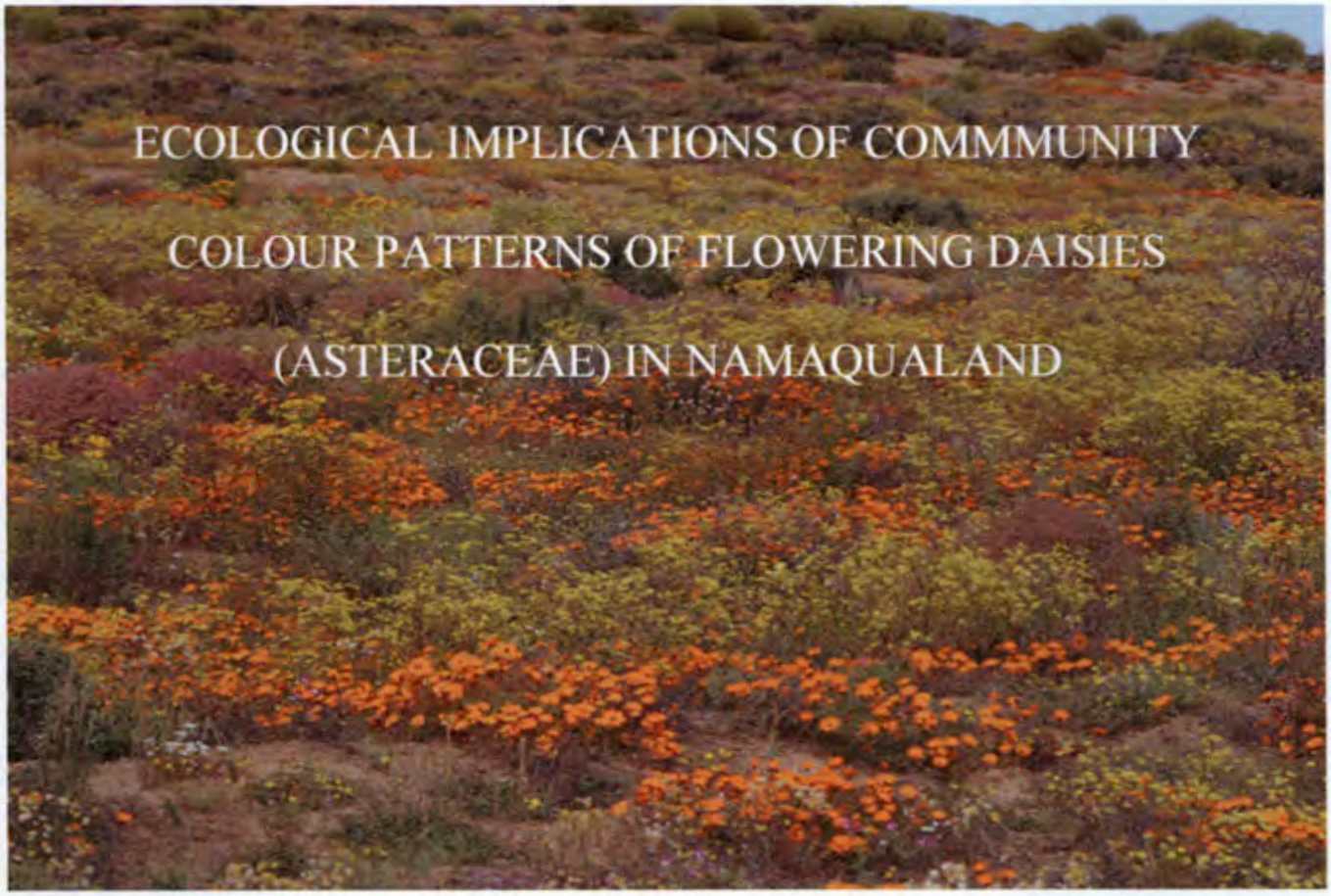

ECOLOGICAL IMPLICATIONS OF COMMMUNITY COLOUR PATTERNS OF FLOWERING DAISIES (ASTERACEAE) IN NAMAQUALAND

Systematics honours project

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Abstract

Plant community structuring is the result of positive (facilitation) and negative (competition) interaction forces acting on species, mediated by phylogenetic constraints. Communities of flowering plants are strongly influenced by their pollinators that in the case of insects are attracted to recognisable colour templates of flowers. Asteraceae (daisies) species in the succulent karoo take part in a simultaneous flowering display over a very short window period, which results in high selective pressure to attract pollinators. We looked at patterns of flower colour in communities of flowering Asteraceae species, using a bee vision model, to determine the dominant interaction force. We also constructed a phylogenetic tree of Asteraceae species to aid us in determining whether there was phylogenetic constraint on flower colour. We found that there are five easily identifiable clusters of flower colours and an equally small number of flower colour templates (combinations of colours in the different flower parts) that contains the majority of the species. Our results showed that facilitation was the strongest interactive force acting in our communities. In addition, there was some form of phylogenetic constraint present. There are many insights into ecological interactions and phylogenetic forces in communities of flowering plants that can be gained by looking at how pollinators perceive flowers.

Introduction

The structure of plant communities is determined by the interactions between the species present in them. Two main opposing forces are thought to govern community structure: competition and facilitation (Callaway and Walker 1997). Competition between species results in differentiation as each species tries to adapt to a niche where competition for resources with other species will be minimal. Facilitation is the result of increased fitness through co-existence with other species. Competition and facilitation often occur together resulting in a complex system of positive and negative interactions between species (see Callaway and Walker (1997) for a synthesis). In addition, phylogenetic constraints influence the ability of species to

adapt to new conditions (Webb *et al.* 2002) while phylogenetic relatedness, as a measure of time since divergence affects the likelihood that species will have evolved new phenotypical traits.

Communities of flowering plants represent a special case of a plant community, where competitive interactions become strongly influenced by the ability of species to attract pollinators and be recognised by them (Chittka *et al.* 1994, Chittka and Kevan 2005). Flowers use several signals to be recognised by pollinators including visual (size, shape, colour) and olfactory, usually with a reward component (pollen or nectar) (Dafni *et al.* 2005). Different pollinators have a wide range of sensitivities to different stimuli (both visual and olfactory) and so will be attracted to different combinations of stimuli, called pollination syndromes (Fenster *et al.* 2004). It is common to find that in plant communities, several species have very similar pollination syndromes (Chittka *et al.* 1994), thus attracting the same type of pollinator (i.e. facilitation). This is because flowering plant species face a trade-off between pollinator quantity and pollinator quality (Aigner 2004), especially in systems where pollinator availability is limited. A flower that shares its syndrome with many other species will increase its chances of being recognised by a pollinator. Pollinators are known to identify a syndrome with a reward (Chittka 1992, Fishbein and Venable 1996) and so it follows that if a pollinator has encountered a syndrome before, it will be more likely to recognise it. However, the problem is that the more species belong to a syndrome, the higher the chances of cross species pollination, which causes a reduction in efficiency as pollen is wasted on other species and stigmas are clogged by foreign pollen (Fishbein and Venable 1996). In the case of closely related species this means that there will be increased gene flow and so there should be strong selection for co-occurring closely related species to diverge in pollination syndrome. Thus, distantly related species may be more likely to converge on a syndrome as a way of increasing the recognisability of a signal without risking hybridization.

For many insect species, visual cues, especially colour, have been identified as the most important cue in floral recognition (Chittka *et al.* 1994, Picker and Midgley 1996, Briscoe and Chittka 2001, Chittka and Kevan 2005). Colour is not an intrinsic property of an object, but rather the interpretation of the reflectance spectra by the visual receptors of a specific organism (Chittka 1992). To be able to quantitatively measure colour we must not only know the reflectance spectra of the object in question, but also the spectral perception of the organism that is seeing that object

(Chittka 1992, Chittka and Kevan 2005). The ancestral state of insect visual receptors is thought to be a trichromatic system with UV, blue and green receptors (Briscoe and Chittka 2001). Most insects are thought to have those three receptors, with deviations from the ancestral state usually being in the form of extra receptors (although loss of receptors can also occur) (Briscoe and Chittka 2001). The most common addition is that of a red receptor, which occurs in some species of Hymenoptera, Coleoptera, Lepidoptera and Odonata (Briscoe and Chittka 2001). Chittka (1992) developed a model that described the visual perception of honey bees. Less is known about the visual systems of other pollinators apart from the number and sensitivity of their visual receptors. Flies (Diptera) can have up to six visual receptors but rarely possess a red receptor (Briscoe and Chittka 2001). Beetles (Coleoptera) have tetrachromatic receptor systems (Briscoe and Chittka 2001), sensitive to UV, blue, green and red parts of the reflectance spectrum.

The succulent karoo is filled every year with a multitude of brightly coloured flowers that defy our idea of an arid landscape. This display is composed of plants of the Asteraceae family, the daisies. Asteraceae is the most speciose family in the succulent Karoo, with almost 300 species (Cowling and Hilton-Taylor 1997) and the largest plant family in the world (Funk *et al.* 2005). As far as it is known, most flowering daisy plants in the succulent karoo take part in a generalised insect pollinated system (Struck 1992, 1994) characterized by a convergent floral structure (Mayer *et al.* 2006). These displays occur over a very short window period, between August and October (Milton *et al.* 1997), when most daisy species flower. Very little work has been done on identifying the major pollinator guilds of daisies in the succulent Karoo, especially annuals. (Struck 1992) conducted one of the few studies of this kind and found that the three families of insects were responsible for the majority of flower visits in the succulent karoo: Hymenoptera (60%), Diptera (20%) and Coleoptera (20%). However, in a recent study Mayer *et al.* (2006) argued that monkey beetles (Hopliini) are the most abundant and efficient flower visitors. Their study looked not only at pollinator abundance, but also pollen loads on visitors, a better measure of efficiency. For bees and monkey beetles, colour is the most important stimulus in floral recognition (Chittka 1992, Picker and Midgley 1996). Thus, understanding the way these insects perceive flowering daisy species can provide a great insight into the way these plant communities are structured

In this study I examined the colour patterns of flowers in several communities of flowering Asteraceae species as a way to answer the following questions:

(1) What are the colour patterns of flowering daisies, as perceived by bees?

(2) What is the strongest ecological interaction force?

(3) How is colour phylogenetically distributed?

In order to answer these questions we sampled several communities of flowering daisy species and determined their colour patterns as perceived by bees. We also constructed a phylogeny of Asteraceae genera, to address whether differences in colour are linked to phylogenetic relatedness.

Methods

Study group

The Asteaceae family was recently described by Funk *et al.* (2005) in a supertree approach that identified 38 tribes, of which 6 are abundantly present in Namaqualand. These are: Anthemideae, Arctoteae, Astereae, Calenduleae, Gnaphalieae and Senecioneae. All tribes have been described as monophyletic, although relationships between tribes are less clear (Funk *et al.* 2005).

Phylogenetic reconstruction

I constructed a tree of Asteraceae genera based on DNA sequences from four, non-coding gene regions: one nuclear, ITS (Baldwin 1992) and three plastid: rbcL (Manen *et al.* 1994), ndhF (Kim and Jansen 1995) and trnL-trnF (Taberlet *et al.* 1991). Unfortunately, it was not possible to obtain sequence data for every species included in the study. Only a few members of each genus are represented in Genbank, so I selected DNA regions which maximised generic coverage (list of species sampled for each gene region shown in Appendix 1). Every genus is represented by at least two gene regions.

The sequences were first automatically aligned using clustalW multiple alignment in Bioedit v.7.09 (Hall 1999) and then manually aligned using MacClade version 4.05 (Maddison and Maddison 2000). Phylogenetic reconstruction was done in Beast version 1.4.6 (Drummond and Rambaut 2006). In order for phylogenetic distances to be as representative as possible of evolutionary distances, branch lengths needed to be proportional to time. Conventionally, this has been achieved by first evaluating the best topology under some optimality criterion, then, in a separate step

using the same methodology to make the branch lengths proportional to time. Drummond and Rambaut (2006) recently introduced a method where branch length information is incorporated into the process of topology estimation in a Bayesian framework. The method uses a relaxed Bayesian clock to make branch lengths proportional to time, while simultaneously estimating the phylogeny in an approach they termed "relaxed phylogenetics".

The MCMC estimations were carried out under the general time-reversible model with gamma distributed variation (four categories used to approximate the gamma distribution) and a proportion of invariant sites. The substitution rate was fixed at 1 and a relaxed clock with an uncorrelated lognormal distribution was used. A fixed substitution rate makes branch lengths proportional to time and is recommended when a strong prior for the substitution rates or root of the tree is not available (as was the case) (Drummond *et al.* 2007). Two independent MCMC chains were run for 10 million states, sampled every 1000th generation, with the initial 1 million states discarded as burn-in. The MCMC samples were examined for convergence using Tracer version 1.4 (Rambaut and Drummond 2005).

The trees produced by Beast were summarised as a 50% maximum clade credibility tree, compiled using TreeAnnotator (Drummond and Rambaut 2006). The phylogenetic tree was rooted in *Arctotis* (tribe Arctoteae) which, in a recent study, was found to be the oldest lineage in the Asteraceae tribes present in Namaqualand (Funk *et al.* 2005) Branch lengths were extracted from the consensus tree and phylogenetic distance (PD) between two genera was defined as the distance from the tip to the first node in common. For all congeneric species PD was set to 0.

Field collecting

Sixteen sites were sampled in Namaqualand (Table 1) in the last week of August and first week of September 2007. A site consisted of a distinct community of flowering daisies, determined by visual inspection. At each site a flower of each of the annual and perennial daisy species with floral displays was collected (summary of species occurrence in Appendix 2) and was kept in water for spectral measurement later on the same day. Vouchers were collected for each species and identified by N.G. Bergh. For species whose spectral reflectance varied across sites, all different forms were measured separately and indicated with the site where they were collected.

Table 1: GPS coordinates and altitude of the sixteen sites were sampling occurred.

Site	Location		Altitude (m)
1	30°26'00"	17°56'21"	587
2	30°09'16"	17°48'48"	579
3	30°33'22"	17°59'44"	226
4	30 28 35	17°56 50	523
5	30°12'17"	17°42'38"	364
6	30°6'49"	17°34'18"	132
7	30°6'51"	17°32'24"	131
8	30°6'50"	17°30'13"	177
9	29°54'42"	17°38'36"	418
10	29°45'60"	17°49'53"	842
11	30°11'0"	18°0'10"	1036
12	30°14'18"	18°3'14"	1051
13	30°11'18"	17°58'42"	966
14	29°14'23"	17°42'36"	873
15	29°10'45"	17°49'14"	903
16	29°25'27"	17°49'18"	844

Colour measurements

Reflectance spectra across the range of wavelengths of visible light, between 300 nm (UV) to 700 nm (red), were recorded using an OceanOptics USB4000 Spectrometer (resolution of approximately 0.37 nm). A diffuse reflectance standard WS-2 was used as white standard. The spectrometer emits a beam of light (1 mm in diameter) at an angle of 45° to the target (ray floret, disc or leaf) and measures the amount of light that is reflected back. The integration time (length of time that the light is shone on the target) was 2,5 seconds, averaged over three measurements. The software SpectraSuite (for Mac OSX) was used to extract the spectral data.

Preliminary measurements suggested that daisy flower-heads often comprise three clear areas of contrasting colour: 1) the disc florets, 2) a band on the ray florets toward the centre of the flower-head and 3) a band around the outer periphery of the ray florets (diagram of flower parts with corresponding reflectance spectra and bee colour space position shown in Figure 3). As a result we took three spectral readings per capitulum if possible. The ray florets were laid flat on sticking tape for spectral measurement. The tips of the disc-florets were cut off and pressed onto sticking tape so that the dominant colour of the disc floret would be exposed. Care had to be taken to make sure that these were lying flat on the sticking tape. When a portion of the disc is not pressed flatly against the tape, it causes the light from the emitter to be reflected at an angle greater than 45° and this increases the amount of reflectance from the light source. The baseline reflectance readings were adjusted for direct reflectance where

necessary (particularly in disc measurements) and to eliminate spurious negative reflectance readings.

Colour hexagon

The way in which flower colour is perceived by a bee is the result of a complex interaction between the illuminating daylight spectrum, the flower's spectral reflectance and the sensitivity of the insect's receptors (Chittka 1996). The standard daylight function D65 (Wyszecki and Stiles 1982) (Figure 1) shows the maximum intensity of light over the reflectance spectrum on a clear day. Looking at the D65 curve, it becomes clear that a receptor that has to detect light over the 300-400 nm range has to be much more sensitive than one from 400-700 nm.

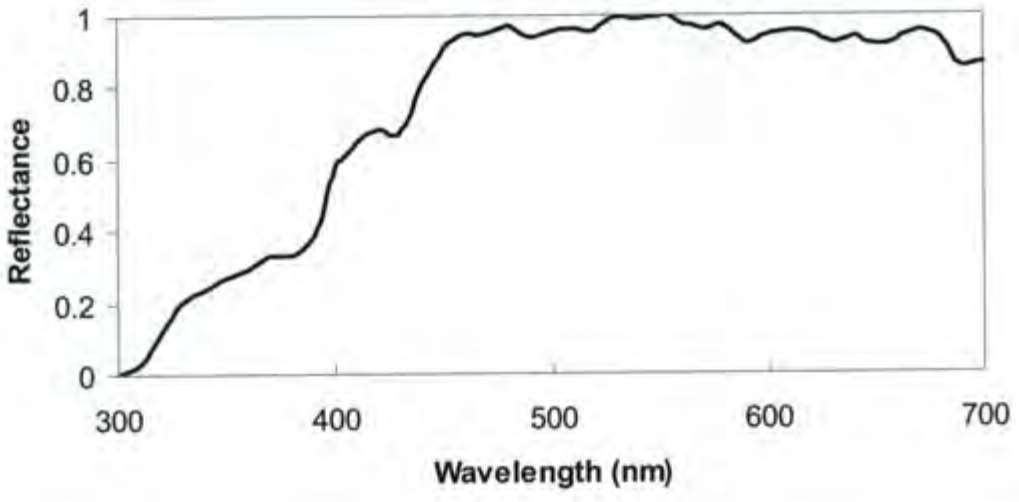

Figure 1: Standard illumination curve D65, normalized to a maximum of 1.

The colour hexagon developed by Chittka (1992) is a model that translates the reflectance spectra of an object into a two-dimension system, representing the relative excitation of the honey bee's spectral receptors. The model was written in Microsoft Excel as follows. First the average reflectance for each nanometre was calculated. Then, every 5th nanometre was extracted and the reflectance values were divided by 100 (so they ranged from 0 to 1). The model then transforms this data into single excitation values for each receptor, based on the absorbance curve of each receptor (Figure 2). The relative excitation values for each receptor are then trigonometrically converted into orthogonal coordinates (Figure 3). The distance between two points

represents how distinct two objects look to a bee. The central circle represents the background colour, against which the visual receptors are adapted to distinguish objects. Most background objects (leaves, rocks, sand) fall in this sector (Chittka *et al.* 1994), so the distance of an object to the origin represents how much that object contrasts with the background. According to (Gumbert 1999) colours separated by an Euclidean distance of less than 0.15 units on the colour hexagon look identical to a bee.

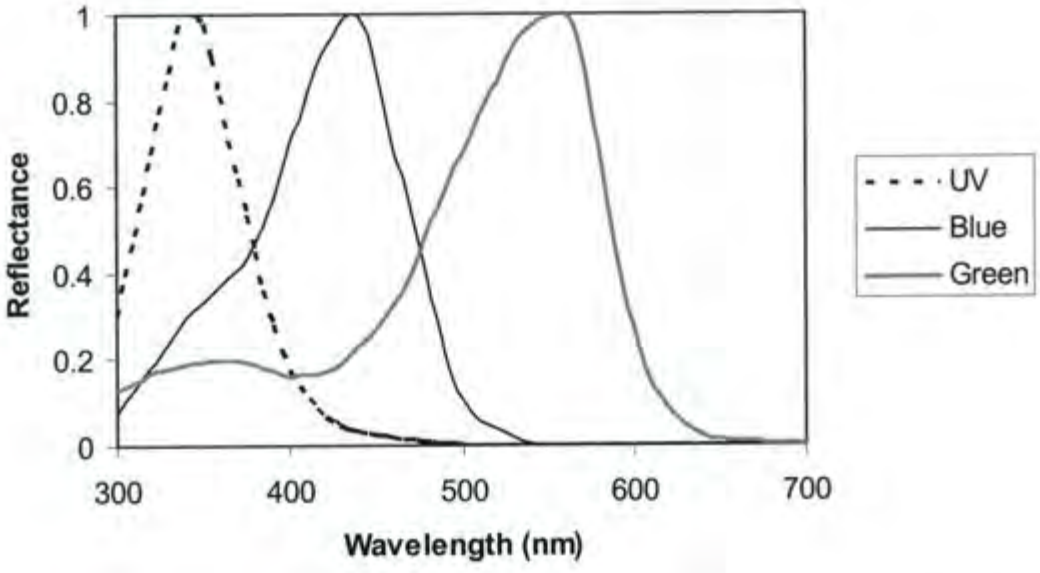

Figure 2: Spectral sensitivity functions for the honeybee UV, blue and green receptors, normalized to a maximum of 1 (from Peitsch *et al.* (1992)).

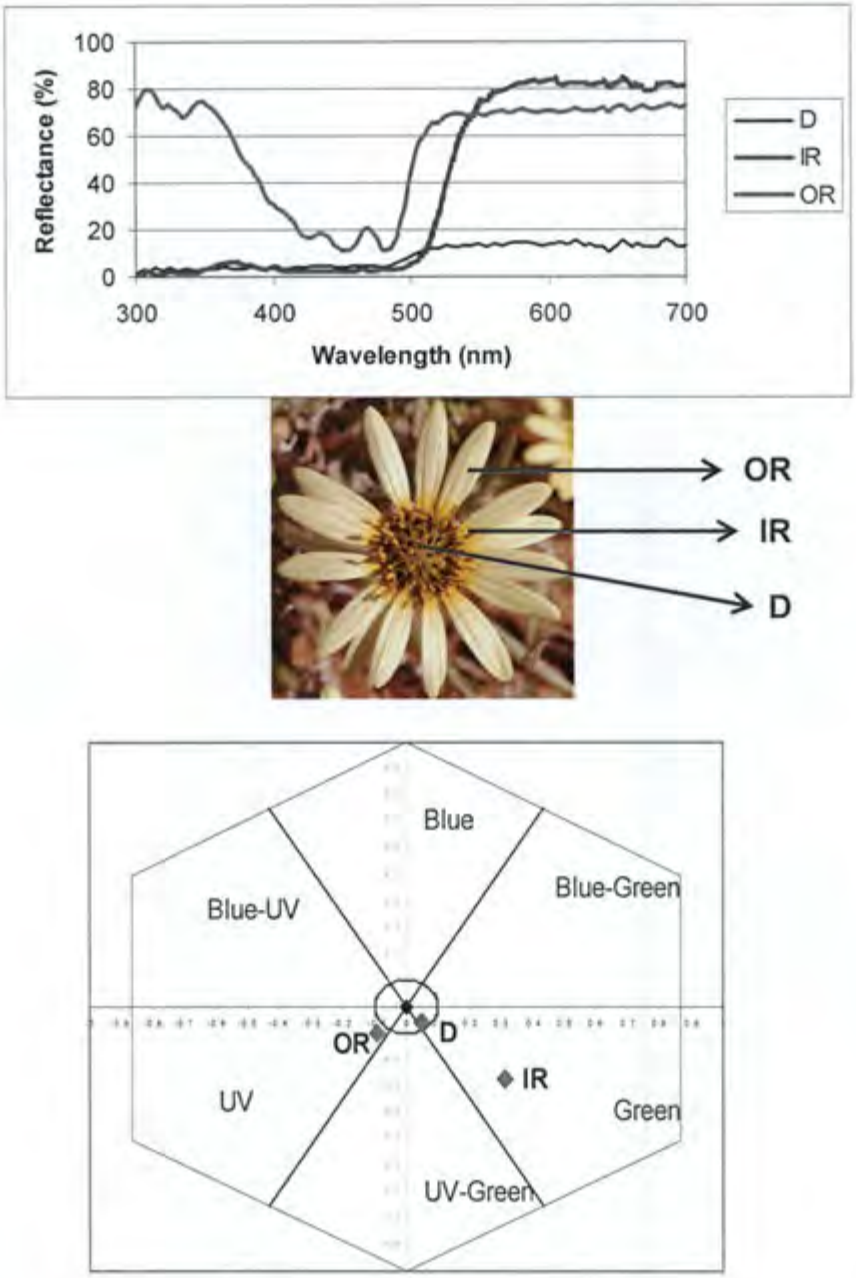

Figure 3: *Arctotheca calendula* flower-head (centre) with the three flower parts shown. Reflectance spectra (top) and corresponding colour hexagon location (bottom) of each part depicted.

In order to determine colour distances among genera several colour indices were chosen. All of the indices were measured as Euclidean distances between two points in bee-vision space or as the sum of the distances. The simplest indices were calculated by comparing pairwise distances between individual flower parts. These were: outer rays (OR), inner rays (IR) and dominant colour. Disc-florets (D) were

ignored as there is very little variation among species. Dominant colour is simply the colour of the biggest flower part (IR, OR or D). The second index was total contrast between the colour bands within an inflorescence, measured as the Euclidean distance between OR and IR and IR and D respectively.

Colour templates

Flower colour templates were designed as a way to represent the different combinations of colours of OR, IR and D. A template is then expressed as the colours of the three floral parts (OR-IR-D). Colours were defined by visually inspecting the distribution of different floral parts in the colour hexagon and determining distinct clusters. Five colour clusters were identified that fell in distinct sectors: green (G), UV-green (UVG), UV, blue (B) and uncoloured (U). Flower parts for all species were then assigned to each colour.

Statistical analysis

A Chi-square test was conducted to determine whether number of genera per template as well as OR and IR colours were greater than expected by chance. Only the five templates with the most number of species were used as most of the templates only had a single species. The expected number of genera was defined as the number species in a category divided by the species to genus ratio for the whole dataset.

Euclidean distance matrices of the xy coordinates in bee space were built for dominant colour, OR, IR and total contrast using NTSYS-pc version 2.1 (Rohlf 1988). Jaccard distance matrices were built for the co-occurrence data for all species using the same program. Assessment of the correlation between phylogenetic distance, colour distance and co-occurrence was done in NTSYS-pc version 2.1 (Rohlf 1988) using a Mantel test. The Mantel test performs an analysis of matrix correspondence based on an assumption of asymptotic normality (Smouse *et al.* 1986). 1000 permutations were used, thus the minimum significance value that can be obtained is 0.001 (if all permutations result in a matrix whose values are significantly greater or smaller than expected). Phylogenetic constraint within a community can be inferred if the slope of the relationship between colour distance and phylogenetic distance is positive, so that species that are more closely related have flower colours that are more similar than expected if the species were randomly assembled. The expected distribution of colours across the phylogenetic space would correspond to a slope of

zero. If the slope is negative then phylogenetic dispersion is present as flower colours of closely related species differ more than expected if the species were randomly assembled.

Results

Phylogenetic reconstruction

The 50% maximum clade credibility tree is shown in Figure 4. All the tribes were recovered as monophyletic with very high PP. Within Arctoteae, the two subtribes Gorteriinae (composed of *Gorteria* and *Gazania*) and Arctotidinae (all the other genera) were also recovered with 100% PP. The relationships between tribes were not so well resolved, except for the relationship between Arctoteae and Senecioneae.

Colour templates

There are 22 different colour templates that are occupied by one or more species out of a total of 50 possible templates (5 OR x 5 IR x 2 disc colours) (Table 2), with five templates occurring in more than 50% of the sites and 12 templates occurring in less than 25% of the sites. Four templates (G-G-G, B-G, UVG-G-G, UVG-UVG-G) contained 62% of all species.

The five more common templates were:

(1) G-G-G (100%): These are the flowers which are all yellow (more commonly), such as *Rhynchopsidium pumilum* or *Leysera tenella*, or orange (*Tripteris sinuata*). Most notably, this template includes all the species with no rays, such as *Cotula spp.* and *Pteronia spp.*.

(2) UVG-G-G (81.25%): These are the yellow and orange flowers that have a UV reflecting OR and no UV in the IR and D (such as *Ursinia nana* and *Dimorphotheca tragus*).

(3) B-G (81.25%): These are the flowers with purple or white rays and yellow D, belonging to the genera *Felicia*, *Senecio* or *Amellus*.

(4) G-U-G (75%): These are the yellow or orange flowers with a black IR and yellow or orange D. *Dimorphotheca pinnata* and the most common form of *Gorteria diffusa* belong to this group.

(5) UVG-G-U (68.75%): These are the yellow or orange flowers with a UV reflecting OR, no UV in the IR and a black centre (*Norlindia amplectens* for example).

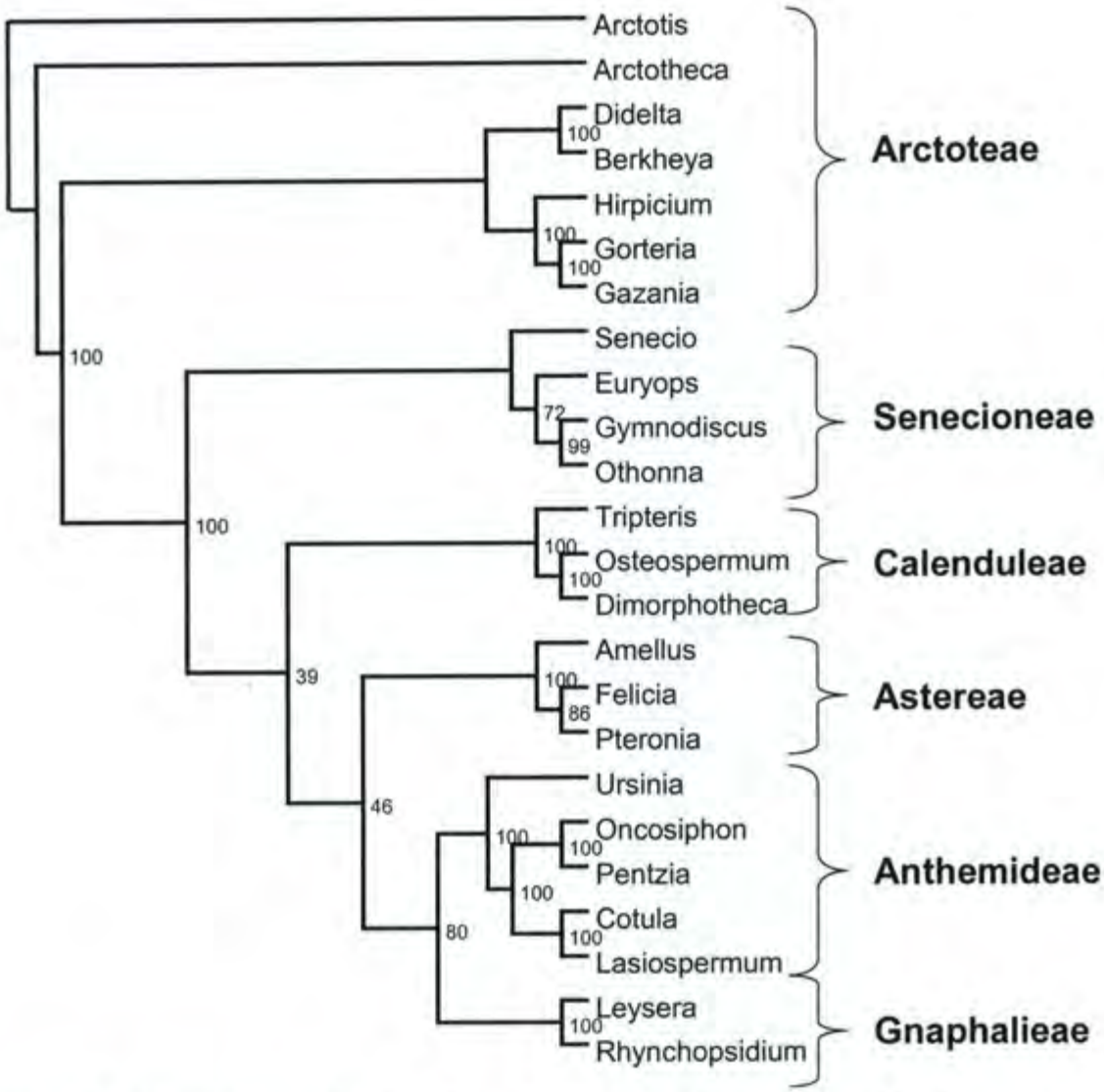

Figure 4: Phylogenetic tree obtained from the four gene regions used. Numbers next to the nodes indicate PP (%) and all 6 tribes also indicated.

Table 2: Summary of templates (OR-IR-D) found in each site. Templates represent colours in hexagon space: green (G), UV-green (UVG), UV, uncoloured (U) and blue (B)

Templates	Proportion of sites	No. of species per template	No. of genera per template	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
B-G	0.8125	11	3	X		X	X	X		X	X	X	X		X	X	X	X	X
B-U-G	0.25	1	1			X			X	X	X								
G-G-G	1	19	12	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
G-G-U	0.1875	2	2		X									X				X	
G-U-G	0.75	4	3	X	X	X	X	X	X	X		X		X	X		X		X
G-U-U	0.1875	1	1									X						X	X
G-UV-G	0.1875	2	2	X										X	X				
G-UVG-G	0.125	1	1							X		X							
G-UV-U	0.0625	1	1														X		
U-U-G	0.125	1	1										X				X		
U-UV-G	0.1875	1	1							X								X	X
U-UVG-U	0.125	1	1											X	X				
UVG-G-G	0.8125	10	6	X	X		X		X	X	X		X	X	X	X	X	X	X
UVG-G-U	0.6875	4	4	X	X	X	X	X		X		X	X		X			X	X
UV-G-U	0.5	1	1		X		X	X				X	X	X	X	X			
UVG-U-G	0.1875	2	2														X	X	X
UVG-U-U	0.375	3	3			X	X		X			X				X			X
UVG-UV-G	0.125	1	1											X				X	
UVG-UVG-G	0.375	7	6			X		X		X		X				X	X		
UVG-UVG-U	0.0625	1	1					X											
UVG-UV-U	0.25	1	1			X		X	X									X	
UV-U-U	0.0625	1	1				X												
				6	6	8	8	8	6	9	4	9	6	8	8	6	8	10	9

Flower colours in bee-vision space

There was an average of 14.8 species per site, with a minimum of 9 species and a maximum of 25. Most species (53.3%) occurred in two sites or less while only 14.7% of species occurred in more than 5 sites. Daisy flower-heads for a representative site are shown in Figure 5, while Figure 6 shows reflectance spectra of different flower colours.

The most common (bee vision space) colour (Table 3) was green, followed by UV-green (UVG) and uncoloured. Blue and UV were the least common colours. When the colours are decomposed into the three flower parts, a different pattern becomes clear. The OR have mostly UVG and green colours; they have a very small proportion of uncoloured and UV. The IR have the most even distribution of colours, with a majority of green followed by uncoloured, UVG, blue and UV. As previously mentioned, discs only had two colours, green and uncoloured.

The distribution of the colours of specific flower parts in bee space is shown in Figure 7. Points that fall in the green sector of the hexagon are either (human) yellow or orange. There does not seem to be any difference to a bee between yellow and orange, as flowers with either of those two colours will occupy seemingly arbitrary points in the green sector. In all cases, distance from the centre is a measure of brightness, so that a brightly coloured object will tend to be farther away from the centre. Purple and white colours appear to look the same to bees. Although white will fall in the blue-green sector and purple in the blue sector, the distance between many purple flowers and the white flowers is less than 0.15 and thus indistinguishable to a bee. There were two clusters of UV-reflecting flowers. The first one was that of yellow and orange flowers, which always fell in the UV-green sector. The second cluster, only contained a handful of species whose flowers had high reflectance values across the entire reflectance spectrum, such as very light yellow or mauve with UV (*Arcotheca calendula* and *Dimorphotheca sinuata* from site 15, respectively). Most (human) black colours fell in the uncoloured sector, although residual UV reflectance caused some to be placed in the UV sector.

Figure 5: Photos depicting a community of flowering daisies (site 3). The first two rows have no UV; bottom two rows all have UV reflectance in outer ray. From left to right, top to bottom: *Dimorphotheca pinnata, Rhynchopsidium pumilum, Cotula coronopifolia, Leysera gnaphalodes, Oncosiphon suffruticosum, Didelta carnosa, Lasiospermum brachyglossum, Amellus microglossus, Norlindia amplectens, Gazania tenuifolia, Senecio cardaminifolius, Monoculus monstrosa* and *Ursinia nana*.

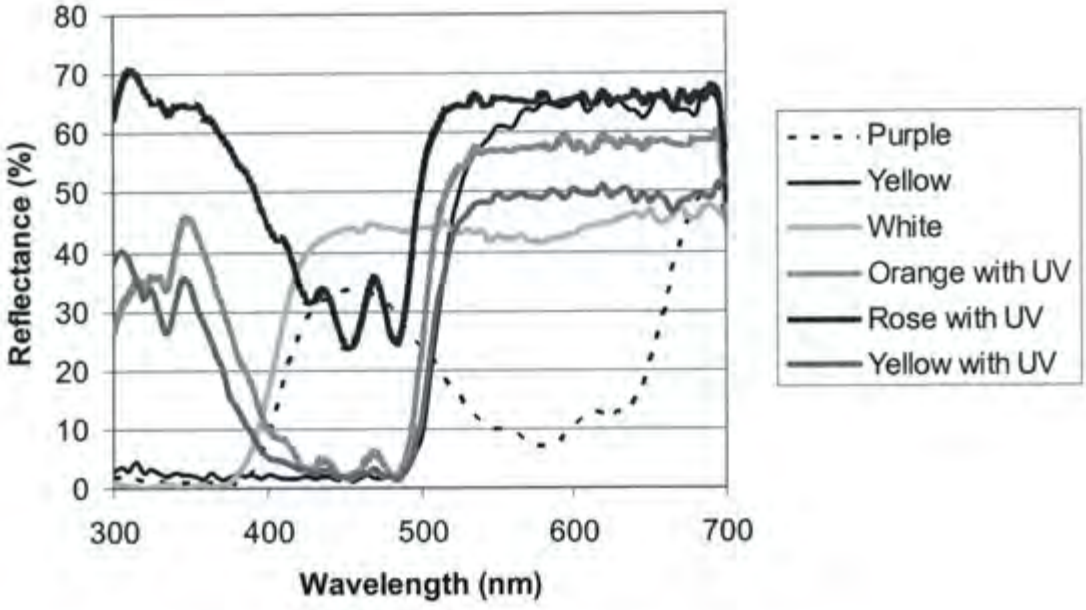

Figure 6: Reflectance spectra of the most common flower colours present.

Table 3: Proportion of colours for sampled flowers. Measurements for all, only outer rays (OR), inner rays (IR) and discs (D).

Bee-space colours	All (%)	OR (%)	IR (%)	D (%)
Blue	9.8	16.1	14.5	0
Green	51.2	32.3	37.1	77.6
UV-green	19.0	43.5	16.1	0
UV	5.4	1.6	11.3	0
Uncoloured	14.6	6.5	21.0	22.4

Astereae and Senecioneae are the only tribes that have ray-floret colours in the blue sector of bee-vision space (Figure 7). With the exception of Astereae and Gnaphalieae, all other tribes have UV-reflective (bee space) green rays. Arctoteae and Gnaphalieae (*Ursinia* only) seem to be the only tribes that have florets in the UV part of the spectrum. Gnaphalieae, Astereae and Senecioneae have only (bee space) green discs. The other tribes have both green and black discs.

The distribution of colours in the different parts of the flower-head (Figure 8) is also indicative of the strategies available to flowering daisies in Namaqualand. Discs are the most conserved part of the flower-head, either being yellow or black. OR have a wide distribution across the hexagon, covering the blue, blue-green, UV-green and black sectors. The IR seem to be the most varied out of all the flower-head parts, showing all the colours that are present in the OR, but also having a strong

presence in the UV space. However, some of these UV IR are in fact black with no UV but due to residual UV reflectance they are placed in the UV sector.

Analysis of the plots with connected IR-OR points in three representative sites (Figure 9) reveals that most flowers produce contrast between IR and OR by combining a green sector part with either a UV,UVG or black part. There are several species per site that have the same green-UVG IR to OR pattern. Other patterns are only reproduced by one or two species per site. There are very few species in any of the three sites that do not have contrast between IR and OR. Of these some, such as *Felicia spp.* instead have a contrasting ray-floret and disc, while others (*Rhynchopsidium pumilum*) have the same colour in all rays and discs

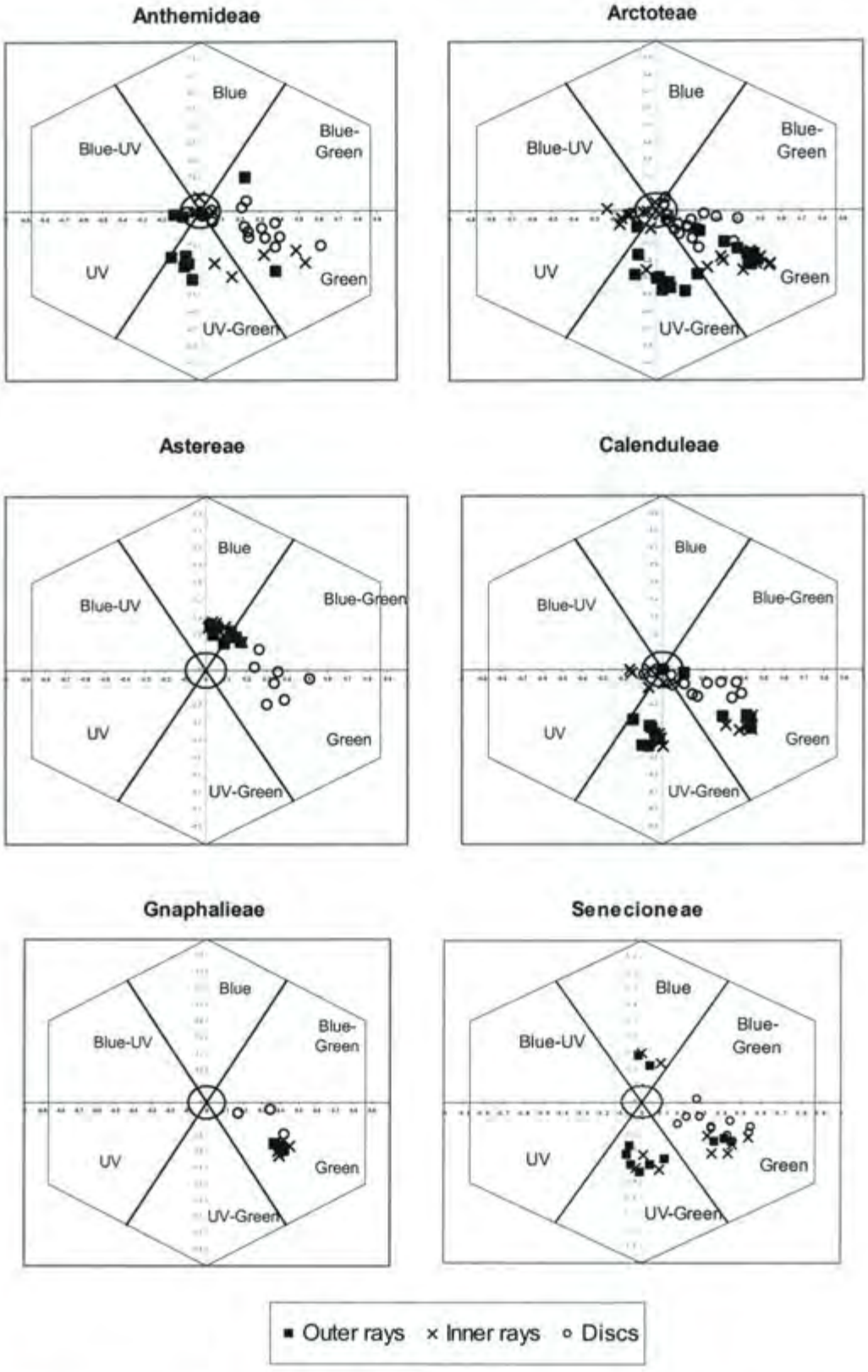

Figure 7: Colour hexagon displaying the loci for different flower parts in all six Asteraceae tribes.

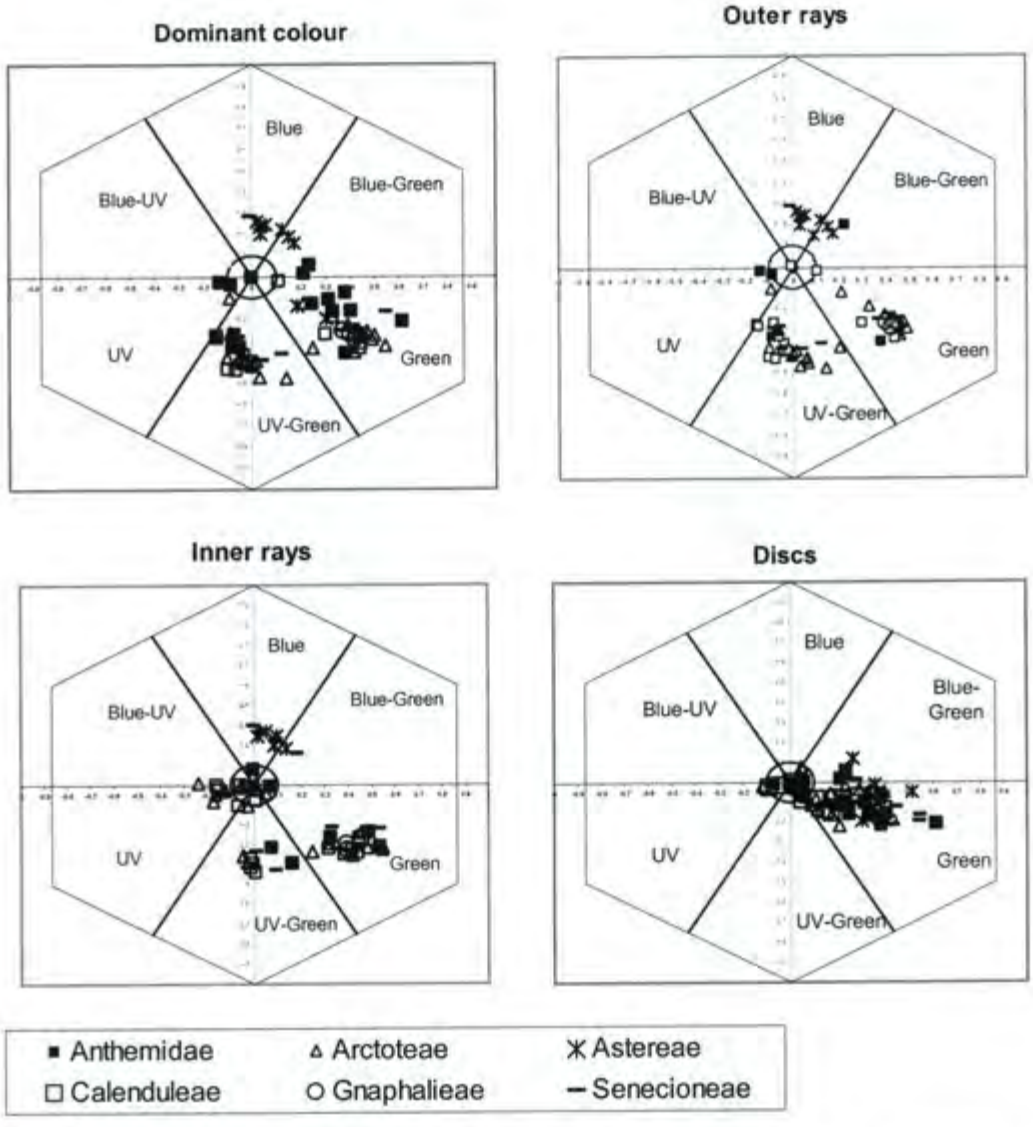

Figure 8: Colour hexagon showing the loci of the different daisy tribes for different parts of the flower.

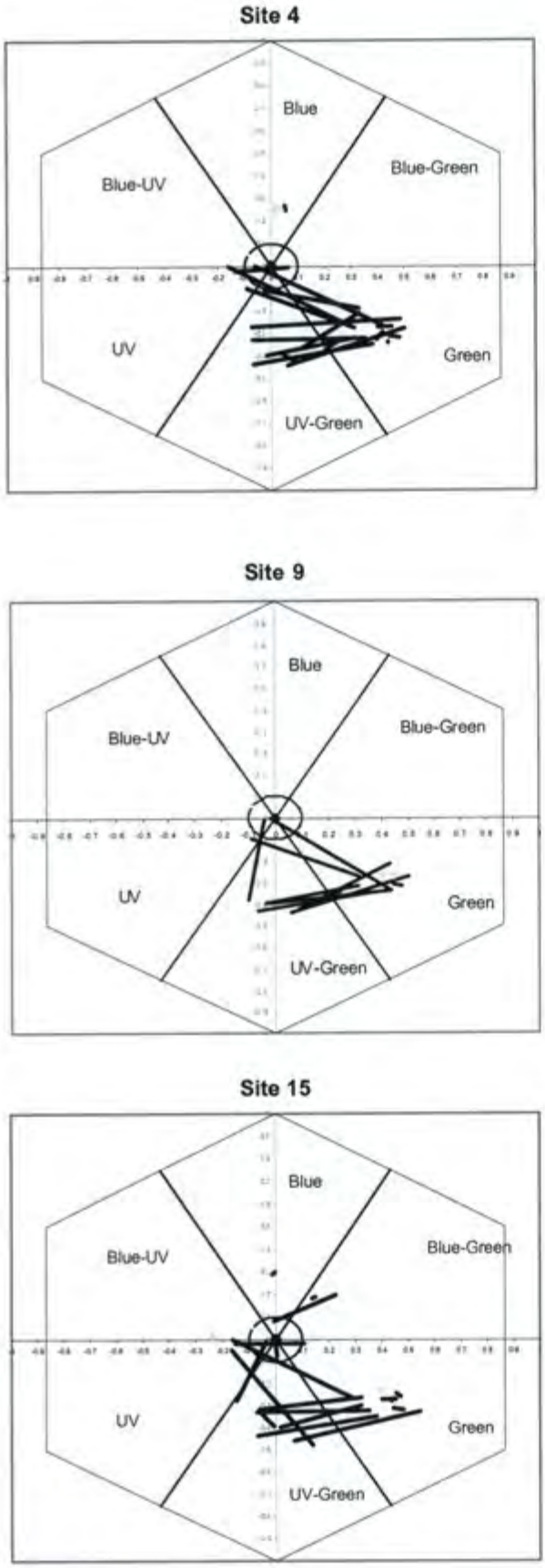

Figure 9: Plot of IR and OR, connected by a line, for three representative sites.

Statistical analysis

The five colour templates with the greatest number of species (G-G-G, B-G, UVG-G-G, UVG-UVG-G and G-U-G) were populated by more genera than would be expected by chance (χ^2=16.91, degrees of freedom=4, $p<0.005$). Colour in OR (χ^2=14.55, df=4, $p<0.01$) and IR (χ^2=10.44, df=4, $p<0.05$) was also found to be distributed across a greater number of genera than expected by chance.

The Mantel tests comparing phylogenetic distances (PD) with colour distance (CD) in bee vision space (Table 5) showed statistically significant positive correlations for the IR and OR, however the correlation coefficients (r) were very low. Co-occurrence was not correlated with PD. Within the Arctoteae and Calenduleae tribes the PD between species was significantly correlated with divergence in bee-vision colour space for the IR, but not for the OR (Table 6). The plot of CD against PD shows that there is high intra and inter generic variation of colour (Figure 10), as species with 0 PD (i.e. belonging to the same genus) have CD that range from 0 to close to the maximum possible.

Table 5: Summary of the Mantel test results. All taxa are included in the analysis.

Test	r	p-value
Dominant colour against PD	0.14	0.001
IR CD against PD	0.12	0.001
OR CD against PD	0.18	0.001
Total contrast against PD	0.10	0.001
Co-occurrence against PD	0.014	0.239

Table 6: Summary of Mantel test results for Arctoteae and Calenduleae tribes.

	Arctoteae		Calenduleae	
Test	r	p-value	r	p-value
OR CD against PD	0.02	0.36	0.07	0.21
IR CD against PD	0.17	0.03	0.36	0.008

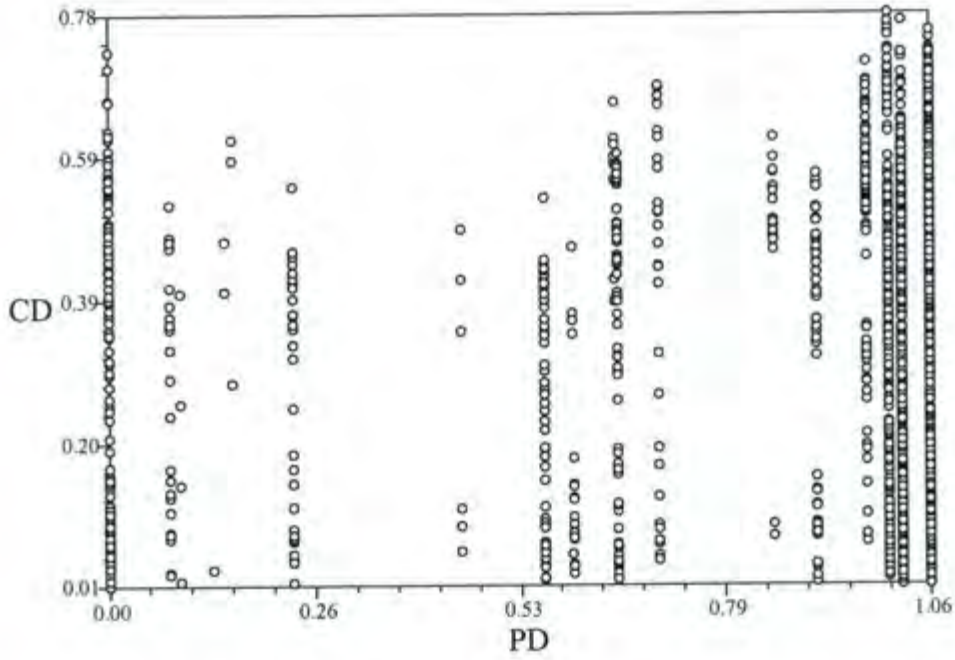

Figure 10: Plot of outer ray colour distance (CD) against phylogenetic distance (PD). All Mantel plots looked very similar so only one plot is shown.

Discussion

Understanding the way insects perceive flowers can provide great insights into the ecological interactions and evolutionary history of plant communities. No study, to our knowledge, has attempted to infer dominant ecological interactions by looking at phylogenetic relationships between plants and insect perception of flowers. In this study we have shown that there are distinct clusters of colours in bee vision space, with as few as five templates representing the majority of patterns found in our study area. The main interactive force acting in communities of flowering Asteraceae species in Namaqualand is facilitation. Most of the species of plants (62%) were aggregated into only four colour templates. This demonstrates that many species clearly belong to a pollination syndrome (as defined by colour only) that contains many other species. The fact that there were more genera per template than expected by chance means that there is strong selection for closely related species (such as those that belong to the same genus) to diverge in flower colour as a way of avoiding gene flow. This indicates that trait evolution in the species under study is convergent.

This study shows how certain flowers that look identical through our eyes have completely distinct signatures to a bee (yellow flowers with or without UV

reflectance), while things that are clearly different to us (yellow and orange or purple and white flowers) look similar to a bee. Some colours deviated from their expected location in the vision space, such as black objects falling in the UV sector due to residual reflectance values on the UV part of the spectrum. The uncoloured space in the bee-vision model is perhaps its most interesting. Although flowers that reflect over the whole spectrum (near white or mauve, with UV) fall in the UV sector, they are usually very close to the uncoloured sector and so should not be distinguishable from background objects to a bee. This is a remarkable feature that would warrant a study of whether species with such flowers are pollinated by bees at all. Alternatively, these flowers may not be using their OR to advertise themselves and instead use the contrast between the IR and D. However, the problem in this case is that these species do not have much contrast between IR and D.

While many species varied among different communities, the most dominant colour templates were found in most communities. This indicates that species have adapted to fill different colour pollination syndromes. As previously noted, species belonging to a colour template were likely to be more distantly related than expected by chance. This suggests that there is strong selection for closely related species to diverge in flower colour, possibly in order to avoid hybridization.

We found that there is a degree of phylogenetic constraint on flower colours in Asteraceae and some tribes explore the flower colour space much more than others. This is only noticeable if looking at flower colour as perceived by bees. The tribes Astereae and Senecioneae for example, appear to have very similar flower colours (purple, yellow and white) but the former do not have any UV reflecting flowers, while the latter do. The statistical analyses between CD and PD indicated that there is phylogenetic constraint, but the low correlation coefficients mean that phylogenetic distance only explains a very small proportion of the variance in flower colour. The analysis at the tribal level was affected by the taxonomic scale at which the phylogenetic distances were obtained, meaning that analyses were only possible on tribes with several genera (in this case Arctoteae and Calenduleae). OR colour for both tribes appears to be completely unrelated to phylogenetic distance, while IR colour appeared to be more phylogenetically constrained in Calenduleae ($r=0.37$) than in the rest of the Asteraceae family. This could be because, in these two tribes, OR are under stronger selective pressure as a result of them being the biggest "advertisers" of a flower. Thus, species can more easily change IR colour, as a way of increasing

contrast without "loosing membership" of a particular pollination syndrome. This increased contrast may be a way to make a flower more noticeable by pollinators at shorter distances, while still belonging to the same "dominant colour syndrome".

It is unavoidable that summarizing the entire visible reflectance spectrum of an object in two dimensions (the colour hexagon) results in loss of information. However, when comparing flowers with several colours (such as daisies) another problem arises. The appearance of a flower is a function of the contrast between its different parts (in our case the OR, IR and D). So, it is not enough to obtain the position of each part in colour space, but also the distance between them. If one adds the distance between the points of all the flower parts, a measure of total contrast can be obtained. However, in doing so, the relative position of the points in the colour space is lost and pairwise comparisons between species are just a measure of the flower contrast and cannot distinguish between species with similar contrasts but completely different flower colours. So, we are constrained in the way we can analyse the data. We can either look at pairwise distances of specific flower parts (e.g. OR) or at the contrast levels. The former method will result in not capturing the contrast of a flower while the latter will result in not being able to distinguish between flower colours. Recently, (Endler and Mielke 2005) proposed a system that allows the use of multivariate statistics to investigate "patterns of colour among sets of points and differences between sets of points". Their methods would be ideal to analyse the kind of data that we obtained, but were outside the scope of this study.

The vision model used in this study has only been shown to work in trichromatic Hymenoptera species (Chittka 1992). However, monkey beetles are thought to have a bigger role in pollination in Namaqualand than bees do (Mayer *et al.* 2006). Thus we are limited in the inferences that we can make as we do not know if flowers form different patterns in beetle vision. It is not known how tetrachromatic insects (such as beetles) interpret colour (Chittka and Menzel 1992). However, the red end of the spectrum looks very similar to all but black flower parts in the species sampled in this study (see the 600-650nm range in Figure 6). Thus it may be that the extra receptor that beetles have does not help them discriminate more colour patterns than bees.

The taxonomic level at which this study was conducted (genus) limited the analyses on the relationship between phylogenetic colour distances. This study would

have benefited from a comprehensive species level phylogeny as there is great variation in colour within genera.

In this study, the appearance of flowers was tested only for bee perception. However, there is nothing preventing the model from being applied to another trichromatic organism, such as certain fly species. Unfortunately, beetles, considered the most important pollinators of Asteraceae (Mayer *et al.* 2006) are tetrachromatic and so their visual system cannot be depicted using the colour hexagon. A solution to this could be to use an alternative model of colour vision developed by Endler and Mielke (2005) which can analyze the visual perception of tetrachromatic organisms and thus would be ideal to study beetles and tetrachromatic flies. Spectral sensitivities have been determined for several Diptera and Coleoptera species (Briscoe and Chittka 2001) so they could be used to test floral perception in these two insect orders.

It would be interesting to know whether different floral templates have different sets of pollinators. If this was the case, then flower colour could be used as a proxy for identifying (or narrowing down) pollinators. However, before these inferences can be made a much greater knowledge of pollinators for several representative species is needed.

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Appendix

Appendix 1: Details of the species representative of each genus, for the four gene regions, obtained from GenBank. Number of base pairs (bp) for each gene region also indicated

rbcL (1399 bp)

Species	Genbank Accession no.	Reference
Amellus sp.	AM234845	(Forest *et al.* 2007)
Arctotheca calendula	AM234848	(Forest *et al.* 2007)
Berkheya rigida	AM234854	(Forest *et al.* 2007)
Didelta spinosa	AM234865	(Forest *et al.* 2007)
Dimorphotheca pluvialis	DMPCPRBCL	(Kim *et al.* 1992)
Euryops speciosissimus	AM234870	(Forest *et al.* 2007)
Felicia bergeriana	FEICPRBCL	(Kim *et al.* 1992)
Foveolina tenella	AM234871	(Forest *et al.* 2007)
Gazania splendens	GZACPRBCL	(Kim *et al.* 1992)
Gorteria diffusa	AM234875	(Forest *et al.* 2007)
Gymnodiscus capillaris	AM234876	(Forest *et al.* 2007)
Lasiospermum bipinnatum	AM234885	(Forest *et al.* 2007)
Leysera gnaphalodes	AM234886	(Forest *et al.* 2007)
Oncosiphon suffruticosum	AM234894	(Forest *et al.* 2007)
Osteospermum jucundum	AM234897	(Forest *et al.* 2007)
Pentzia incana	AM234899	(Forest *et al.* 2007)
Pteronia divaricata	AM234905	(Forest *et al.* 2007)
Rhynchopsidium sp.	AM234908	(Forest *et al.* 2007)
Senecio burchellii	AM234911	(Forest *et al.* 2007)
Tripteris clandestina	AM234918	(Forest *et al.* 2007)
Ursinia sp.	AM234920	(Forest *et al.* 2007)

ITS (473 bp)

Species	Genbank Accession no.	Reference
Amellus strigosus	AF046942	(Noyes and Rieseberg 1986)
Arctotheca calendula	DQ889629	(Funk *et al.* 2007)
Arctotis fastuosa	AY504705	(Funk *et al.* 2004)
Berkheya spinosissima	AY504710	(Funk *et al.* 2004)
Cotula cinerea	AY603260	(Funk *et al.* 2004)
Didelta spinosa	AY504717	(Funk *et al.* 2004)
Euryops pectinatus	AF459964	(Pelser *et al.* 1989)
Felicia clavipilosa	AY193799	(Eastwood *et al.* 2004)
Gazania tenuifolia	AY504720	(Funk *et al.* 2004)
Gorteria diffusa	AY504722	(Funk *et al.* 2004)
Hirpicium echinus	AY504724	(Funk *et al.* 2004)

Oncosiphon grandiflorum	AY127679	(Watson *et al.* 2004)
Othonna sedifolia	DQ915866	(Sombra Staeheli *et al.*)
Pentzia dentata	AY127681	(Watson *et al.* 2004)
Pteronia incana	AF046947	(Noyes and Rieseberg 1986)
Senecio lineatus	AF459939	(Pelser *et al.* 1989)

ndhF (2312 bp)

Species	Genbank Accession no.	Reference
Arctotheca calendula	DQ889661	(Funk *et al.* 2007)
Arctotis fastuosa	AY504747	(Funk *et al.* 2004)
Berkheya spinosissima	AY504752	(Funk *et al.* 2004)
Cotula lineariloba	AF153644	(Watson *et al.* 2000)
Didelta spinosa	AY504759	(Funk *et al.* 2004)
Dimorphotheca pluvialis	L39438	(Kim and Jansen 1995)
Felicia bergeriana	L39445	(Kim and Jansen 1995)
Gazania tenuifolia	AY504762	(Funk *et al.* 2004)
Gorteria diffusa	AY504763	(Funk *et al.* 2004)
Hirpicium echinus	AY504764	(Funk *et al.* 2004)
Oncosiphon grandiflorum	AF153648	(Watson *et al.* 2000)
Osteospermum muricatum	L39440	(Kim and Jansen 1995)
Pentzia dentata	AF153649	(Watson *et al.* 2000)
Senecio mikanioides	L39435	(Kim and Jansen 1995)
Ursinia nana	L39441	(Kim and Jansen 1995)

trnL-trnF (855 bp)

Species	Genbank Accession no.	Reference
Arctotheca calendula	DQ889645	(Funk *et al.* 2007)
Berkheya spinosissima	AY504792	(Funk *et al.* 2004)
Cotula cinerea	AJ748786	(Oberprieler 2004)
Didelta spinosa	AY504799	(Funk *et al.* 2004)
Dimorphotheca sinuata	AF100518	(Bayer *et al.* 2000)
Euryops virgineus	AF100517	(Bayer *et al.* 2000)
Felicia filifolia	AF318929	(Bayer *et al.* 2002)
Gazania tenuifolia	AY504802	(Funk *et al.* 2004)
Gorteria diffusa	AY504804	(Funk *et al.* 2004)
Hirpicium echinus	DQ444807	(McKenzie *et al.* 2006)
Leysera gnaphalodes	AF100473	(Bayer *et al.* 2000)
Pentzia flabelliformis	AF100519	(Bayer *et al.* 2000)
Rhynchopsidium pumilum	AF100474	(Bayer *et al.* 2000)
Senecio lineatus	AF100515	(Bayer *et al.* 2000)
Ursinia trifida	AF452507	(Bayer and Cross 2003)

Appendix 2: Species occurrence per site. Numbers next to species names indicate site for species which had different reflectance spectra in different locations.

Species	Count	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Amellus alternifolius	1														X		
Amellus coilopodius	2						X	X									
Amellus microglossus	4			X			X			X						X	
Arctotheca calendula	8		X		X	X				X	X	X	X	X			
Arctotis adpressa	3	X										X	X				
Arctotis cf. acaulis	2		X													X	
Arctotis cf. fastuosa	2					X				X							
Berkheya fruticosa 10	3				X					X						X	
Berkheya fruticosa 11	1										X						
Cotula barbata	4		X									X	X	X			
Cotula coronopifolia	6	X	X	X	X			X		X							
Cotula leptalea	3									X	X					X	
Cotula microglossa	3	X			X									X			
Didelta carnosa	7			X	X			X	X	X					X		X
Didelta spinosa	4				X					X	X			X			
Dimorphotheca pinnata 5	5			X	X	X	X	X									
Dimorphotheca pinnata 15	3														X	X	X
Dimorphotheca polyptera	1															X	
Dimorphotheca sinuata	13	X	X		X			X	X	X	X	X	X	X	X	X	X
Dimorphotheca tragus	2											X				X	
Euryops multifidus	1														X		
Euryops tenuissimus	1													X			
Felicia australis	8	X	X		X						X		X	X	X		X
Felicia bergerana	8		X		X			X	X			X		X	X		X
Felicia cf. dregei	2											X	X				
Felicia hirsuta	1															X	
Felicia merxmuelleri	1							X									

Foveolina dichotoma	6			X		X	X	X	X	X							
Gazania heterochaeta	2															X	X
Gazania krebsiana	1														X		
Gazania leiopoides	5		X					X		X		X	X				
Gazania lichtensteinii	1															X	
Gazania tenuifolia	4			X			X	X	X								
Gorteria diffusa 5	6	X	X		X	X									X		X
Gorteria diffusa 8	1							X									
Gorteria diffusa 10	1									X							
Gorteria diffusa 16	2										X					X	
Gymnodiscus linearifolia	2											X	X				
Hirpicium alienatum 10	2							X		X							
Hirpicum alienatum 16	1															X	
Hirpicium echinus	1															X	
Lasiospermum brachyglossum	4			X		X	X									X	
Leysera gnaphalodes	3				X						X				X		
Leysera tenella	5			X		X								X		X	X
Monoculus hyoseroides	3		X			X					X						
Monoculus monstrosa	4			X				X						X	X		
Norlindhia amplectens	10	X	X	X	X	X		X		X	X					X	X
Oncosiphon grandiflorum	1							X									
Oncosiphon suffruticosum	4			X	X		X			X							
Osteospermum rigidum	1																X
Othonna cylindrica	2				X					X							
Othonna humilis	2														X	X	
Othonna macrophylla	1													X			
Pentzia incana	5				X						X			X	X	X	
Pteronia glabrata	1								X								
Rhynchopsidium pumilum	8			X	X	X			X	X	X				X	X	
Senecio abruptus	1									X							
Senecio arenarius	1											X					

Senecio cardaminifolius	9	X		X	X	X					X		X		X	X	X
Senecio cf. eriobasis	1													X			
Senecio cinerascens	4				X									X	X		X
Senecio sp.	3											X		X		X	
Tripteris aghillana	2													X			X
Tripteris microcarpa	3			X			X			X							
Tripteris oppositifolia	3				X					X							X
Tripteris sinuata var sinuata	4	X			X					X					X		
Tripteris sinuata var. linearis	1															X	
Ursinia anthemoides subsp. anthemoides	2				X								X				
Ursinia anthemoides subsp. versicolor	1				X												
Ursinia cakilefolia 13	2											X	X				
Ursinia cakilefolia 6	1					X											
Ursinia calenduliflora 14	2									X				X			
Ursinia calenduliflora 16	2															X	X
Ursinia chrysanthemoides	2		X									X					
Ursinia nana	5			X						X					X	X	X
Total number of species per site		9	12	15	23	12	8	15	7	23	13	13	11	17	18	25	16