

FACTORS DETERMINING VISUAL DETECTION DISTANCE TO REAL FLOWERS BY BUMBLE BEES

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Abstract—Plants use visual signals to attract pollinators and direct them to their flowers. Visual capabilities of bees have been extensively studied mostly using artificial paper models. However, there is no empirical determination of the maximal detection distance (MDD) or minimal subtended visual angle (MSVA) of real flowers. Using a six armed radial maze, we tested MDD and MSVA of 12 types of natural and manipulated real flowers by bumble bee (*Bombus terrestris*) workers. Bees were initially trained to obtain sugar solution at target flowers that were presented at close range on a mobile divider at the back of one of the six arms. Bees were individually marked and tested. For bees that passed the short range test, we gradually increased the distance of the target flower, until the number of successful choices reached chance level, indicating that they could not see the target flowers. The results show that MSVA of flowers is correlated with flower diameter but not with MDD. The variation in MDD to natural flowers by bumble bee workers can be best predicted by: $MDD = \text{flower coloured area} / (\text{contour line} * \text{green contrast})$. Contour line length determines flower dissectedness. Full circular flowers can be detected from longer distance than dissected flowers with identical diameter. We hypothesize that dissected flower shapes might be compensated by their higher attractiveness for bees. Empirical determination of real flower MDD and MSVA is important for studying bee foraging behaviour, pollinator induced evolution of flower traits and validation of neurophysiological visual models.

Keywords: Bumble bee; detection distance; flower area; flower contour; green contrast; minimal subtended visual angle

INTRODUCTION

Pollinators visit flowers for their reward (e.g. Heinrich 1975), and plants use olfactory and visual signals to advertise their reward, attract pollinators and direct them to their flowers (Chittka & Raine 2006; Dafni et al. 1997; Menzel & Shmida 1993; Schaefer et al. 2004). Sprengel (1793, in Hasse, 1996) recognized the importance of floral size and shape in "catching the eye of the insect from afar". Flower colour and shape recognition are based on visual perception and learning as shown by the classical work of Karl von Frisch (1914) and others (Hertz 1929). Kugler (1934) was the first to experimentally determine the distance from which bumble bees detect artificial flowers and change their flight direction to visit them, showing that large flowers attract bees from longer distances than small ones.

In accordance to 'honest signalling' principle (Armbruster et al. 2005; Keasar et al. 2006), large flowers are more conspicuous and detectable from longer distances (Chittka & Spaethe 2007) and they generally contain more nectar or pollen rewards than small flowers (Blarer et al. 2002; Fenster et al. 2006; Herrera 2005; Martin 2004). Pollinators typically prefer large over small flowers, visit them more frequently and pollinate them more efficiently (Conner & Rush 1966; Elle & Carney 2003; Fenster et al.

2006). Consequently, the fitness of plants with larger flowers, which can be detected from longer distances by their pollinators, should be higher than that of plants with smaller flowers. Thus pollinators could generate a selective pressure for increased flower size via pollinator mediated selection (Galen & Newport, 1987).

However, in addition to the effect of pollinator mediated selection, the evolution of flower size can also be constrained by selection imposed by plant mating systems (Goodwillie et al. 2010), interactions with flower and plant herbivores, resource costs (Galen, 1999), and a suite of other abiotic and biotic agents of selection that together may shape floral evolution (Krizek & Anderson, 2013). Variation in flower size and other floral traits, within a species, can promote reproductive isolation and ultimately speciation (Krizek & Anderson, 2013).

Giurfa et al. (1996) determined the minimal subtended visual angle (MSVA) that (for full circular paper targets) corresponds directly to maximal detection distance (MDD) from which honeybee (*Apis mellifera*) workers can detect them. By varying target diameter, Giurfa et al. (1996) showed that visual angle is the only factor needed to estimate single full circular target resolution independent of target distance and size. They found that MSVA from which honeybees could detect full circular targets was 5° for targets that provide chromatic and green receptor contrasts to their background, and 15° for targets of similar size, with chromatic but no green receptor contrast. Visual angle is a

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very important parameter in general vision science and it is used by researchers because the actual size of a stimulus is confounded by viewing distance. Thus, targets providing green contrast, irrespective of their chromatic contrasts, could be detected from longer distances than corresponding targets with only chromatic contrast to their background (Giurfa et al. 1996; Giurfa & Vorobyev 1997; Lehrer & Bischof 1995). From these data, models and simulations were constructed to determine the distances from which honeybees could detect flowers, depending only on flower diameter and colour (Giurfa & Vorobyev 1997; Menzel et al. 1997; Vorobyev et al. 1997).

Differing from honeybees, bumblebees are able to detect targets containing both green and colour receptor contrasts at a visual angle of 2.3° and stimuli that contain only colour contrast at a visual angle of 2.7° (Dyer et al. 2008). Recently, Dyer et al. (2016) determined these values also for Australian stingless bees, 9.5° for targets that presented both chromatic- and green-receptor contrasts, and 9.3° for targets presenting only chromatic contrast. These differences between honeybees, bumblebees and stingless bees may have evolved because these bees operate in different environments, but more probably may be due to the efficient dance language that helps honeybee workers to find flowers, whilst bumblebees and stingless bees search individually and thus may require better colour acuity to find small individual flowers (Dyer et al. 2008; Bukovac et al. 2013; Dyer et al. 2016).

Many studies on bee vision have focused on perception, neurobiology, memory and learning mainly of honeybees and bumblebees (Chittka & Raine 2006; Menzel & Giurfa 2006; Srinivasan 2010; Avarguès-Weber et al. 2011; Avarguès-Weber & Giurfa, 2014; Hempel de Ibarra et al. 2014; Hempel de Ibarra et al. 2015). The roles of size, shape and pattern in floral advertisement and their perception by pollinators were reviewed by Lehrer & Bischof (1995), Dafni and Kevan (1997), Dafni et al. (1997), Neal et al. (1998), Zhang et al. (2004), Kaczorowski et al. (2012), Avarguès-Weber et al. (2011), and Hempel de Ibarra et al. (2015). Critical examination of the literature shows that most of the studies used full circular coloured papers, or other artificial targets, but not real natural flowers. Streinzer et al. (2009), and Dyer et al. (2007) examined the detection of real flowers by bees, but did not determine their MDD or MSVA.

Studies using circular artificial targets, which have used diameter and colour as the most important shape parameters to determine MSVA and consequent MDD, seem to be insufficient for inferring to flower-like dissected shapes. Therefore, Ne'eman and Kevan (2001) explored MDD using blue paper flower models with dissected outlines instead of circles. They demonstrated that target coloured area, rather than the diameter of its enclosing circle, is the single most important parameter that affects MDD. Ne'eman and Kevan (2001) showed that MDD is positively correlated with squared target coloured area and negatively with the target contour length (perimeter, cm). MDD to radial dissected targets, which present longer contour lines, is shorter than to non-dissected targets of similar diameter.

MDD to a full circular target with a given diameter is equal to that of a dissected target with the same area but double diameter. Therefore, MSVA cannot be used for calculating their MDD to dissected targets and flowers. Markedly lacking, however, are experiments on the distance from which bees can detect natural flowers of different sizes and shapes, and their correspondent MSVA.

Contour length has been recognized long ago as important for bee vision and behaviour. von Frisch (1914) showed that bees could discriminate between shapes that differed greatly in their contour lengths but could not discriminate between simple shapes, such as triangles, squares and circles that had similar contour lengths. In spontaneous attraction experiments bees were found to prefer shapes with higher contour lengths (Hertz 1929). Anderson (1977) studied the degree of similarity, perceived by the honey bee, between a training shape and each of a set of test shapes. He found that shape area and contour density (the ratio between the length of outline of a shape and the area it encloses) explained the observed degree of similarity perceived by the bees. Bees are capable of much more elaborate pattern detection, differentiation and learning than was suspected in these early experiments (Avarguès-Weber et al. 2011; Srinivasan 2010). In recent papers it was discovered that honeybees have the ability to group similar stimuli into categories. They learned to distinguish between images belonging to different categories: star-shaped flowers of different colours, images of flowers that were nearly circular in shape of different colours, images of plant stems of various shapes, and images of landscapes (Zhang et al. 2004). Honeybees are able to discriminate Monet paintings from Picasso ones by extracting and learning the characteristic complex visual information inherent to each painting style (Wu et al. 2013). Individual free flying honeybees can learn to use size relationship rules to choose either the larger or smaller stimulus as the correct solution in a given context, and subsequently apply the learnt rule to novel colours and shapes (Avarguès-Weber et al. 2014).

While the visual and cognitive capabilities of bees have been extensively studied using artificial models and simulations, empirical determination of actual MDD to real flowers and their correspondent MSVA, which has a crucial ecological effect on foraging bees and pollinator mediated selection on flower size is missing.

Our aim is to determine, for the first time, MDD, to natural flowers by bumble bee workers. In addition we construct an empirical regression model as a practical tool for predicting MDD to real flowers using parameters of flower shape and colour. Empirical determination of flower MDD and MSVA are important for studying bee foraging behaviour, pollinator mediated evolution of flower size shape and colour, as well as validation of psychophysical and neural models established for flower shape and colour.

MATERIALS AND METHODS

Apparatus

Following other researchers (e.g. Lehrer et al., 1995; Simonds & Plowright, 2004; Plowright et al. 2011;

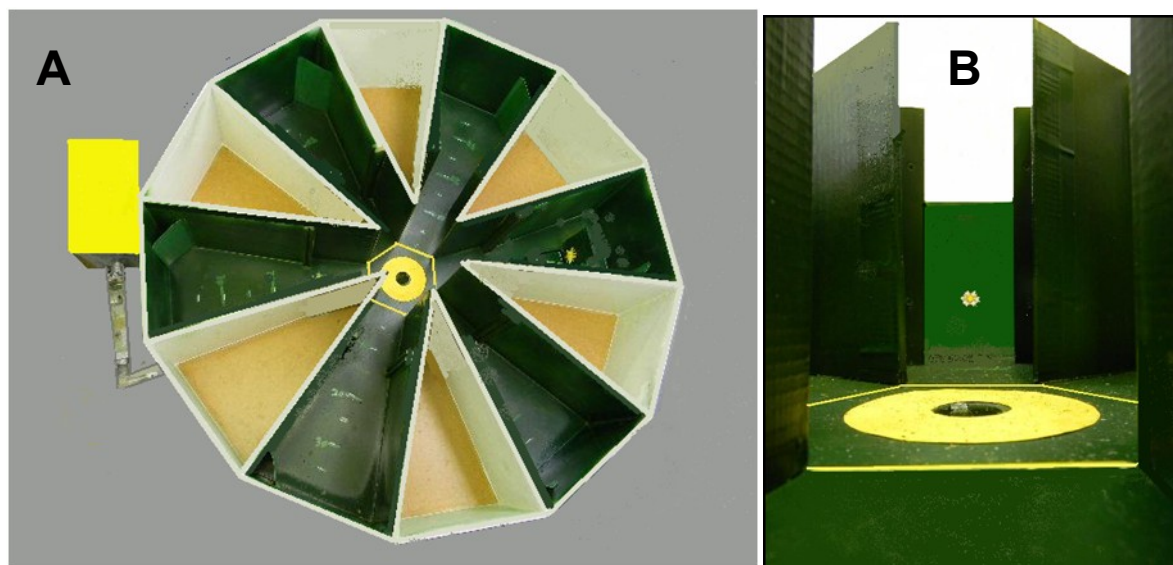


FIGURE 1(A). General view of the six-armed radial maze (100 cm in diameter). A bumble bee colony (yellow box on the left) was connected with a transparent walkway and a plastic tube going under the maze floor to the entrance hole in the maze centre. At one distal corner of each arm there was a small open headed compartment (indicated by arrows) into which fresh flowers of the tested species were inserted. (B) A 'bee view' of the central emergence hole in the maze floor and the target flower (indicated by an arrow) presented on a mobile stand in the rewarding arm.

Thompson & Plowright 2014) we used a six armed radial maze (100 cm in diameter) (Fig. 1A). We used this type of maze because rejection of the null hypothesis of random choices can be achieved with lower number of attempts than in the commonly used dual choice Y shaped maze without losing statistical significance, as explained later (Bukovac et al. 2016).

A bumble bee colony was connected to the maze with a transparent Plexiglas® walkway that led to the entrance hole located in the centre of the maze floor. The walkway had three sliding shutters for controlling bee traffic so that single numbered bees could be identified and let one by one into the maze. A yellow circle was painted around the entrance, to help bees navigate back home. A yellow line connecting the entrances to the six arms marked the borders of the 'decision chamber'. When crossing this line to the rewarding arm first, the bee's choice was considered as successful. An error was recorded if the bee crossed the line to enter an unrewarding arm first. The decision line was 10 cm from the entrance hole and it served as the baseline for measuring the distance of the target flowers. Each arm was 5 cm wide at its proximal and 15 cm at its distal end; the maze was 20 cm high and covered by removable UV-transmitting Plexiglas® plates. The maze was painted dark green, and only in one arm a flower was vertically presented and 50% (weight/volume) sugar solution offered as reward. The flower was mounted on a vertical dark green cardboard fixed to a mobile stand (Fig. 1B). The flower's stem was inserted through a hole into a tube with fresh water located behind the cardboard. At the base of the stand, 5 ml of sucrose solution was presented in a small covered compartment, invisible by the bees from a distance. In a preliminary experiment four bees successfully discovered the uncovered reward from 15 cm (7/9, 10/11, 5/5, and 9/10 correct/total choices), but failed to do so with covered reward (1/8, 2/10, 1/6, and 3/10 correct/total choices); for significance level see 'data

analyses'. Small marks at five cm distances on the floor at the central line of each arm were used to determine the distance of the target flower from the decision line during the experiments.

Using natural flowers in a maze could introduce a difficulty to separate between visual and olfactory attractions of the bees to the flowers. We are confident that in our radial maze, which consisted of a single enclosed space and the random changes of the arms caused equal distribution of the flowers' fragrance in all the maze's arms. However, to ensure equal distribution of the fragrance in the maze, in one distal corner of each arm there was a small open headed but hidden compartment into which fresh flowers of the tested specie were inserted (Fig 1 A).

The maze was lit with high frequency lighting (TMS 24F lamps with 4.3 kHz ballasts; (Philips, Eindhoven, The Netherlands) fitted with fluorescent Activa day light tubes.

Flower types

We used nine flower species (Tab. 1) that were freshly collected each morning (April-May 2012) in the gardens of Queen Mary University of London campus and in the nearby Victoria Park. The flowers were presented vertically, thus seen by an entering bee as if it looked at a natural flower in an 'en face' position. Inflorescences of *Bellis perennis* were presented unmanipulated, but were also manipulated to produce a 16 and 8 petal flower-like shapes; flowers of *Argyanthemum frutescens* were manipulated to produce a 10 petal flower-like shape and flowers of *Spartium junceum* were presented in an 'en face' and profile positions; in total we used 12 flower presentations differing in the following shape parameters (Tab. 1): (1) As a general indication for flower size, we calculated the diameter of the flower-enclosing circle. (2) The area of the dominant colour of the flower (petals). (3) The contour line (perimeter) of the

TABLE 1. Shape parameters: flower (coloured) area, average diameter and the contour line (perimeter) of all tested flowers and the value of the green-receptor contrast between petal colour and the green background of the radial maze against which the flowers were presented to the bees.

Flower	Area (mm ²)	Diameter (mm)	Contour line (mm)	Green-receptor contrast
<i>Allium cristophii</i>	111	17	128	0.379
<i>Aubrieta deltoidea</i>	314	20	74	0.221
<i>Argyranthemum frutescens</i> 10	606	30	234	0.451
<i>Bellis perennis</i>	112	12	47	0.499
<i>Bellis perennis</i> 8	134	16	142	0.499
<i>Bellis perennis</i> 16	242	22	259	0.499
<i>Cotoneaster microphyllus</i>	105	12	61	0.463
<i>Escallonia macrantha</i>	151	15	63	0.255
<i>Ranunculus bulbosus</i>	174	15	55	0.468
<i>Solanum crispum</i> star	190	21	126	0.427
<i>Spartium junceum</i> (en face)	510	26	117	0.379
<i>Spartium junceum</i> (profile)	288	25	113	0.379

coloured area. (4) The value of the green receptor contrast between the dominant petal colour and the green background of the radial maze, against which the flowers were presented to the bees. Floral morphological parameters were measured from calibrated photographs using "CellProfiler 2.0" cell image analysis free software (Massachusetts Technological institute).

Colour Analysis

Spectral reflectance functions of the flowers and the background was measured by using a spectrometer (Ocean Optics, Dunedin, FL, S2000 with a deuterium-halogen light source). The colour parameters (relative excitation values in the bees' UV, blue, and green receptors, colour contrast, and green contrast) were calculated according to Backhaus (1991) using the colour hexagon (Chittka, 1992). The relative amount of light absorbed by each photoreceptor colour type is:

$$P = R \int_{700}^{300} I s(\lambda) S(\lambda) D(\lambda) d\lambda \quad [\text{Eq. 1}]$$

$I s(\lambda)$, is the spectral reflectance function of the stimulus. $S(\lambda)$, is the spectral sensitivity function of the receptor (we used the functions of Peitsch et al. (1992) for the *B. terrestris* UV, blue, and green receptors). $D(\lambda)$ is the illuminate (standard neon light filtered through the Plexiglas cover combined with natural daylight). The sensitivity factor R in Eq. 1 is determined by:

$$R = I \int_{700}^{300} I b(\lambda) S(\lambda) D(\lambda) d\lambda \quad [\text{Eq. 2}]$$

$I b$ is the spectral reflection function of the background to which the receptors are adapted (Fig. 2). With this model, it is assumed that the photoreceptors display half their maximal response when stimulated by the light reflected from the adaptation background. When the maximum excitation E_{\max} of the photoreceptors is normalized to 1, the photoreceptor excitation can be described by:

$$E = P / (P + 1) \quad [\text{Eq. 4}]$$

P is the stimulus strength, in units such that for $P = 1$, $E = 0.5$ [i.e., half the maximum potential; for details see Backhaus (1991) and Vorobyev et al. (1997)]. Thus, for the adaptation background, E equals 0.5 in each photoreceptor. Green contrast, then, is the degree to which any given stimulus generates an excitation value different from 0.5 in the green receptor. Because excitation can range from 0 to 1, the maximum green contrast is 0.5. Stimulus brightness is defined as the sum of all three photoreceptor excitations, so it can have any value from 0 to 3. Because the background, by definition, has a brightness of 1.5, brightness contrast can have any value up to 1.5. For calculation of hexagon colour loci from receptor excitation values, see Chittka (1992). The background colour locus lies in the centre of the colour hexagon. The distance from the centre to any of the hexagon's corners is unity. Perceptual colour differences of stimuli in the colour hexagon is described by a sigmoidal relationship with colour distance (Dyer & Chittka 2004) perceived as differently coloured.

Pictures and relative reflectance curves of the flowers are presented in Fig. 2. The location of the colours of all studied flowers are presented in a bumble bee colour-hexagon (Fig. 3), where the dark green radial maze background is located at the centre, and the distance of each flower from the centre indicates its colour contrast with the background (Chittka 1992).

Bicoloured patterns are detected by bees worse than single coloured and patterns with higher L-contrast in their outer than in their inner pattern are better detected than that with a reverse L-contrast distribution (Hempel de Ibarra et al. 2001). However, because of their small size we could not measure the central parts of the bicoloured flowers and measured only the colour of the dominant peripheral petals. It is generally accepted that 'nectar guides' can be seen only

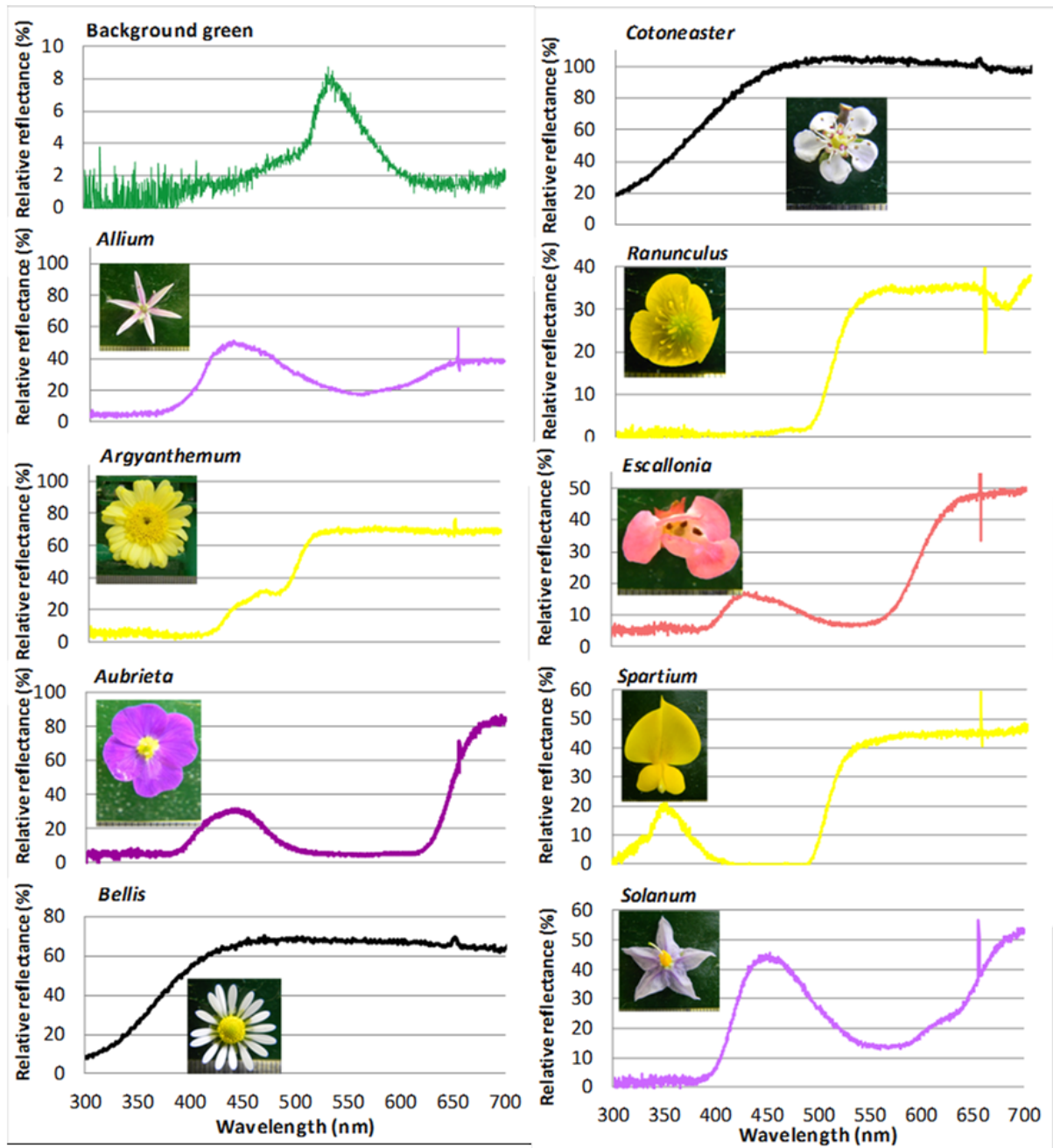


FIGURE 2. The relative reflectance curves of all studied flower species: *Allium cristophii*, *Argyranthemum frutescens*, *Aubrieta deltoidea*, *Bellis perennis*, *Cotoneaster microphyllus*, *Escallonia macrantha*, *Ranunculus bulbosus*, *Solanum crispum* and *Spartium junceum*. For bicoloured flowers (with yellow centres) the reflectance of the petals' major colour was measured.

from short distances and thus should have no or minimal effect on MDD.

Bees, training and experimental procedure

A bumble bee (*Bombus terrestris*) colony was purchased from Syngenta Bioline Bees, The Netherlands. The colonies were kept in dark wooden boxes and the bees were reared in a dark, colour-devoid environment, until training began.

Foraging bees were first allowed to freely enter the maze for two days to acclimatise. During acclimatization, the bees were pre-trained to feed at the feeding stand with a yellow paper target (5 cm in diameter) placed close to the decision

line. Bees that were observed to successfully find the reward were marked with numbered tags for identification. Marked bees were then trained to forage at the feeding stand with a flower target at a distance of 5 cm from the decision line. During a single training and testing session, we worked with up to seven bees, but only one bee was permitted to enter the maze at a time. After each foraging bout, the target was randomly moved to another arm, using a random number chart. The training period enabled bees to associate the sucrose reward with the flower target. The randomisation of the target placement and rewarding arm ensured that bees were prevented from linking the reward to any particular arm

(spatial learning). After a minimum of three training foraging bouts, the bees were tested. Only bees that successfully passed the test from 15 cm, were tested with gradually increasing distances to determine MDD to any tested flower type. All the flowers were tested with sister bees of one colony.

After entering the maze, each bee was observed until it arrived at the feeding stand. Bees that entered the rewarding arm first, were considered to have a successful trial. Bees that after entering crossed first a decision line of an empty arm were considered to have an error. All bees were left to find the target and feed before returning to the colony. After every foraging bout the target was randomly moved to another arm and the distance increased with 10 cm steps, until all individual bees' performance decreased to chance levels. At this stage the target stand was moved 5 cm back toward the decision line and retested. MDD was determined as the average between the longest distance from which a bee could see the target and the shortest one from which it failed. After the MDD was determined for each target, the colony was once again permitted to freely enter the radial maze, so that more bees could be recruited through training and marking. Each target was tested with five to nine bees.

MSVA was calculated from MDD and the average flower diameter (D): $MSVA = 2\arctan(D / 2MDD)$.

Data analyses

The binomial test was used to determine, whether or not, the target could be detected by a given bee from any tested distance. The probability of random choice ($1/6$) is 0.17, but to be on the safe side, we used the probability threshold of 0.2. In each session (testing a flower from a given distance), each bee was tested during up to 12 trials. In order to be successful in a session (choices not to be random at a 95% confidence level) a bee had to be successful for at least in $4/5$, $4/6$, $4/7$, $4/8$, $5/9$, $5/10$, $5/11$, and $6/12$ successful/total choices.

To establish a model for the best prediction of MDD we used linear regression with the following parameters as independent variables: diameter of enclosing circle, coloured area, contour line (perimeter), green receptor contrast and colour distance between flower and the background. We used Kolmogorov-Smirnov test for testing normal distribution of the data. SPSS 19 (IBM Corp., 2010) was used for all statistical analyses.

RESULTS

Flower types

Morphological shape parameters and green receptor contrast of all flower species used in the experiment are presented in Tab. 1, the relative reflectance curves are presented in Fig. 2, and the locations of the studied flower species in the bumble bee colour hexagon in Fig. 3.

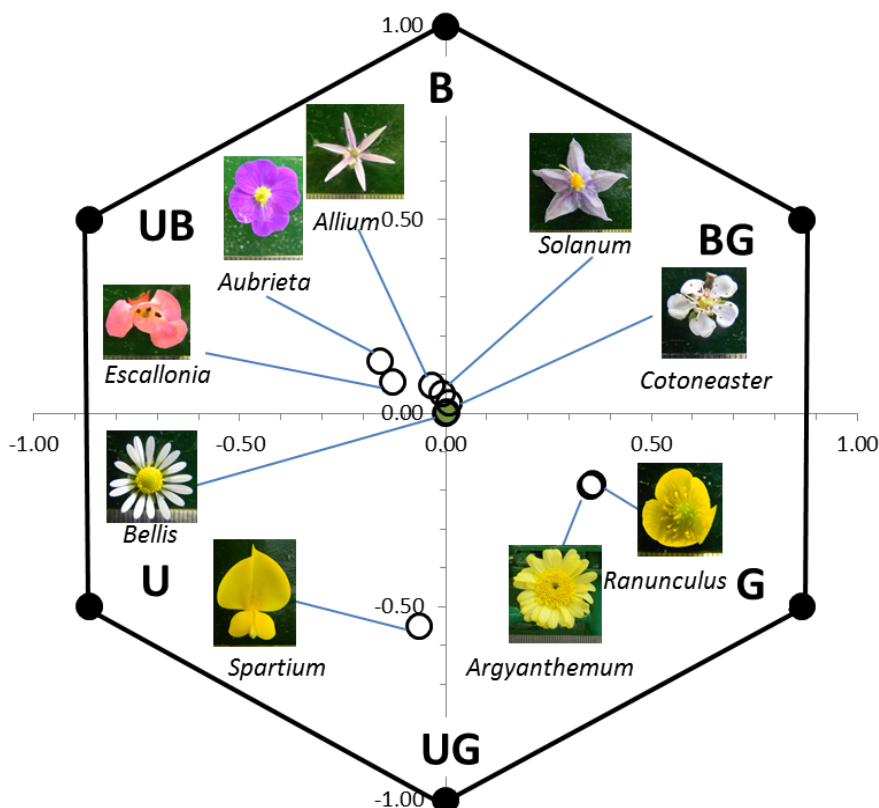


FIGURE 3. The location of the colours of studied flowers (*Allium cristophii*, *Argyranthemum frutescens*, *Aubrieta deltoidea*, *Bellis perennis*, *Cotoneaster microphyllus*, *Escallonia macrantha*, *Ranunculus bulbosus*, *Solanum crispum* and *Spartium junceum*) in the bumble bee colour-hexagon. The dark green radial maze background is located at the centre and the distance of each flower from the centre indicates its colour contrast with the background (Chittka 1992).

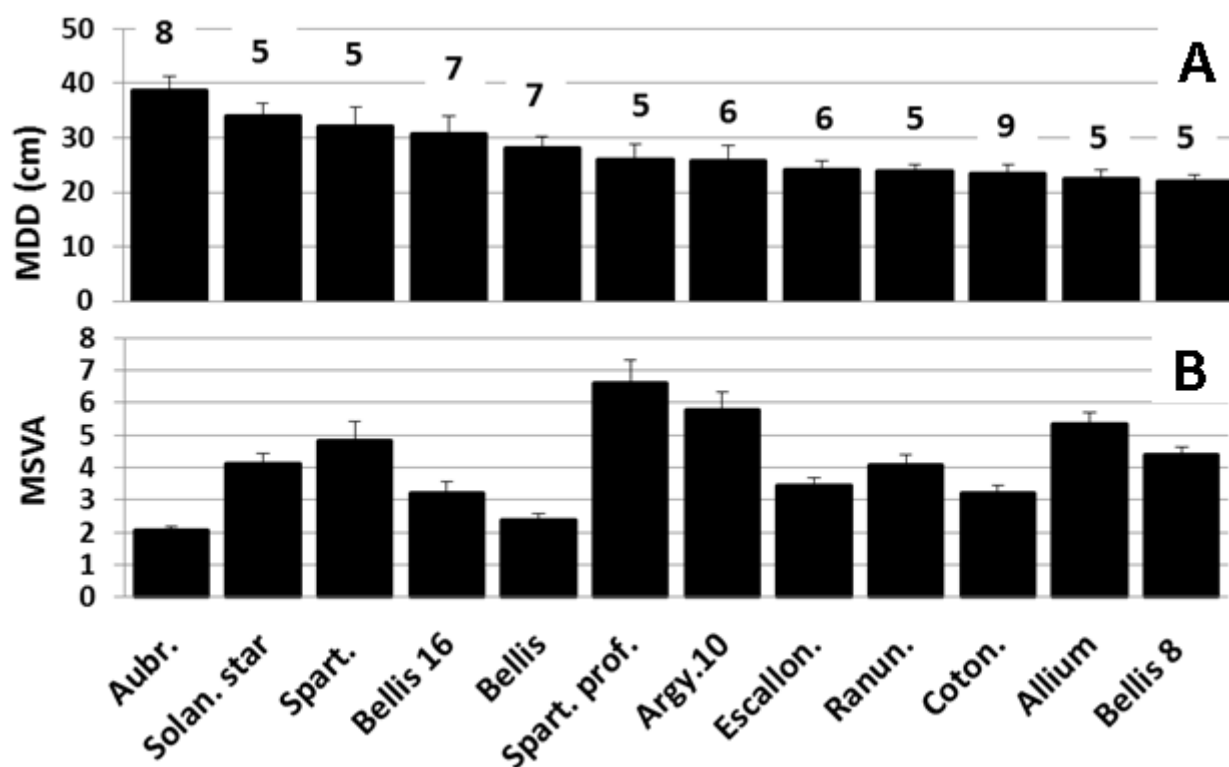


FIGURE 4. The average (\pm SE) maximal detection distance (MDD) (cm) (A), and the minimal subtending visual angle (MSVA) (B) of the studied flower types: *Aubrieta deltoidea* (Arbur.), manipulated *Solanum crispum* (Solan. star), *Spartium junceum*I (en-face) (Spart.), manipulated *Bellis perennis* (Bellis 16), reduced diameter *Bellis perennis* (Bellis), *Spartium junceum*I (profile) (Spart. prof.), manipulated *Argyanthemum frutescens* (Argy.10), *Escallonia macrantha* (Escallon.), *Ranunculus bulbosus* (Ranun.), *Cotoneaster microphyllus* (Coton.), *Allium cristophii* (Allium), manipulated *Bellis perennis* (Bellis 8). The number of tested bees for each flower is indicated above the bars in A. Manipulated flowers have reduced number of petals as indicated by the number following their name in the horizontal axis. The visual parameters of all flower types are presented in Tab. I.

Maximal detection distance and minimal subtending visual angle

For each tested flower type, the average percentage of correct choices as a function of the distance made by *Bombus terrestris* workers; the correspondent binomial probability (P -value) that the number of correct choices was higher than 20% of the total number of trials (average = 7, range 5-9) and the number of bees tested for each flower type are presented in Appendix I. The distance from which P value exceeded 0.05 was determined as the MDD for each flower type and was used for calculation of its MSVA. The MDD of each individual tested bee to each of the tested flowers and their main traits is presented in Appendix II.

The average diameter (\pm STD) of the 12 tested flower was 1.9 ± 0.6 cm. The MDD from which flowers were detected by bumble bee workers ranged from 22.0 cm to 38.8 cm with an average (\pm STD) of 27.6 ± 5.2 cm (Fig. 4A), and the differences among flower types were significant (ANOVA, $F_{11,63} = 6.458$, $P < 0.001$).

We used linear regressions to find the best significant model for predicting MDD as a function of flower shape and colour traits as well as various combinations of them (Tab. 2). The results prove that and MDD cannot be predicted by: (1) Flower diameter (cm); (2) contour density

(contour line length/area) (cm^{-1}); (3) flower area (cm^2); (4) flower area divided by flower contour line (cm); (5) Green receptor contrast between the flower and background; (6) Euclidean colour distance between the flower and the background in bumble bee colour hexagon), none of which was significant (Tab. 2).

Multiple linear regression using uncorrelated predicting variables (flower area, flower contour line, green receptor contrast and chromatic distance) was also not significant ($F_{4,11} = 0.564$, $P = 0.697$), as were Pearson correlation coefficients between MDD and each of the variable presented in Tab. 2.

The only significant regression mode for prediction of MDD to the flowers (Fig. 5) was:

$$\text{MDD} = A / (C * GC) \quad [\text{Eq. 4}]$$

A = floral coloured area (cm^2), C = floral contour line (cm), and GC = degree of floral green-receptor contrast to the background.

The average MSVA (\pm STD) for the tested flowers was 4.12 ± 1.36 cm (Fig. 4B), but it was not correlated with MDD (one tailed Pearson correlation $r = -0.409$, $P = 0.094$).

Independent variable	$F_{1,11}$	P	R^2
Diameter (cm)	3.245	0.102	0.1153
Contour density (contour line /area) (cm ⁻¹)	1.403	0.264	0.1238
Area (cm ²)	1.399	0.264	0.1228
Area/contour line (cm)	3.245	0.102	0.2450
Green receptor contrast	0.708	0.420	0.0661
Euclidean colour distance (hexagon)	1.797	0.210	0.1523
Area / (contour line *green contrast) (cm ²)	6.176	0.032	0.3822

TABLE 2. Results of regression tests (F , P and R^2) of MDD as dependent variable and various flower shape and colour parameters as independent variables ($N = 12$). Green-receptor contrast was calculated between flower and the green maze background.

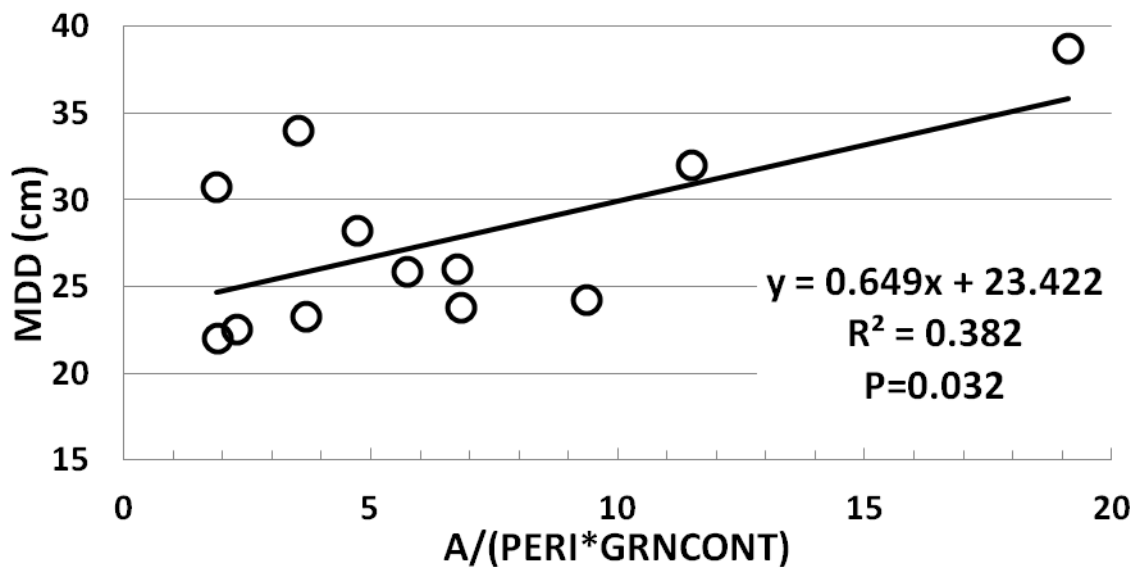


FIGURE 5. Maximal detection distance (MDD) (cm) as a function of floral area (A cm²) divided by floral contour line (C) and the degree of floral green contrast (GC). Regression line formula, its significance (P) and the proportion of explained variability are presented (R^2).

MSVA of the flowers was correlated with average flower diameter (one tailed Pearson $r = 0.605$, $P = 0.0185$), and it was negative correlated with relative flower area (the proportion of actual flower area out of an area of a circle with a diameter equal to the flower average diameter) (one tailed Pearson $r = -0.545$, $P = 0.034$). MSVA can be predicted from its significant regression line with the average diameter of the flower: $MSVA = 0.142 * \text{Diameter} + 1.371$ ($F_{1,10} = 5.766$, $R^2 = 0.366$, $P = 0.037$).

DISCUSSION

Here we present empirical data for determination of MDD to various intact or manipulated real flowers by *Bombus terrestris* workers and their MSVA. To the best of our knowledge, this is the first empirical measurement of MDD to natural flowers, or their correspondent MSVA. The average diameter of the tested flower was almost 2 cm, the average MDD by bumble bee workers was only 28 cm. That is in agreement with the predictions of a previous theoretical model that used photographs of flowers that bees can visually detect flowers only from close range (Vorobyev et al., 1997). The average MSVA was 4° and it was not

correlated with MDD. The lack of correlation is apparently because of differences in shape and green contrast of flowers that shared similar diameters. Therefore, and because MDD is the parameter that dictate the behaviour of bees in the field, and thus affects their foraging efficiency as well as the selection they impose on floral traits, we propose that when discussing detectability distances to real flowers MDD is the most relevant and important variable, as is MSVA for neurophysiological models.

MDD to flowers under natural conditions is probably even shorter than those obtained here under laboratory conditions. This is mainly because bees naturally approach flowers from a variety of angles from which the perceived area of the flowers is smaller than the *en face* view tested here which, in most cases, presents flowers at their maximal area (Spaethe 2001). In addition, natural backgrounds are not uniformly green, as our experimental maze, but are commonly multi coloured and heterogeneous, which can make visual detection harder.

The diameter and colour properties of circular paper targets has been commonly used as the only variable for calculating MSVA (α_{\min}) by bees (Chittka & Raine 2006;

Giurfa et al. 1996; Giurfa & Vorobyev 1997; Lehrer & Bischof 1995; Menzel et al. 1997; Menzel & Giurfa 2006; Vorobyev et al. 1997) and its corresponding MDD. However, compound non-radial and deeply dissected flowers have no single clear diameter. Therefore, Ne'eman and Kevan (2001) examined the MDD to artificial human-blue paper targets with various dissected flower shapes by honeybee workers. They demonstrated that the diameter could not explain the variation in MDD to flower shapes differing in their diameter and degree of dissectedness. Instead they found that the product of coloured flower area squared (cm^4) over its contour length (perimeter) provided the best significant predictor for the MDD to human blue paper models by honeybees (Ne'eman & Kevan, 2001).

Here, working with bumble bees and natural flowers that vary simultaneously in their size, shape and colour, we found that this index was insufficient for predicting MDD (Tab. 2). This may be due to the effects of the green receptor contrast on the visual system of bees in detection of far targets and in determination of vision fields. For the detection of a full circular target with a green-receptor contrast, honeybee workers employ a system having a MSVA of $\alpha_{\min} = 5^\circ$, and a target image must excite a minimum of seven ommatidia to elicit detection. The MSVA of similar targets that lack green-receptor contrast is $\alpha_{\min} = 15^\circ$, and the minimal number of ommatidia that must be excited is 59 (Chittka & Raine 2006; Giurfa & Vorobyev 1997; Hempel de Ibarra et al. 2002). For bumble bees, targets lacking green-receptor contrast have MSVA of $\alpha_{\min} = 2.7^\circ$, and the minimal number of ommatidia that must be excited is seven, and for the detection of a target with a green-receptor contrast, bumble bee workers employ a system having a MSVA of $\alpha_{\min} = 2.3^\circ$ and target image must excite a minimum of three ommatidia to elicit detection, and this varies with bee size (Spaethe and Chittka, 2003). Bumble bees can see the same flower from larger distance than honeybees, especially flowers with relatively little green receptor contrast (Spaethe & Chittka 2003; Dyer et al. 2008; Kapustjansky et al. 2010).

Green colour receptor contrast has been shown to be important for detection of distant targets, while chromatic contrast for near targets (Giurfa et al. 1996). Green receptor contrast also improves the detection of three spatially separated coloured discs, by honeybees and bumble bees, compared with discs that presented chromatic but no green-receptor contrast (Wertlen et al. 2008).

Here, we determined MDD to natural flowers, by bumble bee workers, and propose an empirical regression model for predicting MDD from parameters of flower shape and colour. To do so, we used 12 natural flower types that varied much in their morphology (Tab. 1) and colour (Fig. 2), and determined MDD to each of them by bumble bee workers. Based on previous studies, by applying linear regression, we tested the ability of: flower diameter, contour density, coloured area, area/contour line, green contrast, euclidean colour distance (cm) to predict MDD, none of which yielded significant results. The only significant regression model for predicting MDD by bumble bee

workers to natural and manipulated flowers is presented in (Eq. 4) and Fig. 5.

For a bee to detect a visual stimulus a minimal number of ommatidia must be excited and this depends on stimulus size (Dyer et al., 2008; Giurfa & Vorobyev, 1997). It is thus reasonable to assume that flower area is the most important factor affecting MDD (Eq. 4). Ne'eman and Kevan (2001) found that only target area was not sufficient for their explaining model and only the area squared provided a significant linear model, which is not in concert with current models of bee vision. Bukovac et al. (2016) demonstrated that even for simple colour disc detection in a standard Y-maze, the current models of visual detection seem inadequate but such behaviour fits modelling of what flower colours have actually evolved. Their finding might indicate that flower detection is more complex than previously thought.

When two flowers have equal areas but one has a longer contour line it will inevitably be also more dissected than a flower with a shorter contour line. Although sharing the same diameter or area a dissected flower will probably fully stimulate less ommatidia and consequently will have also a longer MDD by bees relatively to a less dissected flower. This explains the location of the contour length in the denominator of our regression model (Eq. 4).

While chromatic contrast is used for short range vision of details, the green contrast signal is used as far-distance flower detection, and therefore it would always be detected before chromatic contrast during an approach flight (Spaethe & Chittka 2003). The green contrast between the flowers, used as targets in this study, and their background ranged from 0.255 to 0.499 (Tab. 1), where maximum green contrast is 0.5 (Spaethe et al. 2001). The green contrast in all our tested flowers is within the range of the colours tested by Spaethe et al. (2001) that have used the colour hexagon model as we did. High green contrast is essential for distance detection, and its values are always smaller than 0.5; these facts explain the location of the value of the green contrast in the denominator of our proposed linear regression model for predicting MDD (Eq. 4). This indicates that green contrast may have a quantitative effect, which contradicts other studies that proposed a threshold effect (Giurfa et al., 1996), which to the best of our knowledge was never explicitly determined. A threshold effect should not lead to an increase in the distance from which an object can be detected. Working with artificial flowers, Spaethe et al. (2001) found a significantly negative correlation between mean search time and green contrast value of small flowers (< 8 mm). Searching time of *Ophrys heldreichii* flowers and its coloured paper models by male solitary bees from close range (< 30 cm) was negatively correlated with green receptor contrast between flower perianth and background Streinzer (2009). These two cases may also indicate a quantitative rather than threshold effect of green contrast on bee behaviour. Recently it was discovered that sufficient input to the green photoreceptor channel is needed for honeybees to learn and use size relationship rules to choose the larger or smaller stimulus as the correct solution in a given context, and subsequently apply it to novel colours and shapes (Avarguès-Weber et al. 2014).

Our model (Eq. 4) is significant, but it explains only 38.2% of the variation in the MDD data, implying that there are additional variables, not included in the model, which affect MDD. One such main factor, in addition to measurement errors, is the variation in body size of bumble bees. In bumble bees, eye optics is correlated with body size, and an increase of 33% in bumble bee body size is accompanied by 100% increase in precision of single object resolution, since larger bees have larger eyes with better spatial resolution (Spaethe & Chittka 2003). Thus, bee body size is a major candidate for explaining additional part of the variation in MDD to flowers by bumble bee workers.

Our results clearly show that dissected flower shape reduces detection distance by bees, which could negatively affect its visitation rate by pollinators and probably also the plant fitness. If so, why are star shaped and other dissected radial flowers so common? Floral nectar guides are colour markings on flower corolla that guide pollinators to the location of nectar reward in flowers. Therefore, pollinators spend less time in finding and extracting the nectar, they increase visitation rate and increase pollination probability of flowers that have nectar guides (Dafni & Kevan 1997; Dafni et al. 1997; Dafni & Guirfa 1999; Waser & Price 1985). In addition to the response of experienced bees to nectar guides, flower-naïve bumble bees are attracted to the margins of flowers and follow them using the radial flower shape itself to guide them toward the reward (Lunau et al. 2006). Leonard and Papaj (2011) proved that star shaped flowers and flowers with radial nectar guides are preferred by bumble bees, and this preference continues even after reward stopped. When detected, dissected shape presents radial signals that are more attractive to bees and direct them to the reward located in the centre of the flower. Conventional nectar guides can be detected only after a bee has landed on the flowers, while star shaped corolla can be seen from longer distance than nectar guides on a flower of similar size. Dafni and Kevan (1997) proposed that dissected margins of flowers may guide scanning pollinators to flower rewards. An analysis of the Israeli flora indicated that small flowers (< 5 cm²) have disproportionately longer contour lines and more highly dissected shapes. Thus, small flowers may compensate for their reduced detectability by having highly dissected edges vis-a-vis large flowers (Dafni & Kevan, 1997). To this point we hypothesize that the reduced detectability of radial dissected flowers might be compensated by their higher attractiveness for bees and that nectar guide should be less frequent in dissected than in non-dissected radial flowers.

Conclusion

To conclude, we present an empirical linear regression model that can be used for predicting MDD to natural flowers by bumble bee workers using flower coloured area, the length of its contour line and the value of green receptor contrast between the flower reflectance and the green background (Eq. 4). This model requires refinements using more flower types differing in their shapes and colours as well as more near to natural testing background colour and illumination conditions. Further development can also be scaling up the model from individual flowers to inflorescences. Developing procedures to determine MDD in

the field is most important but a difficult future task. Such improvements are essential for better understanding of bees' foraging behaviour under natural conditions, where various plant species co-flower.

Empirical determination of MDD and MSVA to real flowers is most important and should be used as a novel parameter for bee foraging behaviour, pollinator induced evolution of flower size shape and colour, as well as validation of psychophysical and neural models established for flower shape and colour.

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APPENDICES

Additional supporting information may be found in the online version of this article:

APPENDIX I. The average percentage of correct choices as a function of the distance by *Bombus terrestris* workers, and the number of bees tested for each flower type in each distance for all studied flower types.

APPENDIX II. Maximal detection distance (MDD) of all tested individual bees (each line presents a single bee) to all tested flowers; flower (coloured) area, average diameter and the contour line (Perimeter) of all tested flowers and the value of the green-receptor contrast between petal colour and the green background of the radial maze against which the flowers were presented to the bees. See also Tab. 2.

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