Sci Nat (2015) 102:4
DOI 10.1007/s00114-014-1256-1

ORIGINAL PAPER

Insect vision models under scrutiny: what bumblebees (*Bombus terrestris terrestris* L.) can still tell us

Francismeire Jane Telles · Miguel A. Rodríguez-Gironés

Received: 14 July 2014 / Revised: 9 December 2014 / Accepted: 15 December 2014

Abstract Three contending models address the ability of bees to detect and discriminate colours: the colour opponent coding (COC) model, the colour hexagon (CH) model and the receptor noise-limited (RN) model, but few studies attempt to determine which model fits experimental data best. To assess whether the models provide an accurate description of bumblebee colour space, we trained bees to discriminate four colour pairs. The perceptual distance between the colours of each pair was similar according to the CH model but varied widely according to the COC and RN models. The time that bees required to select a flower and the proportion of correct choices differed between groups: decision times decreased as achromatic contrast increased, and the proportion of correct choices increased with achromatic contrast and perceptual distance, as predicted by the COC and RN models. These results suggest that both chromatic and achromatic contrasts affected the discriminability of colour pairs. Since flower colour affects the foraging choices of bees and foraging choices affect the reproductive success of plants, a better understanding of which model is more accurate under each circumstance is required to predict bee behaviour and the ecological implications of flower choice and colour.

Keywords Colour discrimination · Colour hexagon · Colour opponent coding · Achromatic contrast · Receptor noise-limited · Search time

Communicated by: Sven Thatje

Electronic supplementary material The online version of this article (doi:10.1007/s00114-014-1256-1) contains supplementary material, which is available to authorized users.

F. J. Telles (✉) · M. A. Rodríguez-Gironés
Department of Functional and Evolutionary Ecology, Estación Experimental de Zonas Áridas (EEZA-CSIC), Carretera de Sacramento, s/n, La Cañada de San Urbano, 04120 Almería, Spain
e-mail: meirecuesta@gmail.com

Introduction

Like most pollinators, bumblebees use visual information to detect and recognize landmarks and food sources. To maximize foraging efficiency, they require an effective visual system and the ability to associate visual cues with rewards. Several models have been developed to understand how colour vision is processed. Three of them are currently used to study colour discrimination by bees and other hymenoptera: the colour opponent coding (COC; Backhaus, 1991), colour hexagon (CH; Chittka, 1992) and receptor noise-limited (RN; Vorobyev and Brandt 1997; Vorobyev and Osorio 1998; Vorobyev et al. 2001) models. The COC model was originally formulated for honeybees, *Apis mellifera*, while the CH model was developed for trichromatic hymenopteran species and the RN model for a much wider range of taxa, including species with dichromatic and tetrachromatic colour vision. In the remainder of this paper, RN refers to the trichromatic version of the receptor noise-limited model.

The three models assume that colour information is processed via two sets of colour-opponent coding neurons. The COC model used the least-squares method to obtain the set of opponent mechanisms that best fitted honeybee data from a multidimensional scaling experiment (Backhaus et al. 1987). Using this set of opponent mechanisms, we can plot the colour loci of arbitrary colours on a plane. According to the COC model, the perceptual distance between two colours is proportional to the distance between their loci, calculated with the city-block metric (Backhaus 1991). The COC model should provide a reasonably good description of the honeybee colour space, but if different hymenopteran species are endowed with different sets of colour-opponent coding neurones, the COC model might not be applicable to other species. The CH model therefore assumed that the two opponent mechanisms were orthonormal and that perceptual distance between two colours was proportional to the Euclidean distance between their loci

(Chittka 1992). With these assumptions, perceptual distances are independent of the specific choice of opponent mechanisms. The rationale of the CH model was that, when we ignore the set of colour opponent mechanisms used by a species, it might be preferable to describe its colour space using rather non-specific sets of mechanisms than those of another species (Chittka et al. 1992). Finally, the RN model assumes that it is noise at the receptor level, and not the specific information processing rule, that sets the discrimination limit, its parameters are inferred from electrophysiological recordings in photoreceptor cells (Vorobyev et al. 2001), and the model has been used in different experiments to predict chromatic distances between spatially separated stimuli (Hempel de Ibarra et al. 2001; Hempel de Ibarra et al. 2002; Dyer and Neumeyer 2005; Niggebrügge et al. 2009; Martínez-Harms et al. 2014). One common point between the COC and CH model is the way that they deal with the non-linearity of phototransduction. Signals from receptor cells are non-linearly related to the quantum flux that forms the input to the receptor (Naka and Rushton 1966a; Chittka 1996), and both models assume that the phototransduction process is well described by the Naka–Rushton equation (Naka and Rushton 1966a; Naka and Rushton 1966b; Backhaus 1991). By contrast, the RN model assumes linear phototransduction in its linear version (Vorobyev and Osorio 1998) and logarithmic phototransduction in the logarithmic version of the model (Vorobyev et al. 2001).

The three models predict the main features of the honeybee spectral sensitivity data (Vorobyev and Brandt 1997; Brandt and Vorobyev 1997) and explain a number of experimental results (Giurfa et al. 1997; Hempel de Ibarra et al. 2002; Lotto and Chittka 2005; Dyer and Neumeyer 2005; Arnold and Chittka 2012), but the plurality of assumptions of available models could be confusing and lead to erroneous conclusions in ecology and vision research. For example, the ability of pollinators to locate flowers should affect how floral resources are partitioned among pollinator groups (Rodríguez-Gironés and Santamaría 2005). Hence, flower colour may influence both resource partitioning and the selective pressures to which flowers are subject. However, to understand the effect of colour on resource partitioning, we must know with which ease different pollinator species will locate flowers. Likewise, as long as we accept several colour discrimination models, their different assumptions remain putative mechanisms for visual information processing. Falsifying one or more of these models would evidence the models' underlying assumptions to be incorrect, narrowing down the search of putative mechanisms and therefore contributing to our understanding of the bee's visual system.

Due to differences in their underlying assumptions, the models can make different predictions, and it is possible to select a set of colour pairs in such a way that the different models rank their perceptual distances in different orders. If we train bees to discriminate between the two colours of each pair, the proportion of correct choices indicates their perceptual distances, and therefore, we can use these results to evaluate the models.

To select the model that best describes bumblebee (*Bombus terrestris terrestris* L.) colour discrimination, we trained bumblebees to discriminate four pairs of coloured stimuli in the laboratory. The pairs had similar perceptual distances according to the CH model, but varying perceptual distances according to the COC and RN models. Although the COC and RN models have parameters inferred from honeybee behavioural and neurophysiological data, respectively, all three models are commonly used to estimate perceptual colour distances in bumblebees (Gumbert 2000; Kunze and Gumbert 2001; Spaethe et al. 2001;Dyer and Chittka 2004c; Dyer and Chittka 2004a; Dyer and Chittka 2004b; Lunau et al. 2006; Dyer et al. 2008; Martínez-Harms et al. 2010; Arnold and Chittka 2012; Rohde et al. 2013), and it seems reasonable to ask which model is more accurate under our experimental condition.

Materials and methods

Rationale for the selection of colour pairs

Let us draw, in the CH plane, a circle of radius r and centre P. According to the CH model, the perceptual distance between two colours is proportional to the Euclidean distance between their loci in the CH (Chittka 1992). Therefore, the perceptual distance between colours represented by P and Q, where Q is any point on the circle, should be equal to r, regardless of the angle α between the vertical axis and the segment PQ (Fig. 1a).

Knowing the loci of two colours on the CH model, it is straightforward to calculate their perceptual distance according to the COC and RN models (see Online Resource 1). This distance depends on the angle α (Fig. 1b), and the change can be as large as fivefold for the RN model. It follows from Fig. 1b that, if we have two colour pairs, (P, Q_1) and (P, Q_2), such that α_1 is slightly greater than 0° and α_2 around 100°, the chromatic distance between P and Q_1, according to the COC and RN models, should be much higher than the chromatic distance between P and Q_2. Assume that we train a group of bees to discriminate between P and Q_1 and a second group to discriminate between P and Q_2. According to the predictions of the CH model, no difference in the performance of bees between groups should be found. By contrast, if the COC or RN models were correct, performance should be better for bees of the (P, Q_1) group than for bees of the (P, Q_2) group.

Based on these considerations, we have selected four colour pairs in such a way that, when plotted on the CH colour space, all pairs had the same distance, but the line segments joining the two loci of a pair formed different angles with the

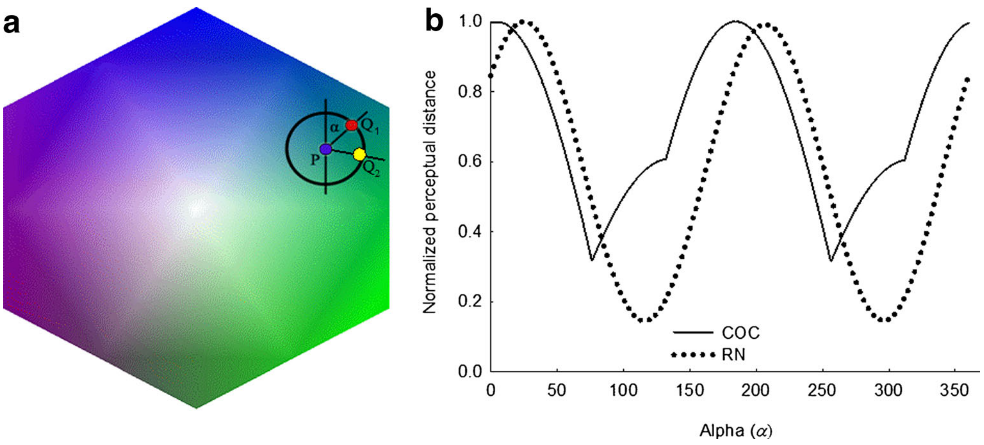


Fig. 1 **a** According to the colour hexagon (CH) model, the perceptual distance between colours represented by points *P* and *Q*, where *Q* lies on a circle of radius *r* centred at *P*, is independent of the angle, α, between the *PQ* segment and the vertical axis. **b** Colour distance between points *P* and *Q* (α) according to the colour opponent coding (COC, *solid line*) and receptor noise-limited (RN, *dashed line*) models, plotted vs. the angle that the *PQ* segment makes with the vertical axis, α. Note that the perceptual distance between *P* and *Q* changes with the orientation of the *PQ* segment. The COC and RN models use different perceptual scales. For comparison, distances have been normalised. Colours (both *P* and *Q* (α)) are chosen in such a way that $E_{\mathrm{UV}}+E_{\mathrm{B}}+E_{\mathrm{G}}=1$ when bees are habituated to the background. For this hypothetical example, calculations assume D65 illumination function and green background colour (HKS coloured paper 54 N)

vertical axis (see below). Figure 1 represents a hypothetical example, not the colours used for the experiment; these are specified in the following.

General set-up

Bumblebees, *B. terrestris terrestris*, kept indoors in a single-chamber nesting box (length, width, height 30×20×25 cm), were trained to collect 60 % (*weight*/*weight*) sucrose solution from ultraviolet (UV) transparent artificial flowers (hollow Plexiglass cubes 4×4×4 cm, with 3-mm-thick walls) inside a flight cage (70×70×35 cm) connected to the hive by a gated tunnel. Bees had ad libitum access to pollen within their nest box and were allowed to collect 20 % (*w*/*w*) sucrose solution from a transparent feeder, positioned inside the flight cage, outside experimental sessions. The flight cage was lined with UV-reflecting grey cardboard paper (Canson Mi-Teintes 431, Fig. 2). Incoming light (Fig. 2) was provided by two Philips TL-D90 Graphica 36w/965 white light tubes and one Philips TL-D 36w BLB UV light tube, 75 cm above the cage floor. Light flicker was converted to 1.200 Hz, diffused and homogenized by a single sheet of Rosco 216 (Rosco, Germany) UV-transmitting white diffusion screen.

Computation of colour distances

We measured the spectral irradiance of the light inside the cage and the reflectance spectra of stimuli and background in the range of 300–700 nm (Fig. 2) with an Ocean Optics USB 4000 spectrometer (Dunedin, FL, USA). For all computations, we used the average of three measurements. The absolute irradiance (photons s^{-1} cm^{-2} nm^{-1}) was measured using a cosine corrector (CC-3-UV-S, Ocean Optics, Dunedin, FL, USA) to collect light, which was transmitted through an optical fibre to the spectrometer. A lamp (LS-1-CAL-220, Ocean Optics) of known output was used to calibrate the spectrometer. For the measurements of the background and stimuli, the spectrometer was calibrated with a standard white (Ocean Optics WS-1) and measures were taken using a reflexion probe at 45° to the surface.

Online Resource 1 specifies how we calculated perceptual distances according to the three models. For the computations, we used the photoreceptor spectral sensitivity of *Bombus terrestris dalmatinus* (Skorupski et al. 2007), as Chittka et al. (2001) found no difference between the behavioural preferences of this subspecies and *B. terrestris terrestris*. Green and brightness contrast, contrast of target colours against background and spectral purity were calculated as

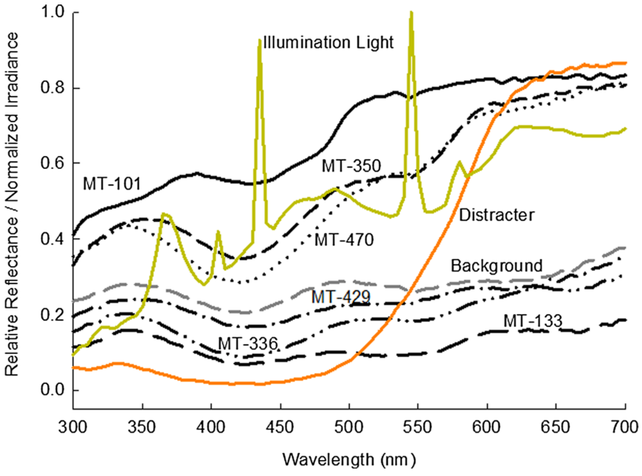


Fig. 2 Spectral reflectance of stimuli and background and normalized irradiance (irradiance reached a maximum of 5.33756×10^{11} photons s^{-1} cm^{-2} nm^{-1} for 545 nm)

specified in Online Resource 1, and the results are presented in Online Resource 2 (Table S1).

Stimuli and perceptual colour distances

Colour stimuli were 7×7-cm squares cut from Canson Mi-Teintes cardboard (98 lb/160-gsm series) and set under the transparent Plexiglass flowers. By combining six different colours (references MT-133, 429, 336, 350, 101 and 470; Fig. 2), we formed four pairs (Table S2, Online Resource 2). Within each pair, one colour was arbitrarily designed as the "A" colour and the other as the "B" colour. According to CH model (Chittka 1992), perceptual distances were similar for all four pairs (mean±SEM=0.0549±0.0007 hexagon units). However, according to the COC (Backhaus 1991) and RN (Vorobyev and Osorio 1998; Vorobyev et al. 2001) models, there were differences in perceptual distances (Online Resource 2, Table S2, Fig. 3); note that, because all colour pairs had similar distances (coefficient of variation=0.06) according to the logarithmic version of the RN model (Vorobyev et al. 2001), we only consider the linear version of this model (Vorobyev and Osorio 1998). For our choice of colour pairs, the predictions of the logarithmic version are indistinguishable from those of the CH model.

Experimental procedure

During sessions, only the experimental subject was allowed to enter the flight cage. The trajectory hive–cage–hive was considered as a foraging bout, and any contact with the surface of a stimulus, regardless of whether the bee only touched it with its front legs or landed on it, was considered as a choice. After every foraging bout, flowers were replaced with new ones and cleaned with 30 % ethanol to remove olfactory cues. To rule

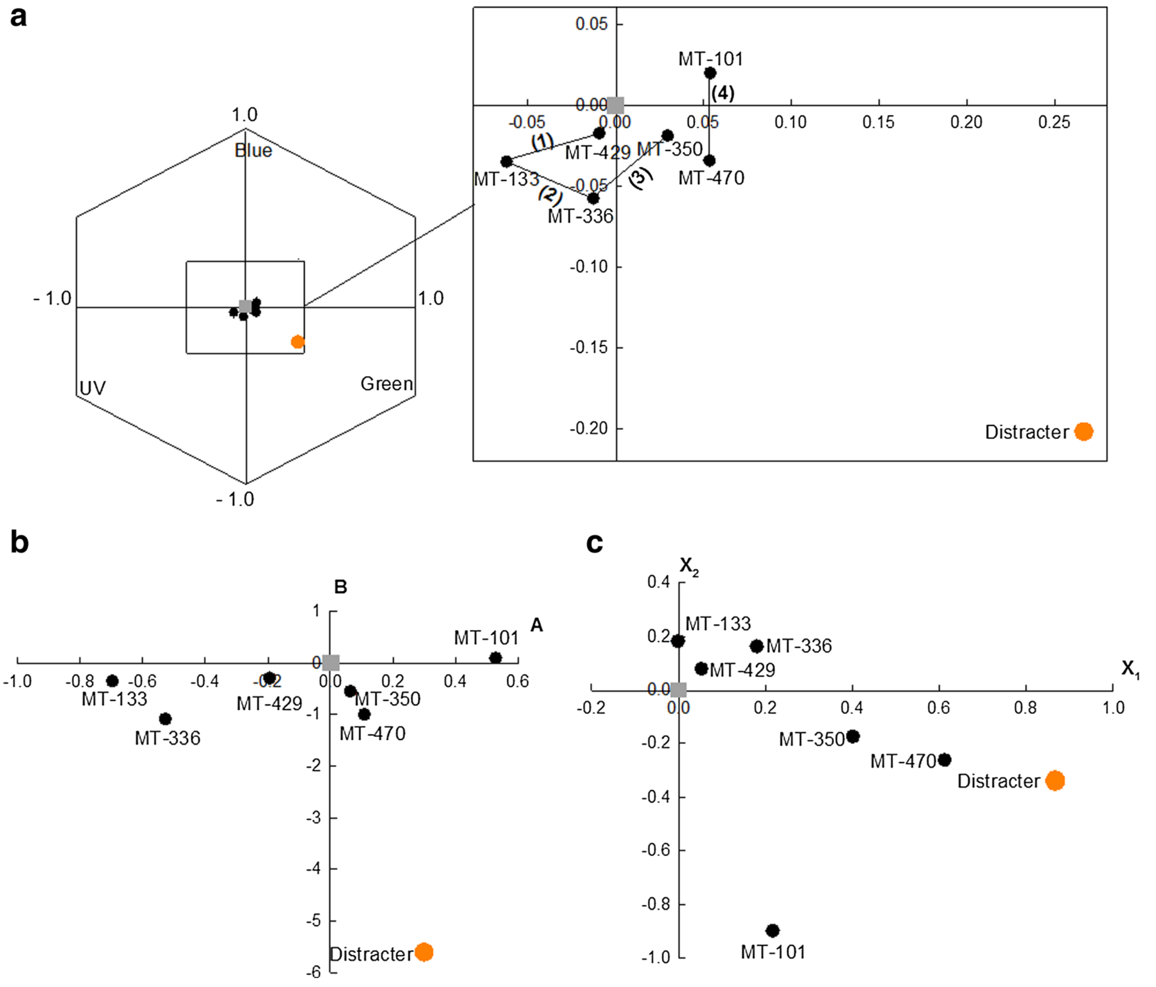


Fig. 3 Loci of stimuli (*black circles*), background (*grey square*) and orange distracter presented during phase I (*orange circle*), plotted on the **a** CH, **b** COC and **c** RN linear colour spaces. Note the detailed magnification of the CH colour space showing the stimuli and background distribution with *black lines* connecting the two colours of each pair (*bold numbers* in parenthesis indicate the group to which they belong). *A* and *B* in **b** represent the two colour opponent coding channels used in the COC model. X_1 and X_2 in **c** correspond to orthogonal axes (for detailed information, see Hempel de Ibarra et al. 2001), calculated with coefficients values of $A=1.104$, $B=1.154$, $a=0.453$ and $b=0.547$, assuming noise to be $E_{UV}=0.74$, $E_B=0.67$ and $E_G=0.61$ (obtained from Fig. 3c, Skorupski and Chittka 2010)

out position learning, the spatial arrangement of the flowers was randomized before each foraging bout.

The experiment was divided into three phases: a pre-training phase was used to discard individuals not using colour as a cue (phase I); during training (phase II), individuals learnt to discriminate between perceptually similar colours, and finally (phase III), their performance was tested over a non-rewarded foraging bout. A total of five individuals were rejected after failing to meet the selection criteria of phase I (see below). Discarding these individuals should not bias the results for two reasons: (1) the task used to discard bees was not the task that they had to perform during the training and testing phases and (2) the five bees had been pre-assigned to the four experimental pairs (two bees to group 3, one bee to each of the other groups). We successfully trained and tested 64 bumblebees (16 per pair), from five different colonies, approximately matched in size (mean±SD length of the left eye, measured as the distance of the longest surface perimeter through the centre: 2.35 mm±0.13 mm) due to the relationship between eye size, optical quality and behavioural ability at target detection and discrimination (Macuda et al. 2001; Spaethe and Chittka 2003; Wertlen et al. 2008). All selected bumblebees did not differ in eye size between groups (generalized linear model (GLM) analysis: $X^2=0.02$; $df=3$; $p=1$). Of the 16 bees tested for each colour pair, eight bees were trained to seek nectar in colour A and the remaining ones in colour B. Bees were assigned to a colour pair in a random order, and therefore, there was no association between colony and colour pair.

Upon entering the flight cage, bees encountered eight target and eight distracter flowers as specified below. From any flower, the nearest neighbours subtended an average angle greater than 8° (bumblebees use chromatic cues to process visual information when objects subtend a visual angle greater than 2.7°; Dyer et al. 2008). The average distance between nearest and most distant flowers was 10.63 and 52.15 cm, respectively. Target and distracter flowers differed in the reward that they offered as well as in their colour. In order to habituate bees to encountering empty flowers before the extinction test (phase III), during phases I and II, four of the target flowers contained 30 μl of 60 % sucrose solution and the other four were empty. All eight distracter flowers contained 30 μl of 0.12 M quinine hydrochloride dihydrate (Sigma) solution during phases I and II.

Phase I: pre-training

Within each group, target flowers were of colour A for half of the bees and of colour B for the other half. Target colours remained the same throughout the experiment (phases I, II and III), while distracter colours changed. During phase I, distracter flowers had the same colour for all bees (Canson Mi-Teintes 553, orange to humans, Fig. 2). This colour was sufficiently dissimilar to all others (mean±SEM distance 0.33 ±0.013 hexagon units, 5.81±0.21 COC units, 0.85±0.12 RN units, Table S3, Online Resource 2) so that any bee should have been able to discriminate between it and the target colour to which it was being trained. Phase I aimed on eliminating bees that were not using chromatic information to locate nectar. In this phase, bees were allowed to complete as many foraging bouts as needed to visit 60 flowers. Individuals that chose target flowers on at least 70 % of visits entered phase II.

Phase II: training

In phase II, bees had to discriminate between the two colours of the pair to which they were assigned. Now, bees pre-trained with colour A were going to find the complementary opponent colour, B, as the distracter flower, and vice versa, with a total of 16 flowers inside the flight cage. Bees completed 15 foraging bouts during phase II. During each bout, bees were allowed to visit as many flowers as they wanted, but we recorded only the first six flowers visited, ignoring revisits to the same previous flower, only being considered a new visit after a different visitation to another flower (already empty or not). Once satisfied, bees flew back to the hive. Upon completion of training, bumblebees were tested in a final non-rewarded foraging bout.

Phase III: testing

Phase III lasted for a single foraging bout with bees visiting as many flowers as they wanted. Flowers had the same colours as in phase II, but they were all clean and empty. This foraging bout was recorded using a video camera for subsequent analysis. Using the Picture Motion Browser (PMB) program (ver. 5.8.02, Sony), we calculated the average time that bees spent in choosing flowers. For each flower visit, we measured the time elapsed since the bee left a flower until it landed on the following one. Dividing the overall time by the number of flowers (including the final one) that the bee approached and inspected during its flight trajectory, we obtained the average time spent per flower during each visit. Taking the median of these values over the duration of phase III, we obtained the "decision time"—or time spent travelling to and inspecting a flower before deciding whether to land on or reject it.

Statistical analyses

A Mann–Whitney *U* analysis, with Bonferroni correction for multiple comparisons ($\alpha_{\text{adjusted}}=0.012$), revealed that, for each colour pair, the proportion of correct choices (Table S4, Online Resource 2) was independent of whether target flowers were of colour A or B (all $p>\alpha_{\text{adjusted}}$). We therefore pooled the data from bees trained to seek nectar at A or B flowers, ignoring this factor in subsequent analyses.

We explored how the acquisition of the discrimination task (changes in the proportion of correct responses through phase II) and final performance (proportion of correct choices during phase III) changed with decision time, colour pair and other parameters that might affect bee choices: brightness (Hempel de Ibarra et al. 2000; Reisenman and Giurfa 2008), green contrast (Giurfa et al. 1996; Giurfa et al. 1997; Giurfa and Vorobyev 1998; Hempel de Ibarra et al. 2001; Hempel de Ibarra et al. 2002; Hempel de Ibarra and Giurfa 2003; Martínez-Harms et al. 2010), colour contrast to the background and spectral purity (Lunau 1990; Lunau 1993; Lunau et al. 1996; Lunau et al. 2006; Rohde et al. 2013; Papiorek et al. 2013).

To analyse the acquisition of the discrimination task, we divided phase II (15 foraging bouts) in three blocks of five foraging bouts each and calculated, for each bee, the proportion of correct choices in each block. Because there were too many explanatory variables to include them all in a single model, we performed several groups of repeated measures analyses of variance on these data. Each group included a subset of explanatory variables. Within each group, we started with the full model, which included all the explanatory variables of the group and interaction terms, and systematically removed non-significant interactions and variables to find the model with the lowest value of Akaike's information criterion (*AIC*, Akaike 1973). In the first group, we started with a model having block (1 to 3) as within-subject repeated measure, colour pair as between-subjects categorical factor and log-transformed decision time as continuous variable (decision time was log-transformed to improve the linearity of the relationship). This model also included the interactions between block and colour pair and (log-transformed) decision time. The process was then repeated with eight additional groups. For the initial model of these groups, we replaced colour pair with a continuous measure of perceptual distance (as predicted by the COC or RN model—half of the groups with each variable) and added a variable related to the achromatic properties of the pair (brightness contrast, green contrast, spectral purity or colour contrast against the background—each of these variables was combined with the two measures of perceptual distance). Table 1 specifies the full model and the most parsimonious model for each group. Note that this exercise was not repeated with the CH and logarithmic RN models because there was no sufficient variability in perceptual distances of the four colour pairs when calculated with these models (coefficients of variation of 0.04 and 0.06, respectively). From all the models tested, we selected the one with the lowest AIC value and those within two AIC units, and these were the models that we used for hypothesis testing. In the selected model, we estimated *p* values with type II tests.

For the analysis of final performance, we determined, for each bee, the number of correct choices over the first 15 flower visits in the extinction test (phase III). Subsequent visits were discarded because bee behaviour becomes haphazard as the number of empty flowers visited increases (Lotto and Chittka 2005). These data were analysed with generalized linear models (GLMs) with binomial distribution and logit link function. As in the previous case, we fitted nine sets of models to the data, with the same structure described for the repeated measures ANOVAs, except that in the GLMs, we removed the variable "block" (as we only included data from the last trial). In each of these sets, we systematically removed interaction terms and variables to search for the most parsimonious (lowest AIC value) model. Table 2 specifies the full model and the most parsimonious model for each group. We used the most parsimonious models to determine statistical significance, based on type II log-likelihood ratio tests, and used planned contrasts to compare performance on specific colour pairs. Note that, in these models, decision time was not log-transformed. The reason for this is that we used a non-linear (logit) link function.

Finally, we used GLMs with Gaussian distribution and identity link function to investigate whether decision times differed between groups. In the first analysis, we used pair as categorical variable. In the following analyses, which were followed by a post hoc Tukey's honest significant difference (HSD) test for comparisons among groups, we used

Table 1 Model selection for the repeated measures analysis of task acquisition (phase II)

Initial model	Selected model	$AIC_{Initial}$	AIC_{Final}
Block * ColourPair+logTime+Block: logTime	Block+ColourPair	−195.66	−257.41
Block * COC+logTime+Brightness+Block: logTime+Block: Brightness	Block+COC+Brightness	−216.82	−265.10[a]
Block * COC+logTime+GreenContrast+Block: logTime+Block: GreenContrast	Block+COC+GreenContrast	−215.71	−264.32[a]
Block * COC+logTime+SpectralPurity+Block: logTime+Block: SpectralPurity	Block+COC+SpectralPurity	−208.09	−260.12
Block * COC+logTime+CCB+Block: logTime+Block: CCB	Block+COC	−203.22	−261.57
Block * RN+logTime+Brightness+Block: logTime+Block: brightness	Block+RN+Brightness	−218.67	−264.82[a]
Block * RN+logTime+GreenContrast+Block: logTime+Block: GreenContrast	Block+RN+GreenContrast	−219.23	−265.34[a]
Block * RN+logTime+SpectralPurity+Block: logTime+Block: SpectralPurity	Block+RN+SpectralPurtiy	−205.89	−256.66
Block * RN+logTime+CCB+Block: logTime+Block: CCB	Block+RN	−201.92	−261.26

[a] Most parsimonious models

Table 2 Model selection for the generalized linear models (GLM) of the extinction test (phase III)

Initial model	Selected model	$AIC_{Initial}$	AIC_{Final}
ColourPair * Time	ColourPair * Time	284.45	284.45[a]
COC+Brightness	COC+Brightness	285.6	285.6[a]
COC+GreenContrast	COC+GreenContrast	288.42	288.42
COC+SpectralPurity	COC+SpectralPurity	295.45	295.45
COC+CCB	COC+CCB	311.92	311.92
RN+Brightness	RN+Brightness	288.1	288.1
RN+Green Contrast	RN+GreenContrast	286.5	286.5[a]
RN+Spectral Purity	RN+SpectralPurity	304.87	304.87
RN+CCB	RN	315.47	313.49

[a] Most parsimonious models

perceptual distance (calculated with the COC or RN model) and achromatic (brightness or green) contrast.

Statistical analyses were performed with R software (R Core Team 2013).

Table 3 Hypothesis testing for the repeated measures analysis of task acquisition (phase II)

Model	Variables	X^2	*df*	*p* value
Block+COC+Brightness				
	Block	89.19	2	<0.0001
	COC	11.45	1	0.0007
	Brightness	10.90	1	0.001
Block+COC+GreenContrast				
	Block	89.19	2	<0.0001
	COC	13.97	1	0.0002
	GreenContrast	10.13	1	0.0015
Block+RN+Brightness				
	Block	89.19	2	<0.0001
	RN distance	10.39	1	0.0013
	Brightness	10.88	1	0.001
Block+RN+GreenContrast				
	Block	89.19	2	<0.0001
	RN	14.31	1	0.0001
	GreenContrast	11.49	1	0.0007

Only the most parsimonious models are described here

Results

In the analysis of task acquisition during training, the most parsimonious models contained block of trials, perceptual distance and either brightness or green contrast (Table 1). We obtained the same results regardless of whether we used the COC or RN models to estimate perceptual distances. Although the model with RN distance and green contrast had the lowest AIC, the difference in AIC value between this model and those with COC and brightness was less than two units (Table 1). As a result, we cannot conclude that one model fits the data significantly better than the others (Burnham and Anderson 2002), and therefore, we used these four models for hypothesis testing (Table 3).

We obtained qualitatively similar results for the four most parsimonious models. The proportion of correct responses increased with experience (effect of block, Fig. 4), with perceptual distance (as calculated with the COC or RN model) and with achromatic (brightness or green) contrast (Table 3).

Overall, the most parsimonious model for the extinction test (phase III) included colour pair as a categorical factor, decision time and their interaction (Table 2). The model with COC distance and brightness was within two AIC units (ΔAIC=1.15), and the model with RN distance and green contrast was just beyond the two-unit limit (ΔAIC=2.05). Since the "two-unit rule" is an arbitrary criterion and was infringed only by 0.05 units, we used all three models for hypothesis testing.

In the most parsimonious model, there was a significant effect of colour pair, and the proportion of correct choices increased with decision time, while the interaction between the two variables was marginally non-significant (Table 4). In the alternative models, the proportion of correct choices increased with (COC or RN) perceptual distance, as well as with the (brightness or green) achromatic contrast (Table 4, Fig. 5). There was a strong correlation between brightness and green contrast (Pearson's correlation r=0.95, t=40.27, $p \leq 0.001$) thus, the two variables are interchangeable in the analyses and figures.

The contrast analysis revealed that the proportion of correct choices of group 3 was significantly higher than for the other groups (t>3.26, df=60, p<0.002).

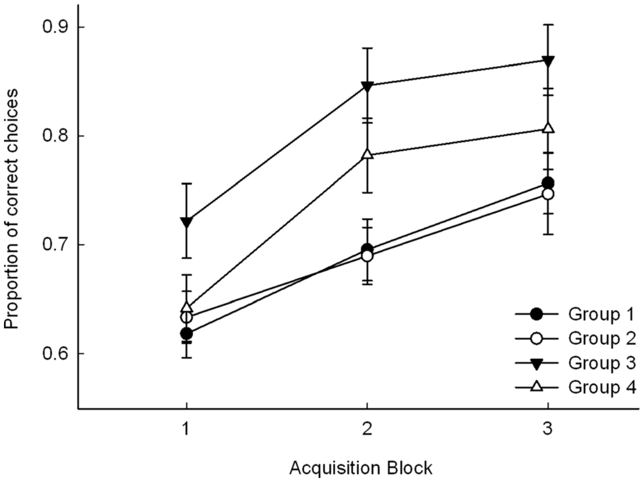


Fig. 4 Acquisition of colour discrimination task during training (phase II): change in the proportion of correct choices as training progresses for the different treatment groups. *Error bars* denote standard errors

Table 4 Results of the generalized linear models (GLM) for the extinction test analysis

Model	Variables	X^2	*df*	*p* value
ColourPair * Time				
	ColourPair	41.71	3	<0.0001
	Time	4.21	1	0.04
	ColourPair: Time	6.88	3	0.08
COC+Brightness				
	COC	28.10	1	<0.0001
	Brightness	28.52	1	<0.0001
RN+GreenContrast				
	RN	35.29	1	<0.0001
	GreenContrast	28.98	1	<0.0001

Only the selected models and their variables are described here

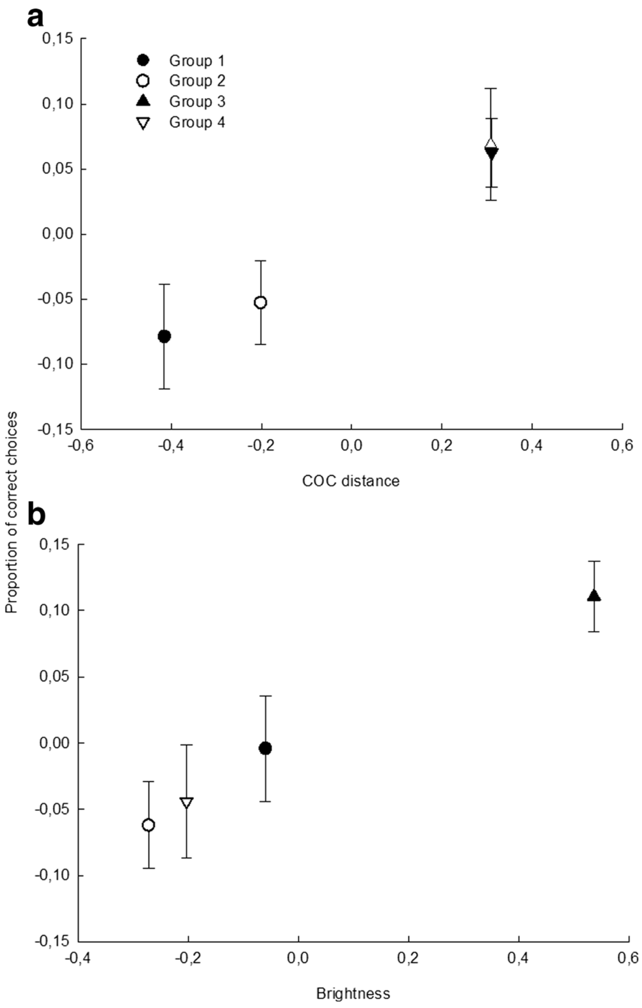


Fig. 5 Partial regression plots showing the relationship between the proportion of correct choices during phase III and **a** COC distance and **b** brightness contrast. In the vertical axis, we show the residuals of the regression of the proportion of correct choices on **a** brightness contrast or **b** COC distance. In the horizontal axis, the residuals of the regression of **a** COC distance on brightness contrast or **b** brightness contrast on COC distance (Moya-Laraño and Corcobado 2008). *Error bars* are standard errors

Concerning the effect of decision time on the proportion of correct choices, after controlling for colour pair, bees with longer decision times were more likely to choose flowers of the correct colour. Since the interaction between colour pair and decision time approached statistical significance, it is important to ask how the relationship between decision time and proportion of correct choices changed between groups. Inspection of the regression coefficients (Table S5, Online Resource 2) showed that the proportion of correct choices increased with decision time for all colour pairs (Fig. S1, Online Resource 2), with the exception of group 3—the group where discrimination reached its maximum value—where increasing decision time had no effect on the proportion of correct choices.

We now ask how the properties of the stimuli affected the decision time. The most parsimonious model included a single variable: green contrast. Nevertheless, the model with brightness contrast and RN distance was within two AIC units (Table S6, Online Resource 2) and must also be considered. Decision time decreased as achromatic contrast increased (Fig. 6b), but somewhat surprisingly, it increased with RN perceptual distance (Fig. 6a), and these effects were statistically significant (Table S7, Online Resource 2). A Tukey HSD test revealed that decision times were higher for bees in group 4 than for all other groups (all $p<0.014$), while there were no significant differences in the decision times of bees trained with colour pairs 1, 2 and 3 (all $p>0.92$).

Discussion

The COC, CH and RN models are often used in the literature to estimate chromatic distances as perceived by bees (Hempel de Ibarra et al. 2002; Reisenman and Giurfa 2008; Benard and Giurfa 2008; Niggebrügge et al. 2009; Martínez-Harms et al. 2010; Rodríguez-Gironés et al. 2013; Wang et al. 2013; Nityananda and Pattrick 2013). Nevertheless, although the models make different predictions, few attempts have been made to determine which model provides the best description of the data (Vorobyev and Brandt 1997; Vorobyev et al. 1999; Hempel de Ibarra et al. 2000), and even less effort has been devoted to determine whether different models should be applied in different circumstances.

Models' predictions can be in agreement with behavioural data, in some cases, for a particular subset of coloured stimuli during a detection or discrimination task, but there are also instances in which they will fail (Hempel de Ibarra et al. 2014).

Most of the studies that use the models to estimate perceptual distances were meant to elucidate the (fine and large) colour discrimination ability of bees (Dyer and Chittka, 2004a), the detectability of colours against complex and neutral backgrounds (Spaethe et al. 2001; Forrest and Thomson 2009), the effect of conditioning protocol on learning rate (Giurfa 2004; Reser et al. 2012) or the effect of aversive

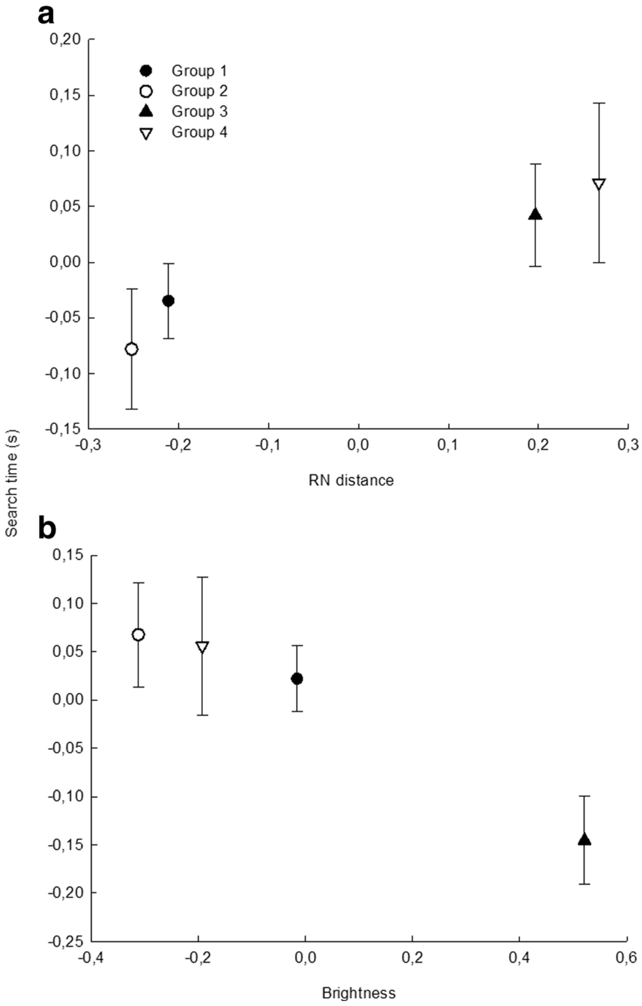


Fig. 6 Partial regression plots showing the relationship between search time and **a** RN distance and **b** brightness contrast. In the vertical axis, we show the residuals of the regression of the search time on **a** brightness contrast or **b** RN distance. In the horizontal axis, the residuals of the regression of **a** RN distance on brightness contrast or **b** brightness contrast on RN distance. *Error bars* are standard errors

stimuli on learning ability (Chittka et al. 2003; Avarguès-Weber et al. 2010). There was sporadic interest in testing model predictions about the detectability of bright and dim objects (Hempel de Ibarra et al. 2000) or under different light conditions (Arnold and Chittka 2012; Dyer and Chittka 2004b), while other studies address questions about innate and spontaneous colour preferences (Giurfa et al. 1995; Lunau et al. 1996; Raine and Chittka 2007; Papiorek et al. 2013) and different learning forms in bees (Giurfa and Sandoz 2012).

In this context, the present experiment constitutes the first attempt to determine which model provides the best description of perceptual chromatic distances in bumblebees, much as Hempel de Ibarra et al. (2000) attempted to determine which model should be used to estimate the detectability of stimuli and Brandt and Vorobyev (1997), using spectral sensitivity data (von Helversen 1972; Bobeth 1979), to test hypotheses about the physiology underlying honeybee spectral sensitivity. Our results show that the ability of free-flying bumblebees to discriminate colour pairs is not well predicted by their perceptual distance, as calculated with the CH model. At the end of the experiment, the proportion of correct choices for different colour pairs ranged from 70 % (groups 1 and 2) to 90 % (group 3), although all colour pairs had the same CH distance (mean±SEM=0.0549±0.0007; Table S2, Online Resource 2). While the predictions of the COC and RN models were somewhat better, they too failed to predict which colour pair bees would find it easier to discriminate: bumblebees were significantly better at discriminating between the two colours of group 3 ($d_{\mathrm{COC}}=1.13$; $d_{\mathrm{RN}}=0.40$) than of group 4 ($d_{\mathrm{COC}}=1.51$; $d_{\mathrm{RN}}=0.78$). These findings demonstrate experimentally that, although current models may be used to obtain rough estimates of perceptual distances, they cannot be used to predict whether bumblebees will discriminate one colour pair better than another.

It has repeatedly been stated that bees ignore brightness differences when choosing target colours subtending a known visual angle (Backhaus et al. 1987; Backhaus 1991; Chittka et al. 1992; Lehrer and Bischof 1995; Vorobyev and Brandt 1997; Giurfa and Vorobyev 1997; Giurfa et al. 1997; Niggebrügge and Hempel de Ibarra 2003; Reisenman and Giurfa 2008; Papiorek et al. 2013). Besides, the idea prevails that honeybees and bumblebees use an achromatic channel when stimuli subtend a small visual angle and a chromatic channel when they subtend a large one (Lehrer and Bischof 1995; Giurfa et al. 1996; Giurfa et al. 1997; Giurfa and Vorobyev 1998; Dyer and Griffiths 2012). Supposedly, bees use the chromatic or achromatic channel depending on the task, but they do not use both channels simultaneously. In our set-up, with 16 flowers randomly distributed on a 70×70-cm surface, the visual angle subtended by the nearest flowers when a bee departed from a flower was typically greater than 8°, and it increased as the bee approached the flower, but also decreased for flowers farther away. Because bumblebees use chromatic cues to process visual information when objects subtend an angle greater than 2.7° (Dyer et al. 2008), we expected them to use chromatic cues in the discrimination task. Nevertheless, both chromatic and achromatic contrasts affected the discriminability of colour pairs—indicating that bees can use the achromatic channel since it is available to the visual system even for subtended large visual angles. It is impossible, however, to infer here whether bumblebees were using both channels at the same time or in a sequence since they were flying around the arena. Because of the strong correlation between green and brightness contrasts among our stimuli pairs, however, it is impossible to determine which achromatic cue bees used.

Careful psychophysics experiments will be required to produce detailed descriptions of the bumblebee colour space. At least within the region of colour space that we used, however, we can conclude that the COC and RN models provide a better description than the CH model of bumblebees' perceptual distances and that bumblebees can use chromatic and achromatic cues to solve a discrimination task even for stimuli that subtend a large visual angle.

Implications for colour processing mechanisms

The visual processing mechanisms that enable colour discrimination are not completely understood. For example, models assume that bee colour discrimination results from comparing the output of photoreceptors using two colour opponent mechanisms. The topology of the colour space results from the choice of unspecified orthogonal colour opponent mechanisms according to the CH model (Chittka 1992), from the choice of a specific pair of colour opponent mechanisms according to the COC model (Backhaus 1991) and from the level of noise of the different receptor types according to the RN model, limiting performance of colour opponent mechanisms (Vorobyev and Osorio 1998; Vorobyev et al. 2001). Our data clearly show that postulating a pair of orthogonal mechanisms (CH model) is not enough to describe the colour space of bumblebees, but differences in the goodness of the fits of the COC and RN models are insufficient to rule out an exclusive effect of receptor noise limiting colour discrimination or the specificity of the colour opponent mechanism adopted by the COC model in the topology of colour space.

If the finding that the COC model provides a good fit to our data suggests that the nature of colour opponent mechanisms must be taken into account to understand colour perception, this suggestion should be taken with care: bees possess several types of colour opponent neurons (Yang et al. 2004) distributed along different regions in the bee brain (Yang et al. 2004; Paulk and Gronenberg 2008), and the implication of these regions in chromatic information processing is still not clear (Mota et al. 2013) and, so, how the different colour opponent neurons are used to produce what seems to be a two-dimensional colour space (Backhaus et al. 1987). On the other hand, the RN model also predicted the bumblebee performance well for the set of colour pairs used in this work, and it could also be that discrimination was limited by the receptor noise mechanism. It is not clear, however, that such limitation is set only by receptor noise, with neural noise being negligible and, with that, the opponent interactions between receptor signals (Vorobyev et al. 2001).

Both the COC and RN models were originally based on experimental honeybee data. Because honeybees and bumblebees perform differently in colour discrimination and detection tasks (Dyer et al. 2008; Wertlen et al. 2008; Morawetz and Spaethe 2012) and it has been suggested that the two species could differ in the way that they process ommatidia signals at the neural level (Wertlen et al. 2008), it is somewhat surprising that both models provide a relatively good description of the bumblebee colour space. These finding suggest that the two species could process colour information in a similar way. Since honeybees and bumblebees belong to the same subfamily (Apinae), it is possible that the CH model provides a better description of the colour space of more distantly related species. Nevertheless, as the COC and RN models made similar predictions for our colour pairs, we cannot rule out the possibility that noise at the receptor level has a strong effect on perceptual distances. If this were the case, the goodness of fit of the COC model to our data would be a spurious consequence of our choice of colours, since the RN model assumes that the noise in receptor mechanisms is dominant and discrimination of signals does not depend on how the receptor signals are combined to form opponent mechanisms (Vorobyev et al. 2001).

Regardless of whether perceptual distances are determined by the choice of colour opponent mechanisms, receptor noise or a combination of both, the role of chromatic and achromatic cues in discrimination tasks may have to be reconsidered and more data should be collected before attempting to produce a new descriptive model of bee colour space and topology.

The positive correlation between decision time and performance has been demonstrated by many authors (Spaethe et al. 2001; Chittka et al. 2003; Dyer and Chittka 2004b; Skorupski et al. 2006; Chittka and Spaethe 2007; Rodríguez-Gironés et al. 2013), suggesting that bees face a trade-off between increasing accuracy and speed (Chittka et al. 2003; Chittka and Spaethe 2007). Our results are consistent with the existence of this trade-off: within colour pairs, the proportion of correct choices increased with decision time, with the exception of group 3, where discrimination was easiest. Between groups, however, decision times were shortest when the achromatic contrast between target and distracter flowers was largest (Fig. 6b), but they increased with perceptual chromatic distance (Fig. 6a).

The behaviour of a bee is the result of a decision-making process that operates on memory and perception, and in order to predict how bees will respond to different environments, we need to understand perception, learning and decision-making. Likewise, if we are to infer the properties of perception from the results of behavioural experiments, we need to know the decision-making process linking perceptual input to behaviour. In addition, the study of recently discovered colour opponent neurons could give some new insights about the visual information process, within distinct bee brain regions.

Ecological implications

Size and shape can affect the foraging choices of bees and other insects because they can constrain access to the reward (Inouye 1980; Stang et al. 2006; Santamaría and Rodríguez-Gironés 2007). Flower colour has also been proposed to affect the foraging choices of pollinators (Raven 1972; Chittka and

Waser 1997; Rodríguez-Gironés and Santamaría 2004). Also, the combination of flower size and colour has been shown to affect the time that bees need to detect flowers (Spaethe et al. 2001) in a homogenous background. As we have seen, both the probability of selecting the correct flower colour and the time required to select a flower depend on the chromatic and achromatic parameters of the target and distracter flowers. Both factors affect foraging efficiency: inaccurate foragers will waste precious time exploiting suboptimal flowers, and increasing decision time reduces the number of flowers that a bee can visit per unit of time. The optimal balance between increasing accuracy and decreasing decision time will depend on the available options and the need to track changes in resource availability (Dyer and Chittka 2004b; Chittka and Spaethe 2007). At the same time, the balance achieved by foraging bees has important consequences for the reproductive success of plants: flower constancy promotes conspecific pollen flow, reduces pollen loss and stigma clogging (Chittka et al. 1999).

Hence, because flower colour affects the foraging choices of bees and foraging choices affect the reproductive success of plants, and thus the selective pressures to which a plant is exposed within a community (Rodríguez-Gironés and Santamaría 2005; Rodríguez-Gironés and Santamaría 2010), in order to understand and predict changes in flower aspect and abundance through time and space and their ecological implications, we first need to understand how the ability of bees to discriminate between flowers and the time that they require to accomplish this task depend on the spectral properties of the flowers and the learning mechanisms that modulate their behavioural flexibility.

Conclusions

Developing a colour difference formula valid throughout the colour space of bumblebees may be an impossible quest (Chittka and Kevan 2005). This, however, does not mean that we should use available models blindly, without trying to elucidate which model provides the most accurate description in each context. This information will greatly increase the performance and power of available models. The present paper is only a small contribution in this direction, but one that can easily be extended in future experiments.

Acknowledgments We thank Alejandro Trillo Iglesias and Guadalupe Corcobado for their assistance running the experiments, Klaus Lunau for his help in the calculation of spectral purities, four anonymous reviewers for comments, and Agrobío (Almería, Spain), for kindly providing bumblebee colonies. This research was supported by the Spanish Ministerio de Ciencia e Innovación/FEDER (project CGL2010-16795 to M.A.R.G.) and a PhD studentship to F.J.T. from the Spanish National Research Council (CSIC), co-funded by the European Social Fund (JAE: JAEPre033).

Conflict of interest The authors declare that Agrobío has no claim on the scientific procedures of this study.

References

Akaike H (1973) Information theory and an extension of the maximum likelihood principle. In: Petrov BN, Csaki F (eds) Proc. Second Int. Symp. Inf. Theory. Akademiai Kiado, Budapest, pp 267–281

Arnold SEJ, Chittka L (2012) Illumination preference, illumination constancy and colour discrimination by bumblebees in an environment with patchy light. J Exp Biol 215:2173–2180. doi:10.1242/jeb.065565

Avarguès-Weber A, de Brito Sanchez MG, Giurfa M, Dyer AG (2010) Aversive reinforcement improves visual discrimination learning in free-flying honeybees. PLoS ONE 5:e15370. doi:10.1371/journal.pone.0015370

Backhaus W (1991) Color opponent coding in the visual system of the honeybee. Vis Res 31:1381–1397

Backhaus W, Menzel R, Kreißl S (1987) Multidimensional scaling of color similarity in bees. Biol Cybern 56:293–304. doi:10.1007/BF00319510

Benard J, Giurfa M (2008) The cognitive implications of asymmetric color generalization in honeybees. Anim Cogn 11:283–293. doi:10.1007/s10071-007-0112-5

Bobeth H (1979) Dressurversuche zum Farbensehen der Bienen: Die Sättigung von Spektralfarben. 67

Brandt R, Vorobyev M (1997) Metric analysis of threshold spectral sensitivity in the honeybee. Vis Res 37:425–439

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd ed. 488

Chittka L (1996) Optimal sets of color receptors and color opponent systems for coding of natural objects in insect vision. J Theor Biol 181:179–196. doi:10.1006/jtbi.1996.0124

Chittka L (1992) The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. J Comp Physiol A 170:533–543. doi:10.1007/BF00199331

Chittka L, Beier W, Hertel H et al (1992) Opponent colour coding is a universal strategy to evaluate the photoreceptor inputs in Hymenoptera. J Comp Physiol A 170:545–563

Chittka L, Dyer AG, Block F, Dornhaus A (2003) Bees trade off foraging speed accuracy. Nature 424:388

Chittka L, Kevan PG (2005) Flower colour as advertisement. In: Dafni A, Kevan PG, Husband BC (eds) Pract. Pollinat. Biol. Enviroquest Ltd., Cambridge, Ontario, Canada, pp 157–196

Chittka L, Spaethe J (2007) Visual search and the importance of time in complex decision making by bees. Arthropod Plant Interact 1:37–44. doi:10.1007/s11829-007-9001-8

Chittka L, Spaethe J, Schmidt A, Hickelsberger A (2001) Adaptation, constraint, and chance in the evolution of flower color and pollinator color vision. In: Chittka L, Thomson JD (eds) Cogn. Ecol. Pollinat. Cambridge University Press, Cambridge, pp 106–126

Chittka L, Thomson JD, Waser NM (1999) Flower constancy, insect psychology, and plant evolution. Naturwissenschaften 86:361–377

Chittka L, Waser NM (1997) Why red flowers are not invisible to bees. Isr J Plant Sci 45:169–183

Dyer AG, Chittka L (2004a) Fine colour discrimination requires differential conditioning in bumblebees. Naturwissenschaften 91:224–227. doi:10.1007/s00114-004-0508-x

Dyer AG, Chittka L (2004b) Bumblebees (*Bombus terrestris*) sacrifice foraging speed to solve difficult colour discrimination tasks. J Comp Physiol A 190:759–763. doi:10.1007/s00359-004-0547-y

Dyer AG, Chittka L (2004c) Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. J Comp Physiol A 190:105–114. doi:10.1007/s00359-003-0475-2

Dyer AG, Griffiths DW (2012) Seeing near and seeing far; behavioural evidence for dual mechanisms of pattern vision in the honeybee (*Apis mellifera*). J Exp Biol 215:397–404. doi:10.1242/jeb.060954

Dyer AG, Neumeyer C (2005) Simultaneous and successive colour discrimination in the honeybee (*Apis mellifera*). J Comp Physiol A 191:547–557. doi:10.1007/s00359-005-0622-z

Dyer AG, Spaethe J, Prack S (2008) Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. J Comp Physiol A 194:617–627. doi:10.1007/s00359-008-0335-1

Forrest J, Thomson JD (2009) Background complexity affects colour preference in bumblebees. Naturwissenschaften 96:921–925. doi:10.1007/s00114-009-0549-2

Giurfa M (2004) Conditioning procedure and color discrimination in the honeybee *Apis mellifera*. Naturwissenschaften 91:228–231. doi:10.1007/s00114-004-0530-z

Giurfa M, Núñez J, Chittka L, Menzel R (1995) Colour preferences of flower-naive honeybees. J Comp Physiol A 177:247–259. doi:10.1007/BF00192415

Giurfa M, Sandoz J-C (2012) Invertebrate learning and memory: fifty years of olfactory conditioning of the proboscis extension response in honeybees. Learn Mem 19:54–66. doi:10.1101/lm.024711.111

Giurfa M, Vorobyev M (1998) The angular range of achromatic target detection by honey bees. J Comp Physiol A 183:101–110. doi:10.1007/s003590050238

Giurfa M, Vorobyev M (1997) Detection and recognition of color stimuli by Honeybees: performance and mechanisms. Isr J Plant Sci 45:129–140. doi:10.1080/07929978.1997.10676679

Giurfa M, Vorobyev M, Brandt R et al (1997) Discrimination of coloured stimuli by honeybees: alternative use of achromatic and chromatic signals. J Comp Physiol A 180:235–243. doi:10.1007/s003590050044

Giurfa M, Vorobyev M, Kevan PG, Menzel R (1996) Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. J Comp Physiol A 178:699–709

Gumbert A (2000) Color choices by bumble bees (*Bombus terrestris*): innate preferences and generalization after learning. Behav Ecol Sociobiol 48:36–43

von Helversen O (1972) Zur spektralen Unterschiedsempfindlichkeit der Honigbiene. J Comp Physiol 80:439–472. doi:10.1007/BF00696438

Hempel de Ibarra N, Giurfa M (2003) Discrimination of closed coloured shapes by honeybees requires only contrast to the long wavelength receptor type. Anim Behav 66:903–910. doi:10.1006/anbe.2003.2269

Hempel de Ibarra N, Giurfa M, Vorobyev M (2002) Discrimination of coloured patterns by honeybees through chromatic and achromatic cues. J Comp Physiol A 188:503–512. doi:10.1007/s00359-002-0322-x

Hempel de Ibarra N, Giurfa M, Vorobyev M (2001) Detection of coloured patterns by honeybees through chromatic and achromatic cues. J Comp Physiol A 187:215–224. doi:10.1007/s003590100192

Hempel de Ibarra N, Vorobyev M, Brandt R, Giurfa M (2000) Detection of bright and dim colours by honeybees. J Exp Biol 203:3289–3298

Hempel de Ibarra N, Vorobyev M, Menzel R (2014) Mechanisms, functions and ecology of colour vision in the honeybee. J Comp Physiol A 200:411–433. doi:10.1007/s00359-014-0915-1

Inouye DW (1980) The terminology of floral larceny. Ecology 61:1251–1253

Kunze J, Gumbert A (2001) The combined effect of color and odor on flower choice behavior of bumble bees in flower mimicry systems. Behav Ecol 12:447–456

Lehrer M, Bischof S (1995) Detection of model flowers by honeybees: the role of chromatic and achromatic contrast. Naturwissenschaften 82:145–147

Lotto RB, Chittka L (2005) Seeing the light: illumination as a contextual cue to color choice behavior in bumblebees. Proc Natl Acad Sci U S A 102:3852–3856. doi:10.1073/pnas.0500681102

Lunau K (1990) Colour saturation triggers innate reactions to flower signals: flower dummy experiments with bumblebees. J Comp Physiol A 166:827–834

Lunau K (1993) Interspecific diversity and uniformity of flower colour patterns as cues for learned discrimination and innate detection of flowers. Experientia 49:1002–1010

Lunau K, Fieselmann G, Heuschen B, van de Loo A (2006) Visual targeting of components of floral colour patterns in flower-naïve bumblebees (*Bombus terrestris*; Apidae). Naturwissenschaften 93:325–328. doi:10.1007/s00114-006-0105-2

Lunau K, Wacht S, Chittka L (1996) Colour choices of naive bumble bees and their implications for colour perception. J Comp Physiol A 178:477–489

Macuda T, Gegear RJ, Laverty TM, Timney B (2001) Behavioural assessment of visual acuity in bumblebees (*Bombus impatiens*). J Exp Biol 204:559–564

Martínez-Harms J, Márquez N, Menzel R, Vorobyev M (2014) Visual generalization in honeybees: evidence of peak shift in color discrimination. J Comp Physiol A 200:317–325. doi:10.1007/s00359-014-0887-1

Martínez-Harms J, Palacios a G, Márquez N et al (2010) Can red flowers be conspicuous to bees? *Bombus dahlbomii* and South American temperate forest flowers as a case in point. J Exp Biol 213:564–571. doi:10.1242/jeb.037622

Morawetz L, Spaethe J (2012) Visual attention in a complex search task differs between honeybees and bumblebees. J Exp Biol 215:2515–2523. doi:10.1242/jeb.066399

Mota T, Gronenberg W, Giurfa M, Sandoz J-C (2013) Chromatic processing in the anterior optic tubercle of the honey bee brain. J Neurosci 33:4–16. doi:10.1523/JNEUROSCI. 1412-12.2013

Moya-Laraño J, Corcobado G (2008) Plotting partial correlation and regression in ecological studies. Web Ecol 8:35–46. doi:10.5194/we-8-35-2008

Naka KI, Rushton WAH (1966a) S-potentials from color units in the retina of the fish (Cyprinidae). J Physiol 185:536–555

Naka KI, Rushton WAH (1966b) S-potentials from luminosity units in the retina of the fish (Cyprinidae). J Physiol 185:587–599

Niggebrügge C, Hempel de Ibarra N (2003) Colour-dependent target detection by bees. J Comp Physiol A 189:915–918. doi:10.1007/s00359-003-0466-3

Niggebrügge C, Leboulle G, Menzel R et al (2009) Fast learning but coarse discrimination of colours in restrained honeybees. J Exp Biol 212:1344–1350. doi:10.1242/jeb.021881

Nityananda V, Pattrick JG (2013) Bumblebee visual search for multiple learned target types. J Exp Biol 216:4154–4160. doi:10.1242/jeb.085456

Papiorek S, Rohde K, Lunau K (2013) Bees' subtle colour preferences: how bees respond to small changes in pigment concentration. Naturwissenschaften 100:633–643. doi:10.1007/s00114-013-1060-3

Paulk AC, Gronenberg W (2008) Higher order visual input to the mushroom bodies in the bee, Bombus impatiens. Arthropod Struct Dev 37:443–458. doi:10.1016/j.asd.2008.03.002, Higher

R Core Team (2013) R: a language and environment for statistical computing

Raine NE, Chittka L (2007) The adaptive significance of sensory bias in a foraging context: floral colour preferences in the bumblebee *Bombus terrestris*. PLoS ONE 2:e556. doi:10.1371/journal.pone.0000556

Raven PH (1972) Why are bird-visited flowers predominantly red? Evolution (N Y) 26:674

Reisenman CE, Giurfa M (2008) Chromatic and achromatic stimulus discrimination of long wavelength (red) visual stimuli by the honeybee *Apis mellifera*. Arthropod Plant Interact 2:137–146

Reser DH, Witharanage RW, Rosa MGP, Dyer AG (2012) Honeybees (*Apis mellifera*) learn color discriminations via differential conditioning independent of long wavelength (Green) photoreceptor modulation. PLoS ONE 7:e48577. doi:10.1371/journal.pone.0048577

Rodríguez-Gironés MA, Santamaría L (2005) Resource partitioning among flower visitors and evolution of nectar concealment in multi-species communities. Proc Biol Sci 272:187–192. doi:10.1098/rspb.2005.2936

Rodríguez-Gironés MA, Santamaría L (2004) Why are so many bird flowers red? PLoS Biol 2:e350. doi:10.1371/journal.pbio.0020350

Rodríguez-Gironés MA, Santamaría L (2010) How foraging behaviour and resource partitioning can drive the evolution of flowers and the structure of pollination networks. Open Ecol J 3:1–11

Rodríguez-Gironés MA, Trillo A, Corcobado G (2013) Long term effects of aversive reinforcement on colour discrimination learning in free-flying bumblebees. PLoS ONE 8:e71551. doi:10.1371/journal.pone.0071551

Rohde K, Papiorek S, Lunau K (2013) Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours. J Comp Physiol A 199:197–210. doi:10.1007/s00359-012-0783-5

Santamaría L, Rodríguez-Gironés MA (2007) Linkage rules for plant-pollinator networks: trait complementarity or exploitation barriers? PLoS Biol 5:e31. doi:10.1371/journal.pbio.0050031

Skorupski P, Chittka L (2010) Differences in photoreceptor processing speed for chromatic and achromatic vision in the bumblebee, *Bombus terrestris*. J Neurosci Off J Soc Neurosci 30:3896–3903. doi:10.1523/JNEUROSCI. 5700-09.2010

Skorupski P, Döring TF, Chittka L (2007) Photoreceptor spectral sensitivity in island and mainland populations of the bumblebee, *Bombus terrestris*. J Comp Physiol A 193:485–494. doi:10.1007/s00359-006-0206-6

Skorupski P, Spaethe J, Chittka L (2006) Visual search and decision making in bees: time, speed, and accuracy. Int J Comp Psychol 19:342–357

Spaethe J, Chittka L (2003) Interindividual variation of eye optics and single object resolution in bumblebees. J Exp Biol 206:3447–3453. doi:10.1242/jeb.00570

Spaethe J, Tautz J, Chittka L (2001) Visual constraints in foraging bumblebees: flower size and color affect search time and flight behavior. Proc Natl Acad Sci U S A 98:3898–3903. doi:10.1073/pnas.071053098

Stang M, Klinkhamer PGL, van der Meijden E (2006) Size constraints and flower abundance determine the number of interactions in a plant-flower visitor web. Oikos 112:111–121

Vorobyev M, Brandt R (1997) How do insect pollinators discriminate colors? Isr J Plant Sci 45:103–113

Vorobyev M, Brandt R, Peitsch D et al (2001) Colour thresholds and receptor noise: behaviour and physiology compared. Vis Res 41:639–653

Vorobyev M, De Ibarra NH, Brandt R, Giurfa M (1999) Do “white” and “ green ” look the same to a bee? Naturwissenschaften 86:592–594

Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. Proc Biol Sci 265:351–358. doi:10.1098/rspb.1998.0302

Wang M-Y, Ings TC, Proulx MJ, Chittka L (2013) Can bees simultaneously engage in adaptive foraging behaviour and attend to cryptic predators? Anim Behav 86:859–866. doi:10.1016/j.anbehav.2013.07.029

Wertlen AM, Niggebrügge C, Vorobyev M, Hempel de Ibarra N (2008) Detection of patches of coloured discs by bees. J Exp Biol 211:2101–2104. doi:10.1242/jeb.014571

Yang E-C, Lin H-C, Hung Y-S (2004) Patterns of chromatic information processing in the lobula of the honeybee, *Apis mellifera* L. J Insect Physiol 50:913–925. doi:10.1016/j.jinsphys.2004.06.010

Electronic Supplementary Material

Insect vision models under scrutiny: what bumblebees (*Bombus terrestris terrestris* L.) can still tell us

The Science of Nature: Naturwissenschaften

Francismeire Jane Telles[a, *] and Miguel A. Rodríguez-Gironés[a]

[a] Department of Functional and Evolutionary Ecology, Estación Experimental de Zonas Áridas (EEZA-CSIC). Carretera del Sacramento, s/n, La cañada de San Urbano. 04120, Almería, Spain.

[*] Corresponding author (meirecuesta@gmail.com)

Online Resource 1 Calculating Perceptual Distances, Colour and Achromatic Contrasts

The quantum catch of a photoreceptor, q_i (where i = UV, B or G), is an estimate of the number of impinging photons that the photoreceptor absorbs under specific illumination conditions. If $R_i(\lambda)$ is the spectral sensitivity of type-i photoreceptors, then its quantum catch is given by:

$$q_i = k_i \cdot \int_\lambda R_i(\lambda) \cdot I(\lambda) \cdot D(\lambda) \cdot d\lambda, \qquad (1)$$

where $I(\lambda)$ and $D(\lambda)$ are the reflectance spectrum of the stimulus and the spectral distribution of incident light, respectively, and k_i is a scaling factor chosen so that quantum catches equal 1 for the background spectrum, $I_b(\lambda)$, to which photoreceptors are adapted:

$$k_i = \frac{1}{\int_\lambda R_i(\lambda) \cdot I_b(\lambda) \cdot D(\lambda) \cdot d\lambda} \qquad (2)$$

For the colour hexagon, CH (Chittka 1992), and colour-opponent coding, COC (Backhaus 1991) models we must also calculate the excitation level of photoreceptor neurons, E_i, according to the equation

$$E_i = \frac{q_i}{1 + q_i} \qquad (3)$$

COC distance

To calculate the colour distance between two stimuli according to the COC model, for each colour of the pair we first calculate the colour-opponent mechanisms (Backhaus 1991) A and B

$$A = -9.86 \cdot E_{UV} + 7.70 \cdot E_B + 2.16 \cdot E_G \quad (4)$$

$$B = -5.17 \cdot E_{UV} + 20.25 \cdot E_B - 15.08 \cdot E_G \quad (5)$$

If colours 1 and 2 produce excitation values (E_{UV1}, E_{B1}, E_{G1}) and (E_{UV2}, E_{B2}, E_{G2}), from which we can compute (A_1, B_1) and (A_2, B_2) using equations (4) and (5), then the perceptual colour distance between colours 1 and 2 according to the COC model, d_{12}, is given by (Backhaus 1991)

$$d_{12} = |A_1 - A_2| + |B_1 - B_2| \quad (6)$$

CH distance

From the photoreceptor excitations (equation 3), we calculate the hexagon coordinates X and Y,

$$X = \sqrt{3} \cdot (E_G - E_{UV})/2 \quad (7)$$

$$Y = E_B - 0.5 \cdot (E_{UV} + E_G) \quad (8)$$

The CH distance between two colours, E_{12}, is the Euclidean distance between the loci of the colours on the hexagon (Chittka 1992):

$$E_{12} = [(X_1 - X_2)^2 + (Y_1 - Y_2)^2]^{1/2} \quad (9)$$

RN distance

To obtain the RN perceptual distance we do not need to obtain the loci of the two colours of a pair on a hypothetical colour space. Rather, the RN model assumes that perceptual distances are determined by the level of noise at the different photoreceptor channels, e_i. For the particular case of *Bombus terrestris* habituated to daylight, these parameters are $e_{UV} = 0.74$, $e_B = 0.67$ and $e_G = 0.61$ (see Fig. 3c of Skorupski and Chittka 2010). With these noise parameters and the quantum catches, the perceptual distance, $(\Delta S^t)^2$, between colours 1 and 2 is given by

$$\left(\Delta S^t\right)^2 = \frac{{e_{UV}}^2 \cdot \left(\Delta q_G - \Delta q_B\right)^2 + {e_B}^2 \cdot \left(\Delta q_G - \Delta q_{UV}\right)^2 + {e_G}^2 \cdot \left(\Delta q_{UV} - \Delta q_B\right)^2}{\left(e_{UV} \cdot e_B\right)^2 + \left(e_{UV} \cdot e_G\right)^2 + \left(e_B \cdot e_G\right)^2} \tag{10}$$

Two versions of this model have been proposed, corresponding to different definitions of the Δq. For the linear version (Vorobyev and Osorio 1998),

$$\Delta q_i = q_{2i} - q_{1i} \tag{11}$$

while for the logarithmic version (Vorobyev et al. 2001),

$$\Delta q_i = \log\left(q_{2i}\right) - \log\left(q_{1i}\right) \tag{12}$$

Calculating COC from CH Distances

It is possible to express the colour-opponent mechanisms A and B of the COC model (equations 4 and 5) as linear combinations of the colour-opponent mechanisms X and Y of the CH model (equations 7 and 8). Specifically,

$$A = 6.94 \cdot X + 7.70 \cdot Y \tag{13}$$

$$B = -5.72 \cdot X + 20.25 \cdot Y \tag{14}$$

Given P = (x, y), let Q = (x’, y’) be a point a distance r from P, at an angle α with the vertical axis. The coordinates of Q on the (X, Y) plane of the colour hexagon are therefore

$$x' = x + r \cdot \sin(\alpha) \tag{15}$$

$$y' = y + r\cdot\cos(\alpha) \quad (16)$$

From equations 13 and 14, the A and B colour-opponent mechanisms for points P and Q are:

$$A_P = 6.94\cdot x + 7.70\cdot y \quad (17)$$

$$B_P = -5.72\cdot x + 20.25\cdot y \quad (18)$$

and

$$A_Q = 6.94\cdot(x + r\cdot\sin(\alpha)) + 7.70\cdot(y + r\cdot\cos(\alpha)) \quad (19)$$

$$B_Q = -5.72\cdot(x + r\cdot\sin(\alpha)) + 20.25\cdot(y + r\cdot\cos(\alpha)) \quad (20)$$

From equation 6, it follows that

$$d_{PQ} = r\cdot(|6.94\cdot\sin(\alpha)+7.70\cdot\cos(\alpha)| + |-5.72\cdot\sin(\alpha)+20.25\cdot\cos(\alpha)|) \quad (21)$$

Calculating RN from CH Distances

A given point on the CH plane corresponds to infinitely many different reflectance spectra (essentially, the same hue with different brightness; Chittka 1992). To calculate the correspondence between CH and RN distances we must therefore make additional assumptions. In particular, we will assume that colours (both P and Q (α)) are chosen in such a way that

$$E_{UV} + E_B + E_G = 1 \quad (22)$$

For each point (X, Y) on the colour hexagon, this constraint, together with equations 7 and 8, define a system of three linear equations with three unknowns (E_{UV}, E_B and E_G) which can be solved for the photoreceptor excitation values. In other words, given the coordinates of a point on the colour hexagon, equation 22 allows us to calculate the photoreceptor excitation values and, from equation 3, the corresponding quantum catches produced by a colour stimulus. We can therefore calculate the quantum catches corresponding to points P = (x, y) and Q = (x', y'), a distance r from P and at an angle α with the vertical axis. Once we have calculated the quantum catches, the RN distance between the colours corresponding to P and Q is simply obtained from equation 10.

Calculating brightness and green contrast

We calculated brightness and green contrasts as specified by Reser et al. (2012). Let q_{ic} be the quantum catch of photoreceptor i (i = 1, 2, 3 for photoreceptors UV, B and G) when the eye of the bee is stimulated with colour c (equation 1).

Brightness contrast between colours 1 and 2:

$$Br_{12} = \frac{\sum_{i=1}^{3} q_{i1}}{\sum_{j=1}^{3} q_{j2}} \qquad (23)$$

Green contrast between colours 1 and 2:

$$G_{12} = \frac{q_{31}}{q_{32}} \qquad (24)$$

Chromatic contrast of target colours against background

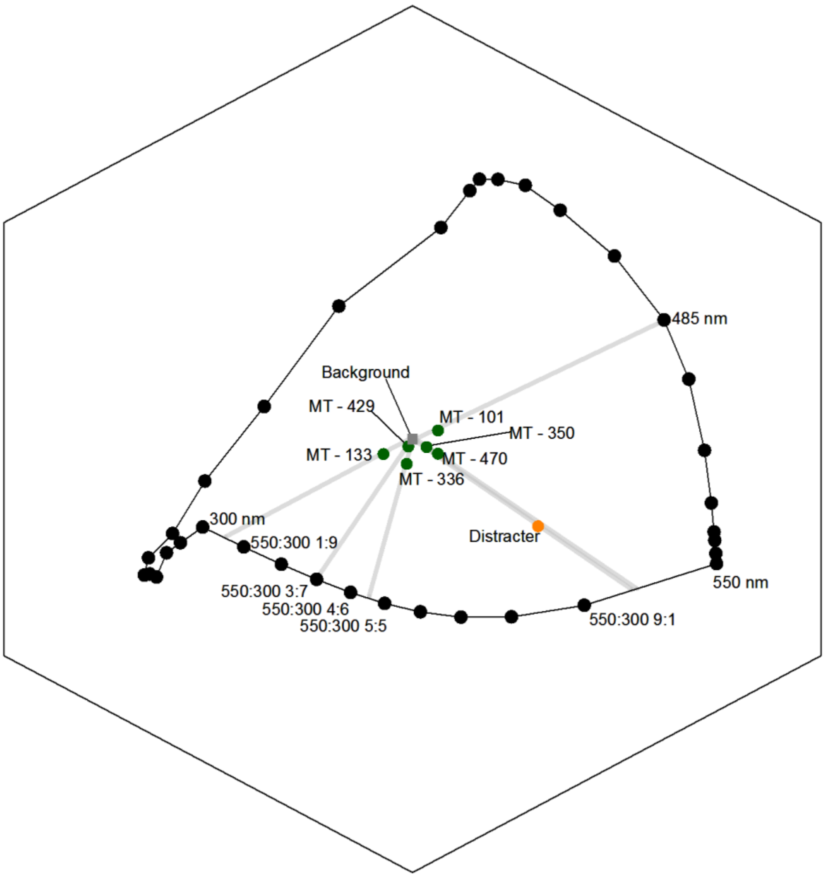
Let (X_c, Y_c) be the colour hexagon coordinates of colour c (equations 7 and 8). The chromatic contrast of colour c agains its background is the euclidean distance between the loci of the colour and the background. Because the coordinates of the background are (0, 0), this is simply $\sqrt{X_c^2 + Y_c^2}$. The contrast between the chromatic contrasts of colours 1 and 2 against the background is therefore

$$CCB_{12} = \frac{\sqrt{X_1^2 + Y_1^2}}{\sqrt{X_2^2 + Y_2^2}} \quad (25)$$

Spectral purity

The spectral purity of colour c is the ratio between its chromatic contrast against the background (as defined above) and the chromatic contrast against the background of its dominant wavelength (Lunau et al. 1996; Rohde et al. 2013) – where the dominant wavelength of colour c is the intersection of the spectral line with the straight line through the centre of the hexagon and the locus of colour c (Fig. S1). Given colours 1 and 2, their spectral purity contrast equals the ratio of their spectral purities.

Fig. S1 Loci of stimuli (green and orange circles), background (grey square) and spectral locus (black circles connected by a line) plotted in the CH space. The stimuli are indicated by their reference names and the corresponding hues are highlighted in grey. The continuous line represents the spectrum locus for bumblebees with the illumination and background colour used in the experiment



References

Backhaus W (1991) Color opponent coding in the visual system of the honeybee. Vision Res 31:1381–1397

Chittka L (1992) The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. J Comp Physiol A 170:533–543. doi: 10.1007/BF00199331

Lunau K, Wacht S, Chittka L (1996) Colour choices of naive bumble bees and their implications for colour perception. J Comp Physiol A 178:477–489

Reser DH, Witharanage RW, Rosa MGP, Dyer AG (2012) Honeybees (*Apis mellifera*) learn color discriminations via differential conditioning independent of long wavelength (Green) photoreceptor modulation. PLoS One 7:e48577. doi: 10.1371/journal.pone.0048577

Rohde K, Papiorek S, Lunau K (2013) Bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) prefer similar colours of higher spectral purity over trained colours. J Comp Physiol A 199:197–210. doi: 10.1007/s00359-012-0783-5

Skorupski P, Chittka L (2010) Differences in photoreceptor processing speed for chromatic and achromatic vision in the bumblebee, *Bombus terrestris*. J Neurosci Off J Soc Neurosci 30:3896–903. doi: 10.1523/JNEUROSCI.5700-09.2010

Vorobyev M, Brandt R, Peitsch D, et al (2001) Colour thresholds and receptor noise: behaviour and physiology compared. Vision Res 41:639–53

Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. Proc Biol Sci 265:351–8. doi: 10.1098/rspb.1998.0302

Electronic Supplementary Material

Insect vision models under scrutiny: what bumblebees (*Bombus terrestris terrestris* L.) can still tell us

The Science of Nature: Naturwissenschaften

Francismeire Jane Telles[a,] * **and Miguel A. Rodríguez-Gironés**[a]

[a] Department of Functional and Evolutionary Ecology, Estación Experimental de Zonas Áridas (EEZA-CSIC). Carretera del Sacramento, s/n, La cañada de San Urbano. 04120, Almería, Spain.

* Corresponding author (meirecuesta@gmail.com)

Online Resourse 2 Additional information about chromatic and achromatic parameters of stimuli used in the experiment, regression coefficients for the proportion of correct choices, model selection and hypothesis testing for the search time and the relationship between proportion of correct choices, decision time (s) and the COC distance during the extinction phase

Table S1 Quantum catch, Green contrast (GC), Brightness contrast, Colour contrast against the background (CCB) and Spectral purity contrast (SP) of target colours used in the experiment

Group	**Colour reference**	**Quantum catch**			**GC**	**Brightness**	**CCB**	**SP**
		UV	**Blue**	**Green**				
Background	MT - 431	1	1	1				
Distracter	MT - 553	0.180	0.117	0.903	1.108	2.500	0.334	0.574
1	MT-133	0.573	0.416	0.414	2.426	2.146	3.639	3.026
1	MT-429	1.047	0.957	1.005				
2	MT-336	0.934	0.719	0.882	2.129	1.807	1.205	1.018
2	MT-133	0.573	0.416	0.414				
3	MT-350	2.005	1.991	2.355	2.669	2.506	1.659	2.690
3	MT-336	0.934	0.719	0.882				
4	MT-101	2.348	3.038	3.234	1.468	1.560	1.107	1.094
4	MT-470	1.672	1.649	2.203				

Table S2 Colour opponent mechanisms according to the CH (X and Y), COC (A and B) and RN_L (X_1 and X_2) models, for the different colours used, perceptual distance for each pair, calculated using the CH, COC and RN (lineal and logarithmic versions) models, and angle α that the segment linking the loci (in the colour hexagon) of the two colours of the pair makes with the vertical axis

Group	**Colour reference**	**Colour opponent mechanisms**						**Angle (α)**	**Perceptual distance**			
		X	**Y**	**A**	**B**	**X_1**	**X_2**		**CH**	**COC**	**RN_L**	**RN_{Log}**
Distracter	MT -553	0.278	-0.208	0.331	-5.815	0.867	-0.339					
1	MT-133	-0.062	-0.035	-0.697	-0.354	-0.001	0.182	71°	0.055	0.55	0.11	0.29
1	MT-429	-0.009	-0.017	-0.195	-0.299	0.053	0.079					
2	MT-336	-0.012	-0.058	-0.528	-1.094	0.180	0.163	294°	0.054	0.90	0.18	0.29
2	MT-133	-0.062	-0.035	-0.697	-0.354	-0.001	0.182					
3	MT-350	0.030	-0.019	0.063	-0.554	0.401	-0.174	227°	0.057	1.13	0.40	0.27
3	MT-336	-0.012	-0.058	-0.528	-1.094	0.180	0.163					
4	MT-101	0.054	0.019	0.527	0.090	0.217	-0.898	180°	0.054	1.51	0.78	0.32
4	MT-470	0.053	-0.034	0.108	-1.002	0.612	-0.263					

Table S3 Perceptual distances between stimuli and distracter during phase I

Group	**Stimuli reference**	**Perceptual distance between distracter and stimuli**			
		CH	**COC**	**RN_L**	**RN_{Log}**
1	MT-133	0.38	6.49	1.01	2.44
1	MT-429	0.34	6.04	0.91	2.29
2	MT-336	0.33	5.58	0.85	2.17
2	MT-133	0.38	6.49	1.01	2.44
3	MT-350	0.31	5.53	0.50	2.12
3	MT-336	0.33	5.58	0.85	2.17
4	MT-101	0.32	6.10	0.86	2.20
4	MT-470	0.28	5.03	0.27	1.98

Table S4 Proportion of correct choices and search time for target colours A and B within groups during the extinction phase (III)

Group	Colour reference	Target colour	Proportion correct choices (%)	Search time(s)
1	MT-133	A	69	1.57
1	MT-429	B	74	1.68
2	MT-336	A	67	1.73
2	MT-133	B	72	1.73
3	MT-350	A	94	1.58
3	MT-336	B	89	1.55
4	MT-101	A	88	1.83
4	MT-470	B	71	2.17

Table S5 Regression coefficients of the GLM for proportion of correct choices (phase III) with group, decision time and their interaction as explanatory variables. Baseline is group 4

Coefficients	Estimate	Std. Error	z value	Pr (>|z|)
Intercept	-0.648	1.046	-0.620	0.535
Group 1	-2.503	2.099	-1.193	0.233
Group 2	2.243	1.494	1.501	0.133
Group 3	0.765	2.354	0.325	0.745
Time	1.159	0.602	1.925	0.054
Group 1 : Time	1.674	1.399	1.197	0.231
Group 2: Time	-1.630	0.912	-1.787	0.074
Group 3 : Time	0.392	1.642	0.239	0.811

Table S6 Generalized linear models with Gaussian distribution to investigate the effect of colour pair, distance (COC and RN) brightness and green contrast on the search time (s)

Initial Model	Selected Model	$AIC_{Initial}$	AIC_{Final}
ColourPair	ColourPair	-10.734	-10.734
COC + Brightness	COC + Brightness	-11.374	-11.374
COC + GreenContrast	GreenContrast	-12.593	-14.333*
RN + Brightness	RN + Brightness	-12.499	-12.499*
RN + Green Contrast	GreenContrast	-12.708	-14.333*

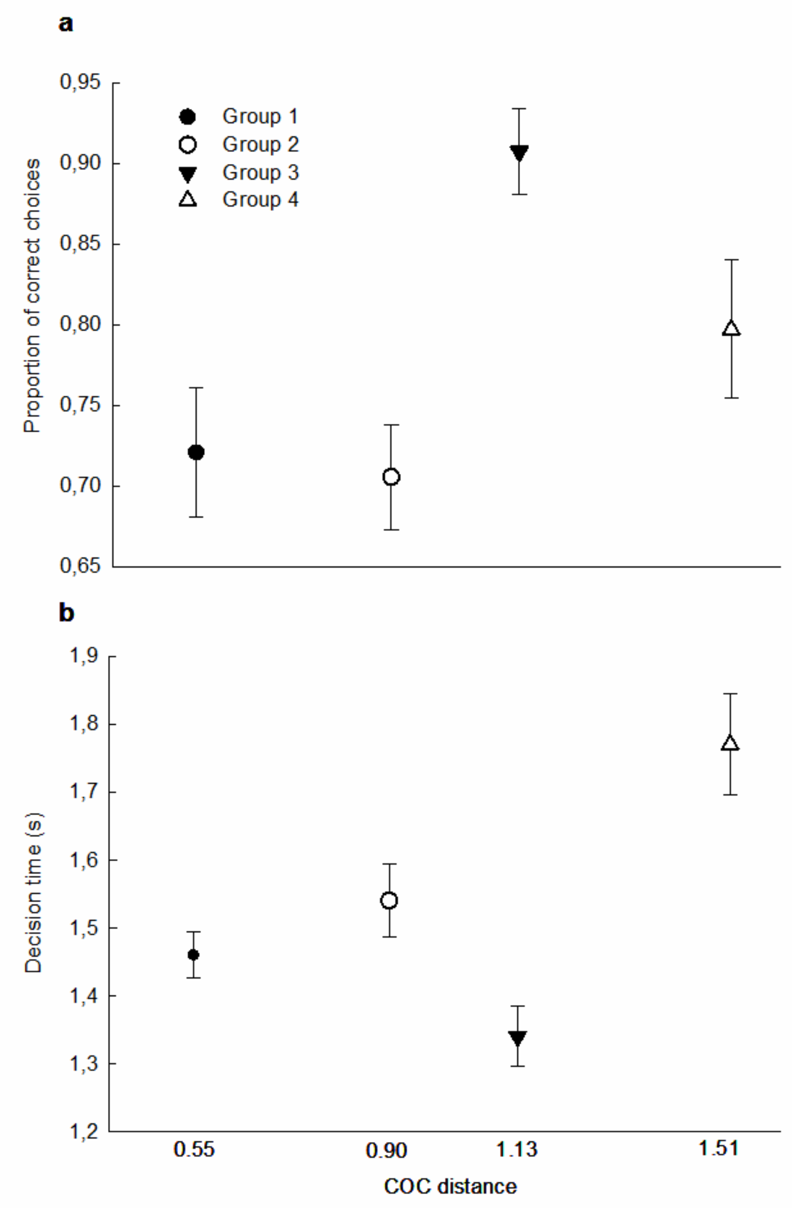
*****most parsimonious models

Table S7 Hypothesis-testing for the GLM analysis with Gaussian distribution of chromatic and achromatic parameters affecting the search time (s). Only the most parsimonious models are described here

Model	Variables	X^2	d.f.	*p*
GreenContrast				
	GreenContrast	27.04	1	<0.0001
RN + Brightness				
	RN	4.87	1	0.03
	Brightness	10.30	1	0.001

Fig. S1 Relationship between proportion of correct choices, decision time (s) and the COC distance during extinction phase (III): Effect of perceptual distance, as predicted by the COC model, on **a** the proportion of correct choices and **b** decision time during phase III. Error bars are standard errors

51


a
Proportion of correct choices
0,95
0,90
0,85
0,80
0,75
0,70
0,65
● Group 1
○ Group 2
▼ Group 3
△ Group 4
b
Decision time (s)
1,9
1,8
1,7
1,6
1,5
1,4
1,3
1,2
0.55
0.90
1.13
1.51
COC distance