the lowest altitude study site, but this increased to 7.9% at the highest altitudes), and this would perhaps lead us to expect that "butterfly colours" might be accordingly more abundant in high alpine locations. Butterflies' innately preferred colours can vary vastly depending on species and individual (Neumayer and Spaethe, 2007) so it is difficult to generalise accurately. However, such colours may appear typically pink, purple and red to humans; from an insect's perspective, often containing very long wavelength reflection, and perhaps also reflection in the UV/violet part of visual spectrum (Lunau and Maier, 1995; Neumayer and Spaethe, 2007).

Bees appear to be especially dominant at low to medium elevations (i.e. below the treeline, in sub-alpine habitats) (Lázaro et al., 2008), their large body size allowing foraging in the relative cold, but their high energetic requirements perhaps restricting their activities at very high elevations (Arroyo et al., 1982). Bees of many species have an innate preference for UV-blue and blue flowers (Giurfa et al., 1995; Raine et al., 2006), but this is easily modifiable by learning, as are the innate preference in many other pollinating insects such as hoverflies and butterflies (Lunau and Maier, 1995); bees also make a significant number of visits to yellow flowers, especially those which appear UV-green to their vision (McCall and Primack, 1992), but this is likely to be learned rather than innate.

There have been previous attempts to document the effects of altitude on flower colours present (see Totland et al. (2000) for a summary) – Weevers (1952) observed that there were more blue flower species in upland areas than in lowland areas (both in Switzerland above 1100m and Java above 1500m), and Kevan (1972) and Savile (1972) observed that flowers in alpine areas and arctic regions (which are climatically similar to alpine areas) tended to consist of a higher proportion of white and yellow species. Many of these earlier studies, however, contain primarily observational recordings that are not well supported by statistical power. More importantly, perhaps, with the exception of Kevan (1972), some of these studies have considered flower colour principally from the human perspective, without fully taking into account the more recent understanding of pollinator visual systems and how these differ from human eyes (Chittka and Kevan, 2005; Chittka and Menzel, 1992; Menzel and Shmida, 1993).

These differences are fundamental: all insects so far extensively tested have UV receptors with a maximum sensitivity between around 330 and 375nm (i.e. in the UV range where human eyes have no sensitivity) – this includes bees and other Hymenoptera, Lepidoptera, Coleoptera, Hemiptera, Diptera, etc. (Briscoe and Chittka, 2001). As discussed in the introduction, bees, which are the most important pollinators in Norway at all but the high alpine elevations (Lázaro et al., 2008), also have blue and green receptors, but lack red receptors. Other insects, including butterflies and flies, have rather different colour vision systems, in some cases more complex than those of bees or humans (Briscoe and Chittka, 2001; Morante and Desplan, 2008). Flies and butterflies both have very variable numbers of photoreceptor types, depending on

species (e.g. five in the case of the housefly, *Musca domestica* (Hardie, 1986) and the butterfly *Papilo xuthus* (Eguchi et al., 1982), three so far identified for the butterfly *Papilo protenor* (Eguchi et al., 1982)). Whether their colour processing behaves as in bees (Backhaus, 1991) is also not completely understood, but research indicates that flies' integration of photoreceptor signals can be entirely different to that of bees (Morante and Desplan, 2008; Troje, 1993).

We sought to investigate whether the flower community growing at high altitude has a different pollinator-relevant colour composition to that of lower altitude areas, by using a data set collected along a transect in the Norwegian Dovrefjell mountains from 700 to 1600m elevation. This is of especial interest in light of the recent study by Lázaro et al. (2008), in which plant communities at different elevations in southern Norway were surveyed for floral colour and morphology, and this was combined with visitation data. The study found evidence of association between traits (including colour) and pollinator, showing that flowers in alpine areas generally seem to be visited by pollinators that could be predicted according to the pollination syndrome hypothesis. Thus it seems that the predominance of pollinator types (and subsequently the main foraging strategies in evidence) varies with elevation and could potentially have strong effects on which plant species are most abundant.

In our study we consider flower colours as seen by their pollinators, firstly using the well-studied model of bee colour vision, secondly using a model of fly colour vision, and also using the raw reflectance spectra of the flowers, thereby considering their colours without bias towards any vision system. As bees are important pollinators in most European habitats, including those in Lázaro et al. (2008) (though much less so at higher elevations), and as bee vision is very well characterised and understood (Chittka and Raine, 2006), it seems reasonable to focus first on the colours as seen by a bee – as spectral sensitivities of bee photoreceptors are well-conserved throughout the taxon, it is reasonable to use the extensive knowledge of *Apis mellifera* as a good approximation for how colours would look to honeybees, bumblebees or solitary bees alike. However, in acknowledgement that bees are only one of several pollinating species present, we also considered how the colours might appear to a fly (using a model of the blow fly (*Lucilia* sp.) as described in Troje (1993); this is not a pollinator, but no other fly colour vision model is currently available). Additionally, given that insect vision can be so

variable and complex, we analysed the raw spectral properties of the flowers. This encompasses wavelengths invisible to humans (<400nm) but does not impose a particular visual system on to the results.

According to the pollination syndrome hypothesis, we would predict a change in the proportions of colours at different elevations, according to the dominant pollinator groups present. This is expected to include a general decrease in species bearing colours associated with bee pollination, i.e. bee-blue and UV-blue, at the highest altitudes. These colours generally correspond to the human colours blue and violet, but it is important to note that there is no absolute correspondence between bee and human colour categories, which highlights the importance of moving away from an anthropocentric view of flower colours. The pollination syndrome hypothesis would also predict an increase in the proportion of bee-blue-green and green species at high elevations in accordance with the increasing importance of fly pollinators. Overall, therefore, one would anticipate a gradual decrease in UV-blue and blue flowers and an increase in blue-green and green ones with elevation, in accordance with the shift from bee-dominated to fly-dominated pollination. This is supported by the findings of McCall and Primack (1992), who observed that purple and yellow (to humans) flowers were the most visited colours in lowland woodland (primarily by bees), whilst yellow and white were the two most visited colours in alpine tundra (most visits being by flies), with blue-purple flowers being much less frequently visited. Absence of such a pattern would indicate that flower colours are not determined only by pollination syndromes and that other factors may be affecting which colours are optimal.

These predictions could, however, be complicated by the evolutionary history of plants present in the communities we studied. To investigate whether phylogeny was significantly linked to the colours of flowers we also took evolutionary history into account, building a phylogenetic tree of the species and testing whether phylogenetic distance correlates with differences in colours, and whether there is an interaction between altitude and phylogeny that affects flower colour. We found that closely related flowers do tend to be more similar in colour than chance would predict, which is unsurprising considering not all plant lineages possess the pathways to make all flower pigments.

Conversely, our study finds no evidence for a significant effect of elevation on flower colour as seen by bees or as considered without bias to a vision system; nor was there a combined effect when both altitude and phylogenetic distance were considered as possible predictive variables for flower colour. Thus we conclude that whilst high altitude does not result in a different flower colour composition compared to lower altitudes, the evolutionary history of flowers is, as would be expected, an important determining factor in their colour. It appears that pollinator selection alone cannot account for the colours of flower species, since the colour composition of these communities does not change even whilst the pollinators are expected to.

MATERIALS AND METHODS

Study sites and data collection

The study site was located in the Dovrefjell National Park in Norway, near to Oppdal. Data were collected in June 1992 in the altitude range 700-1600m a.s.l. (sub-alpine to high-alpine), along a transect starting near Kongsvoll Biological Station and continuing to the Knutshø peaks east of the station. The species of all the plants in flower growing along the transect were noted, along with the altitude at which they were recorded. Spectrophotometer readings from 300-700nm (i.e. including the ultraviolet range) were taken of the flowers of all species present, using the methods described in Chittka and Kevan (2005) and Dyer and Chittka (2004a). (All spectral reflectance functions are available from the Floral Reflectance Database http://www.reflectance.co.uk.) A total of 74 species were sampled from this location and are listed in Appendix II.

Analysis 1: Effect of elevation on bee colour composition of the community

We divided the transect into three elevation ranges: lower altitudes (700-1000m), intermediate altitudes (1000-1300m) and high altitudes (1300-1600m), and recorded which species were found in each, and which spanned more than one range. At this location, the low altitude group corresponds to the vegetation of mountain meadows, stream beds, and some forests (mainly birch); the intermediate group covers the first

zone above the treeline; the high altitude group comprises vegetation growing on rocky, unstable soils. Although the highest mountains in Norway are over 2000m, considering that the mean temperature between June and September in the Sør-Trøndelag region is typically 8°C (Østereng, 2004), the range sampled still extends into regions that can at times be too cold for many pollinator species to fly. Thus the dominant types of pollinators will change significantly within the range sampled (Totland, 1993; Totland et al., 2000), with an increase in muscoid fly species, a decrease in bee and beetle species and possibly an increase in butterfly species with increasing elevation.

We first categorised the flowers by colour as perceived by bee pollinators, since these are one of the two most important pollinator groups in this habitat, according to their loci in the honeybee colour hexagon (Chittka, 1992; Gumbert et al., 1999). Previous studies have indicated that the division of the colour hexagon into six particular categories corresponds well to the actual distributions of flower colours present in nature (Chittka et al., 1994). Thus, we classified flowers as either bee blue, blue-green, green, UV-green, UV or UV-blue (see Appendix II).

We used Microsoft Excel with the Bootstrap add-in (Barreto and Howland, 2005) to investigate whether there is an association between flower colour and elevation. We compared all the species that occurred in the same altitude group or combination of groups (e.g. low and medium) pairwise, counting the total number of times two plant species occurring across the same ranges also shared the same flower colour. This yielded a measure, $N_{\cap}$, of the association between flower colour and altitude ranges of the flowers. Then we reassigned the flower colours across the sample 10,000 times, whilst keeping the altitude range over which each species is found constant, and tracked how $N_{\cap}$ varied with each trial. If particular colours are strongly dominant at some altitudes, $N_{\cap}$ will be disproportionately high.

By noting the number of trials in which either the $N_{\cap}$ value observed in the data set, or a more extreme value (either smaller or larger), was obtained, we could discover whether species growing in particular elevation ranges exhibited increased or reduced probability of sharing the same colour that was obtained by chance in our randomisation trials.

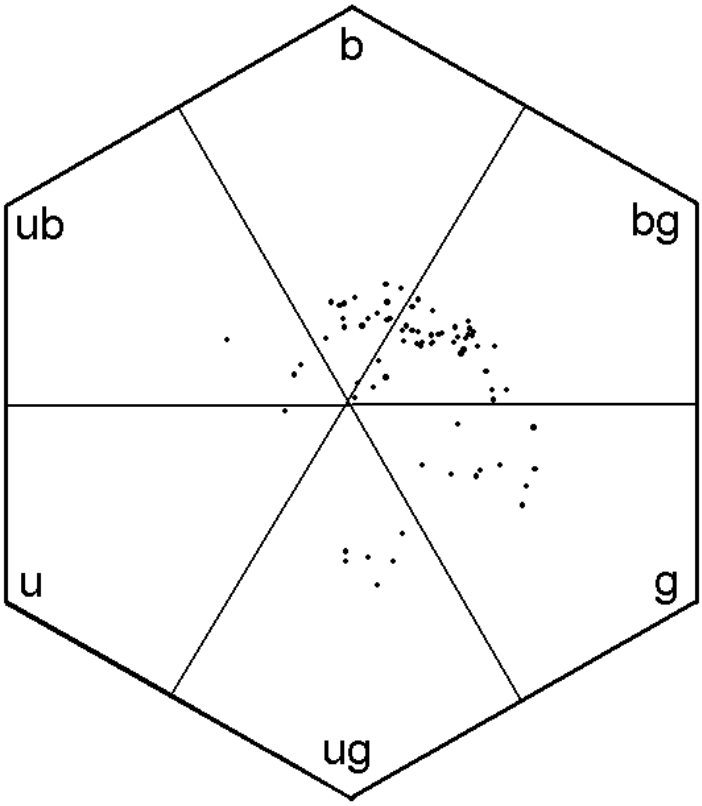


Figure 17. Bee colour hexagon of the species measured. The six segments correspond to the six bee colour categories used in this analysis (b = blue, bg = blue-green, g=green, ug=UV-green, u=UV and ub=UV-blue). Loci are calculated according to the relative stimulation of the three receptor types (UV, blue, green) elicited by the stimulus.

Analysis 2: Effect of elevation on fly colour composition of the community

As bees are not the only pollinators in this habitat, and their importance as pollinators decreases at the highest altitudes, we also looked at patterns in flower colour as seen by flies. The model we used is that of Troje (1993), based on the blow fly *Lucilia* sp., in which stimuli across quite wide spectral ranges are not discriminated, but are discriminated from stimuli in other spectral ranges, with category boundaries at 400 and 515nm. Many dipteran species (Morante and Desplan, 2008; Troje, 1993) have four photoreceptor types, typically referred to as R7p (short-wavelength UV), R7y (longer-wavelength UV/violet), R8p (blue) and R8y (green). The integration system compares relative excitations of the two p-type receptors and the two y-type receptors and the receptor of each pair stimulated most strongly determines the colour the fly perceives. This results in four colour categories, which could be regarded as fly-UV, -blue, -yellow and -purple (purple referring in human vision to a colour where the shortest and longest wavelength receptors are stimulated most strongly), with all stimuli in one category being regarded by the fly as chromatically indistinguishable.

Based on the known receptor sensitivities, and spectral reflectance functions of the flower species, we categorised the flowers into four fly colour groups and then ran a similar randomisation analysis to that used in Analysis 1, once more with 10,000 repeats. This produced a probability distribution of how many species flowering in the same altitude ranges would also share the same colour, were colours randomly assigned to species, which can be compared to the actual number of times this occurred in the real dataset.

Analysis 3: Distributions of absolute flower colours overall by elevation group

Since an alternative possibility is that flowers are selected by abiotic factors, and because fly vision is still incompletely understood therefore cannot be modelled as accurately as bee vision, we also analysed the spectra independently of the consideration of any visual system. We simplified the spectra to the values obtained at 50nm intervals over the range originally measured, and performed a principal components analysis on these data using SPSS for Windows. To test whether the coordinates fell into distinct clusters according to altitude group, we performed a

MANOVA on the points. This provides information on whether the reflectance functions of flowers at the different elevations differ in terms of their physical properties, regardless of the visual system that perceives the flowers.

Analysis 4: Effect of evolutionary history on flower colour

It is possible that phylogeny is a stronger predictor or constraint of flower colour than any selective action of pollinators or abiotic factors within a habitat, as evidenced by the findings in Chittka (1997) that plant families often tend to have flowers of largely two or three bee colour groups, with fewer flowers of other colours. Therefore, it was important not to neglect the existence of phylogenetic constraints of flower colour.

We constructed a phylogenetic tree using existing published DNA sequence information of the *rbcL* gene. This gene has already been extensively used in phylogenetic studies as it is well conserved throughout the angiosperms (Angiosperm Phylogeny Group, 2003; Chase et al., 1993). We used the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/) to search for *rbcL* sequences for the species present in the habitat. When a complete or near-complete *rbcL* sequence was not available for a particular species we recorded, we substituted a sequence from a species of the same genus.

In some cases, no sequence was available for any species in the genus; in these cases we used a close relative from the same family. This is valid provided that the relative is more closely related to the original plant species than to any of the other species sampled in the habitat. Thus, we used a *Dimorphotheca sinuata* sequence in the place of *Antennaria dioica* and a *Platanthera ciliaris* sequence in the place of the two *Dactylorhiza* species. This is justified by Kim and Jansen (1995) and Aceto et al. (1999), which place *Dimorphotheca* and *Antennaria*, and *Platanthera* and *Dactylorhiza* close together on phylogenetic trees. For three species (*Viscaria alpina, Tanacetum vulgare* and *Hieracium* sp.), we were unable to find any appropriate substitute sequences (sequences for other species from the same family were already included in the analysis, but we were unable to find species that were more closely related to the three above species than to the others in their families), so these species remain unresolved in this study and were excluded from the subsequent statistical analysis.

Appendix III lists the species originally recorded in the habitat, and also the data relating to the sequences used to resolve the relationships, including the species from which the *rbcL* sequences were obtained, the accession details of the samples and the relevant references.

We aligned the sequences using PAUP* (Swofford, 2002), then constructed a tree using Maximum Parsimony. We used a heuristic search with the Tree Bisection Reconnection (TBR) swapping algorithm. We performed 1000 replicates for stepwise addition, saving only the 5 best trees from each replicate. The best trees produced were used to create a strict consensus tree, using the two monocot genera (*Tofieldia* and *Dactylorhiza*/ *Platanthera*) to root the tree following the Angiosperm Phylogeny Group (2003), thereby resolving the dataset at the genus level. Two genera (*Saxifraga* and *Silene*) contained three species that were all present at the study site. In order to resolve the relationships between the species within these genera, we used the internal transcribed spacer (ITS) 1 sequences (information provided in Appendix IV).

Using MacClade (Maddison and Maddison, 1992), we then manually substituted the original species names from the habitat in place of the species providing the *rbcL* sequence information. Because of the relatively small number of taxa in our sample, some genera (specifically those genera in the Brassicaceae and Saxifragaceae) were resolved incorrectly according to the current phylogeny published by the Angiosperm Phylogeny Group. In these cases, we corrected the tree in MacClade according to the most recent information of the APG using http://www.mobot.org/MOBOT/APGroup/, moving taxa to their correct branches as given in this resource.

All major lineages contained at least two different bee colours, though the Ericaceae in this sample consisted only of bee-blue and bee-blue-green species. We used MacClade to test whether the distribution of colours with respect to the known phylogeny deviated significantly from random, and also whether species on the tree showed a pattern in their maximum elevations relative to their phylogeny. We tested for random versus nonrandom distribution of traits by shuffling the characters (colour or maximum elevation) 1000 times and testing whether the tree lengths obtained differed significantly from the tree length obtained from the actual data. If the characters are

nonrandomly distributed in the actual tree, the tree length will be significantly shorter than for random reassignments of characters.

To investigate whether there was an interaction between the effects of phylogeny and altitude in determining flower colour, we constructed a distance matrix based on phylogenetic distance between species on the tree. As the sequence data did not perfectly correlate with established trees, we did not use *rbcL* genetic distance in our measure as this would produce anomalous distances; instead we measured distance in terms of the number of nodes in the tree between pairs of species. We created two other similar distance matrices in SPSS, based firstly upon elevation ranges of the species, and secondly on colour distances (both derived from raw spectra and based on calculated Euclidean distances between colour hexagon loci).

If evolutionary history constrains flower colour, one would anticipate that the colour distance matrix would correlate significantly with the phylogenetic distance matrix. Furthermore, if there was a combined effect of phylogeny and elevation on flower colours, an aggregate distance matrix containing combined information on phylogenetic distance and dissimilarity of elevation range would be expected to correlate with a matrix of colour distances – i.e. the closer species are in evolutionary history and elevation range, the more similar their colours. We used the Mantel test from the ade4 package in the R statistical package (R Development Core Team, 2004) with 1000 repeats to test whether this was the case.

RESULTS

The flower colours of the different plant species are shown plotted on a colour hexagon in Figure 17. The colour composition of the flower populations in the different elevation groups is shown in Figure 18; graphs show the bee colours of the flowers, as used in the analyses, and also the species as classified by human colours, for reference.

Analysis 1 results: Effect of elevation on bee colour composition of the community

The commonest bee colour at all altitudes was blue-green (52% of flowers overall), and the proportion of blue-green flowers increased from low to high altitudes, from 45% in the low altitude group to 67% in the high altitude group. Whilst not significant (see below), this trend is in line with predictions based purely on the concept of pollination syndromes – at high altitudes where flies are the dominant pollinator type flowers should, according to this hypothesis, be more likely to be human white, i.e. bee blue-green, than at lower elevations. By contrast, the proportion of bee-blue flowers (usually blue or purple to humans) declined with increasing altitude; only 5% of the flowers recorded above 1300m were blue to a bee's eyes.

The analysis showed no significant tendency for flowers in the same altitude group to share the same colour more often than chance ($p = 0.144$), and this holds when each altitude is considered individually, indicating overall that no flower colour is more dominant than expected at a particular altitude.

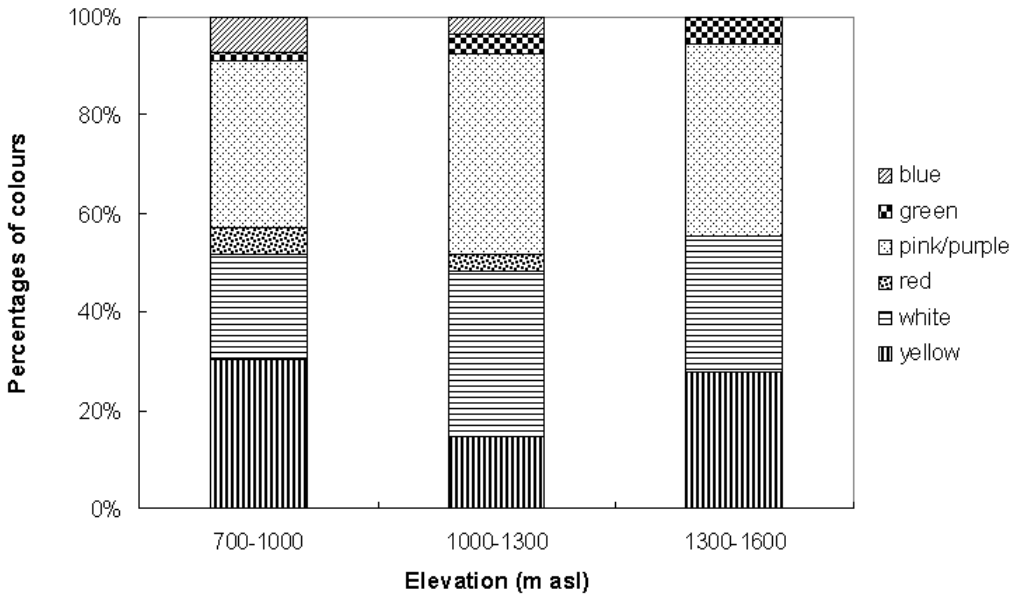
Analysis 2 results: Effect of elevation on fly colour composition of the community

The commonest fly colour categories were "fly-yellow" (50% of species) and "fly-blue" (38% of species); "fly-UV" and "fly-purple" categories contained only 3 and 6 species respectively. Increasing elevation was associated with an increase in the percentage of fly-yellow flowers (51% to 73%) and a decrease in fly-blue and fly-purple flowers (from 36% to 20%, and 8% to 0% respectively). However, these changes are not statistically significant: randomisation analysis revealed no trend for flowers growing in the same altitude ranges to share the same fly colour ($p = 0.594$).

Analysis 3 results: Distributions of absolute flower colours overall by elevation group

The results of the principal components analysis are shown in Figure 19. The distribution of colours from all three altitude groups appear to overlap heavily, and

a)



b)

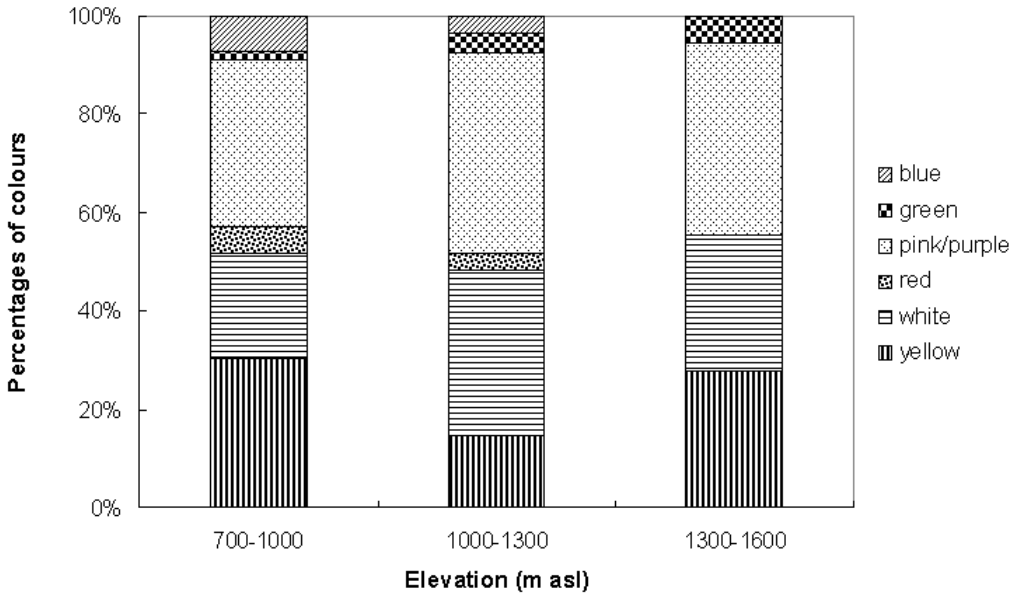


Figure 18. Percentages of different flower colours present at the survey site, with increasing elevation. Flowers are classified on their appearance according to a) the bee visual system and b) the human visual system. (Number of species recorded at each elevation range: 700-1000m, 58; 1000-1300m, 27; 1300-1600m, 18.)

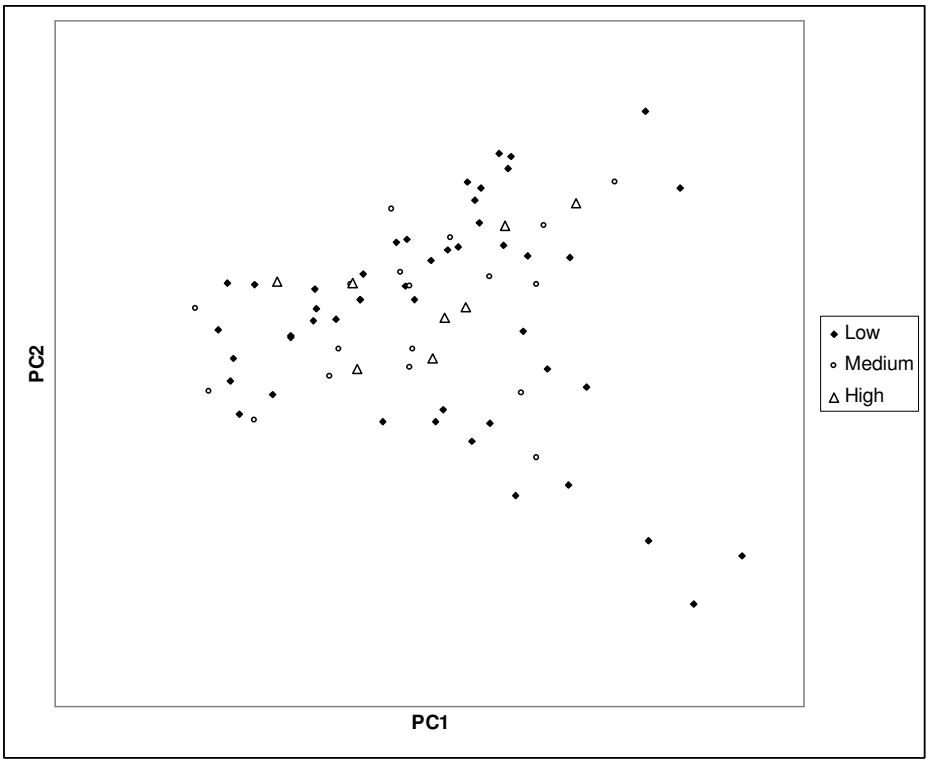


Figure 19. Principal components analysis of spectral reflectance data. Here, flowers are classified as low, medium or high elevation based on the maximum elevation at which they were recorded (<1000m, <1300m and <1600m respectively). Variation accounted for by principal component 1: 45.177%; principal component 2: 25.871%.

indeed the MANOVA reveals no difference between the groups of points ($p = 0.913$), indicating that the communities of plants at all elevations share statistically indistinguishable physical reflectance properties, at least with the sample sizes available to us.

Analysis 4 results: Effect of evolutionary history on flower colour

The phylogenetic tree of the plant species present at the study site is shown in Figure 20, with the colours included for reference purposes. The tree length when maximum elevation was mapped on to the tree is significantly shorter than chance ($p = 0.029$), indicating that growing at high elevations is a trait that occurs nonrandomly with respect to phylogeny. By contrast, in this analysis, the tree length for colour did not differ significantly from random ($p = 0.250$), giving no evidence in this particular analysis of a pattern of colour relative to phylogeny.

When we compared a matrix of phylogenetic distances with a matrix of colour distances (based on bee colour hexagon coordinates), there was a significant correlation ($p = 0.018$), indicating a significant tendency for plant species that are closely related to also have a similar flower colour. However, when colour distance was instead calculated from the raw spectra, this correlation disappeared ($p = 0.174$), suggesting that although flower colour as bees perceive it is constrained by evolutionary history, absolute colour does not seem to be.

However, we found no significant correlation between the matrix of colour distances and the aggregated matrix of phylogenetic distance and dissimilarity in altitude range when the colour distances were derived from raw spectra ($p = 0.123$) or hexagon loci ($p = 0.118$). This indicates that phylogenetic distance across the data set does not interact with altitude to affect flower colour.

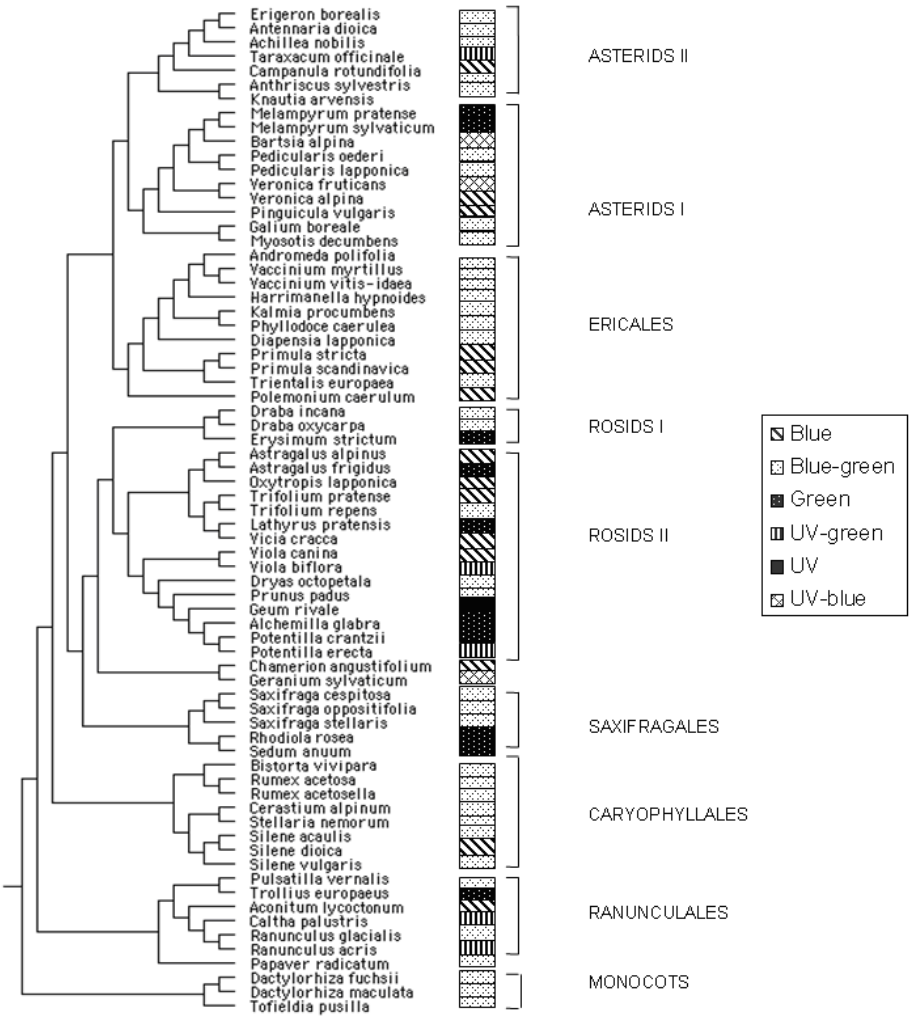


Figure 20. Phylogenetic tree of the species recorded at the study site. We used *rbcL* to resolve relationships to the genus level, and *ITS1* sequences to resolve species within genera where necessary.

DISCUSSION

Previous authors have made observations about the colours of flowers in alpine areas, stating that the colour composition of high-altitude and arctic communities differs from those at lower elevations (Kevan, 1972; Savile, 1972; Weevers, 1952). In this study of flower colours along a single transect in the Norwegian alpine flora, we sought to test whether any of these observations can be supported statistically. Unlike most of the previous authors, we considered the flower colours as they would be seen by insect pollinators, as this better reflects the selective pressures on those flowers, and also analysed them according to their raw spectral properties, a method which makes no *a priori* assumptions about the visual systems viewing the plant species.

A recent study found an association between pollinator type (e.g. bees, flies, butterflies, etc.) and flower colour and other aspects of morphology in Norwegian habitats at various altitudes (Lázaro et al., 2008). Given this, and the fact that the pollinator community changes in composition at different elevations, a possible prediction is that as different pollinator types change in importance at different elevations, and each group is associated with particular colours of flower, then the colours of flowers present should also vary in accordance with these preferences. Although visit frequency taken alone does not perfectly assess an insect's contribution to pollination of a particular plant, visit frequency is one measure of total interaction (Vázquez et al., 2005) and therefore a colour that is associated with more visits from a pollinator is likely also to be receiving more benefit from that pollinator than a flower of another colour visited less frequently. This could apply regardless of whether the colour association is based on innate preferences (Lunau and Maier, 1995) or the result of pollinators learning which flowers are most suitable for them (Raine et al., 2006), given flower morphology and rewards .

However, our analysis provides no evidence for such variation, either for flower colours as perceived by bees or by muscoid flies. Indeed, even when considering the flower colours without any model of insect perception, no differences between the altitude groups emerged; the PCA indicates that flowers from all three groups share statistically

indistinguishable spectral properties. The lack of association between elevation and colour is unlikely to be a result of insufficient data: all species present along our transect were recorded, and the length of the transect spanned sufficient distance that there were substantial changes in the habitat type, from woodland and stream beds to unstable alpine soils. Although the transect began at around 700m, and did not extend to such low elevations as in Lázaro et al. (2008), the change in habitat types suggests a significant change in pollinator composition, such that a change in flower colour composition of the communities could be anticipated.

It known from other studies that evolutionary history constrains flowers' colours (Kalisz and Kramer, 2008; Menzel and Shmida, 1993), since not all families have the biochemical pathways to produce particular pigments. Some plant lineages only contain particular floral pigments and therefore flowers in those groups can only assume a limited range of colours. However, most plant families are ultimately capable of producing a variety of colours (Chittka, 1997). When taken across the whole dataset, there is no evidence of a combined effect of phylogeny and elevation predicting flower colour similarities according to relatedness and shared elevation range.

There are a number of reasons why such a lack of change in the proportions of flower colours with changing elevation, even when the effect of phylogeny is factored into the analysis, may be observed. The first is the phenomenal learning ability of insect pollinators. Even though certain pollinator types have specific innate preferences for colours of flowers (Giurfa et al., 1995; Lunau et al., 1996; Raine et al., 2006), they are able to learn to overcome these preferences easily if a flower is sufficiently rewarding (Menzel, 1985b). Therefore simply because a flower's colour matches the innate preference of a dominant pollinator, this may not necessarily constitute a fundamental selective advantage for the flowers, since the vast majority of pollinator visits will be by experienced individuals (Raine and Chittka, 2007a). The pollination market hypothesis, in fact, advocates that a range of distinct and discriminable colours in a habitat would be most advantageous to plants (Friedman and Shmida, 1995; Gumbert et al., 1999). Even if the diversity of pollinators decreases with elevation, rather than appealing to the innate preferences of those remaining pollinator types, a flower may benefit more from evolving an appearance that is distinctive and recognisable (Chittka et al., 1999).

As stated previously, there is also selection for certain pigments for reasons other than pollinator preference, particularly because of their protective ability for the plant. Examples include defence against desiccation, herbivory or UV damage (Chittka et al., 2001; Fineblum and Rausher, 1997; Mori et al., 2005; Warren and Mackenzie, 2001). There is evidence that in vegetative tissues, production of anthocyanins, which confer blue, purple and red colours on tissues, is induced by UV-B exposure, and that once the plant has been challenged with UV radiation, there is a cross-resistance effect, allowing the plant increased tolerance of extreme cold and drought (Chalker-Scott, 1999). Based on the current knowledge of multiple functions of plant pigments, in particular anthocyanins, it is even conceivable that in some cases the pigments favoured by physical factors conflict with those that pollinators may favour. This would result in an overall observation that colour frequencies at different altitudes do not differ, in spite of various selection pressures favouring particular colours in particular circumstances.

As an example, based on current knowledge of the protective effects of anthocyanin pigments, one might expect that the flowers of plants subjected more often to such extreme environmental conditions would bear such pigments in increased quantities. However, at high altitudes where these conditions are common and so the pigments would be favoured, traditional thinking might suggest that flowers would be principally pollinated by flies and therefore "should" be white or yellow, in line with the pollination syndrome concept (Lázaro et al., 2008). Thus based on pollination biology alone, one might anticipate a significant reduction in, for example, purple flowers (bee blue or UV-blue) with elevation, but not if one considers that purple pigments may serve other functions in floral tissues unrelated to pollination. This could result in a trade-off situation in which flowers must compromise between the colours that appeal to pollinators' innate preferences and those which serve other protective functions, with the possible colours further constrained by the evolutionary history of the flower species. Analogous trade-offs in which traits or behaviours are beneficial in some contexts and disadvantageous in others are relatively abundant in nature, such as in Boeing (2004) in which zooplankton vertical migration in lakes must compromise between the risks of UV damage and predator avoidance.

Overall, our study indicates that the colours of flowers in mountainous areas as elevation increases cannot be predicted with a simple rule, and that the pollinator types

present cannot account for the lack of differences if considered purely within the context of the pollination syndrome concept. However, the colours of alpine flowers are clearly determined by multiple factors, including the floral pigments present in their families and the potential functions of those pigments in cells. We must therefore consider flower colour in the context of plants' evolutionary history, as well the multiple selective pressures on this trait, both biotic and abiotic.

CHAPTER IV

FLOWER COLOUR PHENOLOGY IN EUROPEAN GRASSLAND AND WOODLAND HABITATS, THROUGH THE EYES OF POLLINATORS

INTRODUCTION

In much the same manner as researchers have made observations about the colours of flowers present in alpine and arctic regions, as mentioned and tested in the previous chapter, there have been many observations about the colours of flowers that are present at different times of year. Robertson (1924), for example, stated that plant species with greenish-yellow flowers tend to bloom earlier in the year than ones of other colours; McCann (1986) claimed that spring flowers are most frequently white and late summer flowers more likely to be yellow; Warren and Billington (2005) concluded that there is a significant interaction between flower colour and month, stating that yellow, white and pink/purple flowers are all most abundant in early summer, whilst blue flowers are more or less constant in abundance throughout the flowering season. However, relatively little work has been done to analyse this aspect of phenology statistically, and none at all that considers the flowers' colours as their pollinators see them rather than relying on human classifications, which might be of limited ecological relevance. In this study, we have chosen to analyse the flowers classified by the colours as they appear to the most significant pollinators in the local habitat: bee species (including honeybees, *Apis mellifera*, bumblebees, *Bombus* spp. and diverse solitary bees). We have also considered the colours of flowers based on their spectral properties, independently of any visual system.

Flowering plant species may have evolved in number of ways that reduce competition for pollinators, including separation of flowering in time or space from other species, and evolving a different colour to its neighbours to make the species easier to discriminate by the pollinator and thus secure more conspecific pollen (Heinrich, 1975; Rathcke, 1983; Rathcke and Lacey, 1985; Waser, 1978; Waser, 1983b). However, there is also a trade-off: flowering as part of a large group can attract more pollinators because of a mass display effect (Heinrich, 1975). Therefore the outcome for the flower

can be predicted to be a balance between being visually distinct or physically separate from other species against being too separate and not attracting sufficient pollinators. With regard to phenology, this is reflected in two contrasting hypotheses that predict how biotically pollinated flowers should time their blooming relative to other species in the community. Firstly, it has been suggested that by staggering flowering times, plants can minimise interspecific competition for pollinators and so all species will benefit; secondly, that by synchronising flowering times, all the species will benefit by attracting more pollinators with a mass display effect (Rathcke and Lacey, 1985) (see Martínková et al. (2002) for an overview).

What is often overlooked, however, is the interaction between phenology and the colours of the flowers; it may not be necessary for two plant species to diverge in flowering time if their flowers are of different colours and therefore easily distinguished by pollinators. Many species of pollinators have excellent colour vision and are therefore able to discriminate flowers of different colours with great accuracy (Briscoe and Chittka, 2001; Frisch, 1914; Internicola et al., 2008; Kelber et al., 2003; Kevan and Backhaus, 1998; Menzel, 1985b). The colour vision of Hymenoptera is now well understood and modelled (Backhaus, 1991; Chittka et al., 1992; Daumer, 1958; Frisch, 1914; Menzel, 1975; Menzel, 1985a). Given their good colour vision, the colour preferences of pollinating insects can act as an important selective force in the appearance of entomophilous flowers.

The pollination syndrome hypothesis, as previously discussed, might lead to the prediction that the colours of flowers present at particular times of year should reflect the innate preferences of the dominant guilds of pollinators active at that time. For example, some solitary bees and certain species of bumblebee (especially newly-emerged queens) are most active in early spring (Heinrich, 1976; Herrera, 1988; Macior, 1978). Therefore, one might expect there to be selection for those flowers that bloom around this time to be maximally attractive to bees by producing pigments in "bee colours" (bee blue and UV-blue). By comparison, later in the season more butterflies and hoverflies are active (Bosch et al., 1997; Gutiérrez and Menéndez, 1998; Herrera, 1988), perhaps leading one to expect more of an abundance of the pink/purple ("UV-blue" to bees) flowers considered to be preferred by butterflies, and the white and

yellow (bee "blue-green" and "green") ones that are visited by many syrphids (hoverflies) (Knuth, 1908; Lunau and Maier, 1995) .

However, despite that one might conclude from the predictions of the pollination syndrome hypothesis that pollinating insects will be instinctively drawn to flowers exhibiting particular characteristics such as certain colours, it is well known that insects are plastic in their behaviour. Indeed, there is abundant evidence that many are excellent learners (Chittka and Raine, 2006; Gumbert, 2000; Kelber, 1996; Menzel, 1985b; Zaccardi et al., 2006), able to associate almost any colour with reward. They can therefore potentially take advantage of all the colours of rewarding flowers available in a habitat at a given time. Thus, there may only be minimal advantage from displaying colours preferred innately by the dominant pollinator group at a certain time of year. A better strategy may be to evolve a distinctive colour, to reduce the number of interspecific visits by foraging pollinators and ensure the conspecificity of pollen (Gumbert et al., 1999).

In this study, we investigated whether flowers of particular colours (as seen by bees as well as by human observers, and also considered according to their physical reflectance spectra) tend to bloom at particular times of year. Such a finding might indicate an evolutionary adaptation to a particular guild of pollinators. Alternatively, in a given habitat, flowers of all colours may bloom throughout the year. This observation would instead lend support to the theory that pollination is a market in which flowers compete against one another for pollinators and therefore are under pressure to be different, distinctive and salient more than fulfilling a particular suite of predefined characteristics which are considered to make them best-suited to a certain pollinator species (Heinrich, 1979; Ollerton et al., 2009a; Peleg et al., 1992; Waser et al., 1996).

MATERIALS AND METHODS

Study site and data collection

The data were collected from Unteres Annatal-Lange Dammwiesen, a nature reserve located near Strausberg in Germany, during 1991-1993. Five ecologically distinct sites

were studied at this location, each ca. 500m^2 in area, referred to in this article as "dry grassland", "humid meadow", "roadside", "maple shrub", "hazel woodland". The study sites were visited fortnightly between March and October each year, and any insect-visited flowering species in bloom were recorded (see Gumbert et al. (1999)). Additionally, spectral reflectance readings were taken of all the flowers using a flash spectrophotometer using the protocol described in Menzel and Shmida (1993), Gumbert et al. (1999); see also Chittka and Kevan (2005): this involved directing an electronic flash (including UV light) on to the flower at an angle of 45°, then detecting the light reflected back using a cooled (-60C) photodiode array. The spectrophotometer was calibrated against a BaSO4 standard, and where the measurement area of the spectrophotometer (diameter 10mm) was larger than than the area of a single floral unit, several flowers were "tiled" together, exposing only the flower parts of interest to give a total area larger enough to measure. Where the flower parts were of more than one colour, the dominant colour was considered to be the flower's overall colour. This produces a dataset for each species consisting of the proportion of total light reflected by the flower surface at each wavelength in the bee visible range (300-700nm), at 1nm intervals.

In total, we collected observations for 146 species, from 30 plant families. Some species occurred in more than one habitat, whilst others occurred in only a single habitat. Colours and flowering times of all species observed are included in Appendix V, and are the same as those given in Gumbert et al. (1999). Spectral reflectance data for all species can be found online in the Floral Reflectance Database (http://www.reflectance.co.uk) (Arnold et al., 2008).

Colour categories

Bees (including solitary species such as *Lasioglossum*, and several *Bombus* species) are usually the principal pollinators in these types of habitats in Germany (Raine and Chittka, 2007b; Steffan-Dewenter et al., 2002; Steffan-Dewenter and Tscharntke, 1999). However, other pollinators present include syrphids, beetles and butterflies (Kunze and Chittka, 1996; Steffan-Dewenter and Tscharntke, 1999; Waser et al., 1996). As honeybees and bumblebee species have been shown to have broadly similar colour vision (Briscoe and Chittka, 2001; Peitsch et al., 1992), we calculated flower colour loci

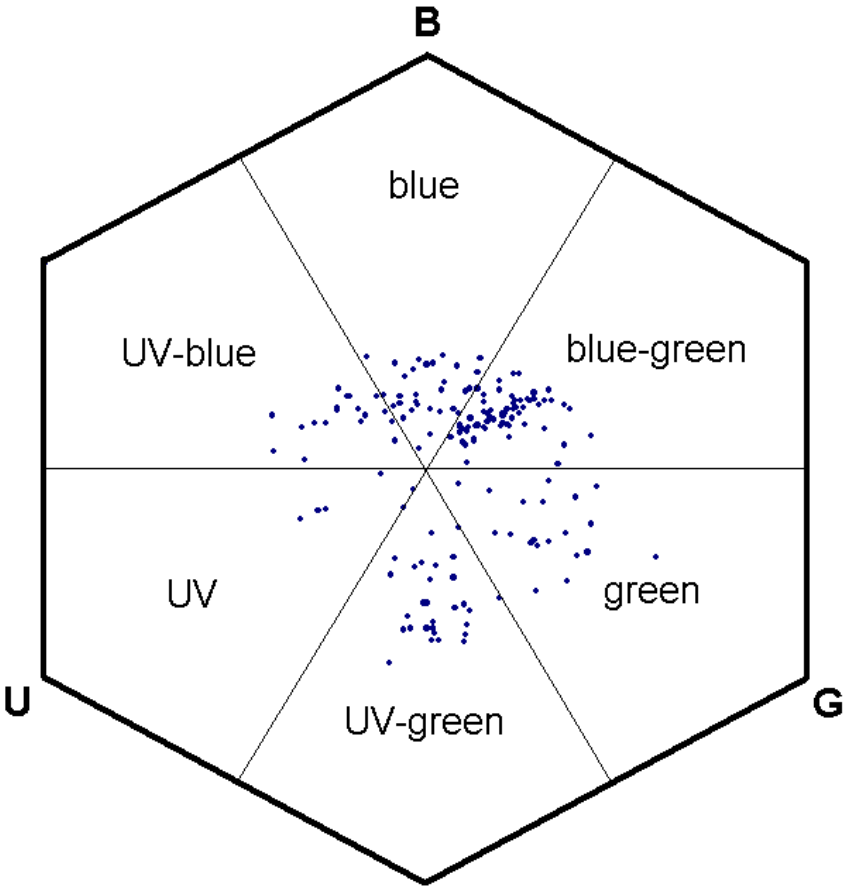


Figure 21. Bee colour hexagon with loci of sample plant species' flower colours plotted. Bees typically have three photoreceptor types, sensitive to blue, green and UV light, and these are indicated at the apices of the hexagon. The hexagon can then be divided into segments, each one corresponding to a different colour category. Loci of individual flowers are shown as points; the habitat contains a range of flower colours. The commonest bee colour for these flowers is blue-green.

as viewed by a honeybee, using the colour hexagon model and the methodology described in Gumbert et al. (1999) and Chittka (1992).

As in the previous chapter, we used the six arbitrary "bee colour categories" to classify flowers as blue, blue-green, green, UV-green, UV or UV-blue in bee colour perception, according to their loci in the colour hexagon.

Statistical analysis: Bee and human colours

Each of the species sampled in the data set was assigned to a colour category based on the appearance of its flowers, either to humans (blue, green, pink, purple, red, white, yellow; judged by the collectors in the field environment and then generalised into the colour category that best describes the appearance) or to bees (blue, blue-green, green, UV, UV-blue or UV-green; calculated from spectral reflectance data). The same species were then categorised as flowering or non-flowering for each month between March and October. Using these data, each species in the data set was compared pairwise with each other species for each month, and the number of cases in which species of the same colour group flowered in the same month was calculated. To test whether this number was greater than would be expected by chance, we elected to use a randomisation approach similar to that described in Rossiter et al. (2005): flower colours were randomly reassigned within habitat and family using *Mathematica* 5.0 (2003) (Wolfram Research, Inc., Illinois, USA). For each randomisation, the number of cases in which species of the same colour group flowered in the same month ($N_{\cap}$) was recalculated for the randomised data. This was repeated 10,000 times, giving a distribution of values to which $N_{\cap}$ could be compared; the proportion of times in which the randomised values equalled or exceeded $N_{\cap}$ is the p value**.**

The analysis was repeated with the plant species classified according to human and bee categories, enabling us to ascertain whether there is a difference in flowering patterns depending on the visual system perceiving them.

As was discussed in the previous chapter, it is important not to neglect the previous observations that flowering characteristics can be affected simply by the plant's evolutionary history. For example, one of the most important predictors of flowering

phenology may simply be the family of the plant (Fox and Kelly, 1993; Ollerton and Lack, 1992). This may not necessarily be an evolutionary constraint *per se*, but certainly some clades seem to have a tendency to flower at similar times of year (e.g. the Asteraceae typically flower later in the year (Ollerton and Lack, 1992)). It has also been noted that some families (e.g. Apiaceae) have a large number of flowers of broadly similar colours (Chittka, 1997; Chittka et al., 1994). This means that any study of this type needs to take such potential correlations into account. Additionally, some particular locations have strongly skewed distributions of flower colour (Goldblatt et al., 1998a; Kevan and Baker, 1983), so it is important to consider the potential influence of habitat in our analysis.

Our statistical approach gave us the options to control for habitat and family, ensuring that ecological and phylogenetic information are preserved and accounted for as necessary. We ran randomisations both with species pooled between habitats, but families still controlled for, and with species pooled between plant families, but with habitats controlled for. We also considered each habitat individually, to ascertain whether there were trends present in some habitats but not others.

Statistical analysis: Spectral properties independent of a visual system

We also considered the plant species' flower colours independent of any visual processing, human or insect. This could indicate any trends in flower colours that were dictated by abiotic constraints, such as drought-tolerance in the height of summer. For the first analysis, we took the raw reflectance spectra of the species present, with all the reflectance values at 25nm intervals between 300 and 700nm. As spectra tend to change smoothly (Chittka et al., 1994), there is little information lost by sampling at a larger wavelength interval than the original spectrophotometer measurements. This provided 17 measurements across the bee visible range for each species, which could be analysed using Principal Components Analysis (PCA) in SPSS for Windows to extract the first two principal components describing variation between the spectra. This was done both for all habitats pooled and for each habitat individually. We divided the species into three groups of broadly similar size (in terms of number of plant species): "early" (blooming in March to May), "mid" (blooming in June and July) and "late" (blooming August to October) in order to compare whether the plant communities at different

times of year had similar compositions of spectra present. We chose to use a smaller number of flowering-times groups for this analysis compared to the month-by-month considerations of flowers in bloom for previous analyses because most species bloom in more than one month successively. Comparing clouds of points (corresponding to flower colours for groups of species) between two consecutive months would cause pseudo-replication and the groups certainly could not be considered to be independent. As the same plant species invariably has the same flower colour in every month of flowering, many of the data points would be the same between months and therefore the chances of finding any significant difference between floral communities in consecutive months would be low.

Several plant species occur in more than one of our broader categories, so it must be acknowledged that the groups are still not entirely independent; however, the analysis can nonetheless indicate whether there are marked changes in the variety of spectral types present in each community at different times of year.

Additionally, we considered whether the differences in phenology between plant species correlate with differences in flower colour, as defined by spectral properties. To do this, we created two matrices in SPSS. The first consisted of the Euclidean distances describing the differences between the plant species' floral reflectance spectra. This was calculated using the spectral reflectance data at 25nm intervals, as for the PCA.

We also calculated a dissimilarity matrix according to the differences between phenological properties of the plant species. To do this, each species was designated as flowering or non-flowering for each month, and the patterns of flowering were compared pairwise between species, with 1 signifying complete synchrony and 0 signifying complete asynchrony of flowering times.

Then, using the R statistical package (R Development Core Team, 2004), we ran a Mantel test to compare the two matrices. If flowers with similar spectral properties also share similar phenological characteristics, a significant correlation between the two matrices would be observed.

RESULTS

The bee colour hexagon loci of all the points used in this analysis are also shown in Figure 21, indicating how the flowers of the different plant species appear to a bee's vision.

The months in which the largest numbers of plant species flowered were June and September (Figure 22 and 23). In the woodland habitats (hazel shrub and maple woodland), flowers generally appeared earlier (Figure 24 and 25), with species blooming in March and/or April comprising 19.2% and 16.7% of total species respectively (compared to 4.7%, 0% and 11.5%, for dry grassland, humid meadow and roadside habitats respectively).

As in previous studies (Chittka et al. 1994), the commonest bee flower colour category was blue-green to bees (typically – but not always – corresponding to human white or pink) and relatively few species are bee-UV (often UV-reflecting red or orange to human eyes, such as the poppy *Papaver rhoeas* L.). White and yellow were the commonest colours when the dataset was categorised by human colour appearance. A first inspection of the proportions of colours as perceived by humans over the year might give the impression of substantial changes from early to later months. In March (and to a lesser extent in April), purple-flowered species appear much more abundant than in later months (Figure 22, bottom), while white-flowered species appear less commonly in these early months. However, it is important to note that very few plant species bloom so early in the year, so the proportions of colours in early months are based on only a small number of species. From May to later months the proportions of different human colours appear largely constant (Figure 22, bottom).

Human colour categories

Our analysis revealed that despite the lower sample sizes in the early months (Figure 22, top), the overall changes in proportions of human colours throughout the year are significant ($p = 0.048$); i.e. species in bloom in the same month are superficially likely to share the same human colour.

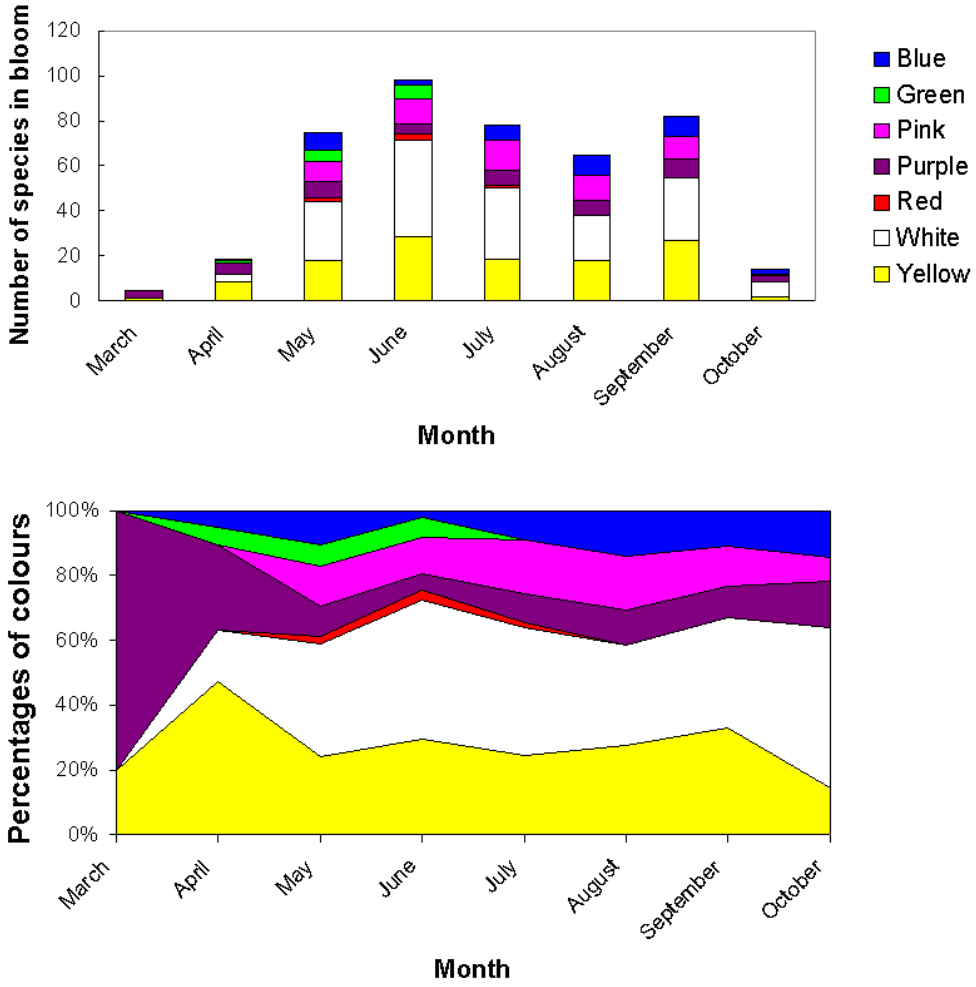


Figure 22. Human colour distributions for all sites combined. Plant species are categorised into flower colour groups according to human judgement. The upper graph shows the absolute counts of species in bloom for all months, whilst the lower shows the percentages of different colours.

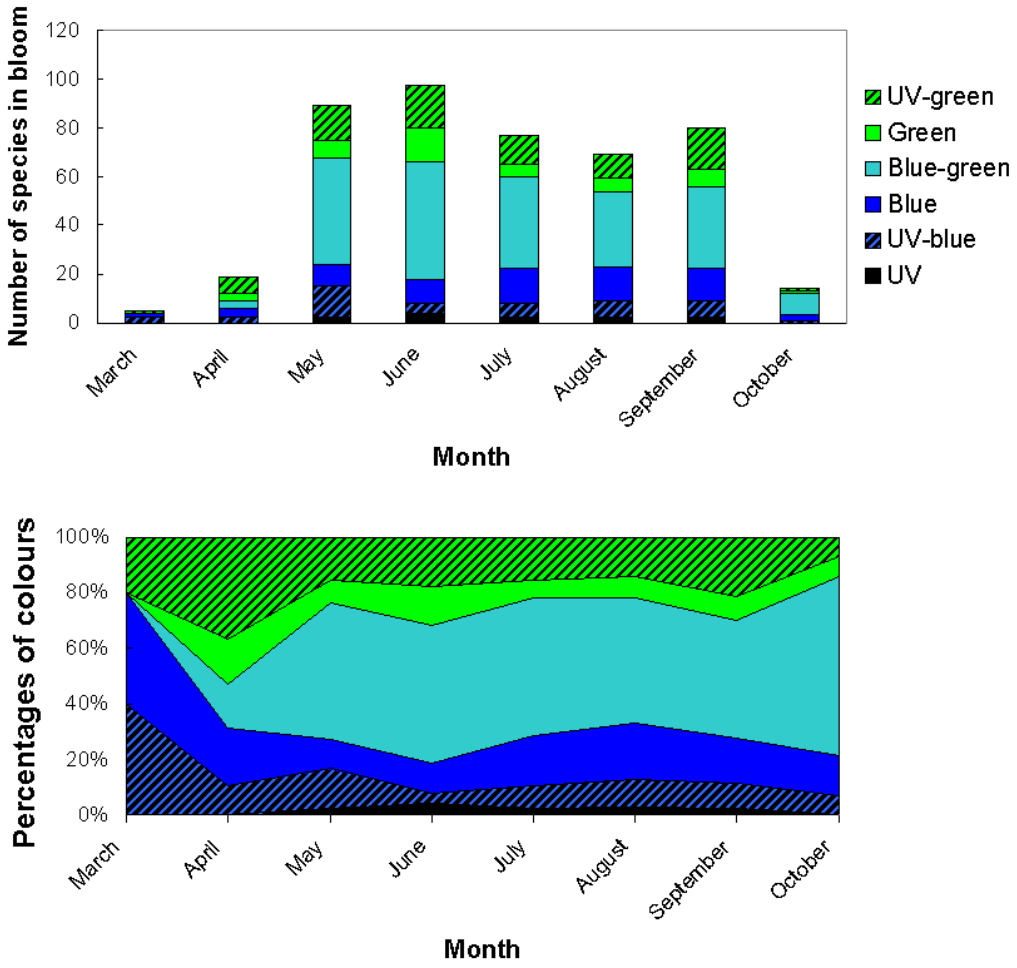


Figure 23. Bee colour distributions for all sites combined. Species are now categorised by colour as they would appear to a bee. The upper graph shows the absolute counts of species in bloom for all months, whilst the lower shows the percentages of different colours.

However, when plant family was controlled for, this apparent trend disappeared (p = 0.2784), indicating that the recorded trend occurs only because plants in the same family tend to have similar traits (colour, as perceived by humans, and flowering time) (Chittka, 1997; Ollerton and Lack, 1992). The trend also disappeared when flower colours were randomised within but not between habitats, controlling for effects of habitat on the dataset (p = 0.1512).

Bee colour categories

For bee colours, likewise, there appears to be a change in relative colour frequencies from early to late months (Figure 23, bottom); in March, UV-blue flower species appear to be more common than in later months, whereas bee green and blue-green flowers appear less common. However, inspection of the sample sizes in the absolute counts (Figure 23, top) once again shows that these apparent temporal changes in flower colour proportions are the result of small sample sizes: there are only half a dozen species that flower in March, in all habitats taken together.

Accordingly, our randomisation approach generated a result that missed the significance threshold (p = 0.0935), indicating no significant tendency for flowers blooming at the same time to share the same bee colour, and this marginal effect vanished entirely when plant family membership was taken into account (p = 0.2608), or when the different habitats were controlled for (p = 0.3099). These findings indicate that flowering time cannot be taken as a significant predictor of bee flower colour, regardless of whether or not the phylogeny of the plants in these habitats is taken into consideration.

Individual habitats

The colour distributions for each habitat are shown in Figures 24 (human colours) and 25 (bee colours). We analysed each habitat separately with the randomisation, once more controlling for possible effects of phylogeny. Regardless of whether the flower colours used were those perceived by bees or humans, no individual habitat showed a significant pattern (Table 2). Therefore, whichever of the habitats is considered, the

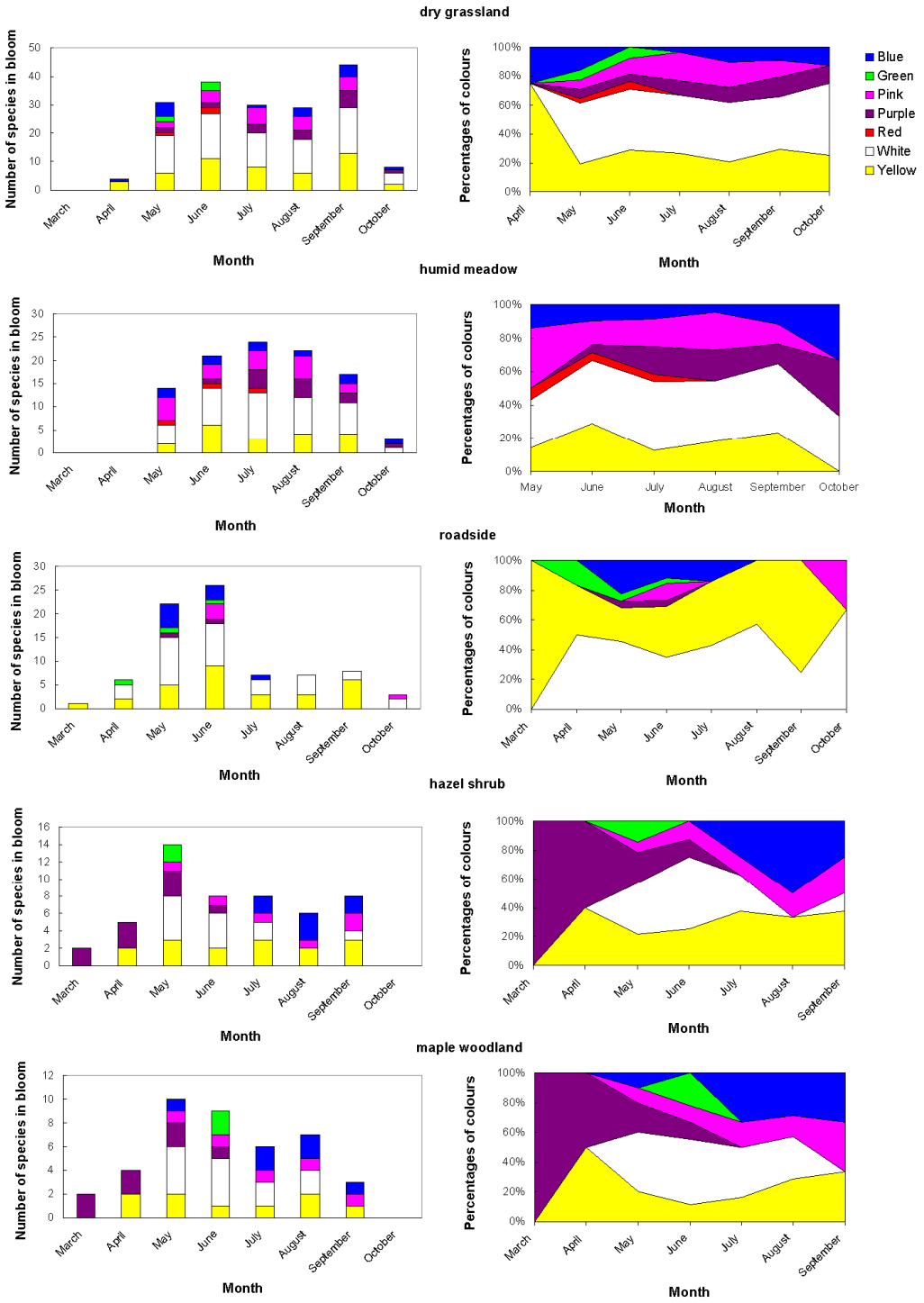


Figure 24. The percentages of different flower colours (as perceived by a human) in the five habitat types throughout the year. Left hand graphs show the absolute counts of flowers in bloom; right hand graphs show the percentages of the different colours present each month.

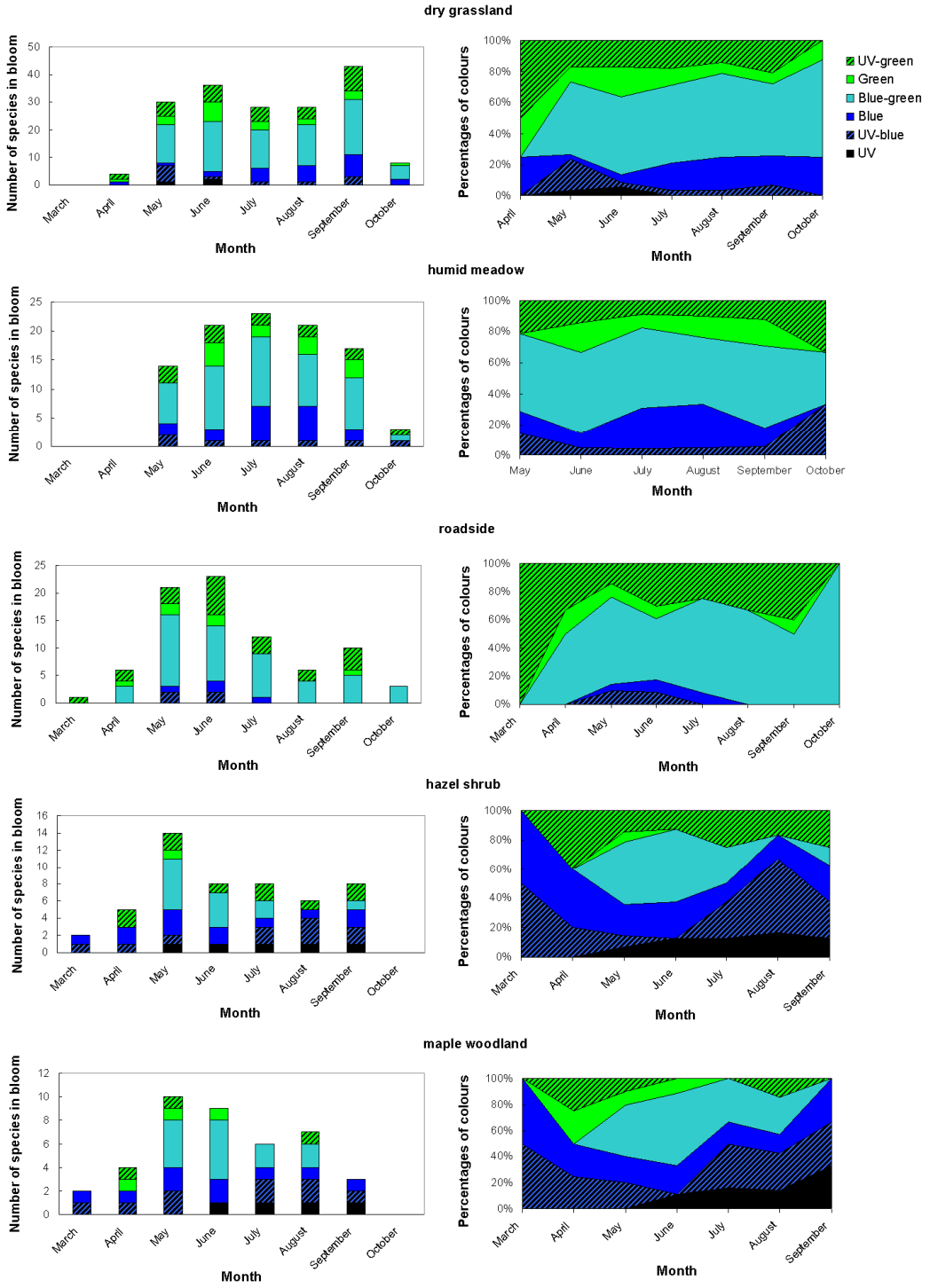
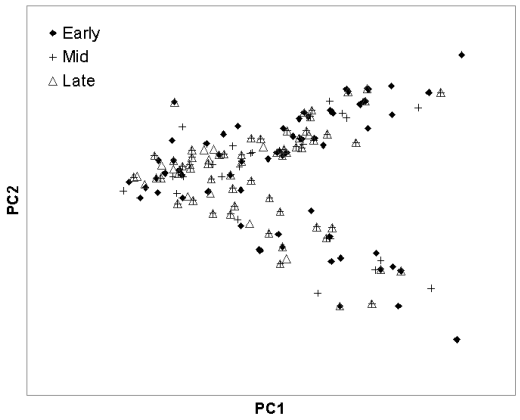


Figure 25. The percentages of flower colours (as perceived by a bee) in the five habitat types throughout the year. As before, left hand graphs show the absolute counts of flowers in bloom; right hand graphs show the percentages of the different colours present each month.

Table 2. Summary of *p*-values for the randomisation tests performed on flower colour trends in individual habitats. The values are the results of randomisation tests investigating whether species in each habitat which share the same colour also share the same flowering phenology. Randomisation tests include a control for evolutionary history.

Habitat	*p*-value for bee colour model	*p*-value for human colour model
Dry grassland	0.2239	0.2886
Humid meadow	0.5943	0.4462
Roadside	0.3057	0.6834
Hazel shrub	0.8566	0.3780
Maple woodland	0.7201	0.7588

a)



b)

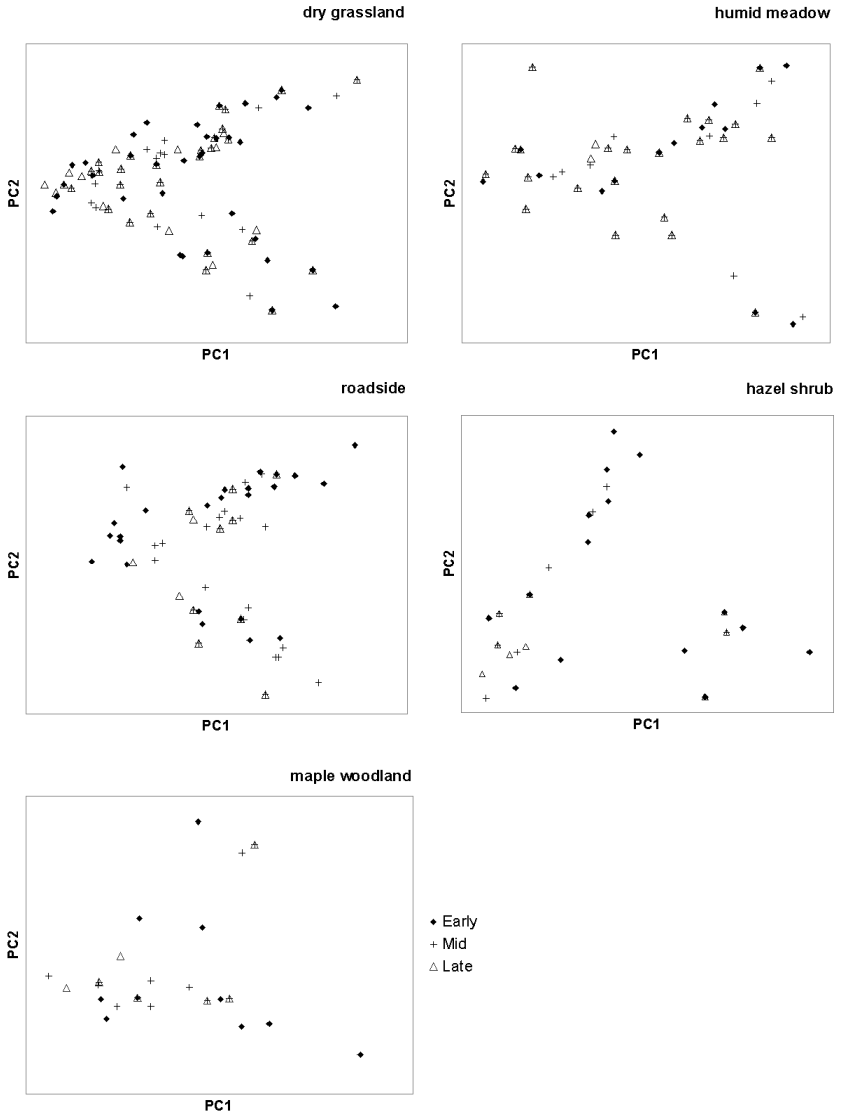


Figure 26. Principal Components Analysis of reflectance spectra for plant species from a) all five habitats combined and b) each habitat individually. Flower species are categorised as early-flowering (March to May), mid-season-flowering (June and July) or late-flowering (August to October).

chances of plant species in bloom in a given month being the same colour to bee or human observers is no greater than chance.

Spectral properties independent of visual system

The Principal Components Analyses, both for the species from all habitats pooled and for the species in each habitat individually, are shown in Figure 26. There appears to be a high degree of overlap between the spectral properties of species blooming at different times of year, and indeed this is supported by the statistics: early-, mid- and late-blooming species overall form statistically indistinguishable groups (Hotelling's Trace, $F = 0.028$, $p = 0.166$, $hdf = 4$, $edf = 460$). When each habitat is taken individually, to discover whether any trends are present in a particular habitat which are masked when data from all five locations are pooled, there is also no statistical difference between the spectra of early-, mid- and late-flowering species (Hotelling's Trace, dry grassland: $F = 0.015$, $p = 0.766$; humid meadow: $F = 0.018$, $p = 0.875$; roadside: $F = 0.061$, $p = 0.416$; hazel shrub: $F = 0.187$, $p = 0.213$; maple woodland: $F = 0.042$, $p = 0.894$).

The comparison of matrices revealed that there was no significant correlation between the spectral properties of flower species and their phenological properties (Mantel test, $p = 0.072$, N=146). The slight trend towards significance, as in the randomisation analysis of human flower colours, may perhaps be caused by a small tendency for closely related flowers to both bloom at the same time of year and possess similar coloured pigments with comparable spectra; however, this effect is not strong enough to pass the significance threshold and there is no definitive evidence that any slight association can exert an effect in a community containing so many species that are only very distantly related.

DISCUSSION

Previous studies have considered the selective forces that determine when a plant should come into flower (Heinrich, 1976; Kochmer and Handel, 1986; Ollerton and Lack, 1992), and whether more species of plants possess particular flower colours at particular times of year (McCann, 1986; Robertson, 1924; Warren and Billington,

2005). The pollination syndrome hypothesis might lead us to expect that if particular pollinator guilds constitute a larger proportion of the total pollinators at certain times of year then those plant species blooming at that time should be more likely to possess the flower colours associated with those pollinators. In our study, we sought to test this, and especially we attempted to disentangle the previous observations based on human colours, and the ecological relevance of these, by modelling flower colours as they are seen by the most important pollinators in our study community, the bees (Gumbert et al., 1999; Steffan-Dewenter et al., 2002), and also by removing the bias of any colour vision system and simply considering the flower colours in the form of their reflectance spectra. Unlike some previous studies (e.g. McCann (1986)), we also address these questions by using robust statistical analyses rather than merely subjective judgements of trends.

Consequently, although superficial examination of the data collected appears to suggest that in some habitats, certain colours of flowers bloom at particular times of year, the statistics show that these observations are largely unsupported. We found no statistically significant evidence that the colours of flowers (as perceived by bee pollinators, or considered in terms of physical reflectance) change throughout the year. We did obtain a single significant finding: a trend for plants flowering in certain months to have the same human colours. This could be taken to be consistent with previous observations of particular human colours dominating at different times of year (McCann, 1986; Warren and Billington, 2005). However, even this one significant result breaks down if the analysis takes into account the phylogeny of the species in the habitats.

Thus our findings support the hypothesis of Heinrich (1975), that selection will tend to favour a variety of colours of flower at any given time of year in order to attract pollinators. It has been shown that several bee species will readily learn to associate any flower colour with a reward (Chittka et al., 1992; Menzel, 1985b) and that many other insect species are similarly capable of associative learning (Kelber, 1996; Kinoshita et al., 1999; Lunau, 1992), and therefore distinctiveness is generally likely to be more of an asset than being any particular colour. Indeed, as also observed in the previous chapter, the majority of pollinators in the field will have learning experience influencing their flower visitation decisions rather than being guided by innate preferences alone. Distinctiveness and detectability are also beneficial in light of more recent experiments

demonstrating that flower constancy only holds over the short term (minutes), as a result of insect memory dynamics (Menzel, 2001; Raine and Chittka, 2005; Raine and Chittka, 2007b): a foraging bee will not necessarily remain loyal to a colour or species of flower indefinitely, and might frequently shift to other species if the previously visited variety is not available in the immediate vicinity. These observations of insect learning and switching behaviour are consistent with our results, which demonstrate a broad range of flower colours present in all habitats studied throughout the year rather than periods in which single flower colours dominate.

We also investigated the phenology of flower colours in different types of habitat, looking at three "open" habitats based largely on grassland, and two "woodland" habitats. Different habitats may have different pollinators and present different foraging conditions for those pollinators, and also present the flowers themselves with different challenges. It is already known that in woodland areas, understorey plants flower earlier (Heinrich, 1976) (see left-hand graphs in Figure 24 and 25), in order to maximise their growth and productivity before the trees come into full leaf and shade them out. The light environment in woodland areas is also distinctive, and this could perhaps impact on pollinators' foraging choices. During much of the year, pollinators in woodland must forage under lower light levels, and also under light that is spectrally different from normal daylight (with a spectral peak around 550nm owing to filtering through green leaves) (Endler, 1993); it is still unknown how this may affect their foraging strategies and colour preferences. For example, some colours of flower may be less salient or harder to discriminate under woodland light than under ordinary daylight, making such colours disadvantageous when the canopy is closed. Whilst it is known that bees at least have good colour constancy and are able to recognise colours accurately under a variety of illuminants (Lotto and Chittka, 2005; Werner et al., 1988), it is also known that their colour constancy is not perfect (Dyer, 1999; Dyer, 2006; Dyer and Chittka, 2004a). The extent to which switching between light habitats while foraging induces "mistakes" (visits to flowers of a plant species that was not the intended target) as a result of imperfect constancy remains to be determined.

However, our results did not provide any evidence of a shift in the flower colours of woodland plant species between early spring (minimal leaf cover) and late spring/summer (more intense leaf cover). There was no trend for woodland flowers

blooming in particular months to share the same colour more often than expected by chance, as one might predict if particular colours dominated at certain times of year and if some colours increased or decreased in importance later in the year. We found no evidence that plant species in these habitats changed in relative frequencies of colours throughout the year, in a way that could be related to the level of leaf coverage. We also found no evidence of shifts in the spectral composition of the woodland plant communities.

Another consideration is that some previous observers may have noted only the abundance of functional floral units of different colours at given times of year, without recording the number of plant species in bloom. This would result in judgements of the "dominant" flower colour based primarily on a the flower colours of just few plant species that happen to occur at very high abundance (e.g. an English bluebell wood in May would appear predominantly blue to human eyes, but the blue effect comprises just one species (Yanney Wilson, 1959)). Our study, however, considers only the diversity of plant species in flower regardless of the numbers of functional floral units per species.

Our results show that previous records of flower colours changing over the year can vary depending on the visual system used to classify flower colours. Plant species that are closely related may share both similar flowering times and similar pigmentation, possibly resulting in apparent abundances of particular colours, as perceived by humans, at particular times of year. However, this pattern is not reflected in the trends in flower colour as perceived by bees that we observed in our study sample, nor is the trend borne out in analyses of the spectral reflectance functions of species in our study sites. Thus our findings demonstrate that we should be wary about drawing conclusions about patterns in flower colour based on human perception alone.

CHAPTER V

FORAGING BEHAVIOUR OF BEES IN PATCHY LIGHT CONDITIONS

INTRODUCTION

As mentioned in the previous chapter, the light environment in any terrestrial habitat is not consistent across space or time. Temperate woodland and hedgerow habitats – in particular during the spring time when the trees and large shrubs are only partially in leaf – present a complex foraging environment for insect pollinators. In these environments, many areas are shaded by leaves for much of the growing season, altering both the overall illuminance and the chromaticity of the light beneath (Endler, 1993). Other patches are open, but will only receive direct sunlight for a small part of the day when the sun is overhead, at other times being lit only by skylight. Furthermore, the spectral content of sunlight and light from the sky varies over the course of the day and between days (Hernández-Andrés et al., 2001; Johnsen et al., 2006).

An animal seeking rewarding flowers or another food source in such a habitat is therefore faced with a considerable visual challenge. Without some way to compensate for changing hues and intensities, colour and brightness information would be unreliable and potentially useless. Simple receptor adaptation enables a basic form of compensation for changing illumination – if a photoreceptor is highly stimulated, it down-regulates its sensitivity to light. This permits some basic compensation for changes in illuminant (Neumeyer, 1981).

However, some animals have now been proven to have more sophisticated colour processing. Honeybees were found in the 1980s to have colour constancy, the ability to discriminate colours correctly under changed illumination (Neumeyer, 1981; Werner, 1987; Werner et al., 1988). These initial experiments were performed with controlled lighting and coloured panels of stimuli; the bees were trained to visit one panel, and then the lighting was changed in its spectral content (e.g. by increasing the blue content) and then the bees were retested to see if they could still choose the correctly-coloured panel or whether they made mistakes. The results of both Neumeyer's (1981)

experiments and Werner's (1988) experiments (which additionally included tests on some stimuli which reflected UV light, under illuminating light with variable UV content) demonstrated presence of colour constancy in bees. Thus, even when the spectral content of illuminating light changes, they are able to learn to distinguish coloured stimuli with a high degree of accuracy.

However, there is an increasing body of evidence suggesting that this ability, as in humans, is approximate rather than perfect. Even the data from Neumeyer's original experiments showed that larger changes in the illuminant caused the bees to make more mistakes when recognising coloured stimuli (Dyer, 1999; Neumeyer, 1981). Furthermore, if bees adapted flawlessly to changed illumination, they would not detect changes in the spectral content of illuminant light and would therefore not be able to use illumination information to influence their behaviour. However, Dyer and Chittka (2004b) and Dyer (2006) demonstrated that bees directly perceived changes in illuminating light. Additionally, Lotto and Chittka (2005) demonstrated that bees could use illumination as contextual cue in foraging tasks. Studies by Dyer (1998; 1999) used data on bees' photoreceptor responses and the reflectances of natural flowers to predict that some flowers would appear to change in colour to bee eyes under altered lighting, demonstrating that bees' colour constancy is only approximate. However, none of these experiments sought to address the responses of bees to short-term changes in illumination that are associated with foraging in patchy light, as they might encounter in nature, i.e. moving rapidly into and out of illumination patches so the illumination surrounding the bee changes over the space of seconds rather than minutes.

Possessing only approximate colour constancy could give rise to a difficult situation for bees foraging in such environments with patchy light, e.g. woodland edges, hedgerows and gardens. Whereas bees may be accurate at discriminating flowers and spotting concealed predators under sunlight, under leaf-shade or skylight they may make mistakes in finding the correct flowers, or even fail to spot flowers with predators (e.g. crab spiders) on them; the same applies when moving from leaf-shade into sunlight patches once more. Some illuminants could therefore be considered by the bees to be more risky, and it may affect their choice behaviour. This can be further complicated by metamerism, when two items with different spectral reflectances which are discriminable under one illuminant become indistiguishable under another (Wyszecki

and Stiles, 1982). For a foraging bee, this could cause misidentification of flowers which in other illuminations would be discriminable (even, perhaps, in spite of shape or odour differences between species).

In the following series of experiments, we sought to test whether an illuminant which simulated leaf-shade was associated with lower accuracy for bumblebees in a colour discrimination task compared to their performance under simulated daylight. We then investigated whether a difference in performance under the two illuminants could lead to an experimentally naïve group of bees behaving differently under the two illuminants, for example, spending more time under one compared to the other, making more mistakes under one than under the other, or altering their foraging strategy, such as becoming more or less flower-constant or preferring particular colours of flower. Flower constancy could conceivably be affected by patchy light as it might alter bees' willingness to probe new species or cause them to make mistakes in species identification.

Through these experiments, we sought to gain a better understanding of how bees forage under patchy light. This will provide us with insight into the limitations of colour constancy, and the biological relevance of failures of colour constancy.

General Methods

Experiments were performed indoors between January 2007 and December 2009. For each experiment, we connected a colony of bumblebees (*Bombus terrestris dalmatinus*) (colonies supplied by Koppert UK Ltd. and Syngenta Bioline Ltd.) to a flight arena (1.2m by 1m) via a plastic tunnel; the tunnel was gated so that only a single bee was released into the arena at a time during the training and testing bouts. Thus bees were always trained and tested individually, with no conspecifics present to provide distractions or cues. Each bee was individually marked with a paint spot on its thorax and was used in only one of the different experiments detailed, so had no prior experience of colour or learning experiments. Between experiments, bees were allowed to forage from a clear (uncoloured) feeder placed on the centre-line of the flight arena, containing sucrose solution; when experiments were not in progress, the arena was

illuminated by the simulated daylight lighting setup detailed below. The colonies were also provided with pollen directly into the nesting box three times per week.

The flight arena consisted of a wooden box with a transparent (UV-transmitting) lid. Lighting was provided by four fluorescent "daylight" tubes (Duro-Test Lighting, Philadelphia, USA) and one UV-blacklight (Maplin Electronics Ltd., UK). Lights were buffered at high flicker-frequency (>1000Hz) and a sheet of UV-transmitting white diffusion (White Light Ltd., London, UK) was placed beneath the lights to prevent the bees from trying to fly towards the fluorescent tubes and to mix the light from the five tubes. This was the default illumination for the arena, and also the illumination in the "daylight" patches during patchy light experiments. We simulated leaf-shade in this setup by using coloured filters placed above the arena to alter the intensity and spectral composition of the incoming light. These filters consisted of a combination of two sheets of green translucent plastic (Acco UK Ltd., Ayelsbury, UK) and one sheet of tracing paper (Simply Stationery, Ackerman Group Plc., London, UK). The transmittance spectra for this and an average spectrum calculated from multiple samples of green leaves (for comparison) are included in Figure 27. We used leaf-shade "patch" filters, which were rectangular sheets one-quarter of the area of the top of the arena; two of these placed in diagonally opposite quadrants of the arena created a setup in which half the arena's area was illuminated by simulated daylight and the other half was illuminated by leaf-shade light (Figure 28). We refer to this later in this chapter as the "Battenberg design" for convenience. Alternatively, a large leaf-shade filter could be placed above the flight arena to illuminate the whole area uniformly with leaf-shade light.

Two colours of stimuli were used throughout the first three experiments, representing two flower colour morphs, or flowers of two different species (and therefore can be considered to be artificial flowers). The positive stimulus (S^+; containing a food reward) consisted of a square 2.4cm x 2.4cm UV-transmitting transparent plastic tile (thickness 4mm) placed over a dark purple square of paper of the same size. The negative stimulus (S^-; containing no reward, or a quinine penalty), where used, was an identical plastic tile but placed over a *pale* purple square of paper. The colours were chosen as they fall within the distribution of colours present in European woodland and were expected to be difficult but not impossible for the bees to learn to discriminate between. Spectra and

colour hexagon coordinates (under normal light) for the stimuli are included in Figure 29.

The tiles contained a small central indent, diameter 2mm, depth 2mm, in which a drop of sucrose reward could be concealed (as bees may use the presence of a visible liquid drop to influence their decision whether to land or not, especially for tasks that are otherwise perceptually difficult). We washed and dried all the tiles after each foraging bout.

The stimuli were placed on glass vials of height 4.2cm, with equal numbers (either 4 or 2, depending on the experiment) in each quadrant of the arena. The arrangement of stimuli within each quadrant was pseudo-randomised after each bout so that the bees could not learn to associate any location in the arena with the predictable presence of either a rewarding or unrewarding stimulus.

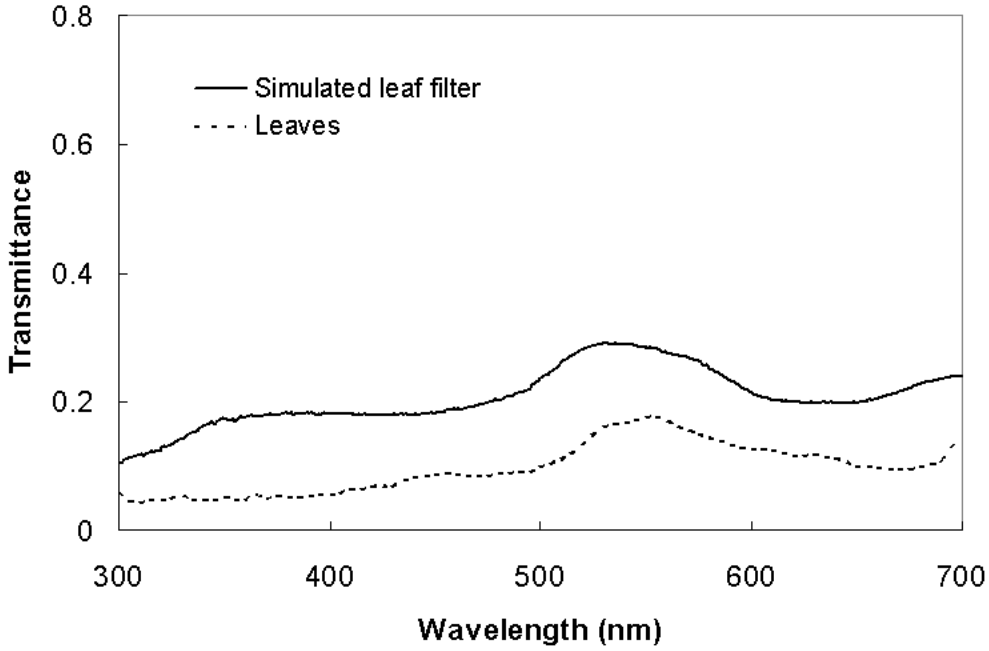


Figure 27. Spectral transmittance of leaves (averaged from multiple samples as in Chittka (1997)) and the leaf filter used in our experiments.

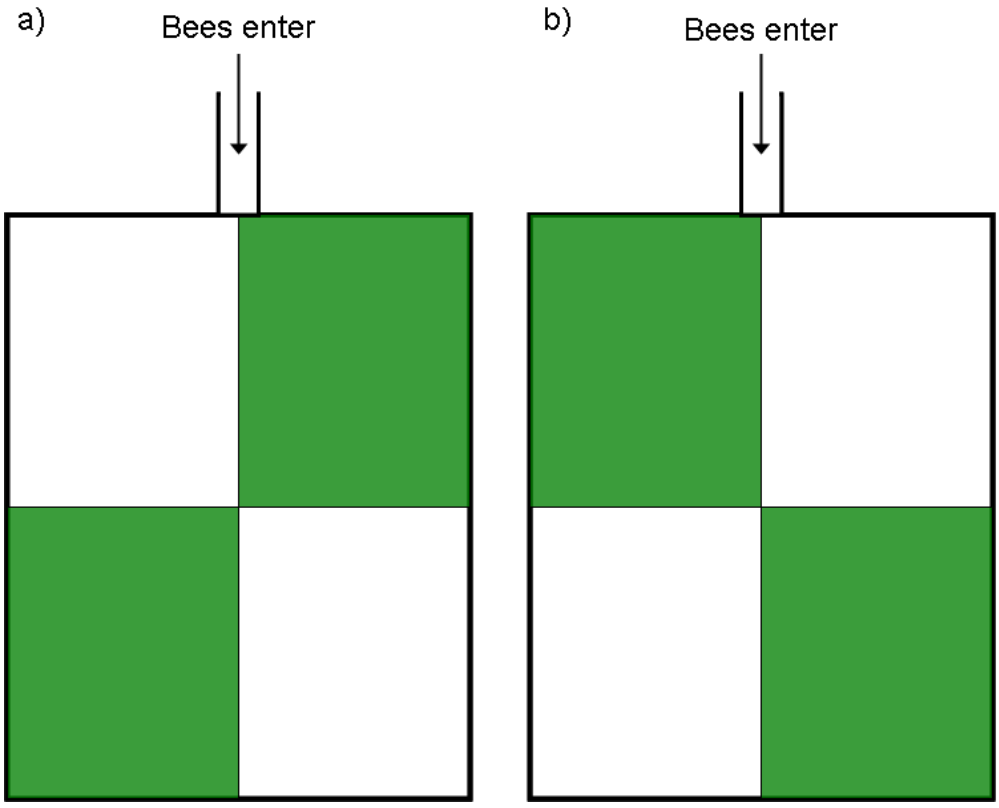
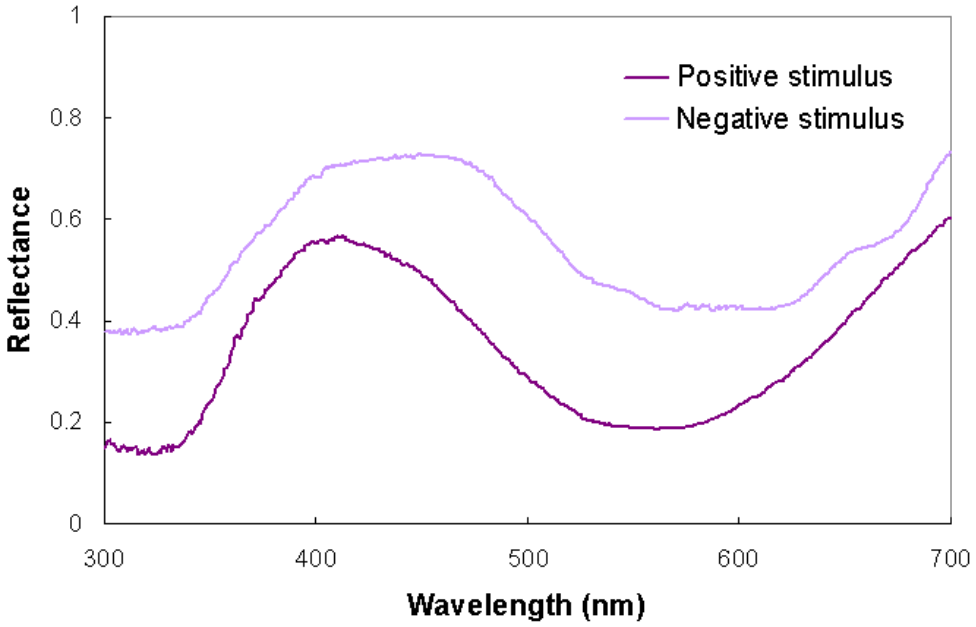


Figure 28. The two alternative patchy light layouts of filters, in the "Battenberg" design. Green patches indicate leaf-shade light filters, whilst the white patches are illuminated by simulated daylight (produced by a combination of "daylight" fluorescent tubes and a UV-blacklight).

a)



b)

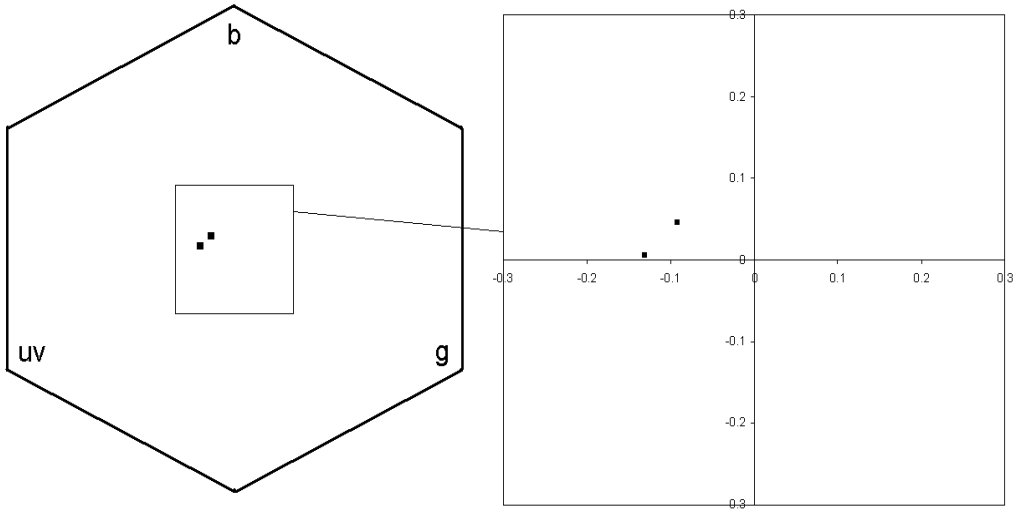


Figure 29. The two colour stimuli, S^+ (dark purple, containing sucrose) and S^- (pale purple, where used, containing quinine), as a) reflectance spectra and b) colour hexagon coordinates under D65 lighting conditions.

EXPERIMENT 1: LEARNING EXPERIMENT

If bee colour constancy is perfect, one would expect bees to learn to discriminate two coloured stimuli as quickly and accurately under one lighting condition as under another. Conversely, imperfect colour constancy may result in a poorer colour learning performance under some illuminants, especially if the illuminant contains low proportions of some light wavelengths relative to others. In this experiment, we used a straightforward colour learning task under two different illuminants to discover whether bees performed equally well under both illuminants or whether there was a difference in learning speed for one of the illuminants.

METHODOLOGY

Each bee in this experiment was only trained and tested under a single illumination condition to control for any bias in prior familiarity with the setup or order effects. Bees were trained and tested under one of two experimental treatments, both of which consisted of uniform illumination (not patchy). Treatment 1 was uniform simulated daylight (no coloured filters); Treatment 2 was uniform simulated leaf-shade, produced by placing the leaf-shade filters beneath the lighting array, so that the entire area above and inside the arena was lit by green-coloured light. We tested 15 bees in each treatment.

In both treatments, a single bee was allowed to forage in the arena containing 8 positive and 8 negative stimuli. The positive stimuli contained a reward of 15µl of 40% sucrose solution, whilst the negative stimuli contained 15µl of 0.013% quinine hemisulphate solution, a known aversive substance to bees commonly used to penalise incorrect choices (Dyer and Chittka, 2004c). The volumes chosen would allow a bee to satiate on sucrose solution within a foraging bout, causing her to return to the nest.

We recorded the bee's landings for 100 visits. If any part of the bee made physical contact with the stimulus, this was counted as a landing. Each time the bee returned to the nest, the plastic tiles were washed, the arena floor was wiped down and the

arrangement of stimuli was pseudo-randomised so that the bees could not use spatial memory to identify the correct artificial flower type.

After 100 training visits, we gave each bee an unrewarded test, in which all the stimuli were washed and replaced in the arena without either sucrose or quinine, and the bee's first 10 landings were recorded. This test controls for any olfactory effect of the sucrose or quinine as the bee cannot use a potential odour to identify whether the stimulus is "correct" or "incorrect"; the outcome therefore depends only on what the bee has already learned in previous bouts. The bee was not allowed to return to the nest until she had completed at least 10 landings.

RESULTS

As could be predicted by the relatively small colour distance between the two stimuli, the bees initially chose between them at chance level under both the daylight and leaf-shade illuminants (Figure 30). In both treatments, the bees gradually learned to prefer the dark purple S^+ stimulus (GLM, $F = 3.579$, $hdf = 9$, $edf = 20$, $p = 0.008$). During the training phase, learning appeared to occur faster under the daylight condition than under the lead shade condition, though this effect fell short of the significance threshold (GLM, $F = 1.763$, $hdf = 9$, $edf = 20$, $p = 0.139$). However, in the unrewarded test at the end of the training period, the bees trained and tested under the daylight condition exhibited a better performance than those under the leaf-shade condition, selecting more of the correct S^+ stimuli (t-test, $t = 1.78$, $df = 28$, $p = 0.043$) (Figure 31).

CONCLUSION

Based on the differences in final performance in the unrewarded test, bees appeared to find colour discrimination more difficult under the leaf-shade condition. This most probably highlights the fallibility of bee colour constancy; if they could correct perfectly for the illumination, there would be no difference between the two groups of bees at any stage in the training and testing process. The decrease in performance could also be a result of the lower light intensity under the leaf-shade filter; it has been shown that the

accuracy of target detection in bees deteriorates under low light conditions (Chittka and Spaethe, 2007; Skorupski et al., 2006). Experiment 3 of this chapter includes a control setup in order to explore whether this is an important contributing factor.

The fact that the effect of illumination on performance was only significant in the final test phase may have one of two explanations: it may be because the differences in accuracy at intermediate training stages were relatively slight and only reached significance when the bees were selecting the correct stimuli well above chance level anyway. Alternatively, it may be that the bees learn both tasks at similar speeds but as their colour constancy is only approximate, they have a "ceiling" performance under some illuminants, and cannot exceed this because of the limits of their physiology in discriminating colours, just as in fine colour discrimination tasks under ordinary lighting, they can never learn perceptually difficult tasks to 100% accuracy (Dyer and Chittka, 2004c).

In any case, we are able to conclude from this experiment that the bees find it harder to discriminate the two colours accurately under simulated leaf-shade light compared to simulated daylight. One must, however, be wary about extrapolating this to all colour pairs (especially ones with a larger perceptual colour difference) under all illuminants. However, it supports existing literature exploring the limitations of colour constancy in bees, and the fact that the leaf-shade condition is an ecologically relevant illuminant which a wild bee may encounter means we should certainly consider the possibility that bees may occasionally misidentify flowers when foraging in woodland and leaf cover.

This may have repercussions for forest understorey plants – although in all environments, flowers are under selective pressure to be highly conspicuous and discriminable, if bees generally discriminate flowers more poorly under leaf-shade relative to daylight, the pressure for species to diverge in colour will be stronger. However, mimicry inside woodlands and forests may perhaps be favoured more strongly, as our data indicate that the bees are more likely to make "mistakes" in such environments and thus potentially visit unrewarding mimic species, making pollination more likely for these species under leaf-shade rather than in open, daylight-illuminated habitats.

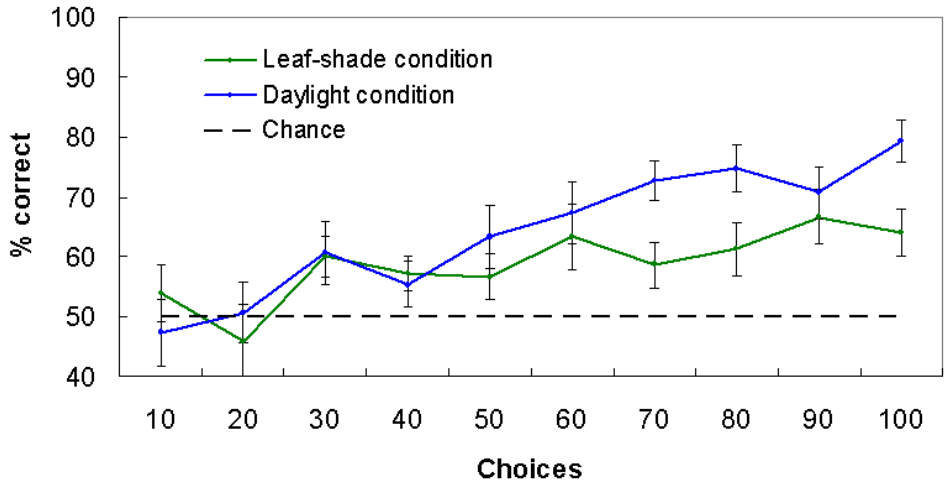


Figure 30. Learning curves for bees under the two lighting conditions, showing the percentage of correct choices per block of 10 flower visits. (Bars = standard error.)

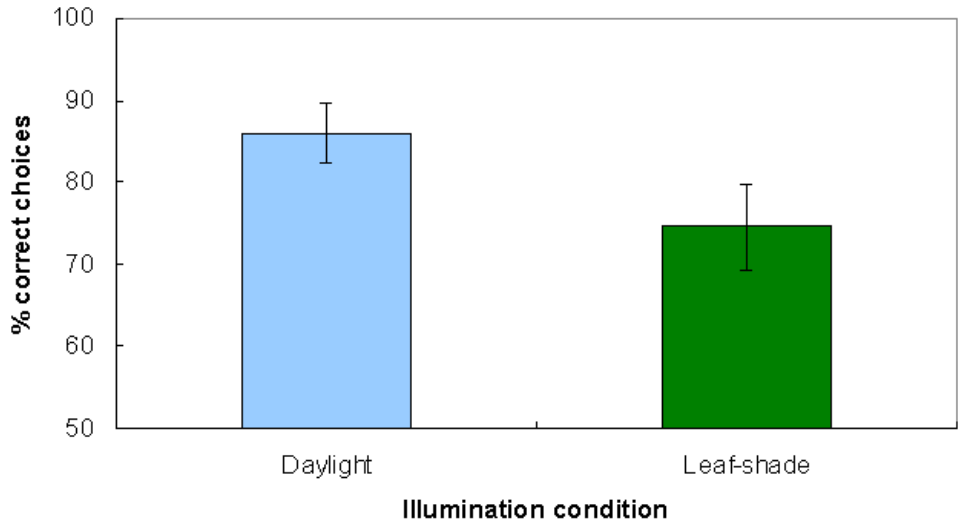


Figure 31. Results of unrewarded test after the differential conditioning training for the two stimuli under leaf-shade and simulated daylight illuminants. The graph shows the % of correct landings under the two illumination conditions, as mean ± standard error for each treatment group. The results show that bees are more accurate at discriminating the two coloured stimuli under simulated daylight than under leaf-shade.

EXPERIMENT 2: PATCHY LIGHT EXPERIMENT

Having established that the bees find one illumination condition more difficult than the other when trained to discriminate between the two stimuli, we investigated their foraging behaviour when they had an opportunity to choose the illuminant in which they preferred to spend the most time and visit the most flowers. It seems likely that they would exhibit some preference for the "easier" illuminant, in which they perform more accurately and make fewer mistakes.

In this experiment we used the "Battenberg" design, in which half of the total arena area was illuminated by simulated daylight and the other half with simulated leaf-shade. As the bees were able to pass freely between the two illuminants, they could choose whether to visit both illuminants equally, or favour one over the other.

The time course of choices for targets and flight behaviour in the two illuminants will also be informative. If the bees avoid the leaf-shade (difficult) illuminant because it presents a higher risk of mistakes, one would expect that the preference for the daylight (easy) illuminant would be relatively weak initially, as the bees have no experience with punishment of errors, and would grow stronger as the bees learn that there is a cost of mistakes. In contrast, an aversion to one of the illuminants that is present at the outset of the experiment is unlikely be related to perceived error rate, given that the bees have no experience with choosing the incorrect stimulus at this point in training. Such a preference must be mediated by some other factor, such as a preference for the higher-intensity illuminant. We investigated these possibilities in a series of control experiments, detailed in the next section of this chapter, in particular the potential effects of familiarity with the illuminant and how light intensity differences between the patches might affect the bees' flight and visitation behaviour.

In addition, there may be a difference depending on the type of learning paradigm used. If both colours of flower are equally rewarding, or the unrewarding flower colour contains only water rather than quinine, there is relatively little incentive for the bees to forage accurately (even in a differential scenario with sucrose versus water, as the energetic cost of moving on to the next flower in a small flight arena is negligible)

(Chittka et al., 2003; Chittka and Spaethe, 2007). This means one would expect the aversion to mistakes and thus to leaf-shade illuminant to develop faster and more strongly in a differential conditioning paradigm with quinine. Conversely, the aversion to leaf-shade light, if present, would remain weaker if there was no or low cost of mistakes, such as in an absolute conditioning paradigm or one in which only water was used on the S^- stimuli.

METHODOLOGY

In these conditions the bees could choose freely whether to fly in both illuminants or just one, and whether to visit the artificial flower stimuli under either or both of the types of illuminant patches.

The green filters were laid on the lid of the arena in the previously described Battenberg design, so that two quarters of the area were illuminated by green light and two quarters by simulated daylight. 16 of the previously described coloured stimuli were used. Parallel horizontal strings (of human-beige colour, 2mm diameter) 7.5cm apart marked out the boundaries of the quadrants in three-dimensions (Figure 32) – foraging bees did not collide with the strings or attempt to land on them, and if the bee crossed one of the strings it could be easily and unambiguously identified as a change of quadrant and therefore recorded as a switch between illuminant patches. This reduced error in human observation of when the bee switched quadrants.

In the Absolute Conditioning treatment, all 16 stimuli were of the dark purple S^+ variety and were rewarded with 10µl of 40% sucrose solution (the smaller volume encouraged the bees to visit all or almost all of the stimuli within a foraging bout). In the Differential Conditioning treatment, there were 8 stimuli of the dark purple S^+ type and 8 of the pale purple S^- type. The S^+ contained sucrose solution; the S^- contained quinine solution. In the Differential Conditioning treatment, the volume of sucrose solution was increased to 20µl so that the bee could satiate within a foraging bout and would return home, and an equal volume of quinine solution was used.

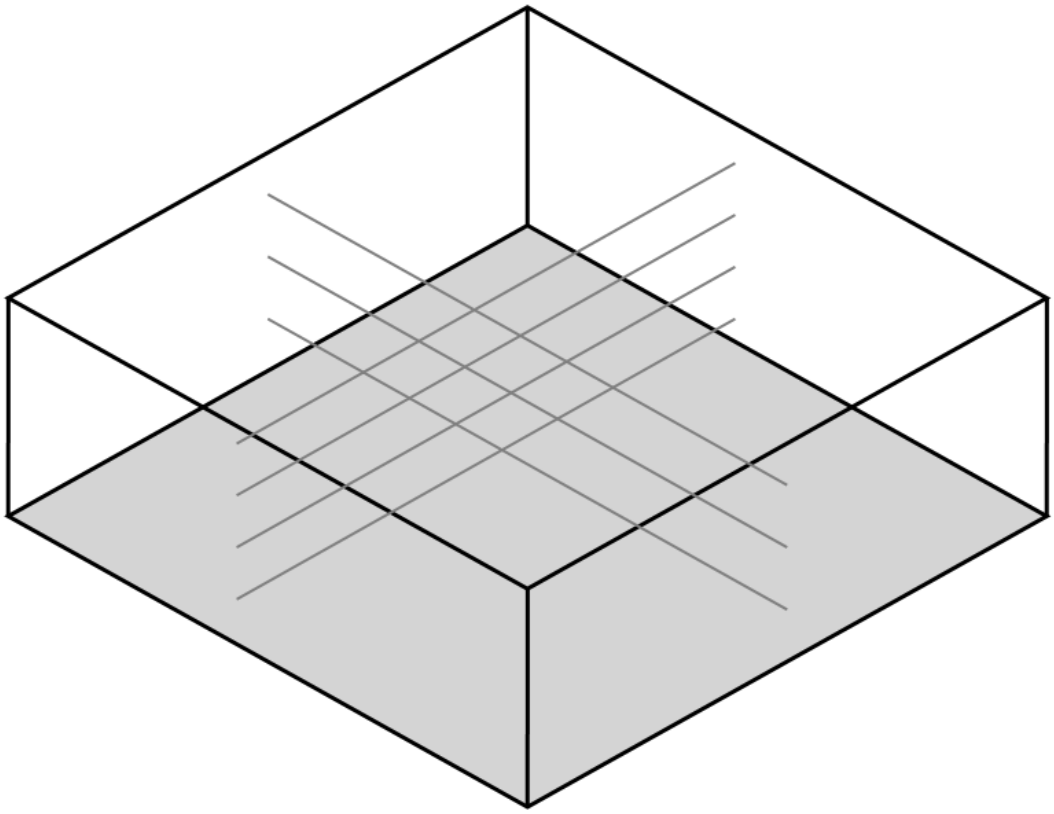


Figure 32. String arrangement crossing arena, dividing it into four quadrants. The purpose of the strings is to ensure accuracy of judging when the bee passes from one quadrant of the arena, and hence illumination patch, to the next. Bees did not land on or collide with the strings during foraging bouts so they are not anticipated to affect performance.

The stimuli were arranged so that there were equal numbers of each colour in each quadrant (i.e. four S^+ per quadrant for Absolute Conditioning; two S^+ and two S^- for Differential Conditioning). The stimuli were pseudo-randomised between bouts, whilst still keeping the numbers in each quadrant the same, and the filters were swapped in a pseudo-random fashion between the two possible Battenberg arrangements shown in Figure 28.

Data recording

Each bee was recorded for five foraging bouts. Using the program ETHOM (Shih and Mok, 2000) on a laptop computer, we recorded each time the bee switched between illuminants (both leaf-shade to simulated daylight and vice versa), when the bee landed on stimuli under the leaf-shade and daylight patches, and whether these choices were correct or incorrect in the Differential Conditioning treatment. The ETHOM program records each event with a timestamp, so that it is possible to calculate how much time the bee spent in different illuminants.

Therefore, the data we acquired allowed us to analyse the proportion of time spent under the two illumination types during each bout, the order of artificial flower visits (including whether each flower was a correct or incorrect choice where relevant, and under which illuminant), and thus analysed the bees' illumination preferences in terms of the time spent in the two lighting types and in terms of where the bees visited flowers. It also allowed us to look at how these preferences and behaviours changed over time. As we collected data both on the flight times under the two illuminants and also the flower visits made, these can be analysed separately, allowing us to consider both behavioural types.

Initial illuminant preferences

We were able to assess the individual bees' initial illuminant preferences by looking at where they made their first ten visits to the coloured stimuli during the first foraging bout, both under Absolute and Differential Conditioning paradigms. A bee foraging in this setup could make all of these ten visits under simulated leaf-shade light, all under

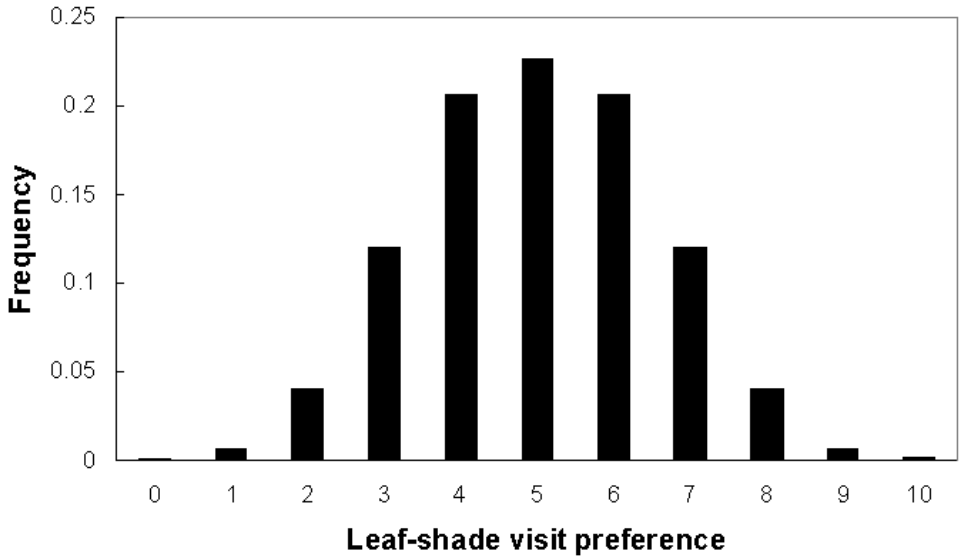


Figure 33. Modelled initial illuminant visit behaviour for a population of bees with no preference for either illuminant. If a population of bees with no illuminant preference were allowed to forage in the arena setup and their first ten choices were recorded, the proportion of choices by each bee in each illuminant would form a Gaussian distribution such as this, with most bees choosing around 5/10 flowers under the leaf-shade illuminant (leaf-shade visit preference).

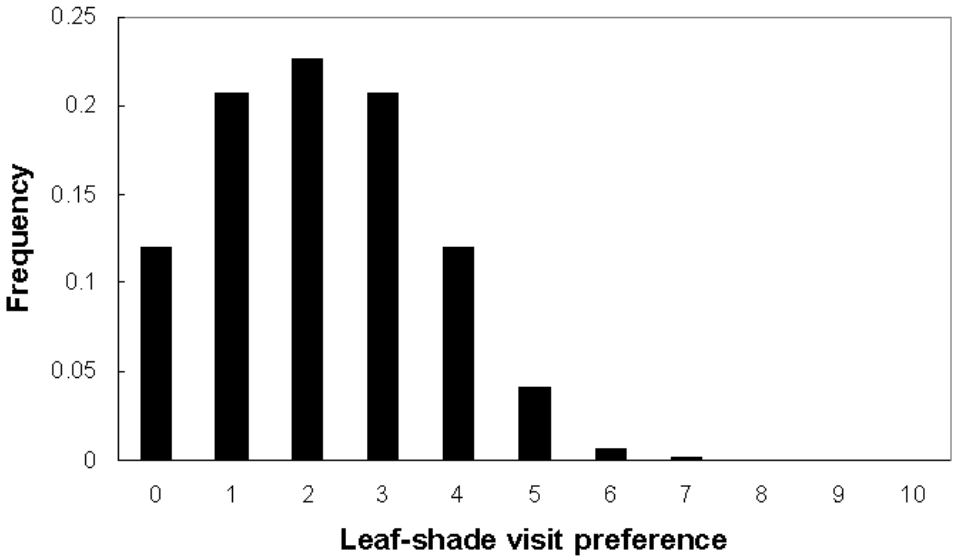


Figure 34. Modelled initial illuminant visit behaviour for a population of bees with a fixed and consistent preference for the daylight illuminant. These are the hypothetical results for a population of bees with a probability of 0.3 of choosing a flower in the leaf-shade illuminant, i.e. a consistent non-exclusive preference for daylight illuminant. As in the previous figure, the proportion of choices by each bee in each illuminant would form a Gaussian distribution, but this time most bees choose around 2/10 flowers under the leaf-shade illuminant.

simulated daylight, or a mixture of both, and the number of visits under green leaf-shade light can be used as an index of initial leaf-shade preference, ranging from 0 (total leaf-shade aversion) to 10 (all choices made under leaf-shade light). If the bees chose artificial flowers randomly, in terms of illuminant, one would anticipate a Gaussian distribution of leaf-shade light preference, with most bees choosing approximately 5 out of 10 of the flowers under leaf-shade illuminant (Figure 33). If the bees avoided the green light with a consistent probability, this distribution would shift towards a lower median number of leaf-shade choices, but remain the same shape (Figure 34).

In contrast, a skewed, non-normal distribution of leaf-shade visit preferences may indicate differing degrees of aversion between individuals. This could, potentially, be correlated with other traits such as overall accuracy in choosing the correct flowers for bees trained and tested in the Differential Conditioning treatment, or eye size (as bees with larger eyes have higher visual acuity and can capture more light, so can forage accurately at a lower light intensity; there is great variability in the size of bumblebee workers (Spaethe and Chittka, 2003) so this may have important effects).

Visit speeds

Using the data collected by ETHOM, one can take the total time a bee spent under one or the other illuminant and divide it by the number of artificial flowers visited during this time under the illuminant. This can be used as a rough proxy for the time taken to find and handle flowers, i.e. "search time". Such a measure will tell us whether, for example, the bee appears to be spending longer flying in the arena per artificial flower visit under the simulated leaf-shade illumination, indicating perhaps that it is taking her longer to locate the stimuli under these illumination conditions.

RESULTS

The result of the learning experiment might lead us to predict that the bees would preferentially avoid leaf-shade light when faced with a free choice of illuminant in a task where mistakes are punished. Presence of an aversion to one of the colours of

illuminant that does not follow this behavioural pattern (i.e. grows weaker with time, or is strong even in bees with no experience of punishment) indicates a different reason for the behaviour. This could, for example, be a preference for high intensity light or an aversion to unfamiliar illuminants.

In terms of flight time, we found that the tendency to fly preferentially in daylight patches was strongest initially (Figure 35). This preference in the first bout was highly significant (paired t-test, Absolute Conditioning: $t = -4.95$, $df = 26$, $p = 0.000019$; Differential Conditioning: $t = -3.36$, $df = 24$, $p = 0.0026$).

However, this preference decreased with time and by the end of the five bouts, the bees were indifferent to which illuminant they flew in (paired t-test, Absolute Conditioning: $t = -0.63$, $df = 26$, $p = 0.267$; Differential Conditioning: $t = -0.78$, $df = 24$, $p = 0.446$ – i.e. in both cases no significant difference in flight times under each illuminant). This effect occurred in both the Absolute and Differential Conditioning treatments, so appears to be independent of the possibility of costly mistakes.

In terms of artificial flower visits, the overall trends are similar (Figure 36): bees initially avoid the green leaf-shade illuminant, preferring to make visits under simulated daylight, but over time become indifferent to illuminant and visit artificial flowers in both patches equally often (paired t-test comparing initial and final preferences, Absolute Conditioning: $t = -5.00$, $df = 25$, $p = 0.0000369$; Differential Conditioning: $t = -4.67$, $df = 23$, $p = 0.000107$).

Initial illuminant preferences

We compared the initial preferences observed in the bees to the modelled distributions described above, to discover whether the bees' preferences appear to be normally distributed (with most indifferent to illuminant or nearly so, and a few bees exhibiting a strong preference for one illuminant or the other). However, both the Absolute and Differential Conditioning data show that initial preferences form a superficially non-normal distribution (Figure 37). The low median preference for the Absolute Conditioning treatment (2/10, versus 5/10 in the random model) suggests that on average, the bees avoid the leaf-shade illuminant. The distribution of preferences,

however, does not significantly differ from normal (Kolmogorov-Smirnov test, Z = 1.201 , p = 0.111). Likewise for the Differential Conditioning treatment, the median preference is 3/10 compared to the model's 5/10, but once again the distribution is not significantly different from normal (K-S test, Z = 0.716, p = 0.684). The two distributions of initial preferences are statistically identical (Mann-Whitney U test, Z = -0.361, p = 0.718), indicating that bees' initial preferences for illuminant are not determined by the presence of "incorrect" or punishing flowers in the paradigm.

Visit speeds

Calculating this approximate measure of "search time" for the bees in the two treatments demonstrates that the average search times in both illuminants decreased between bouts 1 and 5, going from 25.5s per visit overall in bout 1 to 14.2s per visit overall in bout 5 for the Absolute Conditioning treatment and 20.7s per visit in bout 1 to 18.6s per visit in bout 5 for the Differential Conditioning treatment.

However, it also shows that the initial search times for foraging under the leaf-shade illuminant are consistently higher than the search times for the daylight illuminant. In the Absolute Conditioning treatment in the first bout, the bees averaged 58.0s per flower visit under the leaf-shade, and just 24.1s per visit under the simulated daylight (paired t-test on log-transformed data (as raw data were not normal), t = 2.118, p = 0.046). However, by bout 5 the bees took only 15.0s per flower visit in the leaf-shade and 15.2s per visit in the simulated daylight (paired t-test on log-transformed data, t = 1.558, p = 0.131). Likewise, for the Differential Conditioning treatment, the initial time per visit was 30.5s for the leaf- shade illuminant and 19.0s per visit for the simulated daylight (significantly different: paired t-test on log-transformed data, t = 3.135, p = 0.005), reducing to 21.8s and 18.3s per visit respectively by bout 5 (not different: paired t-test on log-transformed data, t = -0.064, p = 0.949), the increased search time perhaps reflecting speed-accuracy trade-offs as this setup contained punishments (Chittka et al., 2003).

The results are indicative of more difficulty or hesitation finding stimuli under the green leaf-shade illuminant when they have no previous experience with it, but a comparable performance at locating stimuli once the bees have experience.

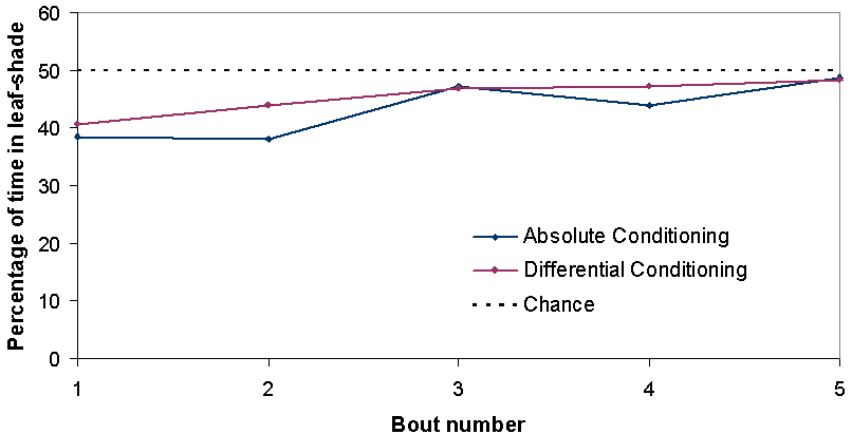


Figure 35. Preference for leaf-shade illuminant in the Absolute and Differential Conditioning treatments. In both cases, the bees initially show a level of aversion to the leaf-shade, spending less than 50% of their time there, but by the end of the training show no preference.

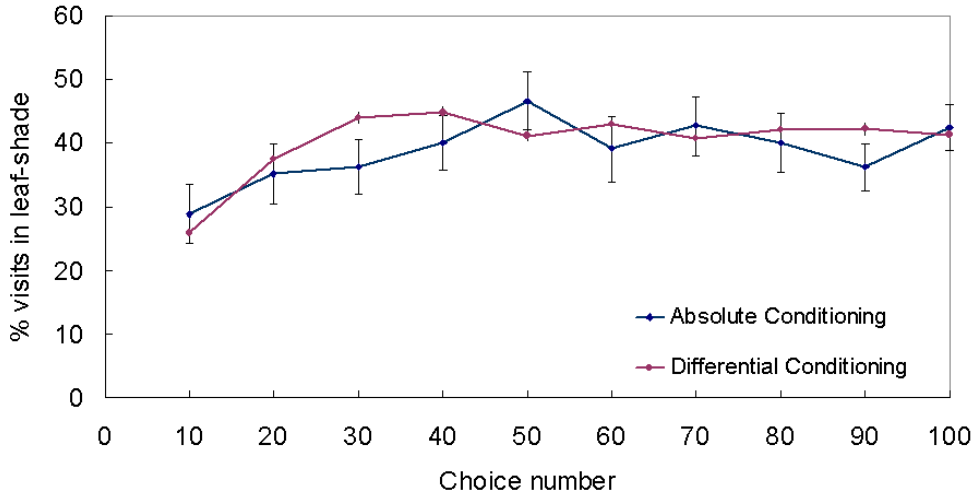


Figure 36. Flower visit data for the Absolute and Differential Conditioning treatments. Over the course of 100 flower choices, initially the bees made less than 30% of their flower visits in the leaf-shade illuminant, but after the 100 visits they have lost this aversion and the number of flower visits taking place under the leaf-shade illuminant is not significantly different from chance. (Bars = standard error.)

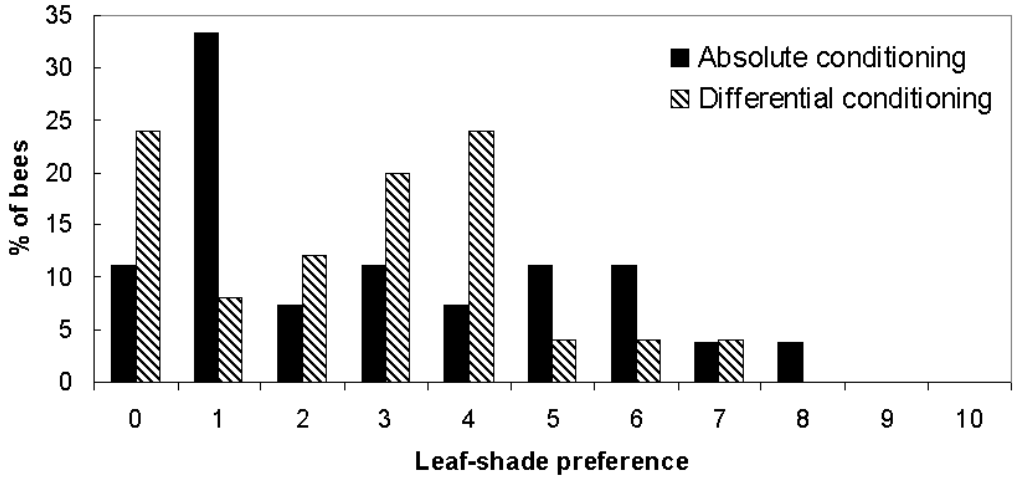


Figure 37. Distribution of initial preferences for visiting flowers in the leaf shade illuminants. Preferences may range between 0 (no visits in leaf-shade) and 10 (all visits in leaf shade) over the first 10 visits each bee makes.

CONCLUSIONS

Perfect colour constancy would require an animal to adapt instantly to moving into a different illuminant, to the point where they would not even be aware of the illumination change. It is clear from this experiment that bees can detect the spectral content of the ambient illumination, and notice when it changes. Furthermore, they are spontaneously choosing to fly in one illuminant more than the other by preference. This requires them to factor illumination information into their chosen foraging routes around the flight arena, potentially modifying routes in order to avoid potentially rewarding flowers because they are in the "wrong" quadrants.

The investigation of the bees' initial illuminant preferences in terms of the flowers they visit confirmed the initial aversion to the leaf-shade illuminant in both the Absolute Conditioning and Differential Conditioning treatments. Although to the eye it appears that both distributions of preferences are non-normal, which would indicate a diversity of illuminant aversion levels among the individual bees, the statistics do not support this conclusion and therefore it is likely that the initial tendency to avoid visiting flowers in this unfamiliar illuminant is a relatively consistent one, with a probability of around 0.7-0.8.

The visit speed data indicates that the bees initially forage relatively inefficiently, perhaps spending a lot of time investigating the arena rather than visiting flowers, or simply spending a lot of time searching for flowers on which to land. With experience, this process becomes more time-efficient, with the number of visits. This is in spite of the fact that experienced bees are less likely to revisit depleted flowers or negative stimuli containing quinine, so the total number of visits they make per bout tends to decrease.

The data also show that the initial search times for flowers under the leaf-shade illuminant are higher. The most likely explanation for this is that the bees find it more difficult to locate flowers under this light relative to daylight when they lack experience foraging in the new illuminant. Under the Differential Conditioning treatment, it is

possible that bees also find it more difficult to assess whether the flowers are “correct” or “incorrect” under leaf-shade. The fact that the behaviour is consistent for both absolute and differential conditioning, however, strongly suggests that it is not just assessing the “correctness” of a flower in leaf-shade that slows down the bee, as then one would not expect this behaviour to persist in the Absolute Conditioning treatment as well. It is therefore most probably simply a difficulty in finding the flowers, either due to the colour shift or the decrease in light intensity (as in Chittka and Spaethe (2003); Skorupski et al. (2006)).

EXPERIMENT 3: CONTROL EXPERIMENTS

Having established that bees exhibit an initial aversion to the leaf-shade illuminant, which is lost with time, the question remains: what causes this preference for one illuminant over the other. As it is present only initially, and over time and with experience the bee becomes indifferent to the illuminant, it cannot be an error-avoidance strategy; the aversion is, in fact, strongest when the bee has no experience of errors, and weakest when the bee has most experience.

This leaves some alternative possibilities:

- the bee avoids the illuminant that is unfamiliar to her
- the bee avoids the illuminant that appears "dimmer", i.e. has a lower light intensity
- the bee avoids illuminants of particular colours

In the control experiments, we examined the first two of these possibilities to see whether either of them was supported by behavioural evidence.

METHODOLOGY

Effect of pretraining

It is possible that the bees' prior level of experience with simulated leaf-shade could affect their behaviour with respect to the leaf-shade illumination patches. In the previous experiments, each bee was naïve in terms of her prior experience with green illumination but had prior foraging experience under simulated daylight illumination.

Therefore we ran a control experiment in which the bees were trained with absolute conditioning as before, but each bee had a minimum of one full bout of foraging under uniform leaf-shade light before the start of the experiment. Therefore, the green light was no longer "unfamiliar" to the bee (although typically she still had more experience with the artificial daylight illuminant). We then proceeded as previously, recording the

bee's choices and flight times under the two illuminants for 5 foraging bouts. The stimulus setup was identical to that in the Absolute Conditioning treatment in Experiment 2.

Effect of light intensity

It is also possible that any differences in bee behaviour under leaf-shade versus daylight could be accounted for not by the chromaticity of the green patches, but by the reduction in light intensity. This would be expected to affect signal-to-noise ratio, meaning that less visual information, and particularly less colour information, is available to the bees.

Therefore we ran an additional experiment to control for light intensity, by balancing the light intensity in daylight and leaf-shade patches by placing a neutral density filter over the arena lid in the daylight patches. Light transmitted through the neutral density filter had a similar illuminance (25.8% of white light) to that transmitted through the leaf-shade filters (18.0% of white light) across the 300-700nm range, so that the two types of illuminant now had similar brightness but one retained essentially the same spectral composition as the original simulated daylight, whilst the other (leaf-shade) was green. Although still not an exact match, one could predict that if a large difference in light intensity accounted for a strong preference for the brighter illuminant over the dimmer one, a *smaller* difference in light intensity would result in a *weakened* preference for the brighter illuminant.

Control for eye size

It is possible that, where there is a demonstrated aversion to leaf-shade light in some bees, the magnitude of this aversion will be determined in part by the bees' visual acuity and/or its ability to function in low lighting. These measures are partly dependent on the size of their eyes and therefore the light that the eyes are able to capture. In bees, visual acuity is dependent on the bee's eye size and the consequent size of ommatidia; this scales relative to the size of the bee's body (Snyder and Menzel, 1975; Spaethe and Chittka, 2003). Body size varies greatly in bumblebees compared to honeybees, sometimes varying by a factor of 10 (Michener, 1974; Spaethe and Chittka, 2003;

Spaethe and Weidenmüller, 2002). Using thorax width as a proxy for eye size can provide us with an approximate estimate of the bee's visual acuity and sensitivity to light (Spaethe and Chittka, 2003), and this should be considered when accounting for variations in bee performance, in particular under low-light conditions or in setups where poor visual acuity or photon capture may compromise performance.

All tested bees from the Differential Conditioning experiment were frozen after the testing, as samples of the total bees used. We then measured their thorax widths using electronic digital callipers (Axminster Power Tool Centre, Axminster). Each bee was measured three times for the width of the thorax just in front of the wings, and the mean of these readings was used as the thorax diameter. We were then able to look for correlations between thorax width and various measures of behaviour.

If it was the case that bees made more mistakes under the leaf-shade light only because of the signal-to-noise ratio, i.e. in lower light intensity it was more difficult to extract useful information from the scene, then bees with larger eyes which can capture more light would be expected to perform better, making fewer mistakes. Equally, if any aversion to leaf-shade light was due to bees avoiding dark patches in which it was more difficult to perform, one might anticipate that this aversion would be stronger in smaller bees with small eyes, who would struggle more under low light conditions.

RESULTS

Effect of pretraining

Bees in this treatment experienced absolute conditioning during the experiment identical to those in the Absolute Conditioning treatment of the previous patchy light experiments, but had received prior experience with green light so that green light was no longer an unfamiliar stimulus. Figure 38 shows the bees' preferences for leaf-shade versus daylight in terms of flight time over the course of the experiment. Although the bees still show a slight initial aversion to the green light (spending only 44% of their time in the green light patches on average), this preference is not significant; the initial aversion to green light appears to have vanished (paired t-test, $t = -1.37$, $df = 14$, $p =$

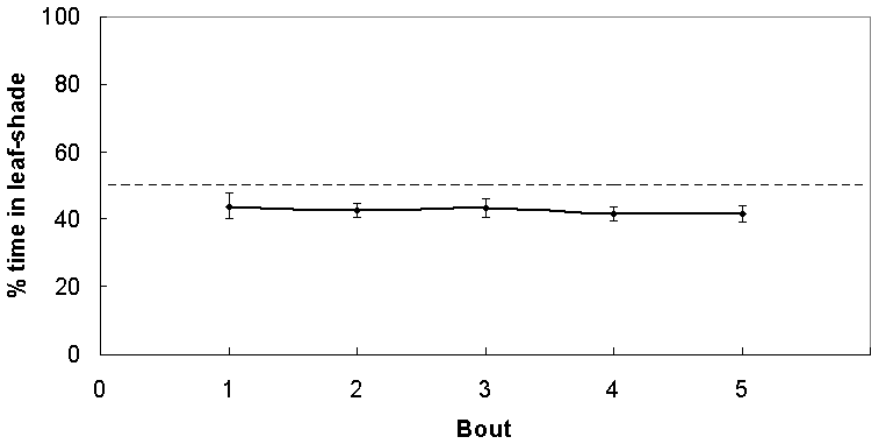


Figure 38. Bee flight time preferences for the leaf-shade patches after prior foraging experience in this illuminant. The aversion to the green leaf-shade patches is no longer significant. (Bars = standard error.)

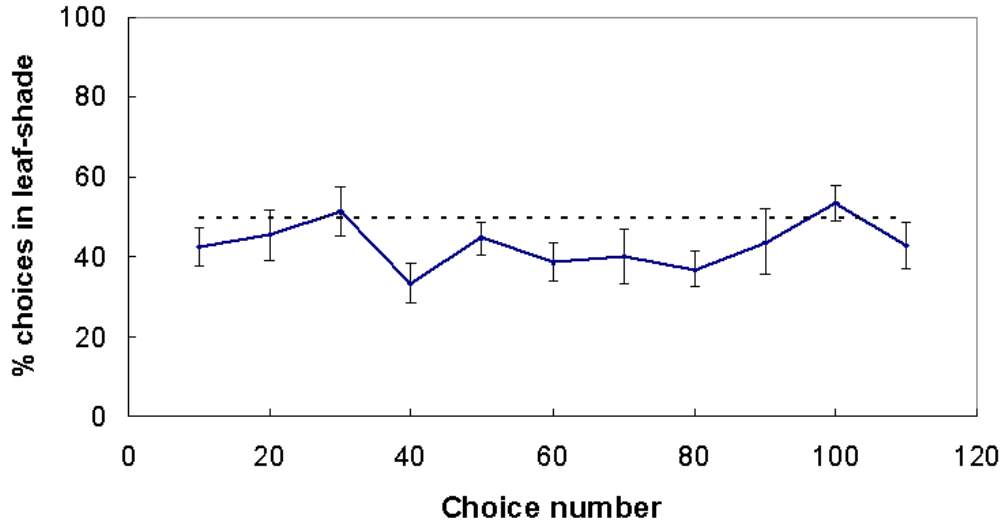


Figure 39. Bee visit preference for flowers in green leaf-shade light after pretraining providing previous foraging experience under leaf-shade illumination. The aversion to leaf-shade light that was observed initially in bees with no prior with the illumination is not present in bees with this experience. (Bars = standard error.)

0.096). This is confirmed by the visit data in Figure 39; the proportion of visits the pretrained bees make under leaf-shade is significantly different to the proportion made by bees with no prior experience of leaf-shade (t-test, 44.2% of visits in leaf-shade versus 28.9%, t = -1.75, df = 36, p = 0.045).

In the previous experiments in which the leaf-shade illuminant was unfamiliar to the bees whilst the simulated daylight was familiar from when they were allowed into the arena to forage outside experimental time, they initially took much longer finding flowers under leaf-shade than under simulated daylight. With pretraining, however, this difference is eliminated: in bout 1, the pretraining control bees visited flowers in the leaf-shade at a speed of 15.2s per visit, and in the simulated daylight at 16.4s per flower visit, which is not a significant difference (paired t-test, t = -0.425, p = 0.339).

Effect of light intensity

Figure 40 shows the bees' preferences for green versus attenuated daylight in terms of flight time. The behaviour of the bees is largely the same as in the original experiment; i.e. the bees initially avoid flying in the green light patches. This preference is smaller than for the initial Absolute Conditioning experiment (41.9% of time spent under leaf-shade, versus only 38.6% in the original Absolute Conditioning experiment), but remains significant (paired t-test, t = -3.80, df = 15, p = 0.0009), and the flower-visiting behaviour, shown in Figure 41, is not significantly different from the original experiment (t-test, t = -0.70, df = 37, p = 0.245). This indicates that although there may be a small tendency for bees to avoid areas with lower light intensity whilst foraging, that does not explain their preference for daylight entirely.

Effect of pretraining: initial preferences

Figure 42 shows the distribution of individual bees' initial preferences for the green leaf-shade light according to their first ten flower choices in the pretraining control experiment, in which bees had prior experience with foraging under the leaf-shade illumination. In this experiment, the bees' median initial preference for the green light

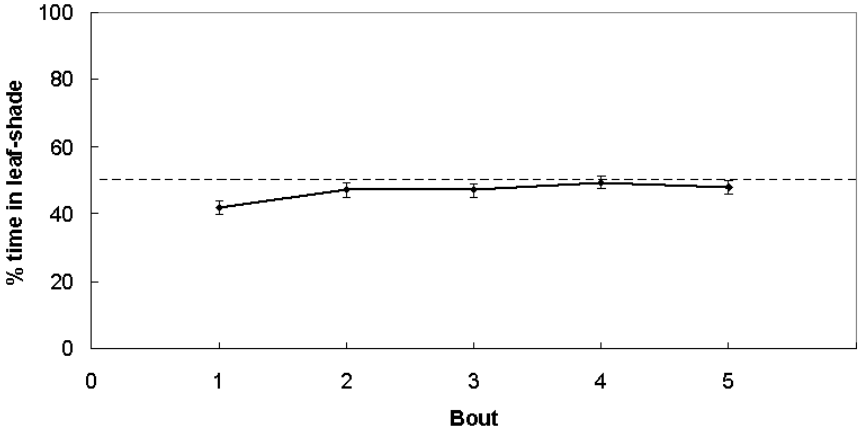


Figure 40. Bee flight time preferences for the leaf-shade patches in the light intensity control, in which leaf-shade and simulated daylight patches are matched for light intensity. The initial aversion to the green leaf-shade patches is retained. (Bars = standard error.)

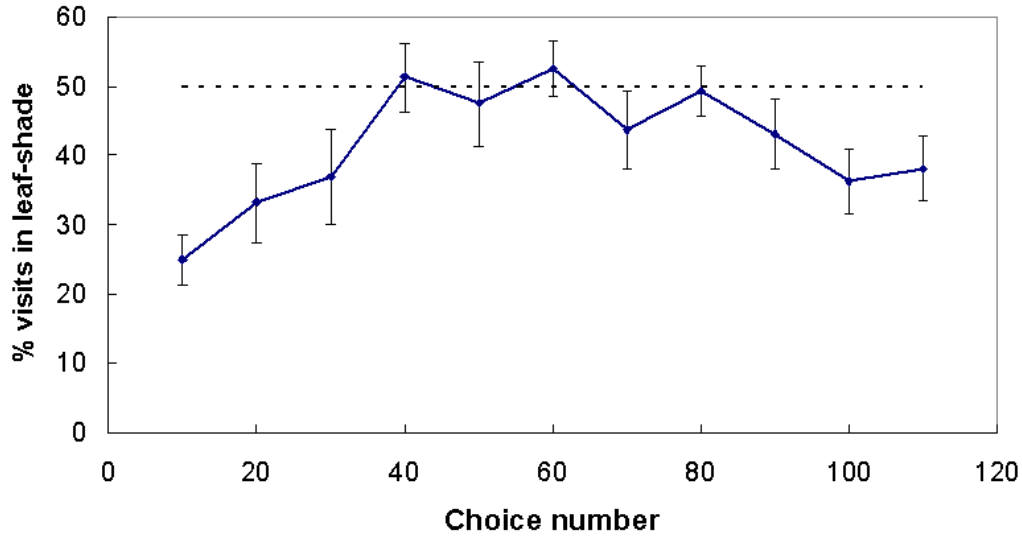


Figure 41. Bee visit preference for flowers in green leaf-shade light when the simulated daylight patches are intensity-matched with the leaf-shade patches. As in the previous experiments, bees exhibit an initial aversion to visiting flowers in the unfamiliar leaf-shade illuminant. (Bars = standard error.)

was 5, which is the same as the median preference produced by the model for bees selecting their illumination randomly. This distribution did differ significantly from the Absolute Conditioning condition of Experiment 1 (Mann-Whitney U, Z = -1.970, p = 0.049), demonstrating that prior experience with the green leaf-shade illumination decreases bees' aversion to it.

Effect of light intensity: initial preferences

The initial visit preferences for this control experiment are shown in Figure 42. The median value for the green light initial preferences was 3 for the light intensity control, in which the "daylight" and "leaf-shade" quadrants matched in light intensity (but not chromaticity) , which is lower than the modelled value of 5 . The distribution of initial preferences does not differ statistically significantly from the comparable Absolute Conditioning results from Experiment 2 (Mann-Whitney U, Z= 0.178, p = 0.858), indicating that the initial aversion to green light cannot be accounted for only by the intensity difference in illumination between the daylight and leaf-shade conditions.

Control for eye size

Thorax widths in the measured bees had a mean value of 5.26mm (range = 4.49 to 5.88mm). Although, as shown in Figure 43, there is a very slight positive correlation between initial visits made under leaf-shade light and thorax width (used as a proxy for eye size) in the bees trained and tested in the Differential Conditioning paradigm, this falls short of significance (Spearman's rank correlation, ρ = 0.078, p = 0.711).

Our data show no evidence that bees with larger eyes perform better under the lower light intensity leaf-shade patches compared to smaller bees: foraging performance under green light was not correlated with eye size (Figure 43; Spearman's rank correlation, ρ = -0.029, p = 0.894) and likewise final preference for green light in fact showed a very slight negative correlation with eye size, which also was not statistically significant (Spearman's rank correlation, ρ = -0.199, p = 0.340).

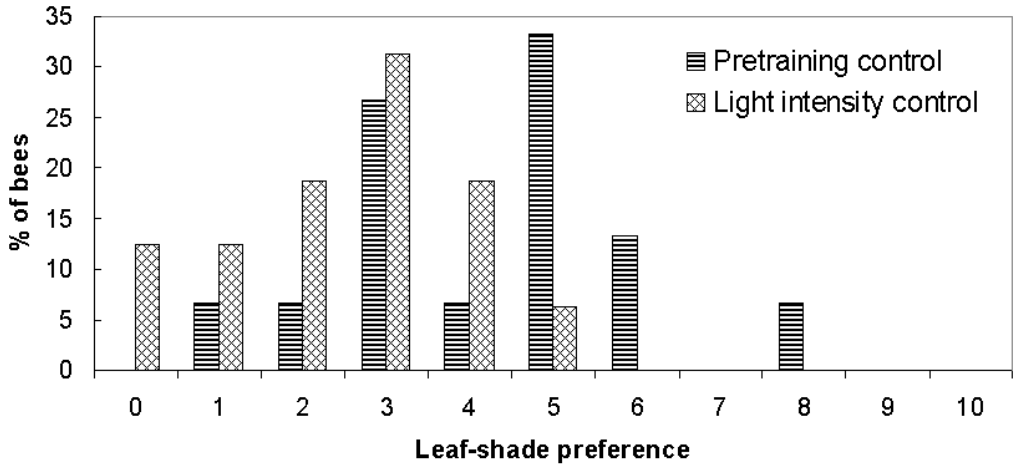
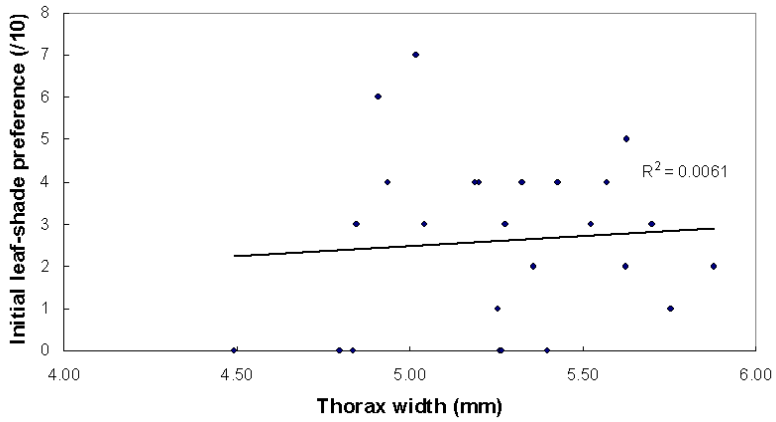
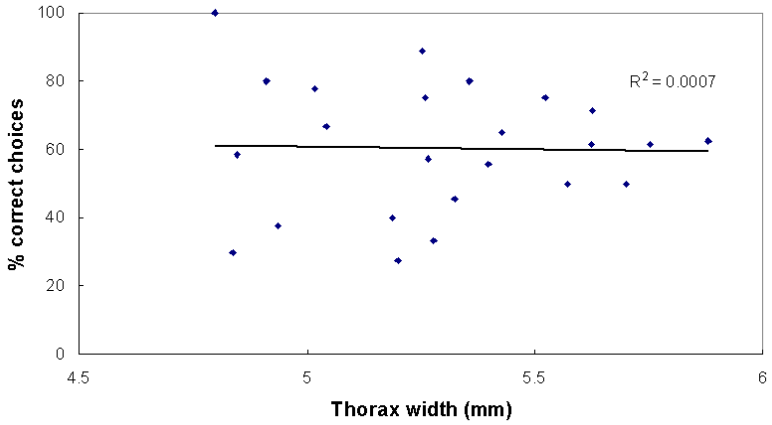


Figure 42. Preferences of the bees for visiting the artificial flowers in the leaf-shade patches versus simulated daylight during their first 10 landings. Bees can make between 0 and 10 of their first 10 artificial flower landings in the experiment on artificial flowers in the green areas; this graph shows the percentages of tested bees making different numbers of landings in the leaf-shade illuminant. These results are for the two control experiments, one controlling for the effect of prior experience (pretraining) foraging under green leaf-shade light, and the other controlling for the reduced light intensity in the leaf-shade patches.

a)



b)



c)

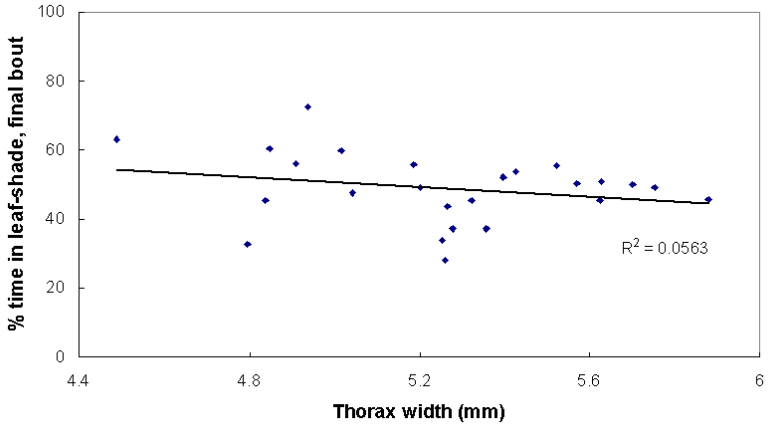


Figure 43. Controls for eye size. Thorax width serves as a proxy for body size and therefore eye size (Spaethe and Chittka, 2003), a metric has implications for bees' visual acuity and foraging under low-light conditions. However, in the Differential Conditioning scenario of Experiment 2, it is not significantly correlated with a) the initial number of visits made to stimuli in simulated leaf-shade during the first 10 choices, b) the total % of correct choices made in the final foraging bout, or c) the amount of time spent under leaf-shade illuminant during the final foraging bout.

CONCLUSIONS

The control experiments demonstrate that initial leaf-shade light aversion seems to be a result of unfamiliarity with the illuminant. It cannot be explained by the lower light intensity present in the green patches. The control for the effect of pretraining also confirms that the reason why bees appeared to spend longer looking for flowers under leaf-shade light in the first experiments was because of the unfamiliarity of the illuminant: in the pretraining control, the bees had to perform the same task, but having prior experience with locating stimuli under leaf-shade illuminant meant that they no longer did so more slowly than under the simulated daylight.

Eye size is not a factor in this leaf-shade aversive behaviour, nor does eye size cause predictable variation in bees' foraging performance under the green light in our experiments. This strongly suggests that bees' visual acuity and the intensity of light are not responsible for any of the behaviours previously noted under leaf-shade, such as a lower accuracy at finding flowers, initial aversion to the illuminant or a slower rate of visiting flowers.

EXPERIMENT 4: FLOWER CONSTANCY UNDER PATCHY LIGHT

This experiment was performed by a final-year undergraduate student, Hin Wai Henry Mak, under my supervision. We designed the experiment in collaboration and he collected the data. I performed the analysis.

Flower constancy is a the behaviour observed in pollinators in which they tend to visit multiple flowers from the same plant species successively, even ignoring potentially rewarding flowers from another plant species (Bennett, 1884; Christy, 1884; Waser, 1986). *Bombus terrestris* is known to exhibit measurable (though not total) flower constancy over the short term (minutes), and this is thought to be constrained by memory dynamics (Raine and Chittka, 2007b). However, this is normally investigated in a meadow environment with relatively spatially consistent light, and many bumblebee species forage in hedgerows and woodland edges where the lighting is spatially inconsistent. If it makes it harder to recognise flowers, this may cause the bees to make "mistakes" when passing from one illuminant to another and visit a different colour or species of flower to the one on which the bee had been foraging previously. Alternatively, the bee may become more conservative in her behaviour and be less likely to probe flowers of new species if they are encountered in an illuminant that is also unfamiliar.

We therefore wanted to investigate how a patchy light scenario affected bumblebee flower constancy. Incomplete (or absent) flower constancy requires bees to sample different flowers. When the lighting is patchy or inconsistent, there are three possibilities as to how this sampling behaviour will be affected:
1. The bees will "sample" different flowers with higher frequency than under uniform light, as the illumination changes reduce their ability to recognise familiar flowers consistently (i.e. cause them to make mistakes).
2. The bees will sample different flowers with lower frequency, as the illumination change creates a riskier environment, in which foraging on a familiar species by preference represents a safer strategy.
3. The bees will correct for the changed illuminant perfectly and their flower constancy behaviour will be unchanged.

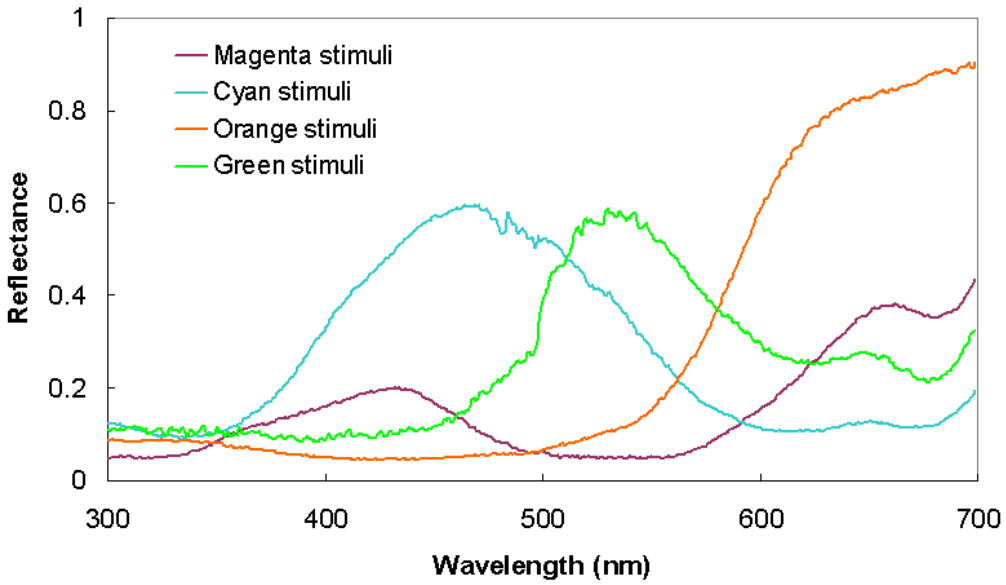
METHODOLOGY

A similar flight arena to previously was used, though the dimensions were smaller (0.7m x 1.1m). As before, 16 artificial flower stimuli were used in an experiment, with two colours of stimuli each time, and these were arranged so that each quadrant of the arena contained two artificial flowers of each colour.

In this experiment, two colour pairs were used, either Magenta/Cyan or Orange/Green. The colour hexagon loci and spectral reflectances for these colours are shown in Figure 44. The colours were chosen because of their clear colour difference in bee colour space (Magenta/Cyan: 0.198 hu; Orange/Green: 0.117 hu) and because the first two colours appear UV-blue and blue to bees respectively, both considered attractive colours to bees with no prior colour experience (Gumbert, 2000), whilst orange and green appear UV-green and green, which are both less attractive. Therefore in both cases the bees should be able to tell the colours apart easily and in neither setup do the bees encounter one very strongly attractive colour versus one that they would typically avoid, a scenario that may bias results. The coloured stimuli were set up as artificial flowers, in the same manner as in previous experiments; the two colours used in each experimental setup represent flowers of two different plant species.

We then released bees which had no prior colour training but which had received absolute conditioning on uncoloured, UV-transmitting plastic tiles into the experimental arena. The arena contained the two types of stimuli, and two quadrants covered by green filters in a Battenberg layout as previously described. All artificial flowers contained 10µl of 40% sucrose solution, allowing the bee to satiate and return home within a foraging bout. Every time the bee touched a flower, it was considered to be a landing, and all the landings made by the bee during a foraging bout were recorded. The bees were allowed to forage until they had made 60 landings (usually taking 3-4 foraging bouts), then they were removed from the apparatus, frozen and their thorax widths measured as a control for eye size, as before.

a)



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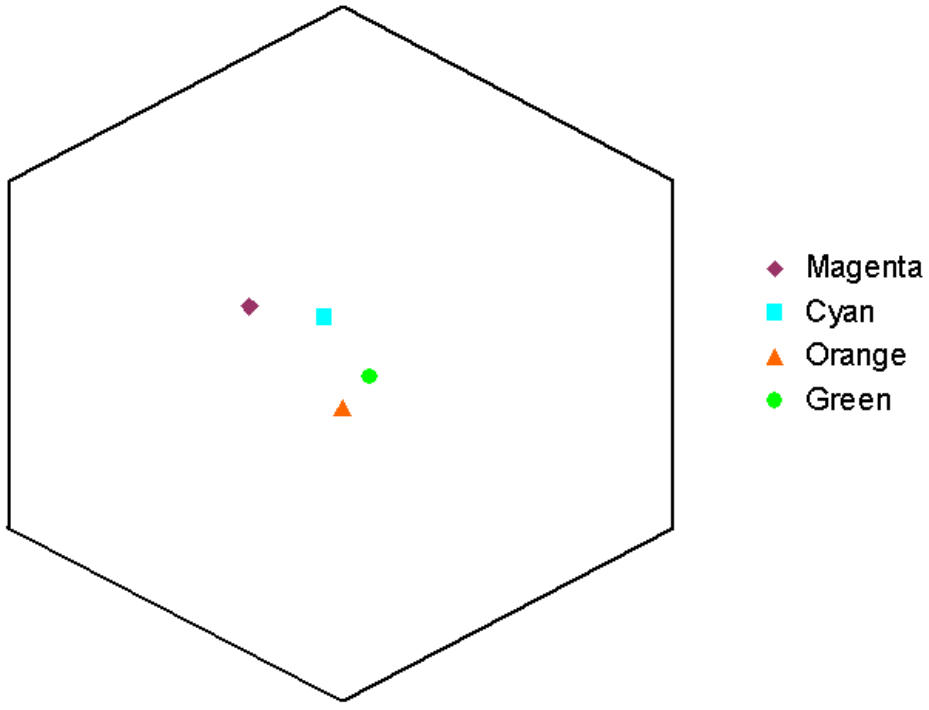


Figure 44. a) Spectral reflectances of the four stimuli used in the flower constancy experiments, and b) their colour hexagon coordinates under simulated daylight illumination.

In total, 21 bees were tested, 11 of them on the Magenta/Cyan colour pair and 10 on the Orange/Green colour pair.

Data analysis

With the data recorded on which colours of flower a bee visited and in what order, it is possible to calculate the bee's flower constancy index (Bateman's Index), as in Gegear and Laverty (2005) and Waser (1986). We were able to do this for the visit data for our experimental bees, and see how it differed overall for the two colour pairs. We were also able to use the same formula to look at switches between illuminants, giving us an index of "illumination constancy" (i.e. fidelity to a single illuminant). Bateman's Index ranges from -1 to 1, with 0 representing random choices (e.g. randomly choosing Magenta or Cyan, or randomly choosing flowers in leaf-shade or daylight illuminant), whilst -1 indicates total inconstancy and 1 indicates total constancy to a species (here, represented by a particular colour of stimulus) or illuminant. If the bees chose flowers entirely at random, both the flower constancy and illuminant constancy indices would be expected to be zero or very close to zero.

Two successive flower visits could be classified into one of four types: same illuminant/same flower type (e.g Magenta versus Cyan) (SI/ST), same illuminant/different type (SI/DT), different illuminant/same species (DI/ST) or different illuminant/different species (DI/DT). If the light colour illuminating a flower makes no difference to whether the bee switches species or not, one would expect that the ratio of SI/ST to SI/DT would be the same as DI/ST to DI/DT – i.e. the bee is as likely to be "disloyal" and switch flower types within the same illuminant as between illuminants. However, if the two ratios differ, it implies that switching illuminant causes the bee to be more or less flower constant than when foraging within an illuminant patch. This might be expected if either the bee makes mistakes in identifying flowers when switching between illuminants, or if the bee is displaying "cautious" behaviour and tending to prefer a more familiar flower type.

RESULTS

Constancy indices

The overall flower constancy index, pooled for all bees in each treatment group, was -0.2959 for the Magenta/Cyan pairing and -0.1392 for the Orange/Green pairing, when illumination is not taken into account. This is indicative of slight flower inconstancy for both colour pairs (some tendency to actively avoid visiting the same flower species twice in succession). Analysis of the ratios of same-type visits to different-type visits supports this, with a significantly higher proportion of visits for both colour pairs being made to flowers of a different type to the same type as the last (Chi-square test, Magenta/Cyan: $\chi^2 = 56.211$, $df = 1$, $p = 6.5 \times 10^{-14}$, Orange/Green: $\chi^2 = 8.786$, $df = 1$, $p = 0.003$).

By contrast, the illumination constancy indices for both colour pairs indicate an above-random degree of fidelity to the illuminant. For the Magenta/Cyan pairing, the illumination constancy was 0.1421 and for the Orange/Green pairing it was 0.0579. Both values are relatively close to random but for the Magenta/Cyan pairing there were nonetheless significantly more visits being made in the same colour of illuminant rather than a different colour (Magenta/Cyan: $\chi^2 = 16.988$, $df = 1$, $p = 0.000038$; Orange/Green: $\chi^2 = 2.197$, $df = 1$, $p = 0.1383$).

Magenta/Cyan pairs

For the Magenta/Cyan colour combination, the bees made 55% of their initial landings on Cyan flowers, and 64% overall were made in daylight patches. These differences did not differ significantly from random (Fisher's exact test, $p = 0.545$). The breakdown of the initial choices of the bees is shown in Table 3.

Throughout the experiment, the bees made slightly more choices (52%) to the Cyan stimuli rather than to the Magenta ones. Although under the leaf-shade illuminant, 54% of visits were made to the Cyan stimuli, this is not a significant preference (Chi-square test, $\chi^2 = 1.524$, $df = 1$, $p = 0.217$).

Table 3. Choice behaviour of bees in the Magenta/Cyan treatment, showing the number of bees choosing each type of artificial flower (Magenta or Cyan, under daylight or leaf-shade) for their first landing

	Daylight	Leaf-shade
Magenta	4	1
Cyan	3	3

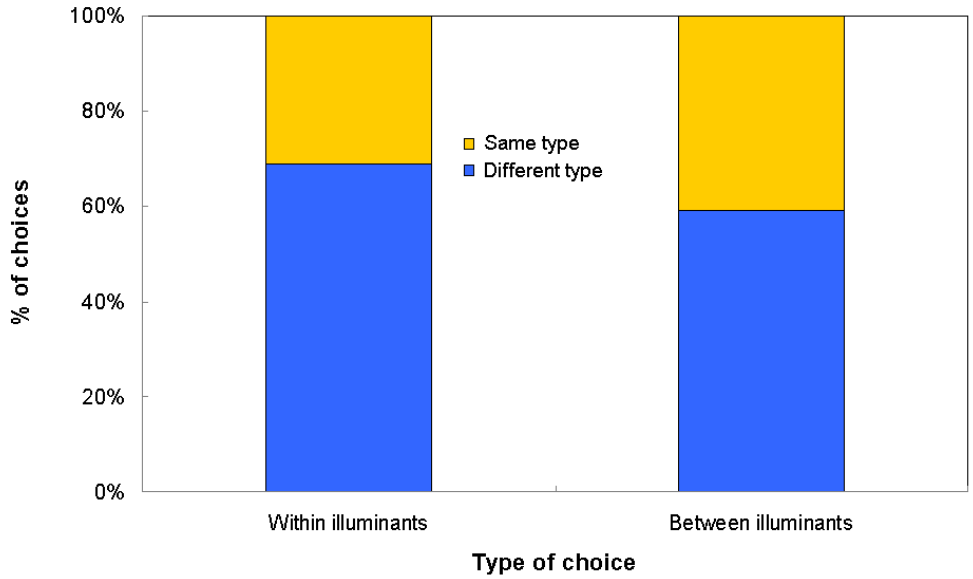


Figure 45. Choice behaviour of bees on equally rewarding artificial flowers of colours Magenta and Cyan, showing the relative frequencies of switches of flower type within and between illuminant patches. Bees choose flowers of a different type significantly more often within an illuminant patch than when switching illuminants for this colour pair.

As demonstrated above, in this experiment, the bees exhibited no flower constancy. However, they switched type within illumination patches more often than between illumination patches (Figure 45) – overall, for this colour pair, 40% of visits involved the bee switching type within an illumination patch. There was a significant tendency for bees to be more flower-constant when switching between illuminants than when foraging within an illuminant (Chi-square test, $\chi^2 = 6.257$, $df = 1$, $p = 0.012$).

Orange/Green pairs

For the Orange/Green pairs, the bees made the vast majority (80%) of their initial choices to the orange stimuli, and 70% of the initial choices in the simulated daylight patches, but these differences are not statistically significant (Fisher's Exact test, $p = 0.533$). The breakdown of initial choices is shown in Table 4.

Throughout the experiment, the bees made more choices (54%) to Orange rather than Green. This preference is not significantly affected by whether the bees are foraging under simulated daylight or leaf-shade (Chi-square test, $\chi^2 = 0.104$, $df = 1$, $p = 0.747$).

Similarly to the Magenta/Cyan colour pair, for Orange/Green pair, a majority (32%) of choices consisted of bees remaining in the same illuminant type but switching type. However, although the proportion of species-switches between illuminants was lower than those within illuminants (Figure 46), this missed the significance threshold for this colour pair (Chi-square test, $\chi^2 = 2.047$, $df = 1$, $p = 0.153$).

CONCLUSIONS

Based on the data collected in this experiment, it appears that bees foraging within an illumination patch will switch between flower type (or in the field, plant species or colour morph) frequently. This is probably a particularly pronounced tendency in a flight arena setup such as this, in which the two flower types have identical morphology and minimal handling times, and the energy expended by landing on a different flower type that may possibly prove unrewarding is very low – so the bees have little incentive to learn flowers of type and remain loyal to that type throughout the bout. In a field

Table 4. Choice behaviour of bees in the Orange/Green treatment, showing the number of bees choosing each type of artificial flower (Orange or Green, under daylight or leaf-shade) for their first landing

	Daylight	Leaf-shade
Orange	5	3
Green	2	0

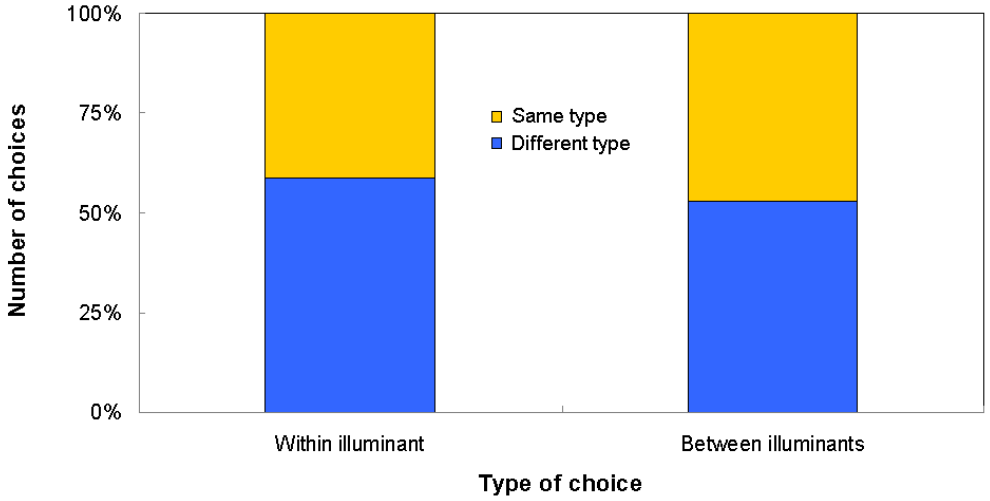


Figure 46. Choice behaviour of bees on equally rewarding flowers of colours Orange and Green, showing the relative frequencies of switches of types within and between illuminant patches. Bees choose artificial flowers of a different type more often within an illuminant patch than when switching illuminants for this colour pair, but this misses the significance threshold.

environment this could work differently, as flowers of different plant species also differ in other dimensionalities: odour, size, morphology, and may accordingly require different handling techniques. These factors will work to reduce switching between flowers from different plant species.

However, based on our findings, when the bees pass into a new illumination, they are more likely to remain with the flower type they most recently visited than they would when foraging in a consistently illuminated patch of the arena. This may be because bees are less likely to engage in further exploratory behaviour in an environment already presenting unfamiliar conditions, and therefore this developed as a risk-aversion strategy.

Given that the cost of foraging in a flight arena is very low, and in particular the flight times between flowers are very small, representing a small risk and low energy expenditure regardless of the choices a bee makes, it is hard to predict whether this behaviour would be maintained in a bee foraging in a more ecological environment such as a woodland edge. Were it to be the case, it may potentially have implications for flowers with a distinctive appearance and low frequency. A bee encountering such a flower in an area of consistent light would be more likely to explore it than if it was encountered whilst the bee was switching illuminant rapidly. This may, possibly, result in selection for rare plant species adapted to living on woodland edges to mimic similar species in order to gain more visits, but for plants better-adapted to living in the centre of meadows or in deep woodland to evolve a more distinctive floral appearance as bees in consistent light will be more likely to land on them without prior experience of the plant species.

DISCUSSION OF BEE FORAGING UNDER PATCHY LIGHT

When foraging in the wild, bees often move in and out of patches of different illuminants, such as daylight and leaf-shade, or solar daylight and skylight (Dyer and Chittka, 2004a; Lotto and Wicklein, 2005; Lythgoe, 1979). Unless they can adapt perfectly to these illuminant changes, this could be expected to affect their perception of colours and possibly their ability to select the correct flowers. We found that indeed, bees selected the correct flower less often under a light condition resembling leaf-shade than under a light condition resembling natural daylight, implying a failure of colour constancy. This finding adds further support to work by Dyer and colleagues (1999; 2006; 2004a), showing that there are limitations to bee colour constancy and under particular circumstances it may be imperfect.

Previous studies such as Dyer (2006) have demonstrated, by varying light conditions during a foraging task, that bees are able to perceive properties of the illuminating light, such as its spectral content, Therefore, the bees would certainly have been aware, in the patchy light conditions in this experiment, that different areas of their foraging environment are illuminated by different types of light.

Based on this information, one would predict that when faced with a choice between visiting flowers in leaf-shade and visiting flowers in daylight (natural or simulated), bees would learn to visit daylight patches preferentially as they are associated with a lower rate of errors. However, this would be a learned behaviour – in the beginning, when the bee has no experience of either incorrect choices or the chances of making mistakes under either illuminant, the bee would have no reason to prefer daylight illuminant over leaf-shade. Our results do not support this hypothesis: although the bees do exhibit an aversion to leaf-shade during the experiment, this aversion is, in fact, stronger in the beginning when the bees have no experience with the stimuli and, regardless of whether there are negative stimuli present or not, it grows weaker over time until the bees are statistically indifferent to the illuminant. This implies that the aversion is based not on avoidance of mistakes, but upon some other behaviour.

One expects that bees would show preferences for illuminants that they consider to be in some way “less risky”, in a similar manner to other behaviours they develop as a

result of increased risk (e.g. Cartar and Abrahams (1996), Harder and Real (1987), Ings and Chittka (2008)). Such adaptive risk aversive behaviour has been recorded in numerous other animal species, both vertebrate and invertebrate (e.g. Gillespie and Caraco (1987), Wunderle and O'Brien (1985)). In this case, the concept of "less risky" could be in terms of their ability to find safe, rewarding flowers based on past experience – which our results do not support – or, alternatively, the illuminants may be considered less risky in terms of their familiarity to the bees. Our data support this hypothesis much better: bees with no initial experience of leaf-shade light avoid it when allowed to choose, but lose this aversion as they gain experience with foraging in the patchy light environment. In contrast, bees that have initial pretraining experience under green light exhibit no significant aversion and their behaviour remains consistent throughout the experiment. Therefore, although the bees do exhibit risk-avoidance behaviour, it would seem to be mediated by lack of familiarity with the illuminant rather than experience of a higher error rate.

This is likely to be a good strategy for bees; unfamiliar illuminants may make it harder to detect threats (predators, etc.) and if bees' colour constancy indeed is imperfect, the bee may lack the ability to anticipate how the illuminant might distort their colour perception of flowers. However, when the bee has more extensive experience with stimulus appearance under an unusual illuminant, she may be better able to predict the appearances of flowers and can estimate the likelihood of the illuminant being associated with risk.

Novelty-aversive behaviour in insects is already documented (Forrest and Thomson, 2009; Lihoreau et al., 2009) and appears to be a trait consistent within individuals at least on a particular day (H. Muller, personal communication). Our results also show individual variation in the strength of the initial aversion to green light, with some bees displaying indifference from the outset even without prior experience of the illuminant, and other bees avoiding unfamiliar illuminants very strongly.

It can be beneficial, in terms of predator and effort avoidance, to display aversion to novel stimuli and contexts - however, for a foraging bee, avoiding novelty, including novel illuminants, may also be detrimental. A bee entirely avoiding the leaf-shade illuminant would miss out on foraging opportunities and may not return to the nest with

a full crop of nectar. However, typically bees made their first flower visit under leaf-shade light before they had exhausted all the flowers under simulated daylight, indicating that the aversion is not absolute. This allows the bees to sample, and gain familiarity with, the novel illuminant.

Bees' reactions to patchy light conditions could have consequences for flower colour evolution, and possibly floral mimicry. With bees more likely to make mistakes under lower-light and unusually illuminated conditions, floral mimics may benefit from growing under leaf-shade and similarly "difficult" light conditions in which bees are more likely to pay visits to them in error. This could be tested by surveying the proportion of floral mimics that tend to grow in shade versus sunlight, relative to the flora in general, in a range of habitats.

Flowers growing in shaded areas may be likely to receive fewer visits from flower-naïve bees relative to flowers in more open spots, assuming that most bees' initial foraging experience will be focused on daylight areas. As bees rapidly learn to overcome the aversion, this effect will be short-lived. However, it is possible that flowers of plant species that grow in shade may benefit from resembling those in open areas in order to attract bees, and species with the plasticity to grow in both shaded and open patches may do best as our experiments provide indications that the bees may be more likely to visit a familiar flower in an unfamiliar illuminant than an unfamiliar flower in an unfamiliar illuminant.

In conclusion, we show here that bees have colour constancy failures under certain conditions, and make mistakes in identifying colours under some illuminants more than others. Bees are able to choose to enter or avoid patches of different illuminants, indicating awareness of the spectral properties of the illuminant and the possibility that some illuminants may be more risky or less desirable than others. They preferentially avoid certain illuminants, but this is mediated by the fact that the illuminant is unfamiliar rather than because it causes colour shifts leading to misidentification of flowers or is associated with lower light intensity, and the aversion is lost with experience. This behaviour may have consequences for the pollination biology of plants in woodland edges and hedgerows, but these are likely to be modest as bees rapidly learn to exploit all available light environments.

CHAPTER VI

THE COLOUR VISION OF MONKEY BEETLES (COLEOPTERA: HOPLIINI) OF THE SOUTH AFRICAN CAPE REGION, AND HOW THIS MAY RELATE TO THE COLOURS OF CAPE FLOWERS.

INTRODUCTION

The reason why flowers are often large and brightly coloured (i.e. contrasting with the background to insect eyes) is to be an attractive advert to pollinators, in order to secure flower visits from pollinators and ultimately transfer of pollen for reproduction. A flower community is a market: plant species compete with each other, displaying flowers that are attractive and salient to pollinators in order to receive more high-quality visits and receive conspecific (rather than heterospecific) pollen (Friedman and Shmida, 1995; Gumbert et al., 1999; Raguso, 2004).

This can be predicted to favour particular floral traits – the flowers must, in some modality, be conspicuous. This could be achieved with large size, colour that is attractive or contrasts well with the background, strong scents or, in many cases, all of these factors. However, as discussed in previous chapters, which colour will result in the highest pollination rates depends on the other plants in the environment and possibly also the pollinator to which the flower is best-adapted.

Introduction to the Cape region and its ecology

The Cape region of South Africa is a species-rich biome. The region includes the Cape Floral Kingdom to the south, extending across the Western Cape near Cape Town and the Succulent Karoo further north, as well as covering Namaqualand in the Northern Cape. These regions all play host to a fascinating and striking diversity of plant and animal life. The region as a whole is characterised by low rainfall, particularly in the north (<150 mm per year in much of Namaqualand, 338 mm per year around

Nieuwoudtville) (Colville et al., 2002; Cowling et al., 1999), but the rain which falls tends to be very reliable, falling predominantly during the winter months (June-August). This provides the soil with sufficient moisture to support a spectacular floral display in the spring (July-September in Namaqualand, continuing through to November further south). The flowers appear for just a few weeks, but because of their simultaneous blooming and bright colours, the effect is noteworthy enough to attract considerable research interest (Cowling et al., 1999).

This bright floral display is well-known throughout the region, though in the wetter Cape Floral Kingdom the display is longer-lasting and has a somewhat different species composition to the Succulent Karoo further north. However, many floral genera (*Ursinia, Dimorphotheca, Gladiolus, Babiana,* etc.) are found throughout the range (Cowling et al., 1999; Goldblatt et al., 1998b). Because of the drought that persists through much of the year, many of the plant species in this region are perennials, remaining dormant through the dry periods and blooming when the rains come. However, numerous species of annual herbs contribute to the flora as well (Cowling et al., 1999).

Many of the flowers (or functional floral units) in the Northern and Western Cape are relatively large in size (*Moraea*, *Dimorphotheca*, *Gazania*, *Babiana*, etc.) and, to human observers, bright colours predominate. To human eyes, the commonest colours typically appear to be shades of yellow and orange (Goldblatt et al., 1998a). However, there are few, if any, studies exploring how the colours are perceived by insects and thus may have evolved under selection by the colour vision of insect pollinators (Johnson and Midgley, 2001; Van Kleunen et al., 2007).

The honeybee *Apis mellifera capensis* is present in the Cape region, as are various species of solitary bee (Melittidae, Megachilidae, etc.), which typically have similar photoreceptor sensitivities (Briscoe and Chittka, 2001; Peitsch et al., 1992). Although other types of pollinator with different colour vision also play an important role in pollination in Northern and Western Cape habitats, using the bee colour hexagon provides a starting point when exploring flower colour here. When plotted in the colour hexagon, many orange and red flowers such as those in the Cape habitats appear UV to bees – a colour not normally associated with bee pollination. The greatest reflectance

values for such species tend to be in wavelengths >600nm, to which bees' photoreceptors are not very sensitive (Chittka et al., 1994; Peitsch et al., 1992).

Although these subjectively-reported observations of flower colours in the Cape region are intriguing, they are not fully supported by species surveys. Here, we investigate whether the region truly does have an unusually large number of bee-UV flowers. And if so, why might so many of these flowers appear bee-UV in colour?

Is it possible that their colour is a signal to a pollinator other than bees? In common with virtually all habitats, South African Karoo and Fynbos contain a diversity of pollinating groups, including vertebrate and invertebrate pollinators. In addition to bees (social and especially solitary), there are long- and short-tongued flies (*Prosoeca* species and *Moegistorhynchus longirostris*, and Muscidae, Tabanidae, etc. respectively), a guild of Scarabaeidae beetles, various butterfly species (in particular, *Aeropetes tulbaghia*), sunbirds (which also serve to pollinate introduced plants from the New World that originally evolved to be pollinated by hummingbirds (Geerts and Pauw, 2009)) and even rodents (Johnson et al., 2001).

Overview of insect pollinators in the Cape and Namaqualand other than Apis mellifera capensis

Beetle pollination. Dafni et al. (1990) observed in the Israeli flora that some long-wavelength reflecting flowers with an open bowl shape and little or no human-detectable scent appear to be pollinated primarily by beetles. Although the colour vision of the beetle species (*Amphicoma* sp.) and the UV reflection of these flowers are still unknown, this would seem to be a comparable situation to that in Namaqualand, with its abundance of large, bright, and open or often bowl-shaped flowers in shades of yellow, red and orange (Goldblatt et al., 1998a). There is a group of potential beetle pollinators as candidates present in the Cape Region that could occupy a similar niche: the monkey beetles (Coleoptera: Scarabaeidae: Hopliini).

These are an important guild of pollinators in South Africa, particularly in the Northern Cape and Western Cape regions (Steiner, 1998). They are now believed to be the sole pollinator of a number of plant species (38% of *Ixia*, 11% of *Romulea* species), and

secondary pollinators of many other species (Picker and Midgley, 1996). Picker and Midgley (1996) identified at least 10 plant families containing one or more putatively monkey beetle-pollinated plant species, and the number of species pollinated by monkey beetles seems to be large, with Goldblatt et al. (1998a) identifying at least 40 species of plant, in 14 genera, pollinated by them.

They are small- to medium-sized beetles (~8-12mm long), often bright in colour, and named for their enlarged hind legs. Their larvae are thought to be mostly slow-growing, soil-dwelling organisms, feeding on the roots of plants throughout the year (Colville et al., 2002). The adult forms, conversely, are most frequently observed in late winter through to early summer (Goldblatt et al., 1998a; Johnson and Midgley, 2001) and are diurnal pollen-feeders (Picker et al., 2004). Their behaviour in terms of flowers is distinctive – they alight on flowers for miscellaneous activities including feeding, mating and overnight rest. Two beetles on a flower may interact (both mating and in some cases, confrontational behaviour to attempt to force one individual off the flower (Goldblatt et al., 1998a)) and often a beetle will spend a long time on a flower – hours, or all night. During this time, they often burrow into the centre of flowers, remaining near-motionless for some time, before eventually relocating to another flower (Goldblatt et al., 1998a). Because of their compact, hairy bodies and burrowing behaviour, they rapidly become covered in a thick layer of pollen (Figure 47), so they are able to transfer a large amount when they move to a new flower (Goldblatt et al., 1998a).

There have been several studies looking at monkey beetle behaviour, flower preference and species diversity, and their response to various environmental challenges (Colville et al., 2002; Goldblatt et al., 1998a; Johnson and Midgley, 2001; Mayer et al., 2006; Steiner, 1998; Van Kleunen et al., 2007). It has been established that there seem to be two groups of monkey beetle species, the guild termed “short wavelength”, and the “long wavelength” group, the former being found largely on blue/white flowers and the latter preferring orange, yellow and red flowers (Goldblatt et al., 1998a; Picker and Midgley, 1996).

Figure 47. Example of monkey beetles (Coleoptera: Scarabaeidae: Hopliini) carrying out mating behaviour on an orange Namaqualand inflorescence (*Gazania* sp.). It is apparent that both individuals have acquired a thick coating of pollen from the flower as it clings to their compact and rather hairy bodies. (Photograph by S.E.J. Arnold.)

Solitary bees. There are many solitary bee species in the region, covering many of the major taxa also present elsewhere in the world (*Anthophora*, *Lasioglossum, Amegilla*, *Andrena*, etc.) and these undoubtedly play an important role in pollinating many plant species (Goldblatt et al., 1998b). Goldblatt et al. (1998b) observed "moderate floral constancy" in *Gladiolus*-pollinating bees, with insects foraging on the same sequence of flowers repeatedly – this suggests they transfer a high proportion of conspecific pollen between flowers. Carpenter bees have some characteristics in common with European and North American bumblebees, namely large body size and the ability to buzz pollinate (Johnson, 1993; Johnson, 2004; Watmough, 1974), and are therefore able to fill an equivalent niche in South African pollination systems. There are also oil collecting bees (*Rediviva*), contributing to the pollination of *Diascia* (Vogel, 1984), some Scrophulariaceae (Manning and Brothers, 1986), and some Iridaceae (Goldblatt and Manning, 2006). Field observations and records of bee-pollinated flora in South Africa indicate that there is no single "bee type" flower colour and that bee-pollinated flowers of a diverse variety of colours are present in Cape and Namaqualand habitats (Goldblatt et al., 2001).

Long-proboscid flies. Fly species such as *Prosoeca* spp. and *Moegistorhynchus longirostris* possess extremely long probosces (6-10cm) (Johnson and Steiner, 1997; Manning and Goldblatt, 1997). This allows them to exploit species such as some *Gladiolus* and *Lapeirousia* species with extremely long corolla tubes. The flies move rapidly from flower to flower, spending a couple of seconds feeding on nectar at each one, often grasping at petal lobes when feeding rather than actually hovering (Goldblatt and Manning, 1999; Goldblatt and Manning, 2000). They are not believed to be flower constant (Goldblatt and Manning, 2000); though they sometimes seem to have a preferred type or form that they visit more often in a short period, but Goldblatt and Manning (2000) describe their foraging as mostly random in nature, switching between forms and colours of flowers.

Flowers pollinated by long-proboscid flies usually appear to have highly saturated and often dark colours to human eyes, e.g. violet, purple, cerise and red. However, they can also can also be pale mauve – especially in the Richtersveld region – and paler markings are common (Manning and Goldblatt, 1996). They are most often, but by no means

uniformly, unscented. They represent a relatively extreme specialisation as they are typically pollinated infrequently or not at all by any other insect species, and many have adaptations to place pollen only on one specific place on the fly's body (Manning and Goldblatt, 1996).

Short-proboscid flies. These flies mostly comprise Muscidae and Syrphidae, and are considered to be predominantly generalist in their flower-visiting preferences. Some, however, are important pollinators of species of plant bearing "carrion" flowers – those which have a odour and sometimes also an appearance resembling decaying animal or plant matter (Johnson and Steiner, 1994).

Lepidoptera. There are examples of some extremely specialised interactions between plants and moth or butterfly pollinators in the Cape region, the most striking example being *Aeropetes tulbaghia*, which visits large, showy, red flowers (Johnson, 1994; Johnson and Bond, 1994). Moth pollination is known to be of importance in these habitats as well, with around 50% of African orchids believed to be pollinated by moths (Dressler, 1981). However, in the fynbos areas of the Cape Region, there is virtually no evidence of specialist hawkmoth pollination (Manning and Snijman, 2002).

Pollen wasps. The pollen wasps (Vespoidea: Masaridae) are abundant and diverse throughout the Cape region (Gess, 1992). The species present in South Africa are generally quite specific in their pollen preferences, most often forage on Mesembryanthemaceae and Aizoaceae (small, low-growing succulent plants with usually fairly flat floral units that are most commonly pinkish or else yellow/yellow-green to human eyes (Struck, 1994)) (Gess and Gess, 1989). Some, however, feed on Asteraceae and Papilionaceae species (Gess, 1992).

What visual capabilities do monkey beetles possess?

In spite of a number of studies examining the behavioural colour preferences and foraging behaviour of monkey beetles, nothing is known about the physiology of their visual systems. Behavioural experiments suggest they are highly sensitive to long wavelengths and able to discriminate orange from red, yellow and blue model flowers (Johnson and Midgley, 2001). Some other beetles have been found to have either three

or four photoreceptor types (Briscoe and Chittka, 2001), in the latter case, with a very long-wavelength receptor with peak sensitivity >600nm.

Evidence for the possession of long-wavelength photoreceptors in some beetle species is compelling in the context of monkey beetles; if these animals have receptors enabling them to see red/orange then flowers which appear UV to bees may appear red/orange and UV-reflecting to the beetles. This would make them highly conspicuous to monkey beetles but not innately especially attractive to bees, implying that the convergence on these colours may have been as a result of beetles being favoured over bees as pollinators, perhaps because beetles can carry higher pollen loads (bees remove a lot of pollen by grooming, and many solitary bee species in South Africa are small) and because the relatively low ratio of bee diversity to plant diversity in the region (Waser and Ollerton, 2006). Adaptation of flower colour to attract other pollinators and deter bees has been previously demonstrated in the case of some hummingbird-pollinated plant species with red flowers (Castellanos et al., 2003), so this strategy is plausible.

Here, we investigate the colour vision of South African long-wavelength guild of monkey beetles, to discover whether they possess specialised red-sensitive photoreceptors. We hypothesise that they should possess some mechanism for seeing and discriminating wavelengths longer than those that can be seen by bees (i.e. enhanced vision in the 600-700nm range), given that they are attracted to flowers reflecting these wavelengths and this would represent an adaptation by the flowers to signal to them more effectively than to bees.

This would provide support for the theories of Dafni et al. (1990) about large, red flowers with open morphology being pollinated by beetles, supplement the existing knowledge of colour vision in beetles, which is still relatively poor, and provide an insight into the selective pressures resulting in the breathtaking floral display in Namaqualand and the Western Cape.

MATERIALS AND METHODS

Spectral reflectance measurements of the Cape flora and consideration of flower colour

In spring 2002, spectral reflectance measurements were collected (by M. P. Powell and V. Savolainen) from sites throughout the Cape Region, from Springbok southwards to Vanrhynsdorp and especially focusing on the areas in and around Namaqualand (the region approximately between Garies and Springbok). Upon arrival at each site, all species present within the area were noted and identified, and spectral reflectance measurements were taken of three flowers per species, from separate plants. Three repeat readings were made per flower, resulting in a total of nine spectral reflectance curves per species, per site. Using these nine curves, one can calculate an average spectral reflectance for each flower type, representing a "typical" example of the species. A list of sites and flowering plant species is given in Appendix VI. The measurements were collected using an Ocean Optics spectrophotometer (Ocean Optics Dunedin, FL, USA), with methodology as described in Chittka and Kevan (2005) and Dyer and Chittka (2004a) (using a Deuterium/Halogen light source and calibrating against a white standard). From these floral spectra we can then calculate colour hexagon coordinates for flowers of all the plant species, using the methodology described in earlier chapters.

As explained in previous chapters, the colour hexagon can be divided into 6 segments. Although arbitrary, this appears to reflect the distribution of flower colours within the hexagon, so would seem justified as a broad way to classify colours in bee colour space (Chittka et al., 1994). Knowing the colour hexagon coordinates for flowers of all the plant species from the study sites, they could then be classified into one of the six bee colour categories, and we could compare the proportion of the flora appearing to be each bee colour with the flora of other world regions. We chose to compare with two tropical datasets, consisting of flowers sampled in Brazil (Chittka, 1997; Chittka et al., 1994) and Costa Rica (collected by M. P. Powell; unpublished), and a dataset from Israel (Menzel and Shmida, 1993), as this covers a similar range of Mediterranean and desert habitats. It might be expected that the Israeli flora would be under similar

selective pressures to those in the Western and Northern Cape of South Africa and therefore that their colour compositions should be more similar than the South African dataset is to either Brazilian or Costa Rican flowers.

Beetle collection

Beetles were collected from two sites in South Africa during September and October 2009. In total, three species were investigated: *Clania macgregori* (4 beetles; 2 male and 2 female), collected from Nieuwoudtville, Northern Cape; *Pachycnema crassipes* (10 beetles; 1 male and 9 female) and *Lepisia braunsi* (4 beetles; 3 putatively male and 1 female), both collected from Darling, Western Cape. These are all relatively common and locally frequent generalist species which therefore have the potential to influence the ecology of multiple plant species.

As the most extensive and high-quality data were obtained from females of *P. crassipes*, this will be the main focus. However, some data providing indications about the colour vision capabilities of all three species were ultimately obtained.

Electrophysiological techniques

The experimental technique employed was similar to that employed in Döring and Skorupski (2007). Beetles were kept refrigerated in the dark at approximately 10°C, with food (horticulturally-cultivated *Gazania* flowers) and a water source available, until required.

Individual beetles were cold-anaesthetised in a -18°C freezer for 5-10 minutes to render them motionless, and then were immobilised using dental wax on a stand. We inserted the indifferent (reference) electrode, made of stainless steel, into the centre of the beetle's thorax, sealing it with silicone sealant. The recording electrode was a silver/silver chloride electrode with the glass microelectrode produced by an Intracel P-97 (Sutter Instrument Company, Novato, CA, USA) from a borosilicate capillary (Sutter Instrument Co.: outer diameter 1.0mm, inner diameter 0.5mm, with filament). The electrode was filled with either insect Ringer solution (recipe as in Federle (2001): NaCl 10.4g/l, KCl 0.32g/l, $CaCl_2$ 0.48g/l $NaHCO_3$ 0.32g/l) or 3.2M KCl solution and inserted

into the retina of the beetle's eye using a Huxley-style micromanipulator (MP85, Sutter Instrument Co.). As the indifferent electrode was placed in the thorax, recordings could be made from either eye.

The electrophysiology equipment we used consisted of a 1401 data recorder (Cambridge Electronic Design Ltd., Cambridge, England), an AxoClamp-2B (Axon Instruments Inc., USA) voltage clamp and an amplifier (NeuroLog; Digitimer Ltd., Letchworth Garden City, England), all connected to a computer running the Spike2 program (Cambridge Electronic Design Ltd., Cambridge, England). The coloured light stimulus was provided by a an IL1 Bentham light source and M300 Bentham monochromator (Bentham Ltd., Reading, UK; using a stabilised power supply 605 from Bentham) for the electroretinogram (ERG) sweeps or a bicolour LED (Maplin Electronics Ltd., UK) for the adaptation experiments.

It proved impossible with this experimental setup to make intracellular recordings from the photoreceptor cells in the beetles' retinas, most likely because of the small size of the cells and the fragility of the tissue, and therefore we focused on measuring extracellular electroretinograms.

Electroretinograms

For this set of experiments, the beetle was dark adapted for a minimum of 30 minutes and typically at least 1 hour. Then the eye was stimulated with 50ms flashes of light from the monochromator, delivered by a liquid light guide (Edmund Optics, UK) at a distance of approximately 1.5cm from the beetle's eye, at 8s intervals. Average intensity of an emitted light at this distance, across all tested wavelengths, was 2.39×10^{-7} μMol.

We stimulated the retina with the following wavelengths of light (in nm): 350, 400, 450, 500, 520, 540, 560, 580, 600, 620, 640, 660. However, the responses at 350nm were mostly of very poor quality with too much noise to ascertain the response accurately. For 540nm, we also took readings using neutral density filters to attenuate the light, a selection from Comar filters with optical density of 0.3, 0.6, 1.0 and 2.0 (Comar, Cambridge, UK), in order to plot an intensity/response curve showing how the response decreased as light intensity was reduced.

For each wavelength, we recorded a minimum of 40 responses in order to calculate an average response. This reduced the effect of random photon noise and helped to make weak responses more detectable.

Adaptation experiments

We performed adaptation experiments using a bicolour LED, which could emit either a red or a green light depending on the polarity of the current passing through the component. The two light colours (red and green) had peak wavelengths of 627 and 565nm respectively, and the emission spectra are shown in Figure 48.

We initially dark-adapted the beetle eye. Then, we used the LED to deliver a series of short flashes (50ms) of either green (565nm) or red (627nm) light, at 1.5s intervals. After recording the beetle's response to these flashes, we light-adapted the eye to constant green light (565nm) for a minimum of 2 minutes before resuming light flashes of the original colour. In a green-adapted eye, it is expected that the ERG response to green flashes would be reduced relative to the green response in a dark-adapted eye, as a result of down-regulation of the sensitivity of the green receptors (Laughlin and Hardie, 1978).

The ERG response to the 627nm flashes after light-adaptation, however, depends on the different photoreceptor types present in the eye. If the beetle possesses only one receptor type in the 500-600nm range, e.g. with a peak around 550nm, the response to red light (627nm) can only be mediated by this receptor. A bright green light at 565nm would light-adapt this receptor and cause down-regulation of its response, so the measured response to red light after adaptation would be reduced by approximately the same relative amount as the green light response. However, if the beetle possesses an additional receptor with a sensitivity of ~600nm, the prediction is that this will be comparatively unaffected by adaptation to green light and will continue to respond to 627nm light flashes at a similar level to a dark-adapted eye.

Therefore, we anticipated that a continuing strong response of the ERG to 627nm light even after adaptation would provide an indication that a "red-sensitive" receptor may be present in the monkey beetle retina.

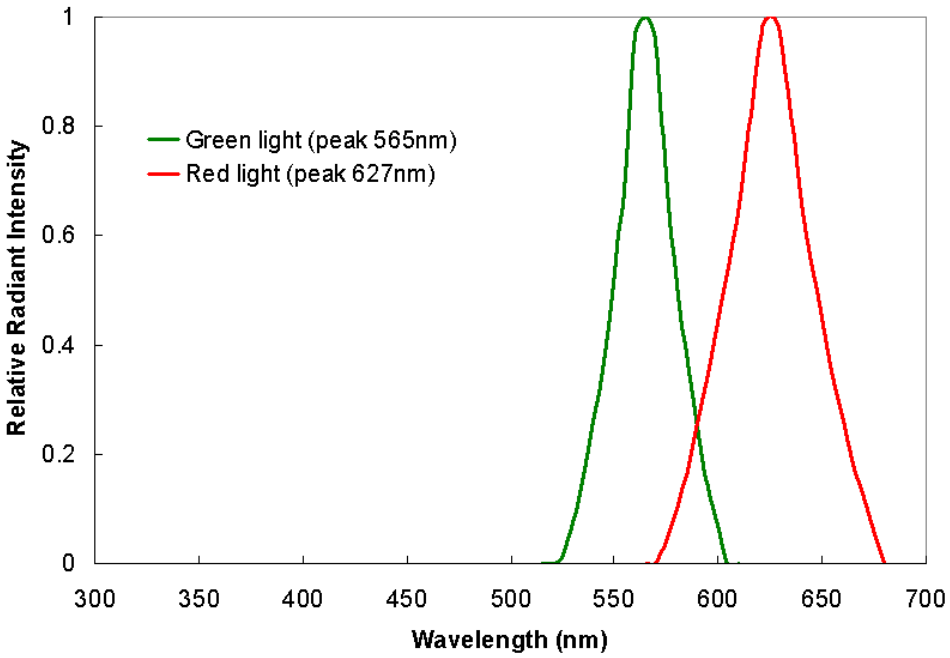


Figure 48. Relative radiant intensity spectra for the two colours of light emitted by the bicolour LED. Data provided by Kingbright to Maplin Electronics Ltd.

Calibration and analysis

The absolute size of the ERG response to different wavelengths of light only provides direct information about the photoreceptors' sensitivity to that wavelength if the monochromator emits all wavelengths at equal intensity. In fact, the photon density of the monochromator is not constancy across all wavelengths (Figure 49) and the highest densities are in the 550-600nm range, with a further peak around 650nm. We therefore used a recent calibration of the monochromator as in Döring and Skorupski (2007) to extrapolate the relative response of the ERG to the different wavelengths of light had the monochromator emitted light of equal photon density throughout the range of wavelengths. We believe such extrapolations to be a valid as the intensity/response curves for the neutral density filter readings were linear in form for the vast majority of the ERGs, implying that the intensity/response was behaving as a linear function at these light levels and halving the light level would approximately halve the response.

RESULTS

Spectral reflectance measurements of the Cape flora

Figure 50 shows the colours of flowers from plant species collected from a selection of sites across the Namaqualand region plotted on the bee colour hexagon, with additional hexagons showing flower colours from some sites in Israel for comparison. Figure 51 shows colour breakdowns averaged for all the sites sampled from each country.

When the proportions of plant species with flowers in the six different bee colour categories are considered, the South African flora differs significantly from the floras of Brazil, Costa Rica and Israel in terms of its colour composition. This is especially evident in the larger number of bee-UV flowers present in the Northern and Western Cape regions of South Africa, much higher than for other locations (ANOVA comparing it to flora from sites in Brazil, Costa Rica and Israel; $F = 6.2451$, $df = 3$, $p = 0.00144$), which can comprise up to 18% of the species present at some sites.

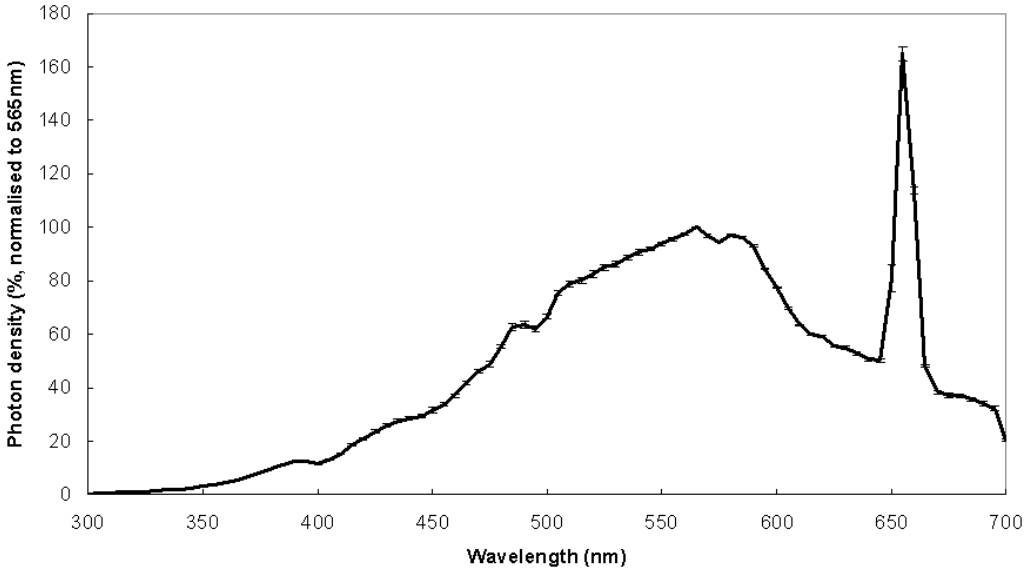
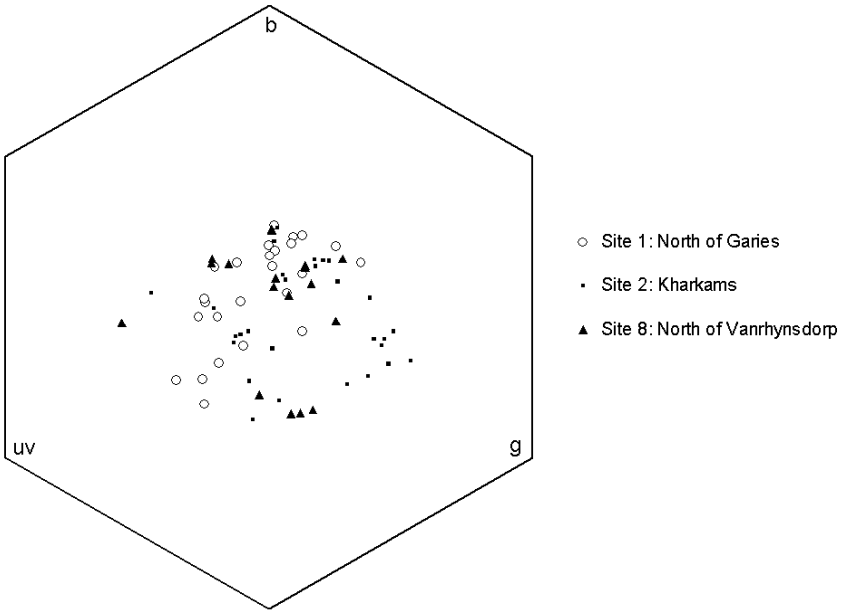


Figure 49. Normalised (to 565nm) photon density emitted by the monochromator used in the experiments. The light emitted is not of consistent intensity or energy for all wavelengths, so the beetle responses were calibrated accordingly. (Measurements performed by T.F. Döring.)

a)



b)

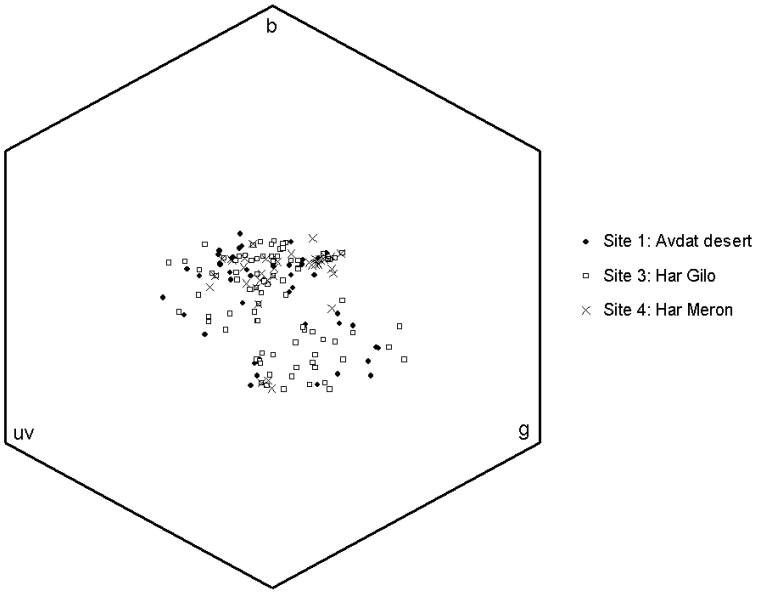


Figure 50. Colour hexagon showing colour distribution of plant species from (a) three sites in South Africa and (b) three in Israel, covering a similar range of arid and semi-arid locations. The three apices marked "b", "g" and "uv" correspond to the three photoreceptor types (blue, green, ultraviolet) present in the honeybee *Apis mellifera*; the more a flower stimulates a receptor type, the closer to the apex the locus falls.

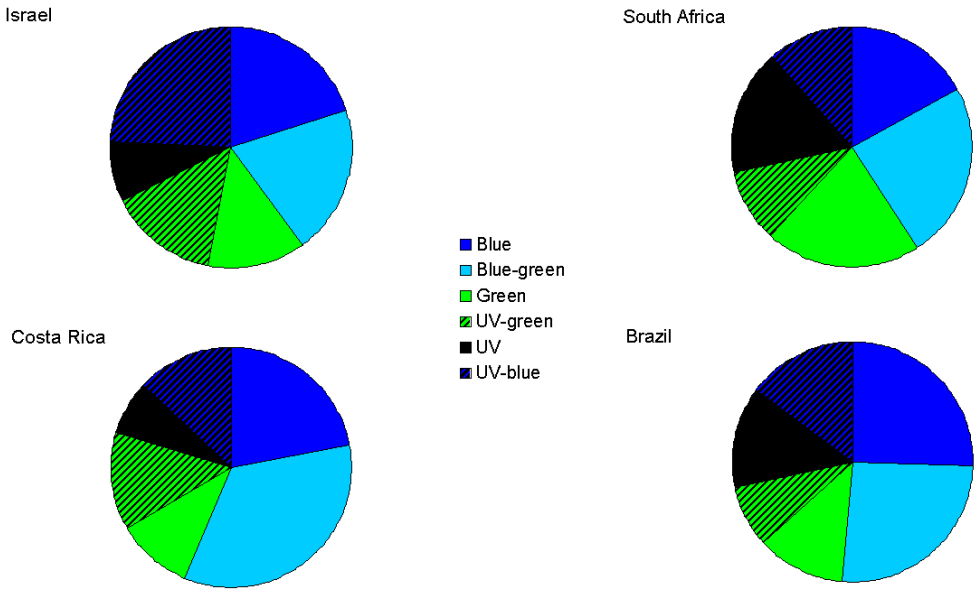


Figure 51. A graphical representation of the proportion of species of each of the six different bee colours, according to their colour hexagon loci, for flowers from four different countries. The proportions are average values calculated from the species recorded at multiple sites. It can be seen that South Africa has a relatively large number of UV flowers (and bee-green ones) and relatively few bee-blue ones; Costa Rica appears to have many plant species with blue-green flowers.

Electroretinograms

For the females of *Pachycnema crassipes* (*N* = 9 individuals), we obtained 22 complete or partial ERGs of sufficient quality to use. We also obtained two usable ERGs from females of *Clania macgregori* (*N* = 2 individuals). The readings taken using the neutral density filters to reduce incident light intensity by known amounts showed an approximate linear relationship between intensity and response at 540nm (Figure 52), and therefore we consider it reasonable to use the measurements of photon density at different wavelengths to calibrate the relative responses of the beetle retinas to the wavelengths. An example of a typical ERG response, an average curve for 57 light flashes at 500nm, is shown in Figure 53.

Pachycnema crassipes

The processed average ERG for females of *P. crassipes* is shown in Figure 54. The recordings we obtained showed consistent peaks in response in the blue-green and red areas of the spectrum and a high response at short wavelengths (violet region) indicating a probable UV peak that we were unable to resolve fully. Thus it appears that the beetles may be either trichromats with three photoreceptor types or tetrachromats, but if they are trichromats, these three photoreceptor peaks are red-shifted relative to those of bees. The approximate sensitivity peaks are located around 520nm and 640nm, with the suggestion of of a third peak around 400nm; it is possible that there is an undetected fourth peak at 370nm or below.

We obtained very few reliable ERG responses at 350nm as we were focusing principally on the long wavelengths, therefore it is possible that the actual peak response of the UV receptor is to wavelengths in the 360-390nm range; however, we lack the data to clarify it. We have many more readings in the 500-660nm region, and consistently obtained stronger relative responses at 640nm than at either 620 or 660nm, indicating a wavelength sensitivity peak here which would facilitate detection of red and orange stimuli by the beetles.

Data from the male *P. crassipes* individual support these findings: although the responses at wavelengths <500nm were not fully resolved, there was evidence of peaks in the blue-green and red ranges, which corresponds well to the those obtained from female beetles (Figure 55).

Clania macgegori

The averaged ERG results obtained from *C. macgregori* are shown in Figure 56. Similarly to *P. crassipes*, there are weak indications of three local maxima, though resolution of the long wavelengths is poorer. This suggests that this species also possesses at least three receptor types, showing maximal sensitivity around 400, 500-520 and >600nm respectively – however, more data are required to confirm which of these peaks are genuine responses and which are simply artefacts caused by noisy responses. In particular, a genuine peak at 640nm would agree with the results for *P. crassipes*, but for these two ERGs there is also an indication of a peak at 600nm (though this could serve a similar function in colour discrimination at long wavelengths).

Adaptation experiments

We obtained results from all three species: *P. crassipes*, *C. macgregori* and *L. braunsi*. (*P. crassipes*, $N = 6$ beetles; *C. macgregori*, $N = 2$ beetles, 1 male, 1 female; *L. braunsi*, $N = 2$ beetles, 1 male, 1 female). All species showed ERG responses to both the green and red LED lights when dark-adapted. We acquired 12 datasets of the green response in *P. crassipes* before and after green adaptation and 11 datasets of the red response for that species. Other species had smaller datasets, for which it would not be appropriate to perform statistical analysis. The graph in Figure 57 shows the percentage change in the post-adaptation response to the two light colours, relative to pre-adaptation response for the tested species, split by sex.

Although the data have a high variance and contain a lot of noise, there is a consistent trend for green response to be reliably reduced by adaptation, as expected. However, the red light response either reduces less or, in fact, increases. We supported this observation for *P. crassipes* with a t-test; the difference between the mean change in

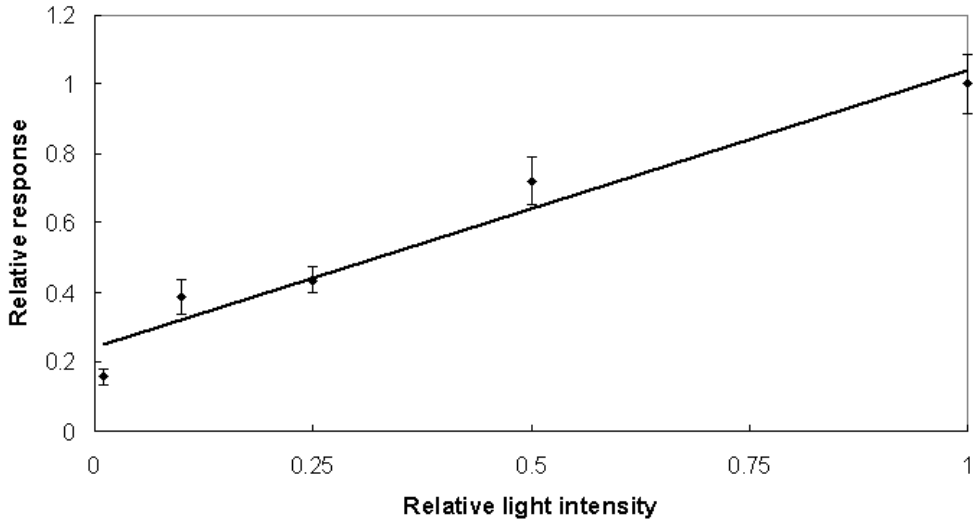


Figure 52. Relative response size to light at 540nm using neutral density filters to reduce the light to 0.5, 0.25, 0.1 or 0.01 relative to the original. "1" is taken to be the maximum light intensity emitted by the monochromator at that distance and wavelength, and the corresponding response recorded from the beetle retina. The response changes linearly in proportion to the intensity. (Bars = standard error.)

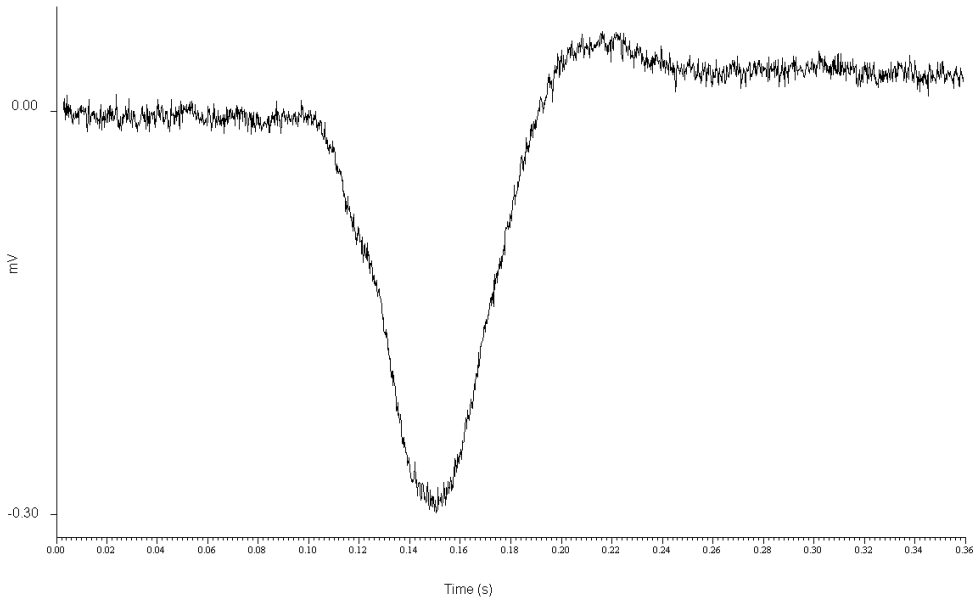


Figure 53. Example of a typical ERG response averaged from the responses to 57 individual light flashes. This curve is taken from a female *P. crassipes* using light of 500nm wavelength.

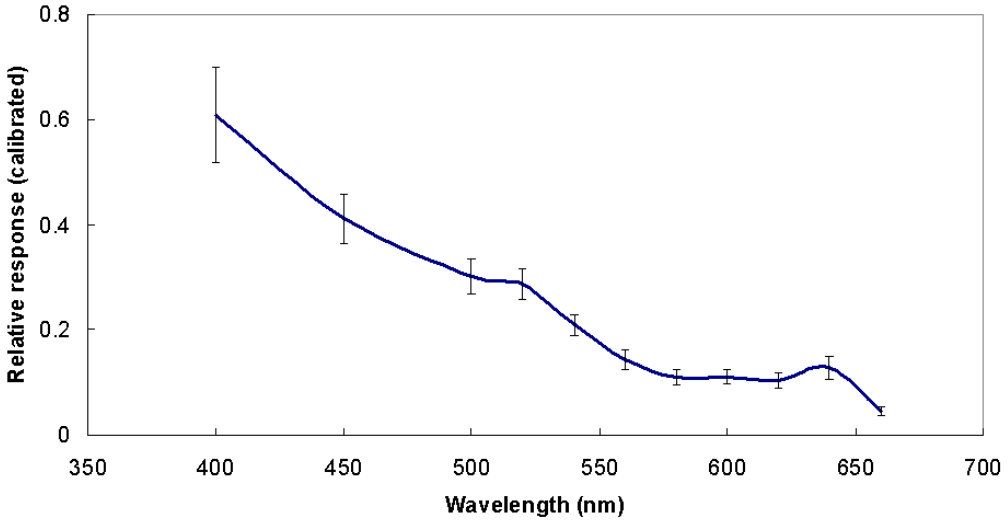


Figure 54. Electroretinogram results for females of *Pachycnema crassipes*. This is the average relative response from 22 complete or partial ERG sweeps in the 400-660nm range for 9 individuals, after calibration. Standard error bars show response variation.

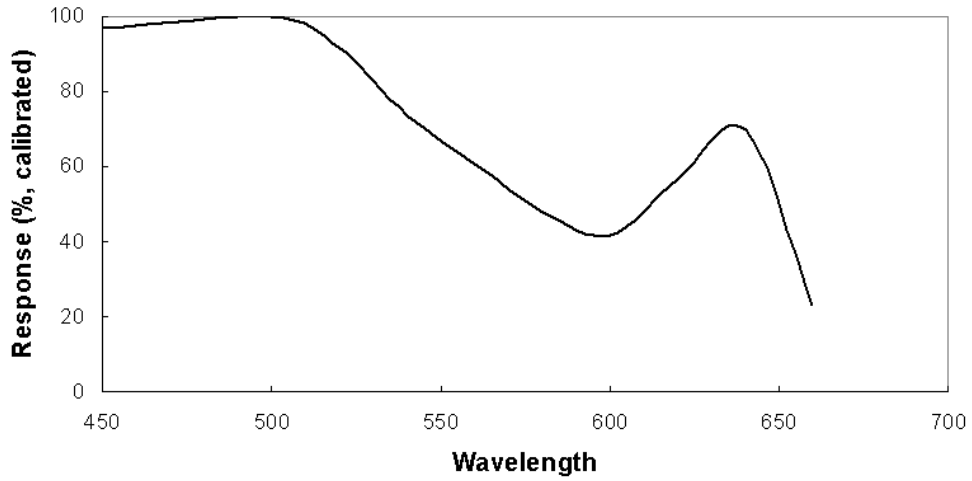


Figure 55. Electroretinogram result for a male of *P. crassipes*. As only one ERG was obtained from a male of this species, the responses are expressed as a percentage of the maximum reading obtained and the data are relatively noisy. However, peak responses at around 500-520nm and 640nm are still indicated.

response to red light and the mean change in response to green light was significant (t-test, $t = 2.200985$, $df = 11$, $p = 0.010529$). This is consistent with the presence of a long-wavelength receptor mediating the red response separately from the receptor mediating the response to green light.

DISCUSSION

Namaqualand and the Cape Floral Kingdom have an unusually large number of relatively scentless, large orange, red and yellow flowers which are not considered to be typical "bee colours" (often appearing UV or green in bee colour space) (Goldblatt et al., 1998a; Johnson and Midgley, 2001). Why this would occur is something of a mystery until one starts to investigate other pollinators in the habitat.

One very important pollinator guild in the Cape habitats is the Hopliini (monkey beetles). The long-wavelength group of monkey beetles are often observed sitting, feeding and mating on orange, red and yellow bowl-shaped flowers, and field studies and surveys have shown that they are important pollinators in the region (Goldblatt et al., 1998a; Mayer et al., 2006; Picker and Midgley, 1996). It therefore seemed likely that their colour vision was somehow better-tuned to long wavelengths than is the case for bees. The most likely possibilities were that either the beetles had an additional photoreceptor with a peak around the 580-640nm part of the spectrum, or else they were trichromats like bees, but their "green-sensitive" long-wavelength receptor had a peak closer to the red than bees'.

Our experiments provide indications that monkey beetles possess at least three photoreceptor types, with peaks found in the violet-blue, blue-green and orange-red parts of the visible spectrum. The short-wavelength receptor could still permit sensitivity to UV reflectance on flowers (though there may be a still shorter-wavelength receptor that we did not detect owing to our primary focus on >500nm sensitivity), whilst the long-wavelength receptor responds robustly to long wavelengths and is very likely to facilitate detection of red and orange flowers in particular.

This is supported both by the electroretinogram sweeps that we obtained, which indicate the likelihood of three peaks in the 400-700nm light spectrum range, and also by the adaptation experiments, in which adapting to green light reduced the response to green light significantly but did not similarly attenuate the response to red light. This would indicate that two separate receptors are responsible for the green and red light responses, presumably the ~520nm receptor indicated by the sweep and the ~640nm receptor respectively.

Such a long-wavelength sensitivity peak coupled with a relatively short-wavelength sensitive "medium-wavelength receptor" is not unprecedented among beetles: ERG evidence from *Carabus nemoralis* and *C. auratus* indicates that these species possess four photoreceptor types with peaks at 348, 430, 500 and 620nm (Hasselmann, 1962). The presence in Hopliini of receptors with approximately similar sensitivities to the medium- and long-wavelength receptors in *Carabus* species is therefore quite plausible. This is further supported by the unpublished discovery of a receptor in *Amphicoma* beetles with a peak sensitivity of 630nm (Briscoe and Chittka, 2001). As their ecology seems to parallel that of monkey beetles in terms of the flowers they visit, it is fitting that their visual physiology appears to follow suit.

Equally, detection of polarised light in another Scarabaeidae species, the European cockchafer *Melolontha melolontha*, is mediated by a photopigment with maximal sensitivity at 520nm (Labhart et al., 1992), so possession of an appropriate photopigment to mediate blue-green sensitivity in this clade is already recorded.

It is likely therefore that the large, open "beetle flowers" in the Cape Floral Kingdom have specifically evolved their colours in order to be more conspicuous to the visual systems of Hopliine beetle pollinators than to bee pollinators. This makes intuitive sense – as the flowering of plants in this region is relatively synchronised and concentrated over just a few months, competition for pollinators is intense. The number of pollinators may be relatively low, and monkey beetles, although slow moving, can fly fairly well and are able to carry large amounts of pollen on their hairy bodies. Therefore, despite that the dominance of bee pollination worldwide (Proctor et al., 1996) might lead on to assume that bee pollination is somehow "optimal", it is unsurprising that some plant species will evolve characteristics that instead favour beetle pollination.

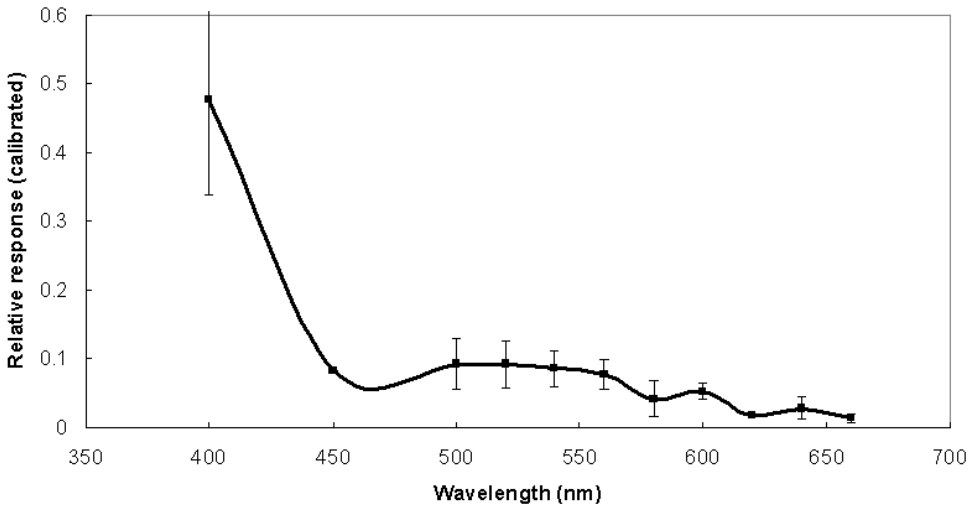


Figure 56. Electroretinogram results for females of *Clania macgregori*. This is the average response from 2 ERGs from 2 females, in the 400-660nm range, after calibration. Standard error bars show response variation. Further resolution is required.

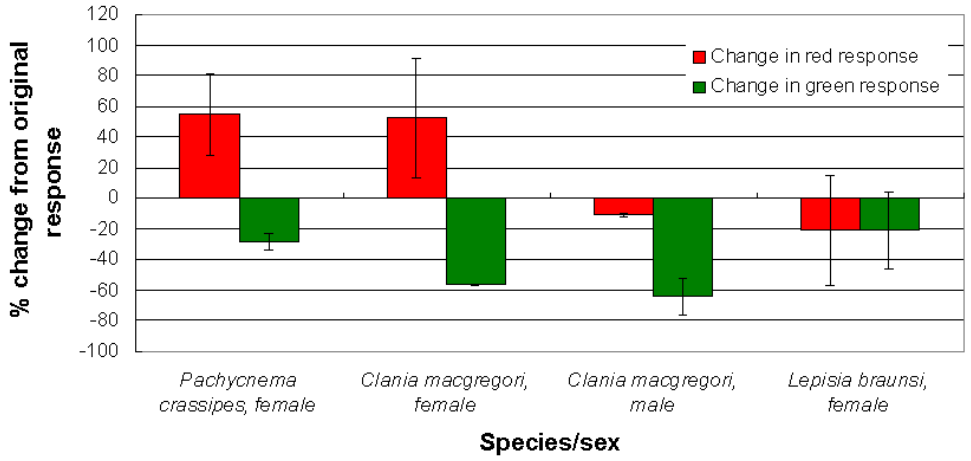


Figure 57. Change in ERG responses to red or green light after adaptation to green light (565nm) for individuals of three monkey beetle species. The red bars indicate the change in red light response after adaptation; the green bars indicate change in green response after adaptation to green light (showing a reduced response, as expected). The changes in red and green response in *P. crassipes* are significantly different, indicative of a red-sensitive receptor mediating the red response. Bars = standard error.

The vision of Hopliini warrants further investigation, both in a physiological context, especially with an eventual aim of obtaining intracellular readings, and also in a behavioural context. Given that there is a relatively large wavelength gap between their medium- and long-wavelength receptors, one would expect that behavioural experiments would demonstrate rather poor colour discrimination in the blue-green to green colour range and somewhat better discrimination of blue shades. The short wavelength detection ability of these beetles is also worthy of further, more detailed exploration, to determine the peak sensitivity of the short-wavelength receptor. Indeed, although we did not find evidence to support the presence of more than one short wavelength receptor, our investigation of the beetles' response to short wavelengths was insufficient to be sure of this. Therefore it would undoubtedly be worthwhile to look further at this part of the spectrum and ascertain whether there are, in fact, two receptors with sensitivities <420nm, similar to the 348 and 430nm receptors in *Carabus*, perhaps one with a peak around 400nm and one which we were unable to detect with our equipment, with a sensitivity of 330-350nm. Such a system could also account for the short-wavelength guild of monkey beetles, as it would enable high colour sensitivity and probably good discrimination of blue and violet flowers, helping to account for the beetles' preference for those.

This study provides evidence relating the unusual and striking colour composition of the South Africa Cape flora to the vision of an important and rather unique pollinator in this habitat. Based on our findings, we think it likely that the large numbers of magnificent long wavelength reflecting flowers in these habitats can be accounted for by a colour vision system in their monkey beetle pollinators which gives them excellent sensitivity to red, orange and yellow stimuli.

CHAPTER VII

DISCUSSION AND CONCLUSIONS

In this thesis I have explored some of the pressures driving flower colour evolution in the context of insect vision, and investigated the behaviour and physiology of some pollinating insects in order to relate this to the flowers they visit. The main contributions my research has made to the field of insect vision and flower colour evolution and

ecology are summarised in Table 5. The results reiterate that there are multiple factors driving and constraining the evolution of flower colours.

Evolutionary history plays a substantial role in what pigments a plant is able to produce in its petals. However, novel pigments do emerge in groups that are typically fairly consistent in colour (e.g. the saturated crimson colour in *Nicotiana forgetiana*, whilst the rest of the genus – including the closely related *N. langsdorfii*, *N. alata* and *N. bonariensis* – are more usually pale coloured, often white or pale greenish-yellow (Kaczorowski et al., 2005)).

An important factor in determining a flower's colour is the selective force of pollinators. Ultimately, the purpose of having a showy flower – an energetically costly ornament, and one which may also attract herbivory (Leege and Wolfe, 2002; Sletvold and Grindeland, 2008) – is to advertise the plant's reproductive organs to pollinators. A pollinator's decision to visit or reject a flower is, however, based on more than just instinctive attraction (Frisch, 1914; Heinrich, 1979; Leadbeater and Chittka, 2005; Menzel, 1985b). Many pollinators in a natural environment have extensive experience with locating flowers, as well as energetic costs and benefits to consider, and therefore have memory and learning experience in operation alongside their innate preferences.

As a result, merely being the colour most innately attractive to an insect pollinator is insufficient to secure reliable and efficient pollination, especially in a competitive environment with many plant species having flowers offering different levels of reward and using different pollination strategies. If one colour of flower offers consistently high rewards, pollinators will learn to favour that colour and visit it more frequently – and possibly with higher fidelity, increasing the transfer of conspecific pollen. If, however, a

Table 5. Major contributions made by this thesis to the field

Research area	**Main findings/contributions**
Flower colour resources	Development of the Floral Reflectance Database. Reference: (Arnold et al., 2008)
Flower colour trends within habitats	Flower colour (modelled through bee or fly eyes, or based on spectra) composition of plant communities does not

	change over elevation gradients. Flower colour (modelled through bee eyes or based on spectra) composition of temperate plant communities does not change over the flowering season. References: (Arnold et al., 2009a, b)
Bee colour constancy	Confirmation that bee colour constancy has limitations and bees perform more poorly at a learning task under some illumination conditions.
Bee foraging in patchy light	Bees choose to avoid some illuminants in favour of other ones, and that this is because of familiarity with some illuminants more than with others. Bees are more likely to switch between flowers of different plant species when foraging within illuminant patches than when switching between patches.
South African flower colours	The colour composition of the Cape region possesses an unusually high number of bee-UV flowers, which mostly appear orange or red to humans.
Colour vision of South African monkey beetles	Females of *Pachycnema crassipes* and *Clania macgregori* possibly have a red-sensitive photoreceptor at around 640nm peak sensitivity, correlating with the colours of flowers they visit.

particular colour of flower ceases to be associated with a good reward (as may happen when there are many insect foragers and flowers become nectar-depleted), many species of pollinator will respond by switching to another, more rewarding, colour (Menzel, 1985b). This has implications for rewarding flowers as well as mimics. Rewarding flowers should have a distinctive colour so they are not confused with non-rewarding species and pollinators can learn to associate them with a reward. Conversely, deceptive flowers usually benefit from resembling a rewarding flower, and their success is often density-dependent – if they become too common, the pollinators will notice that a particular colour is not consistently rewarding and may switch to flowers of another plant species (Anderson and Johnson, 2006).

The context of the flower and its colour is also important. A blue flower in an alpine meadow, for example, is subjected to different selective forces to a blue flower in a rainforest understorey. The pollinators themselves are different, along with their experiences and preferences, but so is the foraging environment – in terms of temperature, humidity, wind speed and light composition and intensity. All of these could affect which colour is optimal and why: pigments may serve a protective function as well as an attractive one, increasing resistance to drought or UV radiation (Chalker-Scott, 1999; Warren and Mackenzie, 2001). On the other hand, a pigment is ineffective as an advertisement for pollinators if it cannot be detected by the visual system of the animal species that the flower relies on for pollen transfer – e.g. UV markings are of no use if the pollinator lacks the ability to perceive wavelengths of light shorter than 400nm.

The plant is therefore faced with complex selective forces and potential trade-offs – pigments may be costly, and although sometimes an intense colour may be beneficial in both the biotic and abiotic context, in other situations this may not be the case. Sometimes, the optimal pigments for protecting against abiotic challenges may be different to those optimal for recognition by pollinators. The plant must produce a combination of pigments to optimise its fitness, taking into account pollinator visitation, vulnerability to pests and herbivory, and physical resistance to climate effects (Arnold et al., 2009b; Warren and Mackenzie, 2001).

In chapter II of this thesis, I provide an introduction to the Floral Reflectance Database, a resource which will prove greatly valuable in future investigations of flower colours. By developing this database of flower colours, other researchers will be able to study the causes and effects involved in pollinator-plant interactions and the evolution of flower appearance in more depth. FReD will continue to expand and develop in coming years, with more spectra and new features being added. The diversity of colour information already available in the database can easily be seen in the colour hexagon summary produced by the search results (Figure 58).

FReD may be of extensive use to researchers in the field of ecology, in studies where floral traits are an essential consideration, and also in some areas of development and genetics – for example, investigating how floral colours are arranged within phylogenies, in polyploid and hybrid species, or comparing various mutant floral strains to wild types in terms of their colour. Such studies will provide further information about the selection pressures exerted by pollinators on flower appearance, whilst bringing in under-explored considerations such as the genetic and developmental elements, as started by Galliot et al. (2006).

What makes FReD unique is the fact that the information on colour provided does not assume a solely human viewpoint. It makes use of the floral reflectance data and bee colour space modelling to provide insight into the appearance of these flowers through bee eyes, and provides the data necessary for them to be modelled in other non-human visual systems as well. This lends an additional level of relevance to conclusions about plant ecology and evolution derived from these data.

Chapters III and IV of this thesis use data from the Floral Reflectance Database to show the results of such trade-offs between abiotic and biotic factors. Despite changing light, weather and temperature conditions over the temporal and spatial scales, and despite changing pollinator compositions within these environments, both studies indicate that the ultimate outcome of natural selection is likely to be a relatively balanced colour composition in plant communities. In both cases, the number of species bearing each colour remains broadly constant throughout the temporal and spatial ranges studied. This observation applies whether colours are considered through insect (bee or fly) eyes

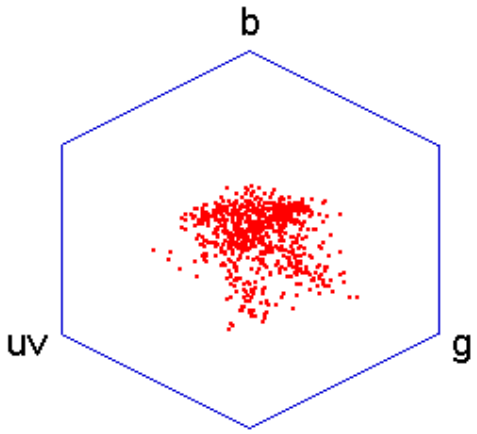


Figure 58. Colour hexagon showing loci generated from all the reflectance spectra currently available in FReD. The spread and range of loci serve to illustrate the diversity of flower colours extant in nature and indeed, the diversity of flowers with spectra available in FReD.

or in the context of physical reflectance spectra of the flowers. Using these biologically-relevant, objective colour assessments gives results that can contradict those of studies using only human colour categories (McCann, 1986; Savile, 1972; Warren and Billington, 2005; Weevers, 1952). Such ecologically-related analyses of flower colour trends in nature are made possible by assembling floral reflectance data in an accessible format with details about the plant species such as their location and elevation above sea level.

The lack of predictable trends in flower colour within many habitats, as revealed in chapters III and IV, informs us about flower colour in a community context. There is clearly no single best colour for flowers to be – or the whole community would tend to converge on that colour and flower meadows would look very different. Indeed, a range of colours seem to be maintained under a variety of conditions; whether it is March, in a woodland; October, in a grassland; or 1000m above sea level on alpine soils, there are typically flowers from all bee colour categories present, and a principal components analysis of the spectra shows that the diversity is maintained throughout all these scenarios. This implies that there is selective pressure on flowers to be diverse and distinctive, which makes sense in the context of pollinator learning and behaviour.

The pollination market hypothesis is the most consistent and logical explanation at present for the colours of flowers present in plant communities, and indeed their other morphological traits. It makes several important and factually-based assumptions:

- flowers of most plant species can be pollinated by more than one pollinator type
- most pollinator species can visit and pollinate more than one flower type
- pollinators are not visiting a single flower colour or type based on innate attraction or instinct, but are able to make decisions about which flowers to visit (Gumbert, 2000; Gumbert et al., 1999)

The theory states that flowers compete with each other, like sellers at a market, for the "custom" of pollinators (Friedman and Shmida, 1995; Gumbert et al., 1999). They can advertise their rewards by being distinctive and conspicuous in various ways: large size, saturated or contrasting colours, strong odours. As in a market, there is no single "best" formula – if the majority of pollinators were to forage one colour or form of flower,

being different will be advantageous as some pollinators will rapidly learn to associate the distinctive form with more consistent rewards due to the reduced competition on that form. Therefore the pressures are to be as salient and easily-recognised as possible, resulting in a range of colours, morphologies and scents amongst flowers in a range of habitats. This agrees well with existing studies of flower morphology and colour worldwide, which have shown that few flowers truly fit into the classical "pollination syndromes" with prescribed sets of traits and that their colour and morphological traits are not reliable predictors of their pollination systems (Ollerton et al., 2009a).

Although the pollination market hypothesis predicts that habitats across the world should all contain a broad range of floral colours and forms, shifts on a local scale do occasionally occur (Goldblatt et al., 1998a; Kevan and Baker, 1983), and individual regions might have varying proportions of species of different colours depending on the plant families comprising the flora, genetic drift and consistent local selection pressures such as unique pollinator groups. This seems to be the case in the Cape region of South Africa; in chapter VI, I performed an analysis of the flower colours at various different sites. It revealed a higher than average number of flowers that appear UV to bees (typically flowers that reflect maximally at long wavelengths, above 620nm), in accordance with previous claims that it has more flowers with red or orange colouration than are seen in other areas. As previously discussed, flowers of all colours are normally present in most habitats, and this was the case for all the floras considered in this chapter. However, in the Cape region the large number of flowers exhibiting a normally uncommon colour (to bees), and the convergence of several unrelated taxa on similar forms does suggest selection by a specific pollinator. This is most likely one that is not present in the fauna of the other habitats whose flora were analysed in this chapter.

Previous studies have linked these flowers to a pollination system involving a group of Scarabaeidae, the monkey beetles (Colville et al., 2002; Picker and Midgley, 1996). This diverse group of insects and the flowers they visit form a system that appears to parallel the *Amphicoma* beetle pollination system in Israel (Dafni and Potts, 2004) but otherwise seems very particular to South Africa. Monkey beetle pollination is unique to southern Africa and therefore will result in differences in the selection pressures and interspecific competition in this region relative to other habitats.

Based on the studies in chapters II and III, and the flora and pollination ecology of South Africa, it is clear that any consideration of floral traits and colour in particular must take into account the insects present throughout the flowering season, their behaviour and their visual capabilities. Flies, for example, perceive colour quite differently to bees, and two colours which are quite distinct to an experienced bee may be entirely indistinguishable to a fly. My experiments – as described in chapter VI – have suggested for the first time that South African monkey beetles, rather than having a generic UV/blue/green trichromatic colour vision system similar to that of many bees, wasps, moths and other beetle species, may possess a colour vision system with enhanced sensitivity in the long wavelengths, apparently mediated by a receptor with a peak sensitivity of over 600nm and possibly as high as 640nm. It seems that the flowers of the region have evolved to take advantage of this sensitivity, having large, open inflorescences in yellow, orange and red colours, forms which are much less common in the flower meadows of Europe, for example.

Electrophysiological techniques have given us insight into the sensory capabilities of insects relevant to their behaviour and ecology, including their vision (Döring and Skorupski, 2007; Peitsch et al., 1992; Skorupski et al., 2007), olfaction (Bhagavan and Smith, 1997; Park and Hardie, 1998; Pope et al., 2004; Raguso and Light, 1998) and neural processing (Giurfa, 2007; Menzel, 2007; Menzel and Müller, 1996). However, extracellular readings used in isolation have their limitations: as they only tell us about the response of the nervous cells in a tissue as a group, rather than of individual receptors or neurons, it is difficult to draw precise conclusions about the behaviour of individual cells or how, for example, a visual response to red light is mediated (Briscoe and Chittka, 2001). For example, one cannot necessarily be certain whether a double-peaked electroretinogram is the result of two photoreceptors with different sensitivities or a single photoreceptor possessing a secondary peak. However, they can certainly provide clear answers on whether an insect has the capacity to detect a particular wavelength of light (or respond to some other stimulus).

We therefore still have much more to discover about the colour vision of monkey beetles, including a more detailed exploration of their UV-vision, and exploration of the colour processing, recognition and categorisation systems they may be using to find flowers. By contrast, the honeybee and bumblebee visual systems are much better

understood, and we are able to ask much more in-depth questions about how they locate, discriminate and categorise flowers in a natural environment. Using this information in concert with the floral reflectance data in FReD and/or reflectance data of stimuli used in behavioural experiments allows us to make testable predictions about behaviours in bees that depend upon colour vision.

Such accurate information is crucial when one takes visual ecology further: even for the same visual system and the same spectral reflectance of the flower, the colour appearance is not an absolute quantity. It depends upon the illuminating light and, for some visual systems, the background against which the object is set. Therefore, a flower appearing one colour – i.e. reflecting certain wavelengths of light – in the centre of an open meadow will reflect a different combination of wavelengths when illuminated by the light present in the shade of a woodland leaf canopy. A pollinator such as a bee seeking such a flower must be able to correct for this difference, or it may struggle to recognise the two flowers as one and the same species. If the pollinator is only partially able to compensate for the illumination change, this may cause it to choose the "incorrect" flower – one that offers no reward, or contains a cryptic predator. This may have repercussions for flowers that grow in variable light environments, if some colours have their appearance altered less, to insect eyes, by changing illumination compared to others.

The natural foraging environment encountered by a pollinating insect is complex and variable, and illuminating light is known to comprise part of this. The light environment in nature changes within a day, between days, and over a spatial scale (Endler, 1993; Hernández-Andrés et al., 2001), and yet it is still important for a foraging insect to locate flowers correctly and identify them in relation to their previous foraging experience. The ability of a pollinator to forage efficiently in a changing light environment is therefore crucial to its success in finding food, and a key question is how effectively they can function in variable and changing illumination – i.e. do they possess colour constancy, and if so, is it perfect or approximate?

Colour constancy has been investigated in a number of species to date, including vertebrates and also some insect species. Although the most extensive body of research is undoubtedly carried out on humans, owing to their ability to communicate their visual

experience and perceptions most accurately, colour constancy has also been studied in goldfish (Dörr and Neumeyer, 2000; Neumeyer et al., 2002), moths (Balkenius and Kelber, 2004), butterflies (Kinoshita and Arikawa, 2000) and stomatopods (Osorio et al., 1997), as well as bees. In goldfish and humans, it has been found, as in bees, to be imperfect (Dörr and Neumeyer, 2000; Lucassen and Walraven, 1996; Neumeyer et al., 2002; Werner, 2006).

The ability of receptors to adapt to the ambient light level could be expected to provide at least a basic level of approximate colour constancy in any animal with receptors whose sensitivities can be up or down-regulated. In humans, it is known that there are also additional levels of neuronal processing (Land, 1977), sampling the light received by different parts of the visual field and using this to estimate the spectral composition of the illuminating light in order to discount it from considerations of the colours of objects (McCann, 1992). There is still much to be discovered about the accuracy and limitations of colour constancy in insects, such as how it depends upon the position in colour space of the stimuli, the spectral content of the illuminating light, depth segmentation and contextual cues.

Imperfect colour constancy, as demonstrated in the research I present in chapter V as well as existing literature (Dyer, 1999; Dyer and Chittka, 2004a; Kevan et al., 2001), has implications for insect foraging. If there are only subtle colour differences present between flowers of different plant species, it could lead to misidentification by insect pollinators under variable illumination. Conversely, large colour differences – to a bee's eyes – between different species will be favoured in changing light contexts as the likelihood of misidentification will be lower. This further reinforces the conclusion, based on the pollination market hypothesis, that flowers need to be as easily discriminated and distinctive as possible (with the obvious exception of deceptive mimics).

There has been no previous work looking at very short-term changes in illumination over small spatial scales in bees previously, or considerations of how this could alter their behaviour. My research in chapter V has provided new insights into bees' response to patchy light in a foraging setting, using colours of illuminant and artificial flowers that are ecologically relevant. Although the small size of a bee flight arena and the

comparatively large rewards provided by most such experiments are a departure from nature, nonetheless the data I have collected about how bees respond to patchy light open up a new area of understanding within the field. The fact that bees are intentionally choosing to spend more time foraging in areas illuminated by a familiar colour of light and making more flower visits there is a new one, though one consistent with what is known of bees' reactions to an unfamiliar physical object (initial avoidance, sampling, and eventual acceptance of the new addition to the environment) (Forrest and Thomson, 2009). Additionally, the finding that bees are more likely to sample flowers of a different species when foraging within a patch of the same illumination, rather than when switching between different lighting conditions, is a novel finding which has implications in the context of bees discovering and pollinating new plant species when foraging in patchy light. It may conceivably result in selection against plant species that occur at low frequencies on the edge of woodlands and other areas with unusual illumination, as most of the encounters bees would have with such flowers would be when changing illumination, when they are less likely to sample the flower than if they encountered it within a patch of familiar and consistent illumination.

All these effects, however, are likely to be relatively subtle in the natural context: the majority of flower visits in the field are made by experienced insect foragers, and so on most occasions, they have prior information about different illumination conditions, the appearance of different flowers in these conditions, and the relative rewards of multiple different flower types. This, and the behavioural plasticity of pollinators and the complexity of factors influencing flower traits, are essential considerations when trying to ascertain which colours or morphologies of flowers should be favoured by selection in different habitats.

FUTURE RESEARCH DIRECTIONS

Floral Reflectance Database still offers much scope for development to increase its applicability and utility for researchers. Foremost, it should acquire the largest possible number of high-quality spectral reflectance datasets from flowers worldwide. At present there is particular need for more data from North America, Australia and Asia to be made available in FReD, but given that there could be as many as 400,000 extant

angiosperm species on earth ((Govaerts, 2001); though see Scotland & Wortley (2003)), there are many possibilities from all areas of the world to gather new floral reflectance measurements.

Even large amounts of floral reflectance data, however, are of limited use unless the flower colours can be considered in the ecologically relevant context of insect colour vision. The colour space models we have for the bee have proven enormously useful in ecological studies. However, bees are not the only pollinators playing a role in ecosystems. We lack a colour space model for hoverflies, a very important pollinator group – if we wish to model fly vision, we must use the model developed by Troje (1993) for blowflies, which rarely perform a pollination service. Acquiring data on the visual physiology and capabilities of hoverflies would allow us to construct a model more relevant to pollination systems. Equally, more information about moth and butterfly pollinators would increase our understanding of the role these diverse groups of insects play in pollination ecology.

More research should also be performed on monkey beetle vision. This thesis presents extracellular recordings, and focuses primarily on long wavelengths. To build a coherent and reliable picture of their vision, we also need more data from the shorter wavelengths (<500nm) and ideally further attempts to gain intracellular readings. More species need to be sampled, including extending the research to male Hopliini, as they have different visual targets (seeking females more than food plants; the females are often very different in colour to the flowers) (J. Colville, personal communication).

Even within the well-characterised field of bee vision there is still much to be done to shed light on their interactions with flowers. We need to explore the limitations of their colour constancy, using different colours of stimuli and illumination in order to establish the conditions under which most mistakes happen, and whether particular parts of bee colour space are associated with poorer colour constancy, as predicted by Kevan et al. (2001). The effects of patchy light on learning behaviours can also be explored much further, especially in the context of foraging and flower visitation – we should discover how it affects bees' navigation and landmark recognition, detection of predators on flowers, and social learning causing bees to visit and prefer new plant species.

CONCLUSIONS

There is no single "best" colour for flowers on a global scale to be. There is, however, likely to be an optimal flower colour for a particular plant species, determined by the pollinator species present, the other flowers in the community and the abiotic challenges. There is a huge diversity of flower colours, both as perceived by humans and, more importantly, as perceived by insects. Just as diverse are the visual systems of the insects that pollinate flowers. As insect vision predates flower colour, it is certain that the evolution of most aspects of flower colour, size and morphology have been driven by pollinator-mediated selection. However, the pollinator visual system does not have to be static over evolutionary time and inevitably is also subject to "fine-tuning" in terms of receptor numbers and sensitivity.

In nature, most pollinators have extensive foraging experience. Although they will always be constrained by the limitations of their physiology – what they can detect, what they are physically able to land on, and which flowers provide them with rewards that are valuable to them – their impressive learning abilities enable them to take advantage of flowers in a variety of habitats and environments, and possessing a variety of characteristics. It is the flexibility of insect foraging strategies as much as the evolutionary plasticity of flowering plants that have helped to account for the enormous colour diversity of angiosperm species present today.

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APPENDICES

APPENDIX I, TO CHAPTER I

List of the Orchidaceae species whose spectral reflectance data were used in the colour hexagon in Figure 6 of the introduction. Human colours were judged in the field by collectors.

Genus	Species	Country	Bee Colour	Human Colour
Bulbophyllum	*falcatum*	West Africa	green	yellow
Cephalanthera	*longifolia*	Israel	blue-green	white
Cirrhopetalum	*cumingii*	Philippines	UV	white-red
Coelogyne	*huettneriana*	Thailand	blue	white
Dactylorhiza	*fuchsii*	Norway	blue-green	purple
Dactylorhiza	*maculata*	Norway	blue-green	pink
Dactylorhiza	*maculata*	Austria	blue	pink
Dactylorhiza	*majalis*	Germany	UV-blue	purple
Dendrobium	*nobile*	Mexico	blue	violet
Dendrobium	*aggregatum*	India/China	UV	yellow
Dendrobium	*hinginianum*	Australia	blue-green	violet
Dendrobium	*loddigesii*	China	blue	violet
Dendrobium	*pierardii*	Burma Himalaya	blue-green	yellow
Epidendrum	*ematophyllum*	Brazil	blue	pink
Epipactis	*atrorubens*	Austria	UV-blue	dark violet
Eria	*pannea*	S.E. Asia	green	orange
Gymnadenia	*canopsea*	Austria	blue	pink
Limodorum	*abortivum*	Israel	UV-blue	violet-white
Maxillaria	*chrysantha*	Brazil	green	yellow
Maxillaria	*variabilis*	Mexico	green	yellow
Miltonia	*cuneata*	China	blue-green	white
Nigritella	*nigra*	Austria	UV-blue	dark brown
Oncidium	*variegatum*	West India	blue-green	white
Orchis	*anatolica*	Israel	blue	violet
Orchis	*galilaea*	Israel	blue-green	violet-green
Orchis	*papilionacea*	Israel	blue	violet-brown
Orchis	*tridentata*	Israel	blue	purple
Phalaenopsis	*schilleriana*	Philippines	blue-green	pink
Phalaenopsis	*stuartiana*	Philippines	blue-green	white
Polystachia	*pubescens*	S.E. Africa	UV-green	yellow
Restrepia	*elegans*	Venezuela	blue	red
Traunsteinera	*globosa*	Austria	blue-green	pink
Zygopetalum	*mackaii*	Brazil	blue-green	pink-white

APPENDIX II, TO CHAPTER III

List of plant species analysed, including colour information (as see by flies, bees, and also human, after categorising the flower colour into one of six human colours) and elevation of collection (in m asl). Species names are as in *Norsk Flora* (Lid and Lid, 2005).

Family	Species name	Bee colour	Human colour	Fly colour	Elevation
Campanulaceae	*Campanula rotundifolia*	blue	blue	p- y+ (“blue”)	700-800
Caryophyllaceae	*Silene dioica*	blue	pink/purple	p- y+ (“blue”)	700-1200
Caryophyllaceae	*Viscaria alpina*	blue	pink/purple	p- y+ (“blue”)	1050-1180
Fabaceae	*Astragalus alpinus*	blue	pink/purple	p- y+ (“blue”)	700-1500
Fabaceae	*Oxytropis lapponica*	blue	pink/purple	p- y+ (“blue”)	700-900
Fabaceae	*Trifolium pratense*	blue	pink/purple	p- y+ (“blue”)	700-900
Fabaceae	*Vicia cracca*	blue	pink/purple	p- y+ (“blue”)	700-800
Lentibulariaceae	*Pinguicula vulgaris*	blue	pink/purple	p- y+ (“blue”)	700-1300
Onagraceae	*Chamerion angustifolium*	blue	pink/purple	p- y+ (“blue”)	700-900
Plantaginaceae	*Veronica alpina*	blue	blue	p- y+ (“blue”)	1100
Polemoniaceae	*Polemonium caerulum*	blue	pink/purple	p- y+ (“blue”)	700-1000
Primulaceae	*Primula stricta*	blue	pink/purple	p- y+ (“blue”)	700-900
Primulaceae	*Primula scandinavica*	blue	pink/purple	p- y+ (“blue”)	1050-1150
Ranunculaceae	*Aconitum lycoctonum* subsp. *septentrionale*	blue	pink/purple	p- y+ (“blue”)	700-900
Violaceae	*Viola canina*	blue	blue	p- y+ (“blue”)	700-900
Apiaceae	*Anthriscus sylvestris*	blue-green	white	p- y- (“yellow”)	700-920
Asteraceae	*Achillea nobilis*	blue-green	white	p- y- (“yellow”)	700-900

Asteraceae	*Antennaria dioica*	blue-green	pink/purple	p- y- (“yellow”)	920-1200
Asteraceae	*Erigeron borealis*	blue-green	white	p- y- (“yellow”)	1400-1500
Boraginaceae	*Myosotis decumbens*	blue-green	blue	p- y+ (“blue”)	800-1000
Brassicaceae	*Draba incana*	blue-green	white	p- y- (“yellow”)	700-1500
Brassicaceae	*Draba oxycarpa*	blue-green	yellow	p- y- (“yellow”)	1600
Caryophyllaceae	*Cerastium alpinum*	blue-green	white	p- y+ (“blue”)	700-1100
Caryophyllaceae	*Silene acaulis*	blue-green	pink/purple	p- y+ (“blue”)	1000-1600
Caryophyllaceae	*Silene vulgaris*	blue-green	white	p- y- (“yellow”)	700-900
Caryophyllaceae	*Stellaria nemorum*	blue-green	white	p- y- (“yellow”)	700-900
Crassulaceae	*Sedum annuum*	blue-green	red	p- y- (“yellow”)	800-900
Diapensiaceae	*Diapensia lapponica*	blue-green	white	p- y- (“yellow”)	1040-1100
Dipsacaceae	*Knautia arvensis*	blue-green	pink/purple	p- y+ (“blue”)	700-900
Ericaceae	*Andromeda polifolia*	blue-green	white	p- y- (“yellow”)	1040-1260
Ericaceae	*Harrimanella hypnoides*	blue-green	white	p- y- (“yellow”)	1040-1160
Ericaceae	*Kalmia procumbens*	blue-green	pink/purple	p- y+ (“blue”)	1040-1160
Ericaceae	*Phyllodoce caerulea*	blue-green	pink/purple	p- y+ (“blue”)	700-1500
Ericaceae	*Vaccinium vitis-idaea*	blue-green	pink/purple	p- y- (“yellow”)	700-900
Ericaceae	*Vaccinium myrtillus*	blue-green	red	p- y+ (“blue”)	1000-1100
Fabaceae	*Trifolium repens*	blue-green	white	p- y- (“yellow”)	700-900
Orchidaceae	*Dactylorhiza maculata*	blue-green	pink/purple	p- y+ (“blue”)	700
Orchidaceae	*Dactylorhiza fuchsii*	blue-green	pink/purple	p- y+ (“blue”)	700
Orobanchaceae	*Pedicularis lapponica*	blue-green	yellow	p- y- (“yellow”)	700-800
Orobanchaceae	*Pedicularis oederi*	blue-green	yellow	p- y- (“yellow”)	920-1600
Papaveraceae	*Papaver radicatum*	blue-green	yellow	p- y- (“yellow”)	900

Polygonaceae	*Bistorta vivipara*	blue-green	white	p- y- (“yellow”)	960-1100
Polygonaceae	*Rumex acetosa*	blue-green	red	p- y- (“yellow”)	700-800
Polygonaceae	*Rumex acetosella*	blue-green	red	p- y+ (“blue”)	700-900
Primulaceae	*Trientalis europaea*	blue-green	white	p- y- (“yellow”)	900-1200
Ranunculaceae	*Pulsatilla vernalis*	blue-green	pink/purple	p- y+ (“blue”)	1000-1200
Ranunculaceae	*Ranunculus glacialis*	blue-green	pink/purple	p- y- (“yellow”)	1600
Rosaceae	*Dryas octopetala*	blue-green	white	p- y- (“yellow”)	900-1500
Rosaceae	*Prunus padus*	blue-green	white	p- y- (“yellow”)	700-800
Rubiaceae	*Galium boreale*	blue-green	white	p- y- (“yellow”)	700-900
Saxifragaceae	*Saxifraga stellaris*	blue-green	pink/purple	p- y- (“yellow”)	1400-1500
Saxifragaceae	*Saxifraga oppositifolia*	blue-green	pink/purple	p- y+ (“blue”)	1400-1500
Saxifragaceae	*Saxifraga cespitosa*	blue-green	white	p- y- (“yellow”)	1100-1600
Tofieldiaceae	*Tofieldia pusilla*	blue-green	white	p- y- (“yellow”)	1400
Asteraceae	*Tanacetum vulgare*	green	yellow	p- y- (“yellow”)	800
Brassicaceae	*Erysinum strictum*	green	yellow	p- y- (“yellow”)	700-900
Crassulaceae	*Rhodiola rosea*	green	green	p- y- (“yellow”)	1160-1600
Fabaceae	*Astragalus frigidus*	green	yellow	p- y- (“yellow”)	700-1000
Fabaceae	*Lathyrus pratensis*	green	yellow	p- y- (“yellow”)	700-800
Orobanchaceae	*Melampyrum sylvaticum*	green	yellow	p- y- (“yellow”)	700-960
Orobanchaceae	*Melampyrum pratense*	green	yellow	p- y- (“yellow”)	700-800
Ranunculaceae	*Trollius europaeus*	green	yellow	p- y- (“yellow”)	900
Rosaceae	*Alchemilla glabra*	green	green	p- y- (“yellow”)	700-1000
Rosaceae	*Potentilla crantzii*	green	yellow	p- y- (“yellow”)	900-1600
Rosaceae	*Geum rivale*	UV	pink/purple	p+ y+ (“UV”)	700-1000

Geraniaceae	*Geranium sylvaticum*	UV-blue	pink/purple	p+ y+ (“UV”)	700-1000
Orobanchaceae	*Bartsia alpina*	UV-blue	pink/purple	p+ y+ (“UV”)	700-1500
Plantaginaceae	*Veronica fruticans*	UV-blue	blue	p- y+ (“blue”)	700-900
Asteraceae	*Hieracium* sp.	UV-green	yellow	p+ y- (“purple”)	900
Asteraceae	*Taraxacum officinale*	UV-green	yellow	p+ y- (“purple”)	700-1000
Ranunculaceae	*Caltha palustris*	UV-green	yellow	p+ y- (“purple”)	900-1000
Ranunculaceae	*Ranunculus acris*	UV-green	yellow	p+ y- (“purple”)	700-1500
Rosaceae	*Potentilla erecta*	UV-green	yellow	p+ y- (“purple”)	700-900
Violaceae	*Viola biflora*	UV-green	yellow	p+ y- (“purple”)	800-1500

APPENDIX III, TO CHAPTER III

Details of the *rbcL* sequences used to build the phylogenetic tree, including accession and citation details for the species providing rbcL sequences. "N/A" indicates that a suitable sequence from a species in the same genus or a closely related genus was not available.

Species measured	Species sequence used	Family	Accession	Citation
Achillea nobilis	*Achillea millefolium*	Asteraceae	EU384938	Panero,J.L. and Funk,V.A. The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. Mol. Phylogenet. Evol. 47, 757-782 (2008)
Aconitum septentrionale	*Aconitum racemulosum*	Ranunculaceae	AY954488	Wang,W., Li,R.-Q. and Chen,Z.-D. Systematic position of Asteropyrum (Ranunculaceae) inferred from chloroplast and nuclear sequences.
Alchemilla glabra	*Alchemilla mollis*	Rosaceae	AMU06792	Soltis,D.E., Morgan,D.R., Grable,A., Soltis,P.S. and Kuzoff,R. Molecular Systematics of Saxifragaceae sensu stricto. Am. J. Bot. 80, 1056-1081 (1993)
Andromeda polifolia	*Andromeda polifolia*	Ericaceae	AF124572	Kron,K.A., Judd,W.S. and Crayn,D.M. Phylogenetic analyses of Andromedeae (Ericaceae subfam. Vaccinioideae). Am. J. Bot. 86, 1290 (1999)
Antennaria dioica	*Dimorphotheca sinuata*	Asteraceae	EU384966	Panero,J.L. and Funk,V.A. The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. Mol. Phylogenet. Evol. 47, 757-782 (2008)

Anthriscus sylvestris	*Anthriscus aemula*	Apiaceae	D44554	Kondo,K., Terabayashi,S., Okada,M., Yuan,C. and He,S. Phylogenetic relationship of medicinally important Cnidium offcinale and Japanese Apiaceae besed on rbcL sequences. J. Plant Res. 109, 21-27 (1996)
Astragalus alpinus/frigidus	*Astragalus membranaceus*	Fabaceae	EF685978	Guo,H., Wang,W., Yang,R., Yuan,Y., Yang,N., Sun,Q. and Yu,J. Identification of Radix Astragali by DNA sequence of its ITS, rbcL, matk, cox1, and NAD1-intron2
Bartsia alpina	*Bartsia alpina*	Orobanchaceae	AF190903	Olmstead,R.G., dePamphilis,C.W., Wolfe,A.D., Young,N.D., Elisons,W.J. and Reeves,P.A. Disintegration of the Scrophulariaceae. Am. J. Bot. 88, 348-361 (2001)
Bistorta vivipara (syn. Polygonum viviparum)	*Polygonum cuspidatum*	Polygonaceae	AB019031	Inamura,A., Ohashi,Y., Sato,E., Yoda,Y., Masuzawa,T. and Yoshinaga,K. Intraspecific sequence variation of chloroplast DNA and a molecular phytogeographic study of Polygonum cuspidatum.
Caltha palustris	*Caltha palustris*	Ranunculaceae	AY395532	Silvertown,J., McConway,K., Gowing,D., Dodd,M., Fay,M.F., Joseph,J.A. and Dolphin,K. Absence of phylogenetic signal in the niche structure of meadow plant communities. Proc. Biol. Sci. 273, 39-44 (2006)
Campanula rotundifolia	*Campanula trachelium*	Campanulaceae	DQ356118	Antonelli,A. Higher level phylogeny and evolutionary trends in Campanulaceae subfam. Lobelioideae: Molecular signal overshadows morphology. Mol. Phylogenet. Evol. 46, 1-18 (2008)
Harimanella hypnoides (syn. Cassiope hypnoides)	*Cassiope mertensiana*	Ericaceae	L12603	Kron,K.A. and Chase,M.W. Systematics of the Ericaceae, Empetraceae, Epacridaceae and related taxa based upon rbcL sequence data. Ann. Mo. Bot. Gard. 80, 735-741 (1993)

Cerastium alpinum	*Cerastium glomeratum*	Caryophyllaceae	AM83542	Manhart,J.R., Hugh,J.H. and Wilson,D. Phylogeny of the Caryophyllales.
Chamerion angustifolium (syn. Epilobium angustifolium)	*Epilobium rigidum*	Onagraceae	AF495763	Levin,R.A., Wagner,W.L., Hoch,P.C., Nepokroeff,M., Pires,J.C., Zimmer,E.A. and Sytsma,K.J. Family-level relationships of Onagraceae based on chloroplast rbcL and ndhF data. Am. J. Bot. 90, 107-115 (2003)
Dactylorhiza maculata/fuschii	*Platanthera ciliaris*	Orchidaceae	AF074215	Cameron,K.M., Chase,M.W., Whitten,W.M., Kores,P.J., Jarrell,D.C., Albert,V.A., Yukawa,T., Hills,H.G. and Goldman,D.H. A phylogenetic analysis of the Orchidaceae: evidence from rbcL nucleotide sequences. Am. J. Bot. 86, 208-224 (1999)
Diapensia lapponica	*Diapensia lapponica*	Diapensiaceae	L12612	Kron,K.A. and Chase,M.W. Systematics of the Ericaceae, Empetraceae, Epacridaceae and related taxa based upon rbcL sequence data. Ann. Mo. Bot. Gard. 80, 735-741 (1993)
Draba incarna/oxycarpa	*Draba nemorosa*	Brassicaceae	NC_009272	Hosouchi,T., Tsuruoka,H. and Kotani,H. Sequencing analysis of Draba nemoroza chloroplast DNA.
Dryas octopetala	*Dryas drummondii*	Rosaceae	U59818	Swensen,S.M. The evolution of actinorhizal symbioses: evidence for multiple origins of the symbiotic association. Am. J. Bot. 83, 1503-1512 (1996)
Erigeron borealis	*Erigeron tenuis*	Asteraceae	EU384973	Panero,J.L. and Funk,V.A. The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. Mol. Phylogenet. Evol. 47, 757-782 (2008)
Erysimum hieracifolium	*Erysimum capitatum*	Brassicaceae	AY167980	Cummings,M.P., Nugent,J.M., Olmstead,R.G. and Palmer,J.D. Phylogenetic analysis reveals five independent transfers of the chloroplast gene rbcL to the mitochondrial genome in angiosperms.

				Curr. Genet. 43, 131-138 (2003)
Galium boreale	*Galium mollugo*	Rubiaceae	AY395538	Silvertown,J., McConway,K., Gowing,D., Dodd,M., Fay,M.F., Joseph,J.A. and Dolphin,K. Absence of phylogenetic signal in the niche structure of meadow plant communities. Proc. Biol. Sci. 273, 39-44 (2006)
Geranium sylvaticum	*Geranium albanum*	Geraniaceae	DQ452884	Fiz,O., Vargas,P., Alarcon,M., Aedo,C., Garcia,J.L. and Aldasoro,J.J. Phylogeny and historical biogeography of Geraniaceae in relation to multiple major increases and decreases in mitochondrial climate changes and pollination ecology. Syst. Bot. 33, 326-342 (2008)
Geum rivale	*Geum macrophyllum*	Rosaceae	U06806	Soltis,D.E., Morgan,D.R., Grable,A., Soltis,P.S. and Kuzoff,R. Molecular Systematics of Saxifragaceae sensu stricto. Am. J. Bot. 80, 1056-1081 (1993)
Hieracium sp.	*N/A*			
Knautia arvensis	*Knautia intermedia*	Dipsacaceae	Y10698	Backlund,A. and Bremer,B. Phylogeny of the Asteridae s.str. based on rbcL sequences with particular reference to the Dipsacales. Plant Syst. Evol. 200 (1997)
Lathyrus pratensis	*Lathyrus pratensis*	Fabaceae	AY395544	Silvertown,J., McConway,K., Gowing,D., Dodd,M., Fay,M.F., Joseph,J.A. and Dolphin,K. Absence of phylogenetic signal in the niche structure of meadow plant communities. Proc. Biol. Sci. 273, 39-44 (2006)

Kalmia procumbens	*Kalmia procumbens*	Ericaceae	U49288	Kron,K.A. and King,J.M. Cladistic relationships of Kalmia, Leiophyllum and Loiseleuria (Phyllodoceae, Ericaceae) based on rbcL and nrITS data. Syst. Bot. 21, 17-29 (1996)
Melampyrum sylvaticum/pratensis	*Melampyrum sylvaticum*	Orobanchaceae	AM503854	Li,M., Wunder,J., Bissoli,G., Scarponi,E., Gazzani,S., Barbaro,E., Saedler,H. and Varotto,C. Development of COS genes as universally amplifiable markers for phylogenetic reconstructions of closely related plant species. Cladistics (2008) In press
Myosotis decumbens	*Myosotis discolor*	Boraginaceae	AY395552	Silvertown,J., McConway,K., Gowing,D., Dodd,M., Fay,M.F., Joseph,J.A. and Dolphin,K. Absence of phylogenetic signal in the niche structure of meadow plant communities. Proc. Biol. Sci. 273, 39-44 (2006)
Oxytropis lapponica	*Oxytropis anertii*	Fabaceae	EF685981	Guo,H., Wang,W., Yang,R., Yuan,Y., Yang,N., Sun,Q. and Yu,J. Identification of Radix Astragali by DNA sequence of its ITS, rbcL, matk, cox1,and NAD1-intron2.
Papaver radicatum	*Papaver* sp. *Goldblatt 12541*	Papaveraceae	AM235045	Forest,F., Grenyer,R., Rouget,M., Davies,T.J., Cowling,R.M., Faith,D.P., Balmford,A., Manning,J.C., Proches,S., van der Bank,M., Reeves,G., Hedderson,T.A. and Savolainen,V. Preserving the evolutionary potential of floras in biodiversity hotspots. Nature 445, 757-760 (2007)
Pedicularis oederi/lapponica	*Pedicularis coronata*	Orobanchaceae	AF206803	Soltis,P.S., Soltis,D.E. and Chase,M.W. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology.
Phyllodoce caerulea	*Phyllodoce caerulea*	Ericaceae	AF419829	Kron,K.A.
Pinguicula vulgaris	*Pinguicula caerulea*	Lentibulariaceae	L01942	Albert,V.A., Williams,S.E. and Chase,M.W. Carnivorous plants: phylogeny and structural evolution. Science 257, 1491-1495 (1992)

Polemonium caerulum	*Polemonium reptans*	Polemoniaceae	L11687	Olmstead,R.G., Michaels,H.J., Scott,K.M. and Palmer,J.D. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of rbcL. Ann. Mo. Bot. Gard. 79, 249-265 (1992)
Potentilla erecta/crantzii	*Potentilla fruticosa*	Rosaceae	U06818	Soltis,D.E., Morgan,D.R., Grable,A., Soltis,P.S. and Kuzoff,R. Molecular Systematics of Saxifragaceae sensu stricto. Am. J. Bot. 80, 1056-1081 (1993)
Primula stricta/scandinavica	*Primula stricta*	Primulaceae	AF394975	Trift,I., Kallersjo,M. and Anderberg,A.A The monophyly of Primula (Primulaceae) evaluated by analysis of sequences from the chloroplast gene rbcL. Syst. Bot. 27, 396-407 (2002)
Prunus padus	*Prunus padus*	Rosaceae	AF411485	Jung,Y.H., Han,S.H., Oh,Y.S. and Oh,M.Y.
Pulsatilla vernalis	*Pulsatilla cernua*	Ranunculaceae	AY954492	Wang,W., Li,R.-Q. and Chen,Z.-D. Systematic position of Asteropyrum (Ranunculaceae) inferred from chloroplast and nuclear sequences.
Ranunculus acris/glacialis	*Ranunculus acris*	Ranunculaceae	AY395557	Silvertown,J., McConway,K., Gowing,D., Dodd,M., Fay,M.F., Joseph,J.A. and Dolphin,K. Absence of phylogenetic signal in the niche structure of meadow plant communities. Proc. Biol. Sci. 273, 39-44 (2006)
Rumex acetosa and acetosella	*Rumex acetosella*	Polygonaceae	D86290	Yasui,Y. and Ohnishi,O. Comparative study of rbcL gene sequences in Fagopyrum and related taxa. Genes Genet. Syst. 71, 219-224 (1996)

Saxifraga stellaris/oppositifolia/ cespitosa	*Saxifraga stellaris*	Saxifragaceae	AF374732	Soltis,D.E., Kuzoff,R.K., Mort,M.E., Zanis,M., Fishbein,M., Hufford,L., Koontz,J. and Arroyo,M.K. Elucidating deep-level phylogenetic relationships in Saxifragaceae using sequences for six chloroplastic and nuclear DNA regions. Ann. Mo. Bot. Gard. 88, 669-693 (2001)
Sedum anuum and Rhodiola rosea (syn. Sedum rosea)	*Sedum rubrotinctum*	Crassulaceae	L01956	Albert,V.A., Williams,S.E. and Chase,M.W. Carnivorous plants: phylogeny and structural evolution. Science 257, 1491-1495 (1992)
Silene acaulis/dioica/vulgaris	*Silene dioica*	Caryophyllaceae	EF646928	Muir,G. and Filatov,D. A selective sweep in the chloroplast DNA of dioecious Silene (section Elisanthe) Genetics 177, 1239-1247 (2007)
Stellaria nemorum	*Stellaria media*	Caryophyllaceae	AF206823	Soltis,P.S., Soltis,D.E. and Chase,M.W. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology.
Tanacetum vulgare	*N/A*			
Taraxacum officinale	*Taraxacum officinale*	Asteraceae	AY395562	Silvertown,J., McConway,K., Gowing,D., Dodd,M., Fay,M.F., Joseph,J.A. and Dolphin,K. Absence of phylogenetic signal in the niche structure of meadow plant communities. Proc. Biol. Sci. 273, 39-44 (2006)
Tofieldia pusilla	*Tofieldia pusilla*	Tofieldiaceae	AJ286562	Bremer,K. Early Cretaceous lineages of monocot flowering plants.
Trientalis europaea	*Trientalis europaea*	Primulaceae	U96655	Anderberg,A., Stahl,B. and Kallersjo,M. Phylogenetic interrelationships in the Primulales inferred fromcpDNA rbcL sequence data. Plant Syst. Evol. (1998)
Trifolium pratense/repens	*Trifolium pratense*	Fabaceae	AY395564	Silvertown,J., McConway,K., Gowing,D., Dodd,M., Fay,M.F., Joseph,J.A. and Dolphin,K. Absence of phylogenetic signal in the niche structure of meadow plant communities. Proc. Biol. Sci. 273, 39-44 (2006)

Trollius europaeus	*Trollius laxus*	Ranunculaceae	AY954486	Wang,W., Li,R.-Q. and Chen,Z.-D. Systematic position of Asteropyrum (Ranunculaceae) inferred from chloroplast and nuclear sequences.
Vaccinium vitis-idaea/myrtillus	*Vaccinium vitis-idaea*	Ericaceae	AF419837	Kron,K.A.
Veronica alpinus/fruticans	*Veronica anagallis-aquatica*	Plantaginaceae	AY034021	Wagstaff,S.J., Bayly,M.J., Garnock-Jones,P.J. and Albach,D.C. Classification, origin, and diversification of the New Zealand hebes (Scrophulariaceae).
Vicia cracca	*Vicia cracca*	Fabaceae	AY395566	Silvertown,J., McConway,K., Gowing,D., Dodd,M., Fay,M.F., Joseph,J.A. and Dolphin,K. Absence of phylogenetic signal in the niche structure of meadow plant communities. Proc. Biol. Sci. 273, 39-44 (2006)
Viola biflora/canina	*Viola philippica*	Violaceae	AB354436	Tokuoka,T. Molecular phylogenetic analysis of Violaceae (Malpighiales) based on plastid and nuclear DNA sequences.
Viscaria alpina	*N/A*			

APPENDIX IV, TO CHAPTER III

Details of the ITS sequences used to discriminate species within genera.

Species	Accession	Citation
Saxifraga cespitosa	AF087604	Conti,E., Soltis,D.E., Hardig,T.M. and Schneider,J. Phylogenetic relationships of the silver saxifrages (Saxifraga, sect. Ligulatae haworth): implications for the evolution of substrate specificity, life histories, and biogeography. Mol. Phylogenet. Evol. 13, 536-555 (1999)
Saxifraga oppositifolia	AF087592	Conti,E., Soltis,D.E., Hardig,T.M. and Schneider,J. Phylogenetic relationships of the silver saxifrages (Saxifraga, sect. Ligulatae haworth): implications for the evolution of substrate specificity, life histories, and biogeography. Mol. Phylogenet. Evol. 13, 536-555 (1999)
Saxifraga stellaris	AF374827	Soltis,D.E., Kuzoff,R.K., Mort,M.E., Zanis,M., Fishbein,M., Hufford,L., Koontz,J. and Arroyo,M.K. Elucidating deep-level phylogenetic relationships in Saxifragaceae using sequences for six chloroplastic and nuclear DNA regions. Ann. Mo. Bot. Gard. 88, 669-693 (2001)
Silene acaulis	U30949	Desfeux,C., Maurice,S., Henry,J.P., Lejeune,B. and Gouyon,P.H. Evolution of reproductive systems in the genus Silene. Proc. R. Soc. Lond., B, Biol. Sci. 263, 409-414 (1996).
Silene dioica	U32568	Desfeux,C., Maurice,S., Henry,J.P., Lejeune,B. and Gouyon,P.H. Evolution of reproductive systems in the genus Silene. Proc. R. Soc. Lond., B, Biol. Sci. 263, 409-414 (1996).
Silene vulgaris	U30969	Desfeux,C., Maurice,S., Henry,J.P., Lejeune,B. and Gouyon,P.H. Evolution of reproductive systems in the genus Silene. Proc. R. Soc. Lond., B, Biol. Sci. 263, 409-414 (1996).

APPENDIX V, TO CHAPTER IV

Phenology tables for the five habitats. Black boxes indicate that the corresponding species was observed in bloom during that month; white boxes indicate that the species was not observed to flower during that month.

Phenology table for the dry grassland.

Family	Species	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	Flower colour	
										Humans	Bees
Apiaceae	*Aegopodium podagria*				■					white	blue-green
	Anthriscus silvestris			■	■					white	blue-green
	Pimpinella major					■	■	■		white	blue-green
	Peucedanum oreoselinum				■	■	■	■		white	blue-green
Asclepiadaceae	*Cynanchum vincetoxicum*			■	■		■	■		white	blue-green
Asteraceae	*Achillea millefolium*				■	■	■	■	■	white	blue-green
	Cirsium arvense					■	■	■		pink	blue-green
	Cirsium oleraceum					■	■	■		white	blue-green
	Cirsium palustre					■	■	■		purple	blue
	Conyza canadiensis					■	■	■		white	blue-green
	Eupatorium cannabinum					■	■	■		pink	blue-green
	Hieracium sabaudum							■		yellow	UV-green
	Matricaria maritima				■					white	blue-green
	Mycelis muralis							■		yellow	UV-green
	Senecio vernalis			■	■					yellow	UV-green
	Senecio viscosus							■		yellow	UV-green
	Senecio vulgaris							■	■	yellow	green
	Sonchus arvensis					■	■	■		yellow	UV-green
	Taraxacum officinale		■	■				■		yellow	UV-green
Boraginaceae	*Lithospernum arvensis*			■						white	blue-green
	Myosotis arvensis			■			■	■		blue	blue-green
	Myosotis hispida			■						blue	blue
Brassicaceae	*Alliaria petiolata*			■	■		■	■		white	blue-green
	Arabis glabra			■	■	■				white	blue-green
	Berteroa incana					■	■	■	■	white	blue-green
	Capsella bursa-pastoris			■	■			■		white	blue-green

	Erysimum cheiranthoides					■				yellow	UV-green
Campunulaceae	*Campanula rotundifolia*							■		blue	blue
	Campanula trachelium					■	■	■		blue	UV-blue
Caprifoliaceae	*Viburnum opulus*			■						white	blue-green
Caryophyllaceae	*Arenaria serpyllifolia*			■						white	blue-green
	Cerastium arvense			■						white	blue-green
	Dianthus carthusianum				■	■	■	■	■	purple	blue
	Holosteum umbellatum			■						white	blue-green
	Melandrium album				■	■	■	■		white	blue-green
	Myosoton aquaticon									white	blue-green
Convolvulaceae	*Calystegia sepium*				■	■	■	■		white	blue-green
Cornaceae	*Cornus sanguinea*				■			■		white	blue-green
Crassulaceae	*Sedum maximum*					■		■	■	white	blue-green
	Sedum sexangulare				■	■	■			yellow	green
Euphorbiaceae	*Euphorbia cyparissias*			■	■					green	green
Fabaceae	*Astragalus glycyphyllos*				■					green	blue-green
	Coronilla varia				■					pink	blue-green
	Trifolium campestre				■					yellow	green
	Trifolium dubium				■					yellow	green
	Vicia sativa			■						purple	UV-blue
	Vicia sepium			■						blue	UV-blue
Geraniaceae	*Geranium robertianum*			■	■	■	■	■		pink	blue
Guttiferae	*Hypericum perforatum*				■	■	■	■		yellow	UV-green
Lamiaceae	*Clinopodium vulgare*							■		purple	blue
	Galeopsis pubescens						■	■		pink	blue
	Galeopsis tetrahit					■				pink	blue-green
	Glechoma hederacea									purple	blue
	Salvia pratensis			■	■			■		purple	UV-blue

	Stachys rectus			■	■			■	■	white	blue-green
Liliaceae	*Allium oleraceum*					■				pink	blue
	Asparagus officinalis			■	■					green	green
	Gagea pratensis		■							yellow	UV-green
	Polygonatum odoratum			■						white	blue-green
Onagraceae	*Epibolium angustifolium*				■	■	■	■		pink	blue
	Epibolium hirsutum					■	■	■		purple	blue
Papaveraceae	*Chelidonium majus*			■	■	■	■	■		yellow	UV-green
	Papaver dubium				■					red	UV
	Papaver rhoeas			■	■					red	UV
	Papaver somniferum									red	UV
Primulaceae	*Primula veris*		■	■						yellow	green
Ranunculaceae	*Ranunculus acris*				■					yellow	UV-green
	Thalictrum minus				■	■		■		yellow	green
Rosaceae	*Fragaria viridis*			■						white	blue-green
	Geum rivale			■						pink	UV-blue
	Geum urbanum			■	■	■	■	■		yellow	UV-green
	Potentilla argentea				■			■		yellow	UV-green
	Potentilla heptaphylla			■						yellow	UV-green
	Rosa canina				■					pink	blue-green
	Rubus caesius				■	■	■	■		white	blue-green
Rubiaceae	*Galium aparine*			■	■	■	■			white	blue-green
	Galium mollugo				■					white	blue-green
	Galium verum				■	■	■	■		yellow	green
Scrophulariaceae	*Linaria vulgaris*							■	■	yellow	blue-green
	Veronica arvensis		■							blue	blue
	Veronica chamaedrys			■						blue	UV-blue
	Veronica spicata						■	■	■	blue	blue

	Veronica prostrata									blue	UV-blue
Solanaceae	*Solanum dulcamara*									purple	UV-blue
	Solanum nigrum									white	blue-green

Phenology table for the humid meadow.

Family	Species	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	Flower colour	
										Humans	Bees
Apiaceae	*Aegopodium podagrarium*				■					white	blue-green
	Anthriscus silvestris			■	■					white	blue-green
	Peucedanum oreoselinum					■	■	■		white	blue-green
	Pimpinella major					■	■			white	blue-green
	Torilis japonica					■	■			white	blue-green
Asteraceae	*Achillea millefolium*					■		■		white	blue-green
	Bellis perennis			■						white	blue-green
	Chamomilla recutita				■					white	blue-green
	Cirsium oleraceum					■	■	■	■	white	blue-green
	Crepis paludosa				■					yellow	UV-green
Boraginaceae	*Myosotis arvensis*				■	■		■		blue	blue-green
	Symphytum officinale					■				purple	blue
Brassicaceae	*Cardamine pratensis*			■						pink	blue-green
Campanulaceae	*Campanula patula*				■	■	■	■	■	purple	UV-green
Caryophyllaceae	*Cerastium arvense*			■	■					white	blue-green
	Cerastium holosteoides			■	■	■	■	■		white	blue-green
	Lychnis flos-cuculi			■	■	■	■			pink	blue
	Stellaria palustris				■					white	blue-green
Fabaceae	*Lathyrus pratensis*				■	■	■	■		yellow	green
	Lotus corniculatus				■	■	■	■		yellow	green
	Trifolium campestre				■		■	■		yellow	green
	Trifolium pratense			■	■	■	■	■		pink	blue
	Trifolium repens				■	■	■	■		white	blue-green
	Vicia cracca					■	■			purple	blue
Lamiaceae	*Ajuga genevensis*			■						blue	UV-blue
	Mentha aquatica						■	■		pink	blue-green
	Mentha arvensis						■			pink	blue
	Prunella vulgaris					■	■			blue	blue
Liliaceae	*Allium oleraceum*					■				pink	blue
Lythraceae	*Lythrum salicaria*					■	■			purple	UV-blue
Onagraceae	*Epilobium hirsutum*						■	■		purple	blue
	Epilobium parviflora					■	■			pink	blue

Polygonaceae	*Polygonum bistorta*			■	■	■					pink	blue-green
	Rumex acetosa			■	■	■	■				red	blue-green
Ranunculaceae	*Ranunculus acris*			■	■	■	■	■	■	■	yellow	UV-green
	Ranunculus repens				■	■					yellow	green
Rosaceae	*Filipendula ulmata*					■					white	blue-green
	Geum rivale			■							pink	UV-green
	Geum urbanum			■							yellow	UV-green
Rubiaceae	*Galium mollugo*				■	■	■	■	■		white	blue-green
Scrophulariaceae	*Veronica chamaedrys*			■	■	■			■	■	blue	UV-blue
Valerianaceae	*Valeriana sambucifolia*					■	■	■	■		white	blue-green

Phenology table for the roadside.

Family	Species	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	Flower colour	
										Humans	Bees
Apiaceae	*Pimpinella major*					■				white	blue-green
	Torilis japonica					■				white	blue-green
Asteraceae	*Achillea milefolium*				■	■	■	■	■	white	blue-green
	Crepis paludosa				■					yellow	UV-green
	Hieracium murorum				■					yellow	UV-green
	Hieracium pilosella				■					yellow	UV-green
	Hieracium sabaudum							■		yellow	UV-green
	Mycelis muralis				■					yellow	UV-green
	Senecio jacobea					■	■	■		yellow	UV-green
	Senecio vulgaris							■		yellow	green
	Taraxacum officinale		■	■						yellow	UV-green
	Tussilago farfara	■	■							yellow	UV-green
Boraginaceae	*Myosotis arvensis*			■						blue	blue-green
Brassicaceae	*Arabidopsis thaliana*			■						white	blue-green
	Berteroa incana					■	■	■	■	white	blue-green
	Capsella bursa-pastoris			■						white	blue-green
	Cardaminopsis arenosa		■	■	■	■	■	■		white	blue-green
Campanulaceae	*Campanula patula*				■					purple	UV-blue
	Jasione montana					■				blue	blue
Caprifoliaceae	*Symphoricarpus albus*				■					pink	blue
Caryophyllaceae	*Arenaria serpyllifolia*			■						white	blue-green
	Cerastium glomeratum			■						white	blue-green
	Cerastium holosteoides				■					white	blue-green
	Holosteum umbellatum			■						white	blue-green
	Silene nutans			■	■					white	blue-green
	Silene vulgaris					■		■		white	blue-green
	Stellaria graminea				■					white	blue-green
	Stellaria holostea			■	■					white	blue-green
Cornaceae	*Cornus sanguinea*				■					white	blue-green
Dipsacaceae	*Knautia arvensis*					■			■	pink	blue-green
Euphorbiaceae	*Euphorbia cyparissias*		■	■	■					green	green
Fabaceae	*Lathyrus vernus*			■						purple	blue
	Trifolium dubium			■						yellow	green

	Trifolium campestre									yellow	green
	Trifolium pratense									pink	blue
	Trifolium repens									white	blue-green
	Vicia hirsuta									blue	blue-green
	Vicia sepium									blue	UV-blue
Guttiferae	*Hypericum perforatum*									yellow	UV-green
Lamiaceae	*Ajuga genevensis*									blue	UV-blue
Ranunculaceae	*Ranunculus acris*									yellow	UV-blue
	Ranunculus repens									yellow	green
Rosaceae	*Agrimonia eupatoria*									yellow	UV-green
	Fragaria vesca									white	blue-green
	Geum urbanum									yellow	UV-green
	Potentilla argentea									yellow	UV-green
	Potentilla reptans									yellow	UV-green
	Prunus padus									white	blue-green
	Prunus spinosa									white	blue-green
	Rubus caesius									white	blue-green
Scrophulariaceae	*Linaria vulgaris*									yellow	blue-green
	Veronica chamaedrys									blue	UV-blue

Phenology table for the hazel shrub community.

Family	Species	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	Flower colour	
										Humans	Bees
Apiaceae	*Aegopodium podagraria*				■					white	blue-green
	Anthriscus silvestris			■	■					white	blue-green
	Torilis japonica					■				white	blue-green
Asteraceae	*Cirsium oleraceum*							■		white	blue-green
Balsaminaceae	*Impatiens parviflora*			■	■	■	■	■		yellow	UV
Boraginaceae	*Pulmonaria obscura*	■	■	■						purple	UV-blue
Brassicaceae	*Alliaria petiolata*			■						white	blue-green
Campanulaceae	*Campanula latifolia*						■	■		blue	UV-blue
	Campanula rapunculoides					■	■	■		blue	UV-blue
	Campanula trachelium					■	■			blue	UV-blue
Caryophyllaceae	*Arenaria serpyllifolia*			■						white	blue-green
Geraniaceae	*Geranium robertianum*			■	■	■	■	■		pink	blue
Lamiaceae	*Galeopsis pubescens*							■		pink	blue
	Stachys sylvatica				■					purple	blue
Liliaceae	*Maianthemum bifolium*			■						white	blue-green
	Paris quadrifolia			■						green	green
	Polygonatum multiflorum			■						green	blue-green
Papaveraceae	*Chelidonium majus*			■		■		■		yellow	UV-green
Ranunculaceae	*Anemone ranunculoides*		■							yellow	UV-green
	Hepatica nobilis	■	■	■						purple	blue
	Ranunculus ficaria		■							yellow	UV-green
	Ranunculus sceleratus			■						yellow	UV-green
Rosaceae	*Geum urbanum*				■	■	■	■		yellow	UV-green
	Rubus caesius				■	■				white	blue-green
Rubiaceae	*Gallium aparine*			■	■					white	blue-green
Scrophulariaceae	*Lathraea squamaria*		■	■						purple	blue

Phenology table for the maple forest.

Family	Species	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	Flower colour	
										Humans	Bees
Apiaceae	*Aegopodium podagraria*				■					white	blue-green
	Anthriscus silvestris			■	■					white	blue-green
	Torilis japonica					■	■			white	blue-green
Balsaminaceae	*Impatiens parviflora*				■	■	■	■		yellow	UV
Boraginaceae	*Pulmonaria obscura*	■	■	■						purple	UV-blue
Brassicaceae	*Alliaria petiolata*			■						white	blue-green
Campanulaceae	*Campanula latifolia*						■	■		blue	UV-blue
	Campanula rapunculoides					■				blue	UV-blue
	Campanula trachelium					■	■			blue	UV-blue
Caryophyllaceae	*Arenaria serpyllifolia*			■						white	blue-green
	Stellaria holostea			■	■					white	blue-green
Geraniaceae	*Geranium robertianum*			■	■	■	■	■		pink	blue
Lamiaceae	*Galeopsis pubescens*									pink	blue
	Stachys sylvatica				■					purple	blue
Liliaceae	*Paris quadrifolia*				■					green	green
Papaveraceae	*Chelidonium majus*			■						yellow	UV-green
Primulaceae	*Primula veris*		■	■						yellow	green
Ranunculaceae	*Anemone ranunculoides*		■							yellow	UV-green
	Hepatica nobilis	■	■	■						purple	blue
Rosaceae	*Geum urbanum*						■			yellow	UV-green
	Rubus caesius					■	■			white	blue-green
Rubiaceae	*Gallium aparine*				■					white	blue-green
Scrophulariaceae	*Scrophularia nodosa*				■					green	blue-green
	Veronica chamaedrys			■						blue	UV-blue

APPENDIX VI, TO CHAPTER VI

Site and species list of flowers sampled from Brazil, Israel, Costa Rica and South Africa, for the comparison of colour compositions of floral communities.

Species	Bee colour	Human colour (if recorded)
Brazil, Location 1: Riberao Preto		
Aeschynomene paniculata	green	red-yellow
Banisteria stellaris	blue	pink
Banisteriopsis laevifolia	blue	violet-brown-green
Bidens gardineri	green	orange
Byrsonima crassa	UV-green	yellow
Cambessedesia ilicifolia	UV-green	yellow
Camptosema ellipticum	UV	red
Chamaecrista nictitans	green	yellow
Chamaecrista sp.	UV-green	yellow
Cochlospermum regium	UV-green	yellow
Desmodium pachyrhiza	blue-green	dark red
Eremanthus sphaerocephalus	UV-blue	violet
Gochnatia barrosii	blue-green	light green
Helicteres brevispira	blue-green	orange
Hyptis pauliana	blue-green	red
Hyptis suaveolens	UV-blue	light violet
Hyptis multibracteata	blue-green	light violet
Jacaranda puberula	blue	dark violet
Lippia lupulina	blue	violet
Lippia pachyrhiza	blue	violet
Lithraea molleoides	green	yellow
Luehea speciosa	blue-green	white-pink
Myrcia uberavensis	blue-green	white-light green
Ouratea nana	green	yellow
Pyrostegia venusta	green	orange
Roupala montana	blue-green	white-green
Serjania lethalis	blue-green	white-light green
Sida linifolia	green	yellow
Stilpnopappus speciosus	blue-green	white-red
Tibouchina stenocarpa	UV-blue	dark violet
Turnera sp.	UV	cream-yellow
Vernonia ferruginea	blue-green	pink
Waltheria indica	UV-green	yellow
Brazil, Location 2: Salvador		
Cuphea sp.	blue-green	yellow-red
Rhizophora mangle	UV-blue	yellow-green
Polygala sp.	blue-green	white
Mimosa sp.	blue	light pink
Lantana hirta	blue	pink
Dalbergia ecastaphyllum	UV	yellow

Brazil, Location 3: Sao Paulo

Abutilon sp.	blue	pink
Achyrocline saturejoides	blue-green	light yellow
Aechmea sp.	UV-blue	pink
Ageratum conyzoides	blue	violet
Aphelandra crenata	UV-blue	violet
Asclepias curassavica	green	yellow
Begonia fischeri	blue	pink
Begonia sp.	blue-green	pink
Billbergia sp.	blue	red
Borreria capitata	blue	pink
Calliandra tweediei	blue-green	red
Canistum cyatiforma	blue	violet
Canna limbata	UV	orange
Cardamine pratensis	UV	light pink
Cestrum sp.	UV-green	yellow
Chrysanthemum leucanthemum	blue-green	white
Cissus sp.	blue	violet
Dichorisandra sp.	UV-blue	dark violet
Dietes sp.	blue-green	light yellow
Dombeya burgessiae	blue	pink
Dombeya wallichii	UV-blue	pink
Emilia sonchifolia	blue	red
Epidendrum imatophyllum	blue	pink
Erigeron sp.	blue-green	white
Erythrina speciosa	UV	red
Eupatorium pauciflorum	blue-green	light blue
Euphorbia milii	UV-blue	red
Euphorbia pulcherrima	UV	yellow
Fuchsia regia	blue	red
Galinsoga parviflora	blue	white
Heliconia velloziana	UV	orange
Hemerocallis flava	UV-green	yellow
Hibiscus rosa-sinensis	UV-blue	white-pink
Impatiens sultani	UV	orange
Ipomoea callida	blue	light violet
Justicia brandegeana	UV-blue	white
Justicia carnea	UV-blue	pink
Justicia rizzini	blue	red
Justicia sp.	UV	red
Lantana camara	blue	pink
Lantana hirta	blue-green	white
Lantana lilacina	blue	pink
Lonicera japonica	blue-green	cream-white
Ludwigia elegans	UV-green	yellow
Malva sp.	UV	red-orange
Malvaviscus arboreus	UV	red
Nemanthus sp.	green	orange
Nidularium sp.	green	light green
Oxalis sp.	blue	light violet
Petasites sp.	blue-green	white-pink
Polygonum capitatum	UV-blue	pink
Pterolepis glomerata	blue-green	light violet
Ranunculus sp.	blue	violet
Rhododendron indicum	blue-green	violet
Rubus rosaefolius	blue-green	white

Sagittaria sp.	blue-green	white
Sanchezia nobilis	UV	yellow
Siphocampylus convolvulaceus	UV	red
Siphocampylus sp.	blue	violet
Solanum sp.	blue	white
Sonchus oleraceus	green	yellow
Stachytarpheta speciosa	blue	violet
Thunbergia grandiflora	blue-green	white
Tibouchina cerastifolia	blue	violet
Tibouchina granulosa	UV-blue	violet
Tithonia diversifolia	UV-green	yellow
Urera sp.	UV-blue	pink
Vernonia scorpioides	blue	pink-white
Vernonia sp.	UV-blue	light violet
Vriesea carinata	green	yellow
Vriesea incurvata	blue-green	orange
Vriesia sp.	UV	yellow
Wedelia paludosa	green	yellow

Israel, Location 1: Avdat desert

Achillea santolina	green	yellow
Amberboa lippi	UV-blue	blue
Anthemis melampodina	blue-green	white
Asphodelus tenuifolius	blue-green	white
Asphodelus aestivus	UV-blue	white
Asphodelus aestivus	blue	white
Asphodelus tenuifolius	blue-green	white
Astragalus amalescitanus	blue	pink-white
Astragalus sanctus	UV-blue	violet-blue
Astragalus tribuloides	blue	violet-white
Calendula arvensis	UV-green	yellow
Centaurea aegyptiaca	blue-green	grey-white
Centaurea ammoncyanus	blue	pink
Colutea istria	UV-green	yellow
Convolvulus althaeoides	UV-blue	pink
Diplotaxis harra	green	yellow
Eremostachys laciniata	blue-green	white
Erodium ciconium	UV-blue	blue
Erodium crassifolium	UV-blue	pink
Erodium laciniatum	UV-blue	pink
Fagonia mollis	UV-blue	pink
Glaucium corniculatum	UV	orange
Gymnocarpus decandrum	blue-green	green-brown
Gynandriris monophylla	blue	violet
Gynandriris monophylla	UV-blue	violet
Helianthemum ventosum	UV-green	yellow
Helianthemum vesicarium	blue	pink
Hyoscamus aureus	green	yellow
Ixiolirion montanum	UV-blue	violet
Lathyrus pseudocicera	UV	orange
Launaea nudicaulis	UV-green	yellow
Leontodon laciniata	green	yellow
Leopoldia longipes	UV	green-brown
Malva sylvestris	UV-blue	pink-white
Matricaria aurea	green	yellow-green
Moricandia nitens	UV-blue	violet

Ornithogalum trichophyllum	UV-blue	white
Papaver hybridum	UV	red
Picris longirostris	green	yellow
Reboudia pinnata	blue-green	white
Retama raetam	blue-green	white
Roemeria hybrida	UV-blue	purple
Salvia lanigera	blue	violet-pink
Scorzonera papposa	UV-blue	lilac
Senecio glauca	UV-green	yellow
Trigonella stellata	green	yellow-green
Tripleurospermum auriculum	green	yellow-green
Zygophyllum dumosum	blue-green	white

Israel, Location 2: Hatzeva desert

Aaronsohnia factorovskyi	green	yellow
Anthemis maris-mortui	blue-green	white
Asteriscus graveolens	green	yellow
Blepharis ciliaris	blue	blue-violet
Centaurea pallescens	blue-green	white-yellow
Echium rauwolffi	UV-blue	pink
Erodium laciniatum	UV-blue	violet
Erucaria boveana	blue-green	white
Fagonia arabica	UV-blue	violet-pink
Haplophyllum tuberculatum	green	green-yellow
Hypecoum imberbe	green	yellow
Kickxia floribunda	blue-green	yellow-white
Limonium pruinosum	blue	violet-pink
Lotus lanuginosus	UV-blue	red
Lycium shawii	blue-green	white
Mesembryanthemum nodiflorum	blue-green	white
Nitraria retusa	blue-green	white
Reaumuria hirtella	blue	white
Tamarix nilotica	blue-green	white-green-pink
Zilla spinosa	blue	white

Israel, Location 3: Har Gilo (Mediterranean)

Adonis microcarpa	UV	red
Ajuga chia	green	yellow
Alcea acaulis	blue	pink
Alkanna strigosa	UV-blue	lilac
Allium trifoliatum	blue	white
Anagallis arvensis	UV-blue	
Anagyris foetida	green	green
Anagyris foetida	green	yellow-brown
Anchusa strigosa	UV-blue	blue-violet
Anemone coronaria	UV-blue	red
Antirrhinum majus	UV-blue	violet
Biscutella didyma	UV-green	yellow
Calycotome villosa	UV-green	yellow
Cardaria draba	blue-green	white
Cercis siliquastrum	blue	pink
Cistus incanus	blue	pink-violet
Cistus salvifolius	blue-green	white
Crataegus aronia	blue-green	
Crepis aspera	green	yellow
Crepis hierosolymitana	UV-green	yellow

Crepis sancta	UV-green	yellow
Crepis sancta	blue-green	green
Crupina crupinastrum	blue	violet
Cyclamen persicum	blue-green	pink
Echium angustifolium	UV-blue	red
Erodium acaule	UV-blue	
Erodium cicutarium	UV-blue	lilac-pink
Erodium malacoides	UV-blue	violet
Euphorbia hierosolymitana	green	yellow-green
Fagonia brugueri	blue	white-violet
Fagonia glutinosa	blue	white-violet
Fumana thymifolia	UV-green	yellow
Geranium molle	UV-blue	violet
Gynandriris sisyrinchium	blue	blue
Gypsophila arabica	UV-blue	white
Hedypnois rhagadioloides	UV-green	yellow
Hirschfeldia incana	UV-green	yellow
Isatis lusitanica	UV-green	yellow
Lamium amplexicaule	blue	violet
Lathyrus aphaca	green	yellow
Lathyrus aphaca	UV	green
Lathyrus blepharicarpus	UV	orange
Lathyrus gorgonii	UV	
Launaea mucronata	UV-green	yellow
Lavatera cretica	blue	
Lotus collinus	UV-green	yellow
Lotus peregrinus	green	yellow
Medicago turbinata	green	yellow
Micromeria nervosa	blue	violet-pink
Onobrychis squarrosa	UV-blue	pink
Onobrychis squarrosa	blue	pink
Ononis natrix	UV-green	yellow
Orchis galilaea	blue-green	violet-green
Orchis papilionacea	UV-blue	violet-brown
Orchis tridentata	blue	
Ornithogalum neurostegium	UV-blue	white
Papaver hybridum	UV	red
Papaver rhoeas	UV	red
Papaver subpiriforme	UV	red
Prunus ursina	blue-green	
Ranunculus asiaticus	green	pink
Ranunculus marginatus	UV-green	yellow
Rhagadiolus stellatus	UV-green	yellow
Ruta chalapensis	green	yellow-green
Salvia dominica	blue-green	
Salvia fruticosa	blue	violet-white
Salvia hierosolymitana	UV-blue	dark red
Satureja thymbra	blue	violet-white
Scrophularia xanthoglossa	UV-blue	dark brown
Senecio vernalis	UV-green	yellow
Silene aegyptiaca	blue	
Sinapis arvensis	UV-green	yellow
Sonchus oleraceus	UV	green-yellow
Tetragonolobus palaestinus	UV	red
Thrincia tuberosa	UV-green	yellow
Tragopogon coelesyriacus	UV-blue	violet

Trifolium stellatum	blue	pink
Trifolium clypeatum	blue-green	white
Trifolium resupinatum	blue	violet
Trigonella caelesyriaca	green	yellow
Trigonella kotscyi	UV-green	yellow
Urospermum picroides	green	yellow
Vicia hybrida	green	yellow
Vicia palaestina	blue	blue-violet
Vicia sativa	UV-blue	violet

Israel, Location 4: Har Meirion (Mediterranean)

Acanthus syriacus	blue	pink-green
Ainsworthia trachycarpa	blue-green	white
Alcea dissecta	UV-blue	pink
Allium neapolitanum	blue	white
Allium nigrum	blue-green	white
Allium trifoliatum	blue-green	white
Anthemis cornucopia	blue-green	white
Anthemis pseudocotula	blue-green	white
Arbutus andrachne	blue-green	white
Asperula libanotica	blue-green	white
Asphodelus aestivus	blue	white
Bellvalia flexuosa	blue-green	white-yellow
Cephalanthera longifolia	blue-green	white
Crataegus azarolus	blue-green	white
Crepis hierosolymitana	UV-green	yellow
Crepis palaestina	UV-green	yellow
Erucaria hispanica	blue-green	pink
Fumaria densiflora	blue	pink
Geranium purpureum	UV-blue	pink
Geropogon hybridus	UV-blue	pink
Hesperis pendula	green	yellow-green
Lamium garganicum	blue	white-pink
Leopoldia comosa	UV-blue	violet
Leopoldia comosa	UV	brown
Limodorum abortivum	UV-blue	violet-white
Linum pubescens	UV-blue	pink
Orchis anatolica	blue	violet
Orchis italica	blue	red-pink
Ornithogalum neurostegium	UV-blue	white
Pisum elatium	UV-blue	pink
Ranunculus millefolius	UV-green	yellow
Salvia hierosolymitana	blue-green	pink
Scandix pectenveneris	blue-green	white
Scilla hyacinthoides	blue	violet
Silene dichotoma	blue-green	white
Symphytum brachycalyx	blue-green	white
Trifolium repens	blue-green	white
Valeriana dioscordiis	blue-green	white

South Africa, Location 1: North of Garies, towards Hondeklipbaai

Didelta spinosa	UV
Dimorphotheca sinuata	UV
Senecio sp.	blue
Ferraria ferrariola	UV-blue
Ferraria uncinata	UV

Lapeirousia silenoides	blue
Pelargonium incrassatum	blue
Microloma sagittatum	blue
Felicia maxmimelleri	blue
Dimorphotheca sinuata	UV
Sperguleria media	green
Ornithogalum polyphyllum	UV-blue
Dorotheanthus bellidiformis	blue-green
Drosanthemum sp	blue-green
Hermannia trifuica	blue-green
Babiana pubescens	blue
Lapeirousia jacquinia	blue
Lyperia tristus	UV
Lebeckia serecia	UV-blue
Heliophila cf. *coronopifolia*	blue-green
Wahlenbergia sp.	UV-blue
Pelargonium praemorsum	blue-green
Sarcocaulon sp.	UV-blue
Leysera tenella	UV
Babiana spiralis	blue
Manulea sp.	UV

South Africa, Location 2: Kharkauis

Heliophila sp.	blue-green
Salvia dentata	blue-green
Cotula cf. *leptalea*	green
Sperguleria media	blue-green
Gorteria diffusa	UV-green
Lebeckia serecia	blue-green
Senecio candaminifolius	UV-green
Heliophila cf. *variablis*	blue-green
Oxalis rescapriae	green
Oxalis obtusa	UV
Oxalis cf. *pescaprae*	green
Moraea miniata	UV
Dimorphotheca sinuata	green
Felicia australis	blue
Arcotheca calendula	UV
Senecio candaminifolius	UV-green
Tripteris hyoeseroides	UV-green
Silene angulata	blue
Babiana curviscepa	blue
Nemesia sp.	UV
Diascia namaquensis	UV-blue
Oxalis namaquensis	green
Trachyandra flexifolia	blue-green
Sperguleria media	blue-green
Rhyncopsidium pumillum	green
Stachys sp.	blue-green
cf. *Zaluzianskya* sp.	green
Bulbine praemosa	UV
Romulea citrina	green

South Africa, Location 3: Springbok, weather station

Zygophyllum cordifolium	UV-green
Bulbine praemosa	UV

Mesembryanthemaceae	UV-blue
Felicia sp.	blue
Grielium grandiflorum/humifusum	blue-green
Albuca maxima	blue-green
Arcotheca calendula	UV
Felicia australis	blue
Oxalis obtusa	UV
Heliophila cf. *variablis*	blue-green
Gorteria diffusa	green
Senecio candaminifolius	UV-green
Dimorphotheca sinuata	green
Leysera tenalla	green

South Africa, Location 4: Soebatsfontein

Sarcocaulon crassicaule	blue-green
Zygophyllum divariatum	UV
Gazania cupsiana	green
Didelta spinosa	green
Lebeckia serecia	blue-green
Herea elongata	blue-green
Montinia caryophyllacea	blue-green
Leysera tenella	green
Arcotheca calendula	UV
Lapeirousia silenoides	blue
Cotula cf. *leptalea*	green
Grielium humifusum	blue-green
Heliophila cf. *amplexicaulis*	blue-green
Romulea citrina	UV-green
Gorteria diffusa	green
Ursinia cf. *calenduliflora*	UV-green
Babiana curvircepa	blue-green
Drosanthemum hispidum	UV-green
Pelargonium praemorsum	blue
Oxalis obtusa	UV
Felicia merymuelleri	blue
Felicia sp.	UV-blue
Hesperantha flexiosa	blue-green
Diascia namaquensis	UV-blue
Crassula cf. *dichotoma*	UV-green
Indigofera sp.	UV-blue

South Africa, Location 5: Kamieskroon

Lupinus sp.	blue
Peliostomum cf. *virgatum*	blue
Leysera tenella	green
Amsinckia calycina	UV-green
Wahlenbergia sp	blue-green
Grielium humifusum	blue-green
Oxalis obtusa	UV
Indigofera sp.	UV-blue
Wahlenbergia sp.	blue-green
Sperguleria media	blue-green
Gorteria sp.	green
Felicia sp.	blue
Cotula cf. *leptalea*	green
Dimorphotheca sinuata	green

Dimorphotheca sinuata	green
Senecio arenaria	blue
Arcotheca calendula	UV

South Africa, Location 6: On N7, 40km south of Garies at junction of Meulsteenberg and Bruintjiehoogte

Mesembryanthemaceae	blue-green
Grielium humifusum	blue-green
Moraea mainata	UV
Didelta cannosa	green
Osteospermum grandiflorum	UV-green
Chlorophytum cf. *angulatum/crassineive*	blue
Oxalis sp.	green
Senecio arenaria	blue
Drosanthemum sp.	UV-blue
Rhyncopsidium pumillum	UV-blue
Osteospermum sp.	green
Zaluzianskya sp.	blue-green
Aizoaceae	blue
Cotula cf. *leptalea*	green
Ornithogalum suaveolens	UV

South Africa, Location 7: On N7, opposite turnoff for Bitterfontein

Tripteris cladestina	green
Ursinia calendulifolia	blue-green
Mesembryanthemaceae	blue-green
Osteospermum grandiflorum	green
Moraea mainata	UV
Heliophila cf. *variablis*	blue-green
Cotula cf. *leptalea*	green
Drosanthemum sp.	UV-blue
Dimorphotheca sinuata	green
Arcotheca calendula	UV
Rhyncopsidium pumillum	green
cf. Asteraceae	UV-blue
Felicia/Senecio sp.	green

South Africa, Location 8: On N7, 30km north of Vanrhynsdorp at junction of Beeswater and Rooiberg

Mesembryanthemaceae	UV
Mesembryanthemaceae	UV-blue
Drosanthemum sp.	UV-blue
Osteospermum grandiflorum	UV-green
Ursinia calendulifolia	blue-green
Senecio arenaria	blue
Suthelandia frutescens	blue
Didelta cannosa	UV-green
Cotula cf. *leptalea*	green
Mesembryanthemaceae	blue-green
Oxalis sp.	blue-green
Peliostomum virgatum	blue
Zaluzianskya sp.	blue-green
Gazania lichtensteini	UV-green
Hypertallis salsoides	blue-green
Lachenalia framesii	UV-blue
Zaluzianskya sp.	blue

Costa Rica, Location 1: Tapanti National Park Sendero Oro pendula

Strelitzia/Heliconia sp.	green	red/yellow
Epidendrum odontochilum	blue-green	
Not identified	UV-blue	red
Not identified	green	red/yellow
Saurauia cf. *montana*	blue	white
Unidentified Acanthaceae	blue-green	off-white
Maxillaria angustissima	blue	
cf. *Gaultheria*	blue	pink (at base)
Stelis sp.	UV-blue	
Alternathera cf. *amoena*	blue-green	white
Unidentified Commelinaceae	UV-blue	
cf. *Aloplectus*	UV-blue	red
Unidentified Asteraceae	blue-green	white
Pilea sp.	green	green

Costa Rica, Location 2: Tapanti National Park Sendero Oro pendula

Oncidium klotzschianum	UV-blue	yellow
Stelis sp.	UV-blue	
Lepanthes disticha	UV-blue	
Saurauia cf. *montana*	blue-green	white
Unidentified Asteraceae	blue-green	white
Cuphea cf. *calophylla*	blue	purple
Not identified	blue	pink
Acmella papposa or oppositifolia	UV-green	yellow
Unidentified Asteraceae	blue	purple
Unidentified Asteraceae	green	yellow
Utricularia cf. *jamesonia*	blue-green	
Trichosalpinx dura	green	yellow
Strelitzia/Heliconia sp.	UV-blue	red/yellow
Masdevallia nidifica	UV-blue	

Costa Rica, Location 3: Tapanti National Park Sendero El Pavo

Stelis guatemalensis	blue-green	
Cleome sp.	blue	pink
Unidentified Melastomataceae	blue-green	white
Unidentified Asteraceae	green	yellow
Unidentified Gesneraceae	UV-blue/blue	red
Solanum sp.	blue-green	white
Pilea sp.	green	green
Centropogon sp.	UV-blue/UV-green	red/yellow
Unidentified Asteraceae	blue	purple
Acmella oppositifolia	UV-green	yellow
Unidentified Asteraceae	blue-green	white
Maxillaria pachyacron	green	
Commelina cf. *erecta*	UV-blue	purple
Strelitzia/Heliconia sp.	UV	red/yellow
Not identified	blue-green	white
Unidentified Asteraceae	UV-green	yellow
Not identified	blue	pink

Costa Rica, Location 4: Braulio Carrillo National Park

Maxillaria campanulata	UV-blue/blue-green	red/yellow
Centropogon sp.	UV/UV-green	red/yellow
Cavendishia complectans	blue	pink

Dioscorea cf. *convolvulacea*	green	green
Not identified	blue	pink
Begonia cf. *carpinifolia*	blue-green	white
Unidentified Asteraceae	green	yellow
Viola stipularis	blue-green/blue	white
Saurauia cf. *montana*	blue-green	white
Alloplectus sp.	blue-green/UV-blue	red/green
Kohleria sp.	green	green
Unidentified Asteraceae	blue-green	purple
Unidentified Gesneriaceae	UV	red
Unidentified Ericaceae	UV-blue	pink
Gunnera sp.	UV	
Heliconia secunda	UV	red
Heliconia lankesteri	green	yellow

Costa Rica, Location 5: San Jorquen de Dota

Oncidium klotzschianum	UV-green	yellow
Columnaea sp.	UV-blue	red
Unidentified Scrophulariaceae	UV	red
Strelitzia/Heliconia sp.	UV	red/yellow
Unidentified Melastomataceae	blue-green	white
Unidentified Asteraceae	UV-green	yellow
Unidentified Asteraceae	UV-green	yellow
Unidentified Asteraceae	blue-green	purple
Unidentified Melastomataceae	blue-green	pink
Cuphea sp.	blue	purple
Mucuma sp.	UV	green
Unidentified Asteraceae	green	yellow
Unidentified Asteraceae	UV	yellow
Cuphea sp.	UV	red
Rubus rosaefolius	blue-green	white
Rondeletia/Gonzalaguenia sp.	blue-green	white
Unidentified Fabaceae	blue-green	
Unidentified Gesneriaceae	UV-blue	red
Unidentified Lamiaceae	blue-green	white

Costa Rica, Location 6: Road from San Pedro

Osmoglossum convallarioides	blue-green	white
Trifolium repens	blue-green	
Unidentified Asteraceae	blue	purple
Unidentified Asteraceae	green	yellow
Justicia sp.	blue	lilac
Erythrina sp.	UV-blue	red

Costa Rica, Location 7: Casa Mata on road from San Isidro to San Jose

Oncidium briolophotum	UV-green	yellow
Masdevallia pictorata	blue	
Oersterdella exasperata	blue/green	
Columnea sp.	UV-green/UV	red
Unidentified Asteraceae	blue-green	purple
Not identified	UV-blue	pink/white
Not identified	blue	pink/white
Unidentified Gesneriaceae	blue-green	red
Cuphea sp.	UV-blue	purple
Cestrum sp.	blue-green	white
Unidentified Asteraceae	blue-green	white

Unidentified Commelinaceae	UV-blue	red

Costa Rica, Location 8: Rancho Montereal nr Liberia, Guanacaste Prov

Encyclia cordigera	blue-green/blue	white/purple
Dalbergia sp.	blue	purple
Combretum fruticosum	green	red
Not identified	green	yellow
Aphelandra sp.	blue-green	brown

Costa Rica, Location 9: Rancho Montereal nr Liberia, Guanacaste Prov

Encyclia cordigera	blue-green/blue	white/purple
Unidentified Asteraceae	blue-green	purple
Heliotropium sp.	blue-green	white
Hyptis sp.	blue-green	white
Cleome sp.	blue-green	white/purple
Psychotria sp.	blue-green	white

Costa Rica, Location 10: Bosque de Los Angeles

Oncidium obryzatoides	UV-green	yellow
Pitcairnea halophylla	blue-green	
Not identified	blue	pink/white
Unidentified Gesneriaceae	blue-green	yellow
Impatiens cf. *walleriana*	blue	pink
cf. *Cuatresia* sp.	blue-green	white
Columnea microphylla	UV	red
Unidentified Gesneriaceae	UV-blue/UV	red/yellow
Rubus rosaefolius	blue-green	white
Unidentified Gesneriaceae	UV-blue	red
Impatiens cf. *walleriana*	blue	pink
Begonia sp.	blue-green	white
Unidentified Asteraceae	blue	purple
cf. *Critonia*	UV-green	yellow
Not identified	blue	purple
Not identified	blue	white

Costa Rica, Location 11: Macizo de la Muerte km 70

Maxillaria falcata	blue	
Cavendishia sp.	UV-blue	
Bomarea sp.	UV-green	
Not identified	blue-green	
Unidentified Ericaceae	UV-blue	
cf. *Pernettya* sp.	blue	
Gaultheria gracilis	blue-green	pink (white bracts)

Costa Rica, Location 12: El Alto de San Juan on road from San Isidro to Dominical

Oncidium dichromaticum	UV	
Oersterdella pinifera	blue-green	
Trifolium sp.	UV-green	
cf. *Brunfelsia*	blue	
Not identified	UV-blue	pink
Mannina deppei	UV-blue	
Pleurothallis homalantha	UV	
Vernonia sp.	blue	
Unidentified Asteraceae	green	yellow
Unidentified Melastomataceae	blue-green	white
Unidentified Asteraceae	blue-green	purple

Unidentified Asteraceae	blue-green	lilac
Unidentified Asteraceae	blue-green	white
Unidentified Asteraceae	blue-green	purple
Miconia trinervia	blue-green	white

Costa Rica, Location 13: Finca Altamira near Santa Elena

Erycina pumilio	UV-green	yellow
Lantana camara	green	yellow/red
Unidentified Melastomataceae	blue-green	white
Unidentified Asteraceae	blue	red/pink
Bouganvilla sp.	UV-blue	pink
Unidentified Asteraceae	blue-green	white
Unidentified Melastomataceae	blue	pink

Costa Rica, Location 14: Finca de Fransisco Cordero near Santa Elena

Macroclinium generalense	UV-blue	
Mitracarpus hirtus	blue-green	white
Psychotria elata	UV	red
Syzygium jambos	blue-green	white
Unidentified Asteraceae	blue	purple
Unidentified Asteraceae	blue-green	purple
Unidentified Asteraceae	UV-blue	red
Cuphea sp.	blue	purple
Impatiens walleriana	UV-blue	pink
Unidentified Asteraceae	green	yellow

Costa Rica, Location 15: La Cicica, El alto de San Juan on route from Dominical to San Isidro

Trichocentrum ascendens	UV-green	
Oesterdella pinifera	blue	
Unidentified Fabaceae	blue	white/purple
Unidentified Caryophyllaceae	blue-green	white
Unidentified Asteraceae	blue-green	purple
Vismia cf. *guianensis*	blue	light brown
Impatiens walleriana	UV-blue	red
Amphitecna sp.	blue-green/green	cream/orange
Unidentified Asteraceae	blue-green	white

Costa Rica, Location 16: La Cicica, El alto de San Juan on route from Dominical to San Isidro

Oncidium dichromaticum	UV	yellow/brown
Passiflora vitifolia	blue-green	
Costus plicatus	UV-blue	
Unidentified Asteraceae	green	yellow
Not identified	UV-blue	
Tripogendra serrulata	blue	red/green

Costa Rica, Location 17: Las Tablas, La Amistad IP

Oncidium klotzschianum	UV-green	yellow
Unidentified Gesneriaceae	blue	pink, white tip
Unidentified Asteraceae	UV-green	yellow
Unidentified Melastomataceae	blue-green	pink-white
Fuchsia paniculata	blue	purple
Begonia sp.	blue-green/blue	white inner, pink outer
Saurauia montana	blue	white

Costa Rica, Location 18: Las Tablas, La Amistad IP

Oncidium cariniferum	blue-green	white lip

Unidentified Gesneriaceae	UV-blue	pink, white tip
Unidentified Asteraceae	UV-green	yellow
Unidentified Asteraceae	blue-green	white
Fuchsia paniculata	blue	purple
Begonia sp.	blue-green/blue	
Phytolacca sp.	blue	purple
cf. *Paliavana*	blue-green	green + purple spots
Schultesianthum megolandrun	blue-green	yellow
Duranta costaricensis	blue-green	pale blue, purple stripe
Solanum sp.	blue-green	white

Costa Rica, Location 19: Las Tablas, La Amistad IP

Oncidium klotzschianum	UV-green	yellow
Fuchsia paniculata	blue	purple
Unidentified Melastomataceae	blue-green	white
Sloanea cf. *faginea*	blue-green	pale red
Unidentified Asteraceae	blue-green	white
Begonia sp.	blue-green	
Elleanthus robustus	blue	purple/white
Unidentified Asteraceae	UV-green	yellow
Sechium sp.	blue-green	green
Unidentified Melastomataceae	blue-green	white
Rubus rosaefolius	blue-green	white
Unidentified Melastomataceae	blue-green	pink/white
Heliconia lankesterei var. rubra	green	
cf. *Paliavana*	blue-green	
Pilea sp.	blue-green	white
Unidentified Asteraceae	blue-green	purple/white
Coboea sp.	blue-green	

Costa Rica, Location 20: On road to Rincon from Palmar Norte, 24km from Rincon

Byrsonima crassifolia	UV-green	yellow
Lantana camara	UV-green	red/orange
Senna sp.	UV	
Unidentified Asteraceae	blue-green	purple
Unidentified Melastomataceae	blue-green	white
Vochysia guatemalensis	UV-green	yellow

Costa Rica, Location 21: On road to Rincon from Palmar Norte, 22km from Rincon

Byrsonima crassifolia	UV-green	yellow
Lantana cf. *trifolia*	blue	lilac
Unidentified Onagraceae	UV-green	yellow

Costa Rica, Location 22: On road from Rincon to Pto Jimenez, 3km from Pto Jimenez

Byrsonima crassifolia	UV-green	yellow
Epidendrum sp.	green	green

Costa Rica, Location 23: Entrance to Refugio Mixta de Vida Silvestre, Carate

Mimosa sp.	green	
Lantana camara	blue	red/orange
Stachyterpheta cf. *jamaicensis*	blue-green	
Terminalia catappa	blue-green	
Citharexylum sp.	blue-green	
Dicliptera (iopus)	green	
Wedelia tribbata	UV-green	

Costa Rica, Location 24: Reserve Forestal Golfo Dulce, on road from Carate to Pto Jimenez, 20km from Pto Jimenez

Byrsonima crassifolia	UV-green	yellow
Anacardium excelsum	blue-green	
Vismia sp.	blue	
Lantana camara	blue	
Mimosa sp.	UV-green	
Unidentified Verbanaceae	blue	

Costa Rica, Location 25: On road from Carata to Pto Jimenez, at junction with Cabo Matapalo (18km from Pto Jim)

Bunchosia cf. *cornifolia*	UV-green	
Unidentified Asteraceae	UV-green	
Unidentified Verbanaceae	blue	

Costa Rica, Location 26: On road to Monteverde Cloud Forest Preserve, c.20km from Monteverde

Stigmaphyllon cf. *ellipticum*	UV-green	yellow
Lantana camara	green	
Russelia sermentosa	UV	

Costa Rica, Location 27: Monteverde Cloud Forest Preserve, entrance to Refugio Eladios

Oncidium klotzschianum	UV-green	yellow
Oncidium parviflorum	UV-green	yellow
Unidentified Verbenaceae	blue	white
Blakea cf. *gracilis*	blue-green	
Conostegia sp.	blue-green	
Not identified	blue	
Impatiens walleriana	blue	

Costa Rica, Location 28: Monteverde Cloud Forest Preserve, Refugio Aleman

Oncidium klotzschianum	UV-green	yellow
Oncidium parviflorum	UV	yellow
Blakea cf. *gracilis*	blue	
Psidium guajava	blue-green	
Hidalgoa termanta	blue-green	
Guzmania sp.	blue-green	