Original Article

Iridescence untwined: honey bees can separate hue variations in space and time

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Iridescence is a phenomenon whereby the hue of a surface changes with viewing or illumination angle. Many animals display iridescence but it currently remains unclear whether relevant observers process iridescent color signals as a complex collection of colors (spatial variation), or as moving patterns of colors and shapes (temporal variation). This is important as animals may use only the spatial or temporal component of the signal, although this possibility has rarely been considered or tested. Here, we investigated whether honey bees could separate the temporal and spatial components of iridescence by training them to discriminate between iridescent disks and photographic images of the iridescent patterns presented by the disks. Both stimuli therefore contained spatial color variation, but the photographic stimuli do not change in hue with varying angle (no temporal variation). We found that individual bee observers could discriminate the variable patterns of iridescent disks from static photographs during unrewarded tests. Control experiments showed that bees reliably discriminated iridescent disks from control silver disks, showing that bees were processing chromatic cues. These results suggest that honey bees could selectively choose to attend to the temporal component of iridescent signals to make accurate decisions.

Key words: ***Apis mellifera*, behavior, color vision, perception, spatial, temporal.**

INTRODUCTION

The beautiful collection of changing colors found in peacock feathers, butterfly wings, or beetle elytra (Loyau et al. 2007; Stavenga et al. 2011; Wilts et al. 2011) are all examples of iridescence: an optical property characteristic of periodic structures, giving rise to a change in hue with the viewing angle, or illumination geometry. This dynamic phenomenon is visually striking to the human eye, and consequently, iridescent color patches have often been considered to serve as dynamic communication signals in animals (Doucet and Meadows 2009; Stuart-Fox et al. 2020). However, this visual effect depends on both the properties of the material as well as the observer's visual system. Whilst from a materials perspective, the degree of iridescence can be quantified as the shift of the reflectance spectrum towards shorter wavelengths (commonly abbreviated as "blue shift" because blue corresponds to wavelengths at the shorter end of the visual spectrum), the biological relevance of iridescence depends on how these changes are perceived by relevant observers.

One obstacle to exploring the biological relevance of iridescence may be our treatment of iridescence as a one-dimensional property. Iridescent color patches change appearance across both space and time and can thus be separated into temporal and/or spatial components, though this separation is not widely appreciated or carefully considered in previous literature on iridescence. For example, movements of the signal, or the observer, can cause the visual angle to shift, resulting in potentially perceivable changes in hue over time. This shift in hue over time can create dynamic visual effects such as flashing or moving color patterns – similar to color shifting security features on banknotes and passports. Alternatively, an iridescent color patch may also contain static color features that show spatial variation independent of temporal changes. For example, if the color patch is large or not perfectly flat, the angle between the signaler, and the observer can differ for certain sections of the patch. This can create a visual effect where the patch may appear as a spatially complex mosaic of colors, analogous to the rainbow of colors seen in a soap bubble. If animals are able to separately attend to the spatial or temporal components of iridescence, we can then ask which components of iridescence are most relevant for signaling or camouflage.

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Observers may process either the temporal or spatial component of iridescence depending on the specific characteristics of their visual system. For example, whether a signal appears as a flash or as a gradually changing color depends on the temporal resolution of the animal's visual system. Alternatively, iridescent color patches may be perceived as a spatially complex mosaic of colors only if the animal's visual system has enough spatial resolution to resolve the different chromatic components of the stimulus. Furthermore, animals may not necessarily attend to the color of the signal, but may instead focus on its other properties such as brightness or motion (Stuart-Fox et al. 2020). The distinction between temporal and spatial components of iridescence is particularly important given that iridescence is widespread in nature and has a multitude of proposed functions (Doucet and Meadows 2009). Iridescent behavioral displays are often simply assumed to be temporally dynamic signals without considering that observers may process and prioritize alternative aspects of an iridescent display such as the spatial collection of colors (Morehouse and Rutowski 2009). This is particularly important in cases where the function of iridescence is unclear, as studying its potential ecological role requires an initial understanding of how such information is processed in the brain.

In this study, we demonstrate the relevance of separately considering the temporal and spatial components of iridescence by investigating if the European honey bee (*Apis mellifera*) can separately attend to only one component to enable accurate decision making. Honey bees are a major model of animal color vision (von Frisch 1914; von Frisch 1967) and enables a detailed understanding of visual processing within the brain. Bee visual perception is enabled by three spectrally distinct photoreceptors contained within the ommatidia, which subsequently project inputs to sequential processing areas including the lamina, the medulla, and the lobula, before integration processing by the mushroom body (Menzel 1974; Hertel and Maronde 1987; Horst et al. 1987; Maddess and Yang 1997; Paulk et al. 2008). The first evidence of chromatic processing occurs in the lobula of the bumble bee brain (which shares a similar visual system to honey bees; Dyer et al. 2011), where there are six anatomically distinct layers of neurons (Paulk et al. 2008). Individual neurons within different layers show responses to stimulus motion, chromatic properties, or both. Thus, the bumble bee brain contains neurons that can process the temporal (motion) and spatial (chromatic) components of iridescence separately, as well as neurons that can jointly respond to both these components.

These insights are well supported by behavioral evidence as bees can be trained to distinguish between colors (Menzel 1967; Reser et al. 2012; Avarguès-Weber and Giurfa 2014), between motion cues (Hori et al. 2007; Mirwan and Kevan 2014), and between the motion of colors (Stojcev et al. 2011) – although this is only possible for small objects at low temporal frequencies (e.g. 6 or 12 hz). This suggests that bees can potentially perceive relatively slow color changes such as those on an iridescent surface. Indeed, previous works have shown that bumble bees can perceive and discriminate between iridescent colors when intensively trained using appetitive aversive differential conditioning (Whitney et al. 2009). Bumble bees have also been shown to have difficulty recognising shapes and objects due to the shifting color and intensity boundaries produced by iridescence (Kjernsmo et al. 2018). However, it is unknown how the temporal or spatial properties of iridescence have contributed to this effect. Overall, bees have the neural capacity to separately process and integrate the temporal and spatial components of iridescence, but it is not clear how they actually perceive these distinct properties within a complex stimulus, and whether they can separately attend to them during decision making.

To determine whether honey bees can separate the temporal and spatial components of iridescence, we test whether bees can differentiate an iridescent disc versus static photographic images of the iridescent patterns presented by the disks. These photographic stimuli contain a similar variety of spatial variation in colors observed in a true iridescent stimulus without the temporal change in hue. This was confirmed using spectral measurements and visual modeling to understand how such stimuli would be perceived by bee observers. If bees can discriminate between the stimuli during behavioral experiments, this suggests that they can selectively attend to the temporal component and separate this from the spatial component. This would be consistent with their neural capacity to process color and motion in independent visual pathways. If bees fail at this task, this suggests that either they are unable to disentangle these components, or that bees primarily process iridescence as a spatial signal and ignore the temporal changes in hue. This outcome would indicate that neurons which respond to chromatic properties are more important in the processing of iridescent stimuli than those that only respond to motion.

METHODS

Stimuli

We used 38 mm diameter metallicized PVC discs coated with an iridescent thin film as our iridescent stimulus (displaying both spatial and temporal components of iridescence). We used the same discs without the iridescent coating as our non-iridescent control (silver) stimulus. This control does not contain chromatic information as it is chromatically grey but displays a strong angular change in intensity (specularity) because it is shiny. To create the distractor stimuli, we took photographs of the iridescent stimulus with a Nikon D7200 DSLR camera and an optical filter transmitting spectral radiation between 400 and 700 nm (Edmund Optics). Photos were taken under direct sunlight on a cloudy and sunny day whilst changing the camera angle to account for the natural variation in illumination likely to occur during behavioral experiments (Stuart-Fox et al. 2020). We then printed the photographs in high resolution using an Epson WF-7520 color printer. The distractor stimuli displayed static representations (only the spatial component) of the wide range of colors produced by the iridescent disc (Figure 1). All stimuli were presented on a 5 × 5 cm grey background and laminated to enable cleaning. Although lamination may add an extra layer of white gloss, it was present in all treatments and controls. Because gloss is independent of the wavelength (Franklin and Ospina-Rozo 2021), it did not alter the chromatic properties of the stimuli.

Spectral measurements of stimuli

We measured the reflectance of our iridescent and distractor stimuli using a PX-2 pulsed Xenon light and a USB 2000+ spectrometer coupled to a goniometer, which we used to control the angle of the collector (Fig. S1). Due to the structural properties of the iridescent stimulus, the iridescence phenomenon was most apparent with a larger area of illumination than provided by the fiber optic in our set-up (<1 mm spot size; Fig. S2). For this reason, we did not use the fiberoptic coupled to the goniometer to control the angle of illumination when measuring the iridescent stimulus. Instead, we illuminated the sample directly by holding the light source such that it uniformly illuminated an area with a diameter of approximately 1 cm (Fig. S1). We arbitrarily chose 15 different positions (i.e. different combinations of azimuth and altitude) for the light source to
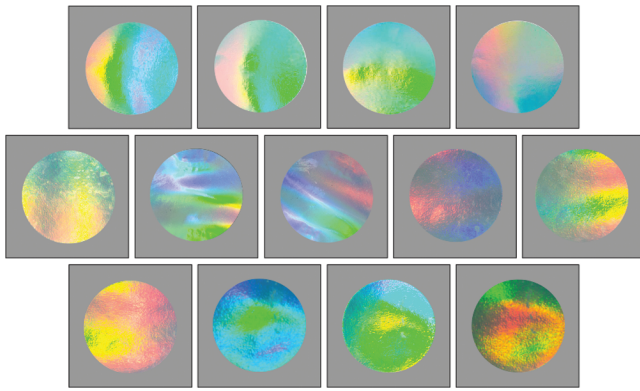
Figure 1
Pool of 13 distractor stimuli.

simulate different angles of direct illumination (i.e. different angles of the sun during behavioral trials). For each position of the light source, we measured reflectance at six angles of the collector (observer), from 5° to 20° from the normal intervals of 2.5°. Due to the artificial nature of our stimulus, there was no difference in the spectral measurements when rotating the sample in the plane parallel to the sample (yaw – Fig. S1). Thus, we kept the orientation of the sample fixed and varied the observer angles in the plane perpendicular to the sample (formed by the axis N and y in Fig. S1), from normal = 0 to a maximum of 20° from the normal. These measurements were made across arbitrary positions on the stimulus surface as the structures producing the iridescent effect are radially symmetrically distributed across the surface.

In contrast to the iridescent stimulus, the pigment-based printed distractors were diffuse, that is they reflect a similar proportion of light at all angles. Therefore, we measured the reflectance of the distractors using a standard set up with light delivered via a fiber optic (point source, 1 mm diameter measurement area) in only one angular combination: 10° light incidence and 10° angle for the collector. To avoid printing artefacts in our spectral measurements, we first sampled the dominant color patches within each distractor and printed them separately to measure their spectra.

Measurements were recorded using the software OceanView 1.6.7. and calibrated against a low reflectance mirror standard (Ocean Optics STAN-SSH) for iridescent samples, and against a diffuse 99% reflectance spectralon standard (Labsphere, North Sutton, NH, USA) for the distractors. The use of different reflectance standards does not have an effect on our comparison of chromatic properties, as color space models, including the hexagon color space used here, exclude intensity information (Chittka 1992). Behavioral data confirm that honey bees appear to largely ignore brightness information in most color processing contexts (Daumer 1956; Menzel 1967; von Helversen 1972; Ng et al. 2018).

To estimate the degree of spectral shifting in our iridescent stimulus, we extracted the spectral location (wavelength at half maximum reflectance) for each angle of the collector for each illumination position using the R package Pavo 2 (Maia et al. 2019). We used spectral location rather than the widely used "peak reflectance" or "wavelength of maximal reflectance" as this produces an equivalent measure of iridescence but is less sensitive to spectral shape (i.e. can be applied to sigmoidal and multi-peaked spectra; see Supplementary Material for further information). For each of the 15 illumination positions, we estimated the spectral shift as the angle of the collector increased from 5° to 20° from the normal by

fitting a linear model with spectral location as the response variable and the angle of the collector as the explanatory variable. We used a linear rather than sinusoidal model (Gruson et al. 2019) because the spectral shift is approximately linear over the limited angular range considered here (5– 20° from the normal) and provides a similar fit (Fig. S3). The slope of these models is negative when there is a shift towards shorter wavelengths. Thus, we report the absolute value of the slope as a statistical estimate of the magnitude of blue shift in the spectral location. We calculated the average blue shift over the 15 illumination angles to derive the average magnitude of blue shifting in the spectral location for every 1° increase in viewing angle in the plane perpendicular to the surface. The average blue shift in the spectral location does not necessarily correspond to a perceivable change in hue; however, it is useful as a reference to compare to stimuli used in other studies with different organisms and measurement protocols.

Perception of temporal and spatial components of the iridescent stimulus

To understand how spectral shifts may be perceived by animals, it is important to consider the natural viewing approach of observers and model chromatic changes in a relevant color space (Renoult et al. 2017; Garcia et al. 2020; Langridge et al. 2021).

As this experiment was conducted with bees, we evaluated whether they sufficiently varied their approach angles to perceive temporal color changes. We video recorded the horizontal movements of 5 bees from above when making 10 landings each towards the iridescent stimulus. We recorded the largest approach from the left and right of the stimulus by measuring the subtended angle from the center of the stimulus with the GIMP measurement tool (The GIMP Development Team 2019). The maximum approach angle was therefore the sum of the largest left and right approach angles (Table S1).

In order to determine if honey bees have sufficient spatial resolution to perceive the spatial component of iridescence, that is the color patches in the iridescent stimulus and distractors, we took photographs of the stimuli, and used the toBeeView R package (Rodríguez-Gironés and Ruiz 2016) to model how they would be perceived at distances of 5, 10, and 20 cm, assuming an acceptance angle of 1.9° (Rigosi et al. 2017) and average interommatidial angles of 1.8° (Land 1997).

Finally, to evaluate if the spectral shifts produced by the spatial and temporal component of iridescence are perceived by the bee as a variation in hue, we modeled the spectra produced by the iridescent and distractor stimuli in bee hexagon color space (Chittka 1992; Fig. S4), using honey bee photoreceptors (Peitsch et al. 1992), and an illumination of 6500 K daylight (Judd et al. 1964). The von Kries transformation was applied to account for receptor adaptation (Chittka 1992; Garcia et al. 2017), and excitation values were used to calculate the coordinates of each stimulus in hexagon color space. To evaluate the perception of temporally changing colors, we modeled the spectra obtained at different angles of the collector, recreating the change that a free flying bee would experience. To evaluate the perception of a spatial distribution of static colors we modeled the dominant color patches within each distractor (Fig. S5).
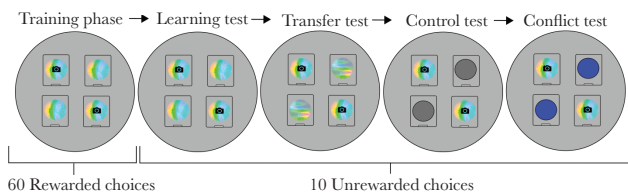
Training

10 free-flying honey bees (*Apis mellifera*) were recruited from a University of Melbourne beehive in March 2020 and marked on

the thorax for identification purposes. Each bee was individually trained to visit a vertical rotating screen (50 cm diameter) containing four hangers (6 × 8 cm), each of them bearing one stimulus and a landing platform for the bee. These hangers could be hung on the various knobs protruding from the screen to vary the spatial location of the stimulus. We used a rotating screen to allow bees to freely approach our stimuli at unconstrained viewing angles. The training phase commenced once bees could land on the platforms of the hangers (Figure 2). Two hangers displayed iridescent disk stimuli, whereas the remaining two presented photographs of the iridescent stimuli. These photo stimuli were randomly selected from a pool of 11 stimuli with a dice (Figure 1; a total pool of 13 but 2 were randomly chosen to be removed at the beginning of each experiment for use in the transfer test). The rotating screen was constantly oriented towards the sun to ensure the stimuli were always under direct illumination. Bees were trained for 60 choices using appetitive differential conditioning (Dyer and Chittka 2004; Giurfa 2004) to visit the iridescent disks presented on the hangers. Bees found a reward of 25% sucrose solution at the hangers displaying iridescent disks, and a drop of water at hangers displaying photographs.

Between each bout (individual bee returning from hive), the photograph stimuli were exchanged with another random pair from the pool of 11 stimuli. The orientation of the iridescent and photograph stimuli was also randomized based on dice roll. The rotating screen and hangers were cleaned with 30% ethanol solution each time a bee made physical contact to remove scent marks. Once the bee had made a correct choice, she was collected from the platform and placed behind an opaque screen. The hanger was then replaced, and the screen was rotated to eliminate olfactory and spatial cues. Once the bee had finished imbibing the sucrose solution, she could either return to the hive and end her foraging bout or make another landing choice.

After the bee made 60 choices, we conducted a non-rewarded learning test of 10 choices (no sucrose present on platforms) with two iridescent stimuli and two photograph stimuli randomly chosen from the pool of 11 training stimuli (Figure 2). The purpose of this test was to determine a baseline of learning towards training stimuli in the context of a non-rewarded test. This allows for direct comparison with performance in subsequent non-rewarded tests. Following the learning test, the bee experienced a transfer test, specularity test (controlling for changes in intensity independently of hue), and then a conflict test, with 1–4 refresher choices conducted between each test to maintain the bee's motivation (Figure 2). Each of these tests (learning, transfer, specularity, and conflict)

were non-rewarded and the order of tests was the same for each bee.

In the transfer test, the bee made 10 choices towards two iridescent stimuli or two photograph stimuli that the bee had never experienced, to determine if the bee was discriminating the iridescent stimuli based on its experience with the pool of training stimuli. In the specularity test, the bee made 10 choices towards two iridescent stimuli or two mirror-like silver stimuli, to determine if bees were choosing iridescent stimuli based on their achromatic properties (i.e. changes in intensity rather than hue). In the conflict test, the bee made 10 choices towards two iridescent stimuli or two blue stimuli (Fig. S6), to determine if the innate preference of blue (Giurfa et al. 1995; Morawetz et al. 2013) would override or conflict with the bee's trained preference for iridescence.

We used a generalized linear model (GLM) with a logit link function to test whether the proportion of correct choices (dependent variable) increased with the number of choices made (continuous predictor variable) across the 60 learning choices. To investigate preferences within each of the test conditions, we used generalized 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 iridescent stimuli differed significantly from the 50% chance level. All GLMMs included bee ID as a random term to account for the repeated measurements recorded from each bee.
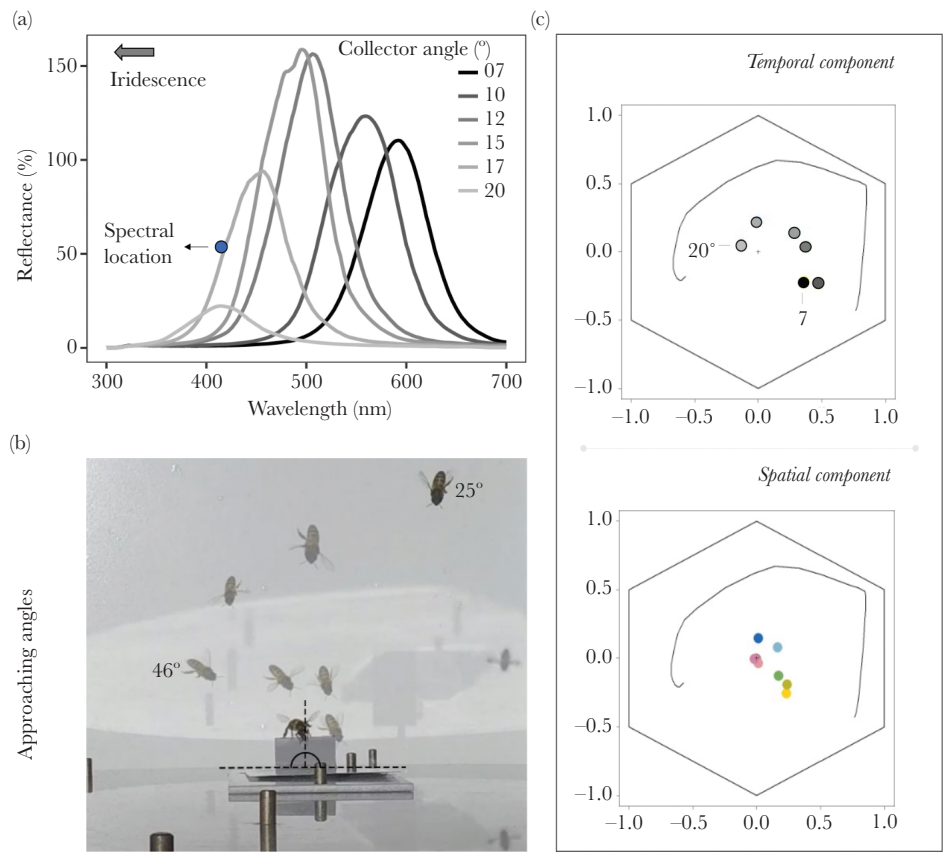
RESULTS
Perception of temporal and spatial components of the iridescent stimulus

Our spectral measurements show a spectral change in the iridescent stimulus even with small angles, displaying a strong blue shift in the spectral location of 11 nm ± 3.9 for every increase of 1° in the position of the collector (Figure 3A). Free-flying bees appear to experience an average angular range of 64° ± 6.8 when approaching the stimulus, and the bees therefore sufficiently vary their approach angle to experience such color changes when viewing the iridescent stimulus (Table 1 and Figure 3B). Our toBeeView models also show that bees have sufficient spatial acuity to differentiate color patches in the iridescent (Figure 4) and distractor (Fig. S8) stimuli. Additionally, both iridescent and distractor stimuli produce varying colors as observed from color modeling (Figure 3C; Fig. S4; Table S1), and differences are further reduced during behavioral trials as both stimuli are displayed in natural illumination (Fig. S4). Overall, our modeling demonstrates that these stimuli were suitable for behavioral experiments as they produce complex color patterns that can be perceived by the bee (spatial component), and the typical flight approach will result in perceivable visual change (temporal component).

Behavioral experiment

We found a significant effect of choice number on the mean proportion of correct choices during the 60 training choices ($z = 2.495$, $P = 0.013$, intercept: 0.395, 95% confidence intervals of the intercept (CIs) = 0.057–0.738, slope = 0.013, CIs of the slope = 0.003–0.023; Figure 5). This suggests that bees learnt to choose the iridescent stimuli as their accuracy significantly increased over time during the training phase. We found a significant difference from chance level (50%) in the mean proportion of correct choices towards the iridescent stimuli (Table 2) in the

Figure 2
Two pairs of distractors are presented vertically on one of four hangers (6 × 8 cm) displayed on a grey rotating screen (50 cm diameter). Bees make a choice by landing on the small platform attached to each hanger where there is either a drop of sucrose or water. Each bee experiences 60 choices in the training phase, and then 10 choices in each of the subsequent non-rewarded tests in the following order: learning test, transfer test, specularity test, and conflict test.

Figure 3

Characteristics and perception of the stimuli. (A) Strong iridescence was quantified by the shift towards shorter wavelengths in the spectral location (wavelength at half maximum reflectance) of one point on the iridescent stimulus as the angle of the collector (representing the viewer) increases. (B) A honey bee will typically view the iridescent stimulus from various angles as it approaches, including the range of angles measured in (A). (C) Honey bee perception of the two components of iridescence represented in hexagon color spaces. The spectral loci (line) represent how bees would theoretically perceive spectrally pure lights (Fig. S7). Temporal component: the perceived color of one point in the iridescent disc changes with the angle (gray shades correspond to the angles in (A)). Spatial component: multiple colors are perceived at the same time, arising from multiple points across the distractor stimuli (each plotted stimulus representing possible patches from distractors; Fig. S5).

Table 1

The mean largest approach angle from the left and right of the iridescent stimulus from video recordings of 5 bees making 10 choices each. Bees experienced a mean range of 59-62° variation in approach angle during each choice (See Table S1 for raw values)

Bee ID	1	2	3	4	5
Mean of largest angle from the left	27.1°	41.6	44.6°	17.6°	26.2°
Mean of largest angle from the right	32.2°	34	16.4°	42.8°	35.4°
Total mean angle range	59.3°	75.6°	61°	60.4°	61.6°

learning test ($z = 5.6$, $P < 0.01$, CIs: 0.71–0.88) and transfer test ($z = 6.1$, $P < 0.01$, CIs: 0.76–0.90), demonstrating that bees could transfer a learned preference for iridescence to a novel stimulus. We also found a significant difference from chance level in the mean proportion of correct choices towards the iridescent stimuli in the specularity test ($z = 4.2$, $P < 0.01$, CIs: 0.63–0.81), and the conflict test ($z = 3.3$, $P < 0.01$, CIs: 0.57–0.77). This showed that bees did not learn to choose the iridescent stimulus based on its high specularity, and that the learned preference persisted even when presented with a salient blue stimulus. Therefore, after 60

choices towards the iridescent stimuli, bees maintained a learned preference towards the iridescent stimuli in all test conditions.

DISCUSSION

Iridescence is a complex phenomenon, and its potential function in communication can be better understood by understanding how it may be perceived. Specifically, iridescence has both temporal and spatial components, and it is important to understand which is most relevant in different communication contexts and what neural capacity might enable such behavioral responses in a particular observer. Here we show that this separation is relevant by demonstrating that honey bees can separately learn the temporal component of iridescence in the presence of a spatially complex distractor. Bees displayed a learned preference towards iridescence in both the learning and transfer tests, confirming that they can perceive and choose iridescent stimuli after training with appetitive differential conditioning. Importantly, the transfer tests involved novel stimuli that contained the elemental colors present in the training stimuli, and thus if only a static instance of the color perception drove decision-making, bees should have chosen between these novel representations at chance level. However, the significant preference for iridescent stimuli that retained the temporal component of color shows that bees were able

to resolve this dimension of color perception. Furthermore, bees were more likely to choose the iridescent stimulus in the specularity test, showing that they were indeed attending to the temporal change in hue rather than absolute stimulus intensity.

These findings in the honey bee are consistent with neuroanatomical and electrophysiological data showing that color and motion can be separately processed in anatomically segregated pathways in the bumblebee brain (Paulk et al. 2008). However, our specularity test indicates that it is the interaction or integration of color and motion that is especially important in iridescence processing. This is because the control stimulus also has temporally varying achromatic properties and attending to temporal change alone would not be enough to effectively discriminate the iridescent disc from the control. Both color and motion must ultimately be integrated in the brain for the bee to identify the moving color patches in the iridescent disc. Therefore, the joint processing of chromatic and motion properties as has been observed in neural responses within layers 5 and 6 of

the lobula is likely to be important in the processing of iridescent stimuli (Paulk et al. 2008). The slow and gradual learning of the iridescent stimuli in our study is also suggestive that the processing of iridescence is multi-dimensional, as multi-modal processing is associated with relatively long learning curves in bees (Giurfa et al. 2001). Thus, bees appear to have a sophisticated neural toolkit for processing chromatic information in complex stimuli.

Our study provides further evidence that bees can perceive iridescence, but does this have any ecological significance? The role of iridescence in plant-pollinator interactions has been debated due to the relatively low salience of floral iridescence (Morehouse and Rutowski 2009; van der Kooi et al. 2014; van der Kooi et al. 2015; Garcia et al. 2019; Garcia et al. 2020), and the use of training regimes in experiments (e.g. appetitive-aversive conditioning; Whitney et al. 2009) that are rarely present in nature. Therefore, the biological relevance of flower iridescence when viewed under natural lighting conditions remains unclear (van der Kooi et al. 2015; Whitney et al. 2016), especially when pollinators may also have strong innate preferences for colors that hierarchically override the processing of other visual traits (Giurfa et al. 1995; Morawetz et al. 2013). Here, we found that bees displayed a learned preference for iridescence over a salient blue stimulus, showing that a learnt preference for iridescence can potentially override innate preferences for specific colors that foraging bees may have. However, our artificial iridescent stimulus showed a large spectral shift towards shorter wavelengths even with a small change in the angle of the collector, the magnitude of which is likely to be much greater than the shifts observed in natural iridescent patches. Thus, iridescence found naturally in flowers may not provide a robust signal that can override the predominant hue of a flower determined by its pigment-based color and surface nanostructures (van der Kooi et al. 2014; van der Kooi et al. 2015; Whitney et al. 2016; Moyroud et al. 2017; Van Der Kooi et al. 2019).

Iridescence is ubiquitous in the natural world; however, as highlighted by the debate over the importance of flower iridescence in plant-pollinator interactions, its functional significance is often unclear. It is important to distinguish whether the primary signal from an iridescent stimulus is a change in hue or/and intensity given that iridescent surfaces are often also highly specular or glossy and that chromatic and achromatic stimuli are processed in largely separate visual pathways (Livingstone and Hubel 1987; Kemp et al. 2015; Sabesan et al. 2016), though in many cases there is evidence that these pathways interact to some degree (Schnaitmann et al. 2013; Kinoshita and Stewart 2020; Pagni et al. 2021). Distinguishing the primary signal of iridescence could be especially relevant for animals such as bees where the processing of chromatic or achromatic signals is more effective under different visual contexts (Giurfa et al. 1996), and where the achromatic processing pathway may directly feed into motor systems to drive rapid responses (Skorupski and Chittka 2010). Our results demonstrate that honey bees were attending to chromatic information and that they can use the temporal component of iridescence as a cue that is distinct from the spatial component.
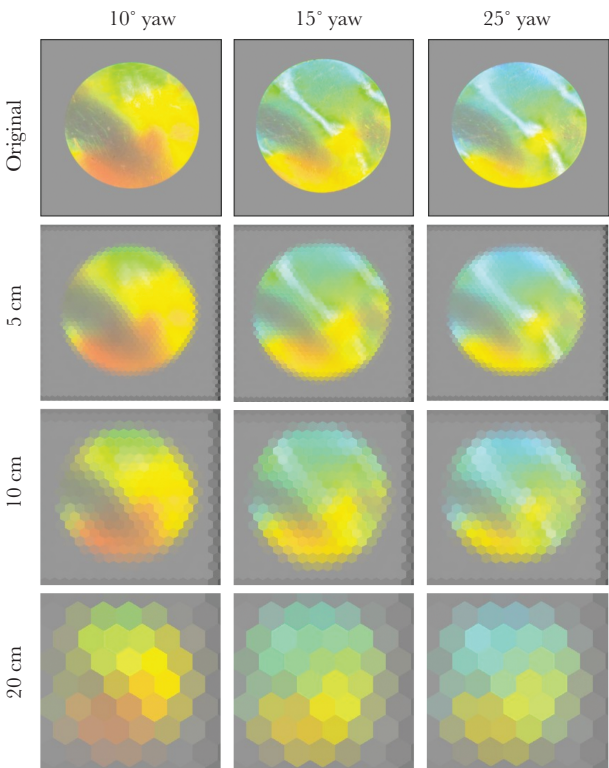
Figure 4
RGB representation of distance effect on spatial acuity of iridescent stimuli for bee vision produced by toBeeView (Rodríguez-Gironés and Ruiz 2016) for distances of 5, 10, and 20 cm, at 10°, 15°, and 25° yaw. Honey bees have sufficient spatial acuity to perceive such moving spatial patterns of the iridescent stimuli even from a distance.

Table 2

The mean proportion and standard error of correct choices by bees towards the iridescent stimuli in each of the 4 test conditions

Condition	Mean proportion of correct choices towards iridescent stimuli (%)	Standard error (%)
Learning test	80	±4.0
Transfer test	84	±3.7
Specularity test	72	±4.5
Conflict test	67	±4.7
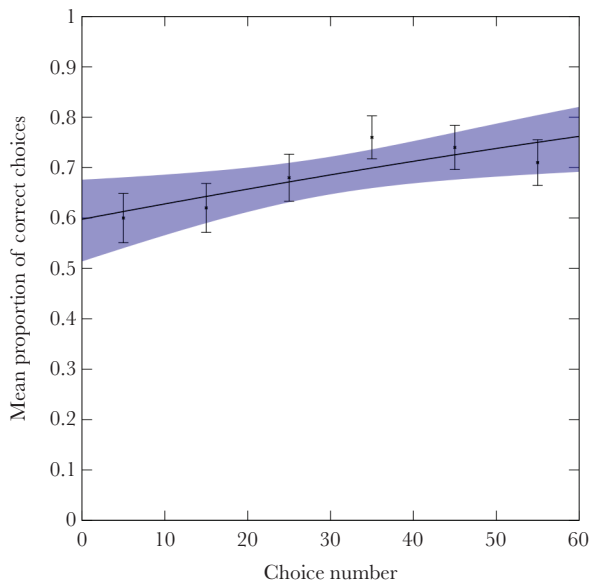
Figure 5

Performance during the training phase as modeled by a generalized linear model (GLM), showing the effect of trial number on the mean proportion of correct choices. The shaded band surrounding the regression line represents 95% confidence interval. Each point represents the mean proportion of correct choices of all bees at each block of 10 choices, with respective standard error bars.

Other animals may use the temporal component of iridescence as a signal or cue provided that the signaler or observer generates sufficient movement to produce the hue changing effect, can perceive the hue change, and has the capacity to process temporal information. Temporal processing is not neurologically trivial and animals have different capacities to process temporal changes (Donner 2021). Further, the effectiveness and reliability of temporal information is highly dependent on the context; for example there is a large literature on the effects of temperature, emotional state, and attention on human time perception (Wittmann 2013), though the effects of such factors on non-human time perception has yet to be adequately explored (Ng et al. 2021). Processing temporal changes in hue also require a sufficiently large working memory which may be limited in some animals such as insects (Zhang et al. 2005), although currently little is known about how working memory interacts with time processing in non-human animals. Interestingly, honey bees may have evolved a capacity to process temporal information for a different type of task – decoding the famous waggle dance (von Frisch 1967; Ng et al. 2021).

Distinguishing between temporal and spatial components of iridescence provides a useful framework for investigating the ecological significance of iridescence beyond the context of plant-pollinator signaling. For example, the temporal component of iridescence is relevant when hue change complements movement or sound; as is the case in male Anna's hummingbirds, *Calypte anna* (Hogan and Stoddard 2018), and male birds-of-paradise *Lawes' parotia* (Wilts et al. 2014). Morphological adaptions that enhance the directionality of an iridescent signal can also serve to produce abrupt switching between hues, rather than slow graded changes, potentially facilitating recognition and memory of the iridescent signal. Alternatively, when iridescence conveys information about an animal's age (Kemp and Macedonia 2006), sex (Brien et al. 2019), or nutritional status (Meadows et al. 2012), the spectral variation of colors at any given time (i.e. the spatial component of iridescence)

may be sufficient to convey such information. In other contexts, both components could be relevant and synergistic. For example, male peacock spiders wave their imperfectly flattened abdomen during courtship (Girard and Endler 2014), and may require both spatial and temporal components of their display to attract the female observer. Future studies should strive to disentangle the spatial and temporal components of iridescence to understand its ecological significance. In all cases, it is important to consider visual and temporal processing systems of the receiver, as these determine whether and how animals process the temporal and/or spatial components of iridescence.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at *Behavioral Ecology* online.

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