FACETS
a multidisciplinary open access science journal

RESEARCH ARTICLE

Why colour is complex: Evidence that bees perceive neither brightness nor green contrast in colour signal processing

Leslie Ng[ab], Jair E. Garcia[a], and Adrian G. Dyer[ac]*

[a]Bio-Inspired Digital Sensing (BIDS) Lab, School of Media and Communication, RMIT University, Melbourne, VIC 3001, Australia; [b]School of Biosciences, University of Melbourne, Melbourne, Parkville, VIC 3010, Australia; [c]Department of Physiology, Monash University, Melbourne, Clayton, VIC 3800, Australia

*adrian.dyer@rmit.edu.au

Citation: Ng L, Garcia JE, and Dyer AG. 2018. Why colour is complex: Evidence that bees perceive neither brightness nor green contrast in colour signal processing. FACETS 3: 800–817. doi:10.1139/facets-2017-0116

Handling Editor: Peter G. Kevan

Received: October 9, 2017

Accepted: May 30, 2018

Published: August 2, 2018

Published by: Canadian Science Publishing

Abstract

Honey bees (*Apis mellifera* Linnaeus, 1758) potentially rely on a variety of visual cues when searching for flowers in the environment. Both chromatic and achromatic (brightness) components of flower signals have typically been considered simultaneously to understand how flower colours have evolved. However, it is unclear whether honey bees actually use brightness information in their colour perception. We investigated whether free-flying honey bees can process brightness cues in achromatic stimuli when presented at a large visual angle of 28° to ensure colour processing. We found that green contrast (modulation of the green receptor against the background) and brightness contrast (modulation of all three receptors against the background) did not have a significant effect on the proportion of correct choices made by bees, indicating that they did not appear to use brightness cues in a colour processing context. Our findings also reveal that, even at a small visual angle, honeybees do not reliably process single targets solely based on achromatic information, at least considering values up to 60% modulation of brightness. We discuss these findings in relation to proposed models of bee colour processing. Therefore, caution should be taken when interpreting elemental components of complex flower colours as perceived by different animals.

Key words: achromatic, *Apis mellifera*, brightness, colour, complex, signal processing

Introduction

Brightness is a key component of primate colour vision; our brain binds together both chromatic and achromatic (brightness) information when interpreting colourful images (Burns and Shepp 1988; Croner and Albright 1999; Clery et al. 2013). Brightness is defined as the attribute of a visual sensation where a stimulus is perceived to be more or less intense, based on the achromatic modulation of photoreceptors by the stimulus (Wyszecki and Stiles 1982). In the early stages of primate visual processing, chromatic and achromatic information appear to be separated into magnocellular (M) and pavocellular pathways (P) respectively (Livingstone and Hubel 1988; Nassi and Callaway 2009). These signals are eventually bound together at a later stage, although where this integration takes place remains unclear and may involve multiple stages (Nassi and Callaway 2009). Therefore, colour perception incorporating brightness as perceived by primate brains appears to be a complex multi-stage process, and may not be experienced by all animals.

The European honey bee (*Apis mellifera* Linnaeus, 1758) is an important model species for understanding animal colour vision. The species has a rich history in colour science and was the second non-human animal after the goldfish to demonstrate a capacity to perceive colour (von Frisch 1913, 1914). Honey bees have a trichromatic visual system with photoreceptors maximally sensitive to 344 nm (ultraviolet (UV)), 436 nm (blue) and 544 nm (green) electromagnetic radiation (Peitsch et al. 1992; Briscoe and Chittka 2001). All three receptors have been identified to contribute to colour perception through colour opponent processes; however, the exact mechanism of how these signals are processed remains unclear (Kien and Menzel 1977; Hertel 1980; Dyer et al. 2011). The green receptor has been identified to drive achromatic visual responses such as shape processing (Lehrer and Bischof 1995; Hempel de Ibarra and Giurfa 2003; Stach et al. 2004; Morawetz et al. 2013) and motion perception (von Hess 1913; Kaiser and Liske 1974; Stojcev et al. 2011). Achromatic perception in the honey bee is, therefore, assumed to be determined by the green contrast of a stimulus against the background (Giurfa et al. 1996; Giurfa and Vorobyev 1998), although in principle, brightness might also be mediated by the sum of photon catch by all three photoreceptors in bees (Spaethe et al. 2001).

The role of brightness as a visual cue has been previously investigated using behavioural experiments (Lunau and Maier 1995; Giurfa et al. 1996; Kelber 2005) and, therefore, might be an important visual signal in bee-pollinated flowers. Brightness perception is the ability to perceive stimulus intensity differences (Wyszecki and Stiles 1982; Reser et al. 2012), where intensity is related to the total amount of energy reflected by a stimulus. To understand whether intensity is a meaningful signal for bee pollinators it is important to test visual perception while isolating other confounding chromatic cues (Reser et al. 2012). The intensity of flower signals can vary depending on colourless copigments such as flavones, flavanols, or organic acids (Miller et al. 2011), although flower intensity can also be modulated by physical properties. Such properties include flower thickness, the dense packing of cells via veins or thin cell layers, the curvature in the epidermal layer, and irregularly shaped granules with high refractive indices (Stavenga and van der Kooi 2016; van der Kooi et al. 2016, 2017). Ecological studies theoretically investigating brightness often involve pollinator-mediated selection experiments, where selection pressures towards specific colour traits are quantified (Caruso et al. 2010; Renoult et al. 2013; Wassink and Caruso 2013; Sletvold et al. 2016). Such studies provide some evidence that brightness may be important for pollinators. For example, Renoult et al. (2013) found that bumble bees drive selection on the brightness component of *Centaurea cyanus* Linnaeus flower colouration. However, these experiments do not reveal how bees are processing these components of complex colour signals. This is partially because achromatic visual channels are difficult to isolate from other visual or colour processing mechanisms that can be used by bees (Giurfa et al. 1996; Morawetz et al. 2013), and natural spectra may potentially modulate several mechanisms in a highly correlated fashion (Koethe et al. 2016). In addition, Renoult et al. (2013) has highlighted a paucity in detection threshold data for achromatic vision in bees. To acquire such data it is important to determine potential detection thresholds to improve our understanding of how bees may first detect the presence of a stimulus against a background, and then, if detection is enabled, how brightness might facilitate the discrimination of a particular target stimulus from an alternative distractor stimulus.

It is surprising that brightness cues in flowers are currently considered important for bees (Caruso et al. 2010; Renoult et al. 2013; Wassink and Caruso 2013; Sletvold et al. 2016), given the classic psychophysical stance that bees do not process brightness as a dimension of perception in their colour visual system. Early behavioural experiments suggested that honey bees ignore brightness cues in colour choice experiments (Daumer 1956; Menzel 1967; von Helversen 1972). Furthermore, this segregation in visual channels is reflected in the separated use of chromatic or achromatic cues in honey bees at different visual angles (Giurfa and Vorobyev 1998). Chromatic cues are used when a colour stimulus subtends a large visual angle of approximately >15° (Giurfa et al. 1996). Alternatively, achromatic cues appear to be used when a stimulus subtends a small angle of approximately 5°–15° (Giurfa et al. 1996;

Giurfa and Vorobyev 1998), although true achromatic stimuli may be difficult to process (Giurfa et al. 1996). Bees appear to use achromatic cues at a large visual angle when green contrast is sufficiently high (Hempel de Ibarra et al. 2000). Therefore, although chromatic and achromatic processing depends on the visual angle subtended by a stimulus to the compound eye, bees may potentially process both these types of information as a combined signal, regardless of the viewing angle.

An additional point to consider is the role of attention and motivation during behavioural experiments. Bees in previous psychophysical studies were not specifically trained to use brightness cues in experiments investigating bee colour vision (von Frisch 1914; Menzel 1967). It is, therefore, possible that bees may have ignored brightness in favor of more salient colour cues in these experiments. This is plausible as there seems to be a hierarchy in the bee visual system where some types of chromatic information are weighted above achromatic information (Morawetz et al. 2013); therefore, it remains unclear whether bees can use brightness in their colour perception when given the proper training. To answer this question, bees need to be trained using a highly motivating appetitive-aversive differential conditioning framework (Dyer and Neumeyer 2005; Avarguès-Weber et al. 2010; Morawetz et al. 2013). This involves the use of both a reward (CS+) and punishment (CS−) while the bee is learning the task (Dyer and Chittka 2004; Avarguès-Weber et al. 2010).

In this study, we aimed to expand the current understanding of honey bee brightness processing by investigating whether bees are able to use brightness cues as a component of colour perception when trained using an appetitive-aversive differential conditioning framework; and if so, we sought to establish detection threshold values for brightness perception. We used the well-accepted definitions of brightness (green contrast, and also brightness contrast), as defined by Spaethe et al. (2001) to reveal whether modulation of brightness over a broad range of values will allow bees to detect achromatic stimuli at a large visual angle.

Methods

Sample collection and measurement

To test our research question, we required a range of achromatic stimuli with low colour contrast. A number of commercially available "colour" cards were measured to find suitable stimuli, and laminated to allow for easy cleaning with 20% ethanol during the experiments (**Figs. S3**, **S4**). These stimuli were measured using an Ocean Optics spectrophotometer (Dunedin, Florida, USA) with a PX-2 pulsed xenon light source with a bifurcated probe kept at a 45° angle relative to normal incidence from the sample to reduce specular reflection. The spectrophotometer was calibrated using a UV-reflecting white standard from the same manufacturer. We first modeled these stimuli in Hexagon colour space (Chittka 1992) using honey bee photoreceptors (Peitsch et al. 1992; detailed in **Supplementary Material 1**). Receptor excitation values were calculated for each stimulus using reflectance data at 10 nm intervals considering an illumination of 6500 K daylight (Judd et al. 1964), expressed as photon flux (Spaethe et al. 2001). Colour contrast was calculated as the Euclidean distance of a stimulus from the adaptation background, and stimuli were selected to have a colour contrast of $\leq$0.05 hexagon units, which represents very low chromatic contrast for bees trained with differential conditioning (Chittka 1992; Dyer and Neumeyer 2005; Dyer et al. 2008). Green and blue contrasts (see below) were calculated as the degree to which the relevant receptors generated an excitation value different from 0.5, which represents adaptation to the background (Spaethe et al. 2001; Morawetz et al. 2013). Brightness contrast was calculated as the sum of UV, blue, and green contrasts (Spaethe et al. 2001).

As a result of this selection process, two 5 cm × 5 cm bee achromatic cards, perceived by humans as pink, and three pairs of 20 cm × 20 cm bee achromatic background cards, perceived by humans as

Table 1. Receptor excitation and contrast values (presented as absolute values) calculated for each stimulus.

	PNK1 against GRY1 (achromatic)	PNK2 against GRY2 (achromatic)	PNK2 against GRY3 (achromatic)	HKS3N against GRY1 (chromatic)	HKS3N against HKS92N (chromatic)	HKS33N against HKS92N (chromatic)
Excitation						
UV receptor	0.77	0.67	0.58	0.11	0.37	0.60
Blue receptor	0.83	0.68	0.60	0.23	0.30	0.64
Green receptor	0.80	0.66	0.59	0.80	0.80	0.42
Contrast						
Colour contrast	0.05	0.02	0.02	0.64	0.48	0.20
Green contrast	0.30	0.16	0.09	0.30	0.30	0.08
Blue contrast	0.33	0.18	0.10	0.27	0.20	0.14
Brightness contrast	0.90	0.52	0.26	0.36	0.03	0.16

Note: The maximum value for both excitation and colour contrast is 1. The maximum value for both green and blue contrast is 0.5. The maximum value for brightness contrast is 1.5. PNK, pink stimulus; GRY, grey stimulus; HKS3N, yellow stimulus; HKS33N, purple stimulus; HKS92N, grey stimulus.

grey, were selected for the experiment. These pink cards were Prisma Favini pink (PNK1; Art spectrum, Brunswick, Australia) and Rose Petal (PNK2; Canson, Annonay, France). The grey background cards were Mi-teintes dark grey (GRY1; Canson, Annonay, France), Flannel Grey (GRY2; Canson, Annonay, France), and Graphite Tiziano (GRY3; Carta Fabriano, Fabriano, Italy). From these pink and grey cards, three card combinations had low colour contrast (≤0.05), but varied in (i) green contrast (ranging from 0.09–0.3; equivalent to 18.0%–60.0% modulation relative to the adaptation background) and (ii) brightness contrast (ranging from 0.26–0.9; equivalent to 17.3%–60.0% modulation relative to the adaptation background; **Table 1**). The yellow stimulus HKS3N (against GRY1) was used as a positive control stimulus, as it has been previously established that this stimulus is readily learned and detected by free-flying bees (Giurfa et al. 1996; Dyer et al. 2008; **Table 1**). For comparative purposes, the bee achromatic stimuli were also modeled using the colour opponent coding (COC) model (Backhaus 1991), the receptor noise-limited (RNL) model (Vorobyev and Osorio 1998; Vorobyev et al. 2001), and compared in terms of brightness contrast based on brightness differences as predicted by the achromatic RNL model (Siddiqi et al. 2004; **Table 2**). The COC model predicted that all three achromatic stimuli were below the detection threshold of 1.47 (Avarguès-Weber et al. 2010). The RNL model predicted that only PNK2 and PNK3 would be below the detection threshold of 1.0 against their respective backgrounds (Vorobyev and Osorio 1998; Vorobyev et al. 2001; Barry et al. 2015). Alternatively, the achromatic RNL analysis predicted that all three stimuli were above the theoretical detection threshold of 1.0 (Siddiqi et al. 2004).

Table 2. Chromatic contrasts calculated using the Hexagon, COC, and RNL colour models.

Stimuli	Hexagon	COC	RNL (chromatic)	RNL (achromatic)
PNK1 against GRY1	0.05	1.20	2.66	11.70
PNK2 against GRY2	0.02	0.28	0.76	2.85
PNK3 against GRY3	0.02	0.41	0.80	5.60

Note: Achromatic contrast was also calculated using the RNL model as per Siddiqi et al. (2004). COC, colour opponent coding; RNL, receptor noise-limited; PNK, pink stimulus; GRY, grey stimulus.

Training

Free-flying honey bees (*A. mellifera*) were recruited from a University of Melbourne research beehive. A total of 10 honey bees were marked on the thorax for identification (ID) purposes and individually tested. Each bee participated in a total of four detection tests, including a positive control priming test that was conducted first and then three subsequent tests. Bees were trained to fly into a Y-maze, which is a standard apparatus for controlling the visual angle in bee experiments (Giurfa et al. 1996; Avarguès-Weber et al. 2010). The Y-maze was illuminated by daylight and covered with a UV-transparent plexiglass ceiling. The entrance of the maze led to a decision point (**Fig. 1**), which further led to the two arms of the maze (10 cm long and 20 cm tall). Bees were trained to fly towards the arm presenting the target stimulus, and a choice was counted when the bee passed the decision line (**Fig. 1**). The target was fixed to a grey pole of the same colour as the background, and was presented at a visual angle of 28°, which mediates colour processing in honey bees (Giurfa et al. 1996). Bees were motivated to detect the target card using an appetitive-aversive differential conditioning framework where 50% sucrose solution was associated with the target (CS+) and deposited on the target pole, whereas 60 mmol/L quinine solution was associated with an incorrect decision (CS−) and deposited on an identical pole. The arm on which the target would be presented was decided randomly by coin toss.

Each bee initially participated in a positive control priming test to ensure that they had learned to use the Y-maze and could perform the detection task. The target for the priming test was the positive control salient yellow stimulus (HKS3N against GRY1 background). Once a bee was able to detect this stimulus above a threshold of 60% correct choices, for a minimum of 10 choices, the bee then participated in three subsequent detection tests. In each test, a bee had to make 20 choices when presented with a specific target stimulus. Each bee was first presented with the stimulus with the highest green contrast against the grey background (PNK1 against GRY1, green contrast = 0.3), then the next highest pair (PNK2 against GRY2, green contrast = 0.16), and finally the pair with the lowest contrast (PNK2 against GRY3, green contrast = 0.09). We used a set order as this promotes learning of perceptually difficult visual tasks in bees (Dyer and Chittka 2004; Dyer and Neumeyer 2005). The equipment was cleaned with 20% ethanol solution to exclude olfactory cues that might be left by the bees. After each choice, the target stimulus would once again be randomly allocated to one of the two arms, and the bee was allowed to return to the hive when satiated.

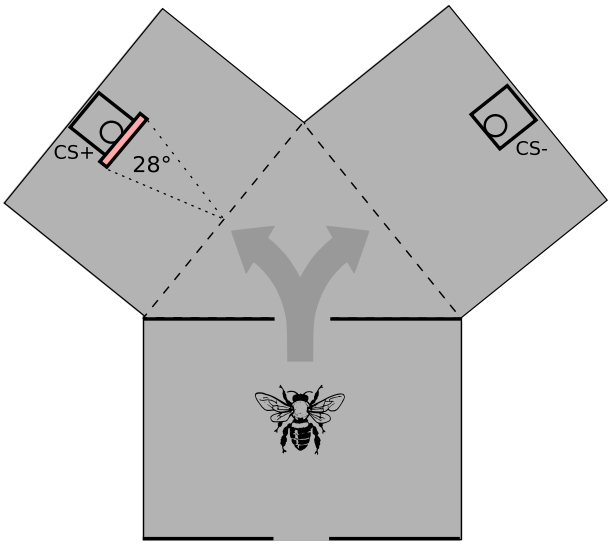

Fig. 1. Bees fly through the Y-maze to reach the decision point. A choice is determined when the bee crosses one of the decision lines (dotted).

Data analysis

We used generalised linear mixed models (GLMMs) with a logit link function only including the intercept as a fixed term to determine whether the mean proportion of correct choices towards the three achromatic stimuli and chromatic positive control stimulus differed significantly from the 50% chance level. Bee ID was included as a random effect term in the models to account for variation in the responses among the bees.

To determine the potential influence of the four main effects (green contrast, colour contrast, blue contrast, and brightness contrast) on the proportion of correct choices made by bees towards the three achromatic stimuli, we formulated six different GLMMs (collectively known as *Models: 1*), where each model included only two of the main effects at a time and a random effect of Bee ID (**Table S2**). Blue contrast was included in this analysis as honey bees have been found to have a blue colour preference that may potentially interfere with achromatic processing (Morawetz et al. 2013). Values for the main effects were standardised prior to the analyses to ensure convergence.

To investigate how the significance of the four main effects could change when including colour stimuli in the analyses, we constructed an additional two sets of models (*Models: 2* and *Models: 3*). These models followed the same structure as *Models: 1* (**Table S2**), differing only in the data set used for constructing them. GLMMs from *Models: 2* were used to test the influence of the four main effects on the proportion of correct choices, when using behavioural data collected from the current study towards the three achromatic stimuli, and also choices towards the yellow control stimulus (HKS3N against GRY1). Therefore, the only difference between *Models: 1* and *Models: 2* was that *Models: 2* also included choices towards the positive control stimulus.

GLMMs from *Models: 3* were used to test the influence of the four main effects on the proportion of correct choices, but included choices towards the three achromatic stimuli, the yellow control stimulus, and choices towards HKS3N (against HKS92N) and HKS33N (against HKS92N) collected by Dyer et al. (2008) for free-flying honey bees using chromatic vision to detect stimuli. Therefore, the only difference between *Models: 2* and *Models: 3* was that *Models: 3* also included choices towards two chromatic stimuli from Dyer et al. (2008). The main effects within each model in *Models: 3* were then graphed separately to provide a qualitative interpretation of potential interaction effects that may occur when using a larger data set.

Extended training control experiment

To confirm our findings for the main group experiment, we individually tested an additional 14 free-flying honey bees using a Y-maze. In this experiment, bees were first provided with training for the salient yellow stimuli HKS3N that contained both chromatic and green contrast at a small visual angle of 10° for 30 decisions with appetitive-aversive differential conditioning to ensure a very high level of motivation and apparatus proficiency. These bees were then trained to try to detect the most salient achromatic stimulus (PNK1 on GRY1 background) at a small visual angle of 10° for 30 decisions. Finally, the bees were trained to detect the most salient achromatic stimulus (PNK1 on GRY1 background) at a large visual angle of 28° for 30 decisions (see **Supplementary Material 1**).

Results

We found no significant difference from the chance level (50%) in the mean proportion of correct choices towards the achromatic PNK1 against GRY1 stimulus ($z = 0.989$, $p = 0.323$, 95% confidence intervals (CIs): 0.466–0.603), the achromatic PNK2 against GRY2 stimulus ($z = 1.271$, $p = 0.204$, CIs: 0.476–0.613), and the achromatic PNK2 against GRY3 stimulus ($z = -0.141$, $p = 0.888$, CIs: 0.426–0.564; **Table 3**); see **Fig. 2**. However, we found a significant difference from the chance

Table 3. The mean proportion and standard error of correct choices by bees towards target stimuli.

Stimulus number	Stimulus type	Mean proportion of correct choices (%)	Standard error (%)
1	PNK 1 against GRY1 (achromatic)	53.5	±3.5
2	PNK2 against GRY2 (achromatic)	54.5	±3.5
3	PNK2 against GRY3 (achromatic)	49.5	±3.5
4	HKS3N against GRY1 (chromatic)	81.0	±2.8
5	HKS3N against HKS92N (chromatic)	87.8	±2.0
6	HKS33N against HKS92N (chromatic)	96.1	±1.1

Note: Choices towards stimulus numbers 5 and 6 were taken from Dyer et al. (2008). PNK, pink stimulus; GRY, grey stimulus; HKS3N, yellow stimulus; HKS33N, purple stimulus; HKS92N, grey stimulus.

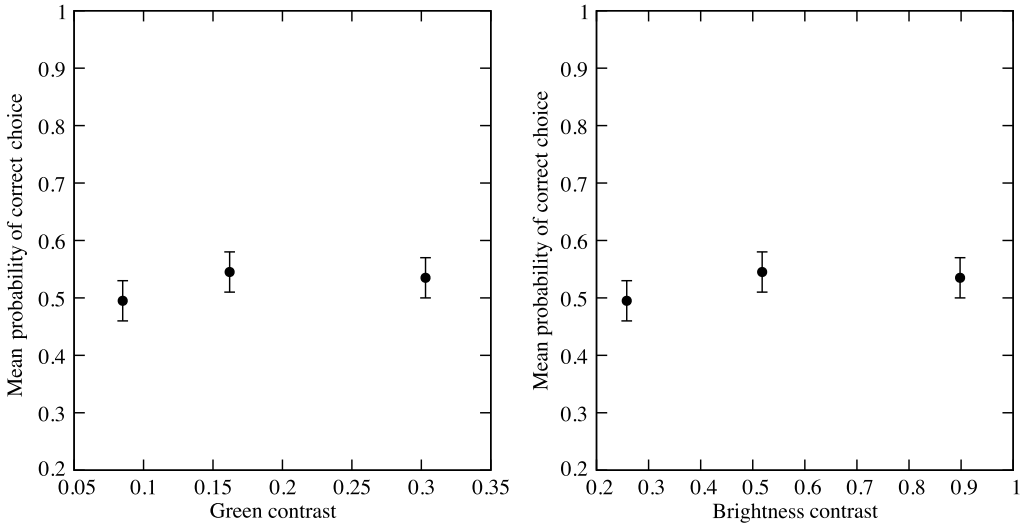

Fig. 2. Mean probability of correct choices for the three achromatic pink stimuli (colour knockouts) considering three different levels of green contrast (left) and brightness contrast (right) with standard errors. The results of neither green contrast nor brightness contrast were significantly different from chance (see the text for details).

level in the mean proportion of choices towards the chromatic positive control stimulus ($z = 5.688$, $p < 0.001$, CIs: 0.726–0.883; Table 3). This indicates that bees have the capacity to perform well in this visual task if they are able to detect the target stimulus.

GLMMs including only achromatic stimuli (*Models: 1*)

When honey bees made choices towards the three achromatic stimuli (PNK1 against GRY1, PNK2 against GRY2, and PNK2 against GRY3), the main effects of green contrast, colour contrast, blue contrast, and brightness contrast appeared to have no influence on the choices that were made. Specifically, none of the main effects from *Models: 1* had a significant effect on the proportion of correct choices (Table 4). These results suggest that honey bees use neither green contrast nor brightness contrast in colour perception. Furthermore, blue contrast does not appear to have any confounding effect on bee choices.

Table 4. The largest *p*-values for the main effects within each generalised linear mixed model (GLMM) (*Models: 1*).

GLMM	Fixed effects	*p*
I	Colour contrast + brightness contrast	>0.309
II	Green contrast + colour contrast	>0.309
III	Green contrast + brightness contrast	>0.414
IV	Colour contrast + blue contrast	>0.309
V	Green contrast + blue contrast	>0.417
VI	Blue contrast + brightness contrast	>0.421

Note: The full data with confidence intervals are in Table S3.

GLMMs including achromatic stimuli + chromatic positive control stimulus (*Models: 2*)

When honey bees made choices towards the three achromatic stimuli (PNK1 against GRY1, PNK2 against GRY2, and PNK2 against GRY3) and the positive control stimulus (HKS3N against GRY1), the main effects of green contrast, colour contrast, blue contrast and brightness contrast appeared to have some influence on the choices. Specifically, for all models in *Models: 2* where colour contrast was included as a main effect, of the two variables only colour contrast was found to be significant (Table 5). However, in models where colour contrast was not a factor, all variables such as green contrast, blue contrast, and brightness contrast were found to be significant. These results show that colour contrast strongly drives the proportion of correct choices made by bees, and without careful choice of stimuli that exclude colour contrast it is possible to run statistical models that produce false positive significant findings for secondary cues or traits like brightness.

GLMMs including achromatic stimuli + chromatic positive control stimulus + Dyer et al. (2008) chromatic stimuli (*Models: 3*)

When considering the choices made by honey bees towards the three achromatic stimuli (PNK1 against GRY1, PNK2 against GRY2, and PNK2 against GRY3), the chromatic positive control stimulus, and the two chromatic stimuli from Dyer et al. (2008), it appears that all four of the main effects (green contrast, colour contrast, blue contrast, and brightness contrast) have an effect on the

Table 5. The largest *p*-values for the main effects within each generalised linear mixed model (GLMM) (*Models: 2*).

GLMM	Fixed Effects	*p*
I	Colour contrast + brightness contrast	<0.001 (colour contrast only)
II	Green contrast + colour contrast	<0.001 (colour contrast only)
III	Green contrast + brightness contrast	<0.001
IV	Colour contrast + blue contrast	<0.001 (colour contrast only)
V	Green contrast + blue contrast	<0.001
VI	Blue contrast + brightness contrast	<0.001

Note: The full data with confidence intervals are in Table S4.

Table 6. The largest *p*-values for main effects within each generalised linear mixed models (GLMM) (*Models: 3*).

GLMM	Fixed effects	*p*
I	Colour contrast + brightness contrast	<0.001
II	Green contrast + colour contrast	<0.001
III	Green contrast + brightness contrast	<0.001
IV	Colour contrast + blue contrast	<0.01
V	Green contrast + blue contrast	<0.001
VI	Blue contrast + brightness contrast	<0.001

Note: The full data with confidence intervals are in Table S5.

proportion of correct choices that the bees made. Specifically, all main effects in *Models: 3* were found to be significant (Table 6). Therefore, it appears that although bees do not use brightness in a colour processing context as shown by *Models: 1*, including chromatic stimuli with high colour contrast into a statistical analysis may artificially inflate the importance of green contrast and brightness contrast, suggesting that strong correlations may exist between colour and brightness in naturally occurring spectra (as shown in the supplementary simulation analysis investigating these correlations; see Supplementary Material 1).

The main effects from each of the six models within *Models: 3* were graphed separately to demonstrate the potential complexity of colour stimuli. Although we did not have the statistical power to test for any interactions, as that was not the primary aim of the study, the intersecting relationships between the main effects within each test (Fig. 3) suggest that interactions are likely to occur if large natural databases of flower stimuli were evaluated. If these interactions are genuine, this would further demonstrate the difficulty in evaluating the elemental components of natural flower signals individually.

Extended training control experiment

In the extended training experiment, which was aimed at testing whether experience might confound the main experiment findings, we observed that even following extensive appetitive-aversive differential conditioning with the most salient achromatic stimulus, bees showed no evidence of being able to process this stimulus (Table S1). This was despite extensive priming to a salient stimulus (HKS3N) and, thus, experience with the experimental apparatus (see Supplementary Material 1).

Discussion

Although brightness and green contrast have been thought to be important cues when considering flower evolution (Smith et al. 2008; Hopkins and Rausher 2012; Renoult et al. 2013; Sletvold et al. 2016), our findings suggest that honey bees do not make use of such information when using colour vision. Bees were unable to detect the achromatic stimuli from the central region of colour space (Fig. S4) when presented at a large visual angle that mediates colour processing, even when brightness and green contrast were modulated over a broad range (Fig. 2). This finding was confirmed following extensive training with the most salient achromatic stimulus (see Supplementary Material 1). Consequently, we were unable to establish precise detection thresholds of achromatic cues in bee colour vision; it appears that bees do not process brightness information for single target visual processing (Daumer 1956; Menzel 1967; Reser et al. 2012; van der Kooi et al. 2018). Our findings are consistent with the colour modeling predictions for both the Hexagon and COC models as the

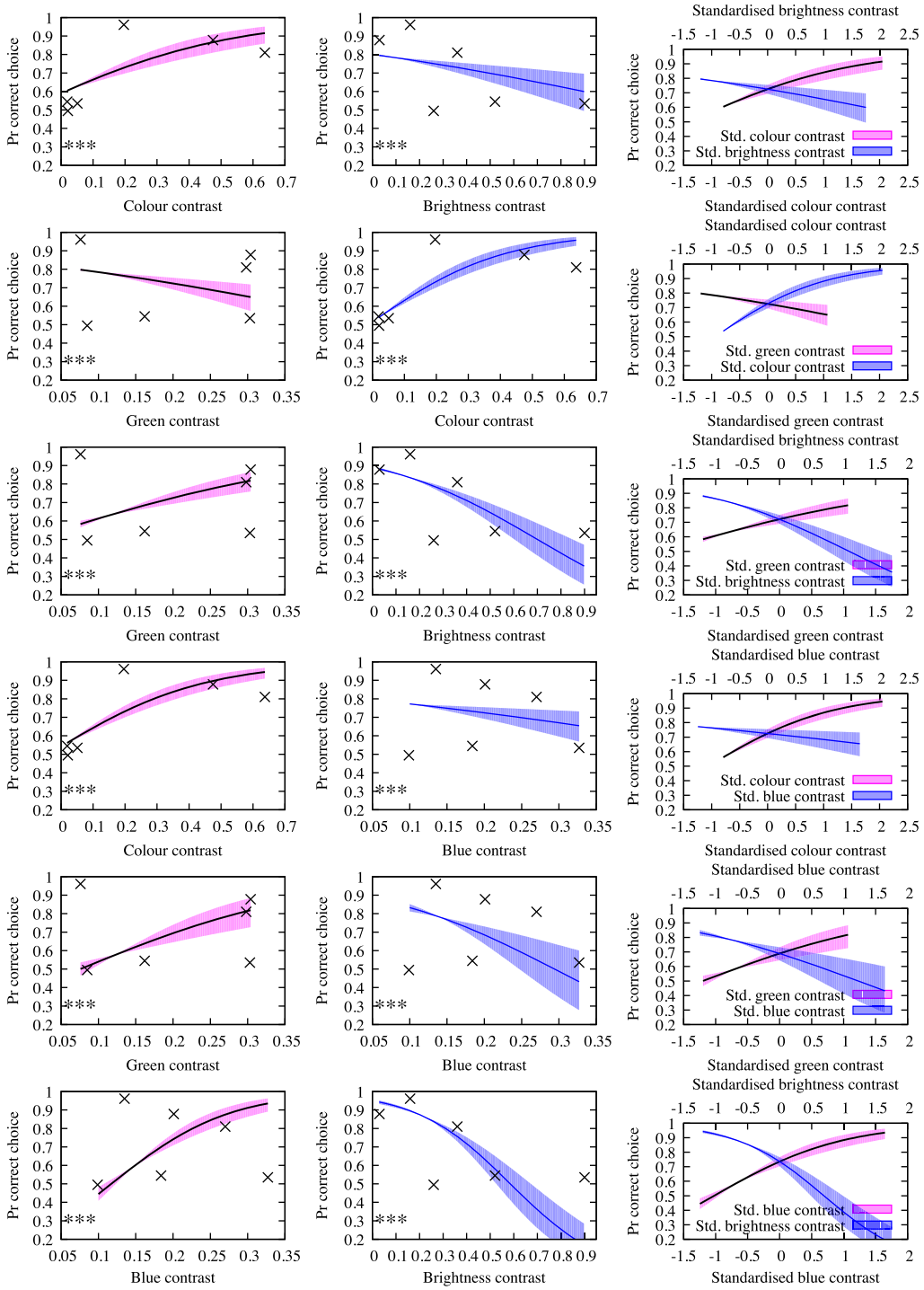

Fig. 3. Main effects of generalised linear mixed models (GLMMs) (*Models: 3*) including both achromatic and chromatic stimuli graphed separately in columns 1 and 2. *** represents $p < 0.001$ for each one of the six models only including the main effects. Each row represents one of the six GLMMs of *Models: 3*. Column 3 represents the main effects within each model graphed together. The coloured band surrounding each regression line is the 95% confidence interval. "×"s represent the mean proportion (Pr) of correct choices towards each stimulus.

stimuli are below the perceptual threshold for honey bees (**Table 2**), but our results are not consistent with the RNL (colour) model, which predicted that bees should have been able to process the most salient achromatic stimulus, and the achromatic RNL model actually predicts that all three stimuli should have been easily processed using "brightness" cues. However, although the RNL-achromatic model is based on combined physiological parameters from several vertebrate species, it has not been experimentally validated for bees. We, thus, show in the current study that RNL modeling is not well validated for bee vision considering achromatic processing, and recent work suggests the model is also poor for colour modeling of bee behaviour (Avarguès-Weber et al. 2010; Garcia et al. 2017b). Thus, if RNL modeling is to be used for other animals it will be essential to conduct behavioural experiments to validate RNL model predictions. The fact that bees seem to be unable to use achromatic cues in colour tasks agrees with recent findings that flowers do not produce more white signals in low intensity illumination environments (Binkenstein and Schaefer 2015), and that flowers rarely reflect more than 50% of the incident light, regardless of dominant wavelength, suggesting that higher reflectance does not increase the conspicuousness of the flowers (van der Kooi et al. 2016). However, bees are tuned to the chromatic signals provided by flowers (Dyer et al. 2012).

Our findings show that caution is required when investigating pollinator-mediated selection of flower colours, as it is possible that the importance of traits such as brightness may be confounded by their strong relationships with colour when processed by the visual system of an insect pollinator. This is likely the reason why brightness contrast and green contrast were found to be significant main effects in GLMMs where chromatic stimuli were included in the data set (*Models: 2* and *3*), as the addition of these chromatic stimuli may be driving the significance of brightness contrast and green contrast as a consequence of being directly correlated with these variables (**Fig. S2**). Therefore, it is possible that the individual relationships between variables in *Models 2* and *3* may be biologically meaningless (**Fig. 3**). Without considering the psychophysical evidence showing that bees cannot actually detect stimuli when chromatic contrast is low (**Fig. 2**), it can be tempting to conclude that brightness and green contrast are both important, as suggested by a simple interpretation of the statistical analysis. Therefore, it is difficult to disentangle the effect of chromatic information from brightness or green contrast when attempting to interpret the importance of chromatic or achromatic traits individually. A qualitative analysis of the main effects from *Models: 3* revealed that potential interactions may exist between all variables of interest. This provides further evidence regarding the complexity of colour as a signal, and the difficulty in disentangling the effects of each factor from the others. These findings call into question whether it is appropriate to investigate these factors individually, as is commonly done in studies exploring flower colours (Smith et al. 2008; Caruso et al. 2010; Hopkins and Rausher 2012; Renoult et al. 2013; Wassink and Caruso 2013; Sletvold et al. 2016). Specifically, colour, by definition, is a construct of an animals' brain (Lennie 2000; Dyer 2012), and quality behavioural data should be carefully considered when attempting to assess colour information processing in animals.

Our findings are supported by early psychophysical and behavioural experiments that suggested that bees ignore brightness cues when processing colour stimuli (Daumer 1956; Menzel 1967). They are also consistent with studies investigating the role of visual angle on honey bee colour processing, as bees were unable to detect the achromatic cues at a large visual angle (Giurfa et al. 1996; Giurfa and Vorobyev 1998). This inability to process the achromatic cues is likely to be independent of potential attentional confounds, as we trained bees using an appetitive-aversive differential conditioning framework. Therefore, it appears that bees do not process brightness as a dimension of colour perception for single target detection. Achromatic information might only be processed separately for special tasks like motion perception (von Hess 1913; Kaiser and Liske 1974; Stojcev et al. 2011). This conclusion is further supported by neuroanatomical and electrophysiological studies that suggest that brightness and colour information are processed in parallel and independent neural pathways in the bee brain (Paulk et al. 2008; Dyer et al. 2011). In the honey bee neuroanatomy, visual information is

passed from photoreceptors in the eye to the lamina, the lobulla, and then to higher colour processing areas (Dyer et al. 2011). The lamina primarily receives input from long-wavelength-sensitive photoreceptors (Menzel 1974; Ribi 1975; Dyer et al. 2011). Alternatively, information received from the short-wavelength-sensitive and medium-wavelength-sensitive photoreceptors are instead passed through the lamina directly to the medulla, and then passed onwards to higher colour processing areas (Dyer et al. 2011). Despite these independent neural pathways, honey bees are able to detect achromatic signals at large visual angles if green contrast is sufficiently high (e.g., black against white; Giurfa et al. 1996). As the brightest stimulus from our study had a green contrast of 0.3, bees may be able detect an achromatic signal with a green contrast greater than this, but such stimuli are also likely to modulate chromatic contrast.

Although honey bees may not use brightness cues when viewing flower colours, the green contrast of a flower in the presence of chromatic contrast appears to allow bees to more easily detect it against the background when viewed from a small visual angle (Giurfa et al. 1996; Giurfa and Vorobyev 1998; Bukovac et al. 2017). However, Giurfa et al. (1996) found that for honey bees, one stimulus (HKS-21N) that lacked chromatic contrast but contained green contrast was poorly processed by bees at a small visual angle, and our control experiment yielded results consistent with this finding (see **Supplementary Material 1**). Colour vision is, therefore, mainly used when bees approach a flower and it subtends a large visual angle (Giurfa et al. 1996). It is also possible that insect pollinators other than bees include brightness as a dimension of colour vision, and it may be these species that drive the selection of brightness cues in flowers. For example, diurnal hawkmoths and *Papilio* butterflies have been found to use achromatic cues when landing on or probing artificial flowers (Koshitaka et al. 2011; Goyret and Kelber 2012). Further research is, thus, required to elucidate how other insect pollinators process brightness cues in floral displays.

The fact that chromatic and achromatic processing appears to occur independently in honey bees poses an interesting question: Why is this separation more pronounced in bees than in other animals such as primates? For primates, it is plausible that brightness may allow for an improved ability to discriminate between stimuli, especially as interactions between colour and brightness can change the appearance of an object under different viewing conditions (Xing et al. 2015). Sexual selection and foraging demands that are uniquely experienced by primates are also likely to have influenced the evolution of a colour visual system including brightness as a dimension (Surridge et al. 2003; Fernandez and Morris 2007). In contrast, honey bees may have little use for brightness information in a foraging context and, therefore, their colour vision may be sufficient for discriminating between flower species. One possible explanation for this segregation is that the inclusion of brightness information in colour processing may confound its initial purpose of solving the problem of lightness constancy (von Campenhausen 1986; Maximov 2000). Achromatic vision has been shown to be less reliable than colour vision under changing light conditions (Maximov 2000; Kelber et al. 2003). This is because changes in illumination can result in large variations in receptor signals (Kelber et al. 2003). Therefore, it has been proposed that the evolution of colour vision was a solution to lightness constancy (von Campenhausen 1986; Maximov 2000). Opponency between two spectrally distinct photoreceptors allows for the ratio of light in a scene to be calculated; therefore, the changing signal of background illumination can be differentiated from other changes in the visual scene (Maximov 2000). If colour vision is indeed a solution to lightness constancy then it would make little sense to feed brightness information into colour processing, as this may result in unreliable output signals. Furthermore, a recent study investigating the dorsal ocelli in honey bees revealed that the ocellar photoreceptors are able to provide information regarding the spectral quality of ambient light conditions to the visual system (Garcia et al. 2017a). This information is then integrated with colour signals from the frontal compound eyes through a direct neural pathway to allow for a highly accurate reconstruction of flower colour. Therefore, it may not be important to process brightness information

captured by the frontal compound eyes, as the required spectral information necessary for solving lightness constancy issues is already provided by the dorsal ocelli.

Conclusion

In this study, we found that honey bees were unable to use brightness information at a large visual angle, which mediates colour processing, even when appetitive-aversive differential conditioning was employed. Therefore, we were unable to establish precise detection thresholds for achromatic cues in bee colour vision; in fact, such a threshold does not appear to exist. This has important implications for ecological studies, as our findings suggest that brightness defined as either green contrast or brightness contrast should not be important when considering how honey bee colour vision has affected flower signal evolution. Brightness contrast and green contrast appear to be both strongly correlated with colour contrast, making it difficult to interpret the effect of brightness in isolation. It would be of value to test for interactions between colour contrast, green contrast, brightness contrast, and blue contrast in other flower pollination vectors in future research to shed more light on this important question.

Acknowledgements

We thank Devi Stuart-Fox, Mani Shrestha, and Scarlett Howard for their valuable comments, discussions, and support for this research. We are also appreciative of insightful comments by two reviewers and the editor on previous versions of our manuscript. AGD acknowledges the Australian Research Council Discovery Project DP160100161 for support.

Author contributions

LN, JEG, and AGD conceived and designed the study. LN performed the experiments/collected the data. LN, JEG, and AGD analyzed and interpreted the data. AGD contributed resources. LN, JEG, and AGD drafted or revised the manuscript.

Competing interests

The authors have declared that no competing interests exist.

Data accessibility statement

All relevant data are within the paper and in the Supplementary Material.

Supplementary material

The following Supplementary Material is available with the article through the journal website at doi:10.1139/facets-2017-0116.

Supplementary Material 1

References

Avarguès-Weber A, de Brito Sanchez MG, Giurfa M, and Dyer AG. 2010. Aversive reinforcement improves visual discrimination learning in free-flying honeybees. PLoS ONE, 5(10): e15370. PMID: 20976170 DOI: 10.1371/journal.pone.0015370

Backhaus W. 1991. Color opponent coding in the visual system of the honeybee. Vision Research, 31(7): 1381–1397. PMID: 1891826 DOI: 10.1016/0042-6989(91)90059-E

Barry KL, White TE, Rathnayake DN, Fabricant SA, and Herberstein ME. 2015. Sexual signals for the colour-blind: cryptic female mantids signal quality through brightness. Functional Ecology, 29(4): 531–539. DOI: 10.1111/1365-2435.12363

Binkenstein J, and Schaefer HM. 2015. Flower colours in temperate forest and grassland habitats: a comparative study. Arthropod-Plant Interactions, 9(3): 289–299. DOI: 10.1007/s11829-015-9369-9

Briscoe AD, and Chittka L. 2001. The evolution of color vision in insects. Annual Review of Entomology, 46(1): 471–510. DOI: 10.1146/annurev.ento.46.1.471

Bukovac Z, Shrestha M, Garcia JE, Burd M, Dorin A, and Dyer AG. 2017. Why background colour matters to bees and flowers. Journal of Comparative Physiology A, 203(5): 369–380. PMID: 28478535 DOI: 10.1007/s00359-017-1175-7

Burns B, and Shepp BE. 1988. Dimensional interactions and the structure of psychological space: the representation of hue, saturation, and brightness. Attention, Perception, & Psychophysics, 43(5): 494–507. PMID: 3380640 DOI: 10.3758/BF03207885

Caruso CM, Scott SL, Wray JC, and Walsh CA. 2010. Pollinators, herbivores, and the maintenance of flower color variation: a case study with *Lobelia siphilitica*. International Journal of Plant Sciences, 171(9): 1020–1028. DOI: 10.1086/656511

Chittka L. 1992. The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. Journal of Comparative Physiology A, 170(5): 533–543. DOI: 10.1007/BF00199331

Clery S, Bloj M, and Harris JM. 2013. Interactions between luminance and color signals: effects on shape. Journal of Vision, 13(5): 16. PMID: 23599420 DOI: 10.1167/13.5.16

Croner LJ, and Albright TD. 1999. Seeing the big picture: integration of image cues in the primate visual system. Neuron, 24(4): 777–789. PMID: 10624942 DOI: 10.1016/S0896-6273(00)81026-0

Daumer K. 1956. Reizmetrische untersuchung des Farbensehens der Bienen. Zeitschrift für vergleichende Physiologie, 38(5): 413–478. PMID: 19349661 DOI: 10.1007/BF00340456

Dyer AG. 2012. Psychophysics of honey bee color processing in complex environments. *In* Honeybee neurobiology and behavior: a tribute to Randolf Menzel. *Edited by* CG Galizia, D Eisenhardt, and M Giurfa. Springer Netherlands, Dordrecht, The Netherlands. pp. 303–314.

Dyer AG, and Chittka L. 2004. Fine colour discrimination requires differential conditioning in bumblebees. Naturwissenschaften, 91(5): 224–227. PMID: 15146269 DOI: 10.1007/s00114-004-0508-x

Dyer AG, and Neumeyer C. 2005. Simultaneous and successive colour discrimination in the honeybee (*Apis mellifera*). Journal of Comparative Physiology A, 191(6): 547–557. PMID: 15871026 DOI: 10.1007/s00359-005-0622-z

Dyer AG, Spaethe J, and Prack S. 2008. Comparative psychophysics of bumblebee and honeybee colour discrimination and object detection. Journal of Comparative Physiology A, 194(7): 617–627. PMID: 18437390 DOI: 10.1007/s00359-008-0335-1

Dyer AG, Paulk AC, and Reser DH. 2011. Colour processing in complex environments: insights from the visual system of bees. Proceedings of the Royal Society B: Biological Sciences, 278(1707): 952–959. PMID: 21147796 DOI: 10.1098/rspb.2010.2412

Dyer AG, Boyd-Gerny S, McLoughlin S, Rosa MG, Simonov V, and Wong BB. 2012. Parallel evolution of angiosperm colour signals: common evolutionary pressures linked to hymenopteran vision. Proceedings of the Royal Society B: Biological Sciences, 279(1742): 3606–3615. PMID: 22673351 DOI: 10.1098/rspb.2012.0827

Fernandez AA, and Morris MR. 2007. Sexual selection and trichromatic color vision in primates: statistical support for the preexisting-bias hypothesis. The American Naturalist, 170(1): 10–20. PMID: 17853988 DOI: 10.1086/518566

Garcia JE, Hung Y-S, Greentree AD, Rosa MG, Endler JA, and Dyer AG. 2017a. Improved color constancy in honey bees enabled by parallel visual projections from dorsal ocelli. Proceedings of the National Academy of Sciences of the USA, 114(29): 7713–7718. PMID: 28673984 DOI: 10.1073/pnas.1703454114

Garcia JE, Spaethe J, and Dyer AG. 2017b. The path to colour discrimination is S-shaped: behaviour determines the interpretation of colour models. Journal of Comparative Physiology A, 203(12): 983–997. PMID: 28866838 DOI: 10.1007/s00359-017-1208-2

Giurfa M, and Vorobyev M. 1998. The angular range of achromatic target detection by honey bees. Journal of Comparative Physiology A, 183(1): 101–110. DOI: 10.1007/s003590050238

Giurfa M, Vorobyev M, Kevan P, and Menzel R. 1996. Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. Journal of Comparative Physiology A, 178(5): 699–709. DOI: 10.1007/BF00227381

Goyret J, and Kelber A. 2012. Chromatic signals control proboscis movements during hovering flight in the hummingbird hawkmoth *Macroglossum stellatarum*. PLoS ONE, 7(4): e34629. PMID: 22529922 DOI: 10.1371/journal.pone.0034629

Hempel de Ibarra N, and Giurfa M. 2003. Discrimination of closed coloured shapes by honeybees requires only contrast to the long wavelength receptor type. Animal Behaviour, 66(5): 903–910. DOI: 10.1006/anbe.2003.2269

Hempel de Ibarra N, Vorobyev M, Brandt R, and Giurfa M. 2000. Detection of bright and dim colours by honeybees. Journal of Experimental Biology, 203(21): 3289–3298. PMID: 11023849

Hertel H. 1980. Chromatic properties of identified interneurons in the optic lobes of the bee. Journal of Comparative Physiology A, 137(3): 215–231. DOI: 10.1007/BF00657117

Hopkins R, and Rausher MD. 2012. Pollinator-mediated selection on flower color allele drives reinforcement. Science, 335(6072): 1090–1092. PMID: 22300852 DOI: 10.1126/science.1215198

Judd DB, MacAdam DL, Wyszecki G, Budde H, Condit H, Henderson S, et al. 1964. Spectral distribution of typical daylight as a function of correlated color temperature. Journal of the Optical Society of America, 54(8): 1031–1040. DOI: 10.1364/JOSA.54.001031

Kaiser W, and Liske E. 1974. Die optomotorischen reaktionen von fixiert fliegenden bienen bei reizung mit spektrallichtern. Journal of Comparative Physiology A, 89(4): 391–408. DOI: 10.1007/BF00695355

Kelber A. 2005. Alternative use of chromatic and achromatic cues in a hawkmoth. Proceedings of the Royal Society B: Biological Sciences, 272(1577): 2143–2147. PMID: 16191627 DOI: 10.1098/rspb.2005.3207

Kelber A, Vorobyev M, and Osorio D. 2003. Animal colour vision—behavioural tests and physiological concepts. Biological Reviews, 78(1): 81–118. PMID: 12620062 DOI: 10.1017/S1464793102005985

Kien J, and Menzel R. 1977. Chromatic properties of interneurons in the optic lobes of the bee. Journal of Comparative Physiology A, 113(1): 35–53. DOI: 10.1007/BF00610452

Koethe S, Bossems J, Dyer AG, and Lunau K. 2016. Colour is more than hue: preferences for compiled colour traits in the stingless bees *Melipona mondury* and *M. quadrifasciata*. Journal of Comparative Physiology A, 202(9–10): 615–627. PMID: 27480640 DOI: 10.1007/s00359-016-1115-y

Koshitaka H, Arikawa K, and Kinoshita M. 2011. Intensity contrast as a crucial cue for butterfly landing. Journal of Comparative Physiology A, 197(11): 1105–1112. PMID: 21847617 DOI: 10.1007/s00359-011-0671-4

Lehrer M, and Bischof S. 1995. Detection of model flowers by honeybees: the role of chromatic and achromatic contrast. Naturwissenschaften, 82(3): 145–147. DOI: 10.1007/BF01177278

Lennie P. 2000. Color vision: putting it together. Current Biology, 10(16): R589–R591. PMID: 10985374 DOI: 10.1016/S0960-9822(00)00632-1

Livingstone M, and Hubel D. 1988. Segregation of form, color, movement, and depth: anatomy, physiology, and perception. Science, 240(4853): 740–749. PMID: 3283936 DOI: 10.1126/science.3283936

Lunau K, and Maier EJ. 1995. Innate colour preferences of flower visitors. Journal of Comparative Physiology A, 177(1): 1–19. DOI: 10.1007/BF00243394

Maximov VV. 2000. Environmental factors which may have led to the appearance of colour vision. Philosophical Transactions of the Royal Society B: Biological Sciences, 355(1401): 1239–1242. PMID: 11079406 DOI: 10.1098/rstb.2000.0675

Menzel R. 1967. Untersuchungen zum erlernen von spektralfarben durch die honigbiene (*Apis mellifera*). Journal of Comparative Physiology A, 56(1): 22–62. DOI: 10.1007/BF00333562

Menzel R. 1974. Spectral sensitivity of monopolar cells in the bee lamina. Journal of Comparative Physiology A, 93(4): 337–346. DOI: 10.1007/BF00606801

Miller R, Owens SJ, and Rørslett B. 2011. Plants and colour: flowers and pollination. Optics & Laser Technology, 43(2): 282–294. DOI: 10.1016/j.optlastec.2008.12.018

Morawetz L, Svoboda A, Spaethe J, and Dyer AG. 2013. Blue colour preference in honeybees distracts visual attention for learning closed shapes. Journal of Comparative Physiology A, 199(10): 817–827. PMID: 23918312 DOI: 10.1007/s00359-013-0843-5

Nassi JJ, and Callaway EM. 2009. Parallel processing strategies of the primate visual system. Nature Reviews Neuroscience, 10(5): 360–372. PMID: 19352403 DOI: 10.1038/nrn2619

Paulk AC, Phillips-Portillo J, Dacks AM, Fellous J-M, and Gronenberg W. 2008. The processing of color, motion, and stimulus timing are anatomically segregated in the bumblebee brain. Journal of Neuroscience, 28(25): 6319–6332. PMID: 18562602 DOI: 10.1523/JNEUROSCI.1196-08.2008

Peitsch D, Fietz A, Hertel H, de Souza J, Ventura DF, and Menzel R. 1992. The spectral input systems of hymenopteran insects and their receptor-based colour vision. Journal of Comparative Physiology A, 170(1): 23–40. PMID: 1573568 DOI: 10.1007/BF00190398

Renoult J, Thomann M, Schaefer H, and Cheptou PO. 2013. Selection on quantitative colour variation in *Centaurea cyanus*: the role of the pollinator's visual system. Journal of Evolutionary Biology, 26(11): 2415–2427. PMID: 24070120 DOI: 10.1111/jeb.12234

Reser DH, Wijesekara Witharanage R, Rosa MGP, and Dyer AG. 2012. Honeybees (*Apis mellifera*) learn color discriminations via differential conditioning independent of long wavelength (green) photoreceptor modulation. PLoS ONE, 7(11): e48577. PMID: 23155394 DOI: 10.1371/journal.pone.0048577

Ribi WA. 1975. The first optic ganglion of the bee. I. Correlation between visual cell types and their terminals in the lamina and medulla. Cell and Tissue Research, 165(1): 103–111. PMID: 1203968 DOI: 10.1007/BF00222803

Siddiqi A, Cronin TW, Loew ER, Vorobyev M, and Summers K. 2004. Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. Journal of Experimental Biology, 207(14): 2471–2485. PMID: 15184519 DOI: 10.1242/jeb.01047

Sletvold N, Trunschke J, Smit M, Verbeek J, and Ågren J. 2016. Strong pollinator-mediated selection for increased flower brightness and contrast in a deceptive orchid. Evolution, 70(3): 716–724. PMID: 26878831 DOI: 10.1111/evo.12881

Smith SD, Ané C, and Baum DA. 2008. The role of pollinator shifts in the floral diversification of *Iochroma* (Solanaceae). Evolution, 62(4): 793–806. PMID: 18208567 DOI: 10.1111/j.1558-5646.2008.00327.x

Spaethe J, Tautz J, and Chittka L. 2001. Visual constraints in foraging bumblebees: flower size and color affect search time and flight behavior. Proceedings of the National Academy of Sciences of the USA, 98(7): 3898–3903. PMID: 11259668 DOI: 10.1073/pnas.071053098

Stach S, Benard J, and Giurfa M. 2004. Local-feature assembling in visual pattern recognition and generalization in honeybees. Nature, 429(6993): 758–761. PMID: 15201910 DOI: 10.1038/nature02594

Stavenga DG, and van der Kooi CJ. 2016. Coloration of the Chilean Bellflower, *Nolana paradoxa*, interpreted with a scattering and absorbing layer stack model. Planta, 243(1): 171–181. PMID: 26369332 DOI: 10.1007/s00425-015-2395-0

Stojcev M, Radtke N, D'Amaro D, Dyer AG, and Neumeyer C. 2011. General principles in motion vision: color blindness of object motion depends on pattern velocity in honeybee and goldfish. Visual Neuroscience, 28(4): 361–370. PMID: 21518470 DOI: 10.1017/S0952523811000101

Surridge AK, Osorio D, and Mundy NI. 2003. Evolution and selection of trichromatic vision in primates. Trends in Ecology & Evolution, 18(4): 198–205. DOI: 10.1016/S0169-5347(03)00012-0

van der Kooi CJ, Elzenga JTM, Staal M, and Stavenga DG. 2016. How to colour a flower: on the optical principles of flower coloration. Proceedings of the Royal Society B: Biological Sciences, 283(1830): 20160429. PMID: 27170723 DOI: 10.1098/rspb.2016.0429

van der Kooi CJ, Elzenga JTM, Dijksterhuis J, and Stavenga DG. 2017. Functional optics of glossy buttercup flowers. Journal of the Royal Society Interface, 14(127): 20160933. PMID: 28228540 DOI: 10.1098/rsif.2016.0933

van der Kooi CJ, Dyer AG, Kevan PG, and Lunau K. 2018. Functional significance of the optical properties of flowers for visual signalling. Annals of Botany. DOI: 10.1093/aob/mcy119

von Campenhausen C. 1986. Photoreceptors, lightness constancy and color vision. Naturwissenschaften, 73(11): 674–675. PMID: 3808080 DOI: 10.1007/BF00366692

von Frisch K. 1913. Weitere untersuchungen über den farbensinn der fische. Zoologische Jahrbücher. Abteilung für allgemeine Zoologie und Physiologie der Tiere, 34: 43–68.

von Frisch K. 1914. Der farbensinn und formensinn der biene. Zoologische Jahrbücher. Abteilung für allgemeine Zoologie und Physiologie der Tiere, 37: 1–238.

von Helversen O. 1972. Zur spektralen unterschiedsempfindlichkeit der honigbiene. Journal of Comparative Physiology A, 80(4): 439–472. DOI: 10.1007/BF00696438

von Hess C. 1913. Experimentelle untersuchungen über den angeblichen farbensinn der bienen. Zoologische Jahrbücher. Abteilung für allgemeine Zoologie und Physiologie der Tiere, 34: 81–106.

Vorobyev M, and Osorio D. 1998. Receptor noise as a determinant of colour thresholds. Proceedings of the Royal Society B: Biological Sciences, 265(1394): 351–358. PMID: 9523436 DOI: 10.1098/rspb.1998.0302

Vorobyev M, Brandt R, Peitsch D, Laughlin SB, and Menzel R. 2001. Colour thresholds and receptor noise: behaviour and physiology compared. Vision Research, 41(5): 639–653. PMID: 11226508 DOI: 10.1016/S0042-6989(00)00288-1

Wassink E, and Caruso CM. 2013. Effect of coflowering *Mimulus ringens* on phenotypic selection on floral traits of gynodioecious *Lobelia siphilitica*. Botany, 91(11): 745–751. DOI: 10.1139/cjb-2013-0112

Wyszecki G, and Stiles WS. 1982. Color science: concepts and methods, quantitative data and formulae. 2nd edition. Wiley, New York, New York.

Xing D, Ouni A, Chen S, Sahmoud H, Gordon J, and Shapley R. 2015. Brightness-color interactions in human early visual cortex. Journal of Neuroscience, 35(5): 2226–2232. PMID: 25653377 DOI: 10.1523/JNEUROSCI.3740-14.2015