Higher iridescent-to-pigment optical effect in flowers facilitates learning, memory and generalization in foraging bumblebees

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Iridescence—change of colour with changes in the angle of view or of illumination—is widespread in the living world, but its functions remain poorly understood. The presence of iridescence has been suggested in flowers where diffraction gratings generate iridescent colours. Such colours have been suggested to serve plant–pollinator communication. Here we tested whether a higher iridescence relative to corolla pigmentation would facilitate discrimination, learning and retention of iridescent visual targets. We conditioned bumblebees (Bombus terrestris) to discriminate iridescent from non-iridescent artificial flowers and we varied iridescence detectability by varying target iridescence relative to pigment optical effect. We show that bees rewarded on targets with higher iridescent relative to pigment effect required fewer choices to complete learning, showed faster generalization to novel targets exhibiting the same iridescence-to-pigment level and had better long-term memory retention. Along with optical measurements, behavioural results thus demonstrate that bees can learn iridescence-related cues as bona fide signals for flower reward. They also suggest that floral advertising may be shaped by competition between iridescence and corolla pigmentation, a fact that has important evolutionary implications for pollinators. Optical measurements narrow down the type of cues that bees may have used for learning. Beyond pollinator–plant communication, our experiments help understanding how receivers influence the evolution of iridescence signals generated by gratings.

1. Introduction

Iridescence is defined as the change in dominant wavelength with the viewing/illumination angle [1]. It is widespread in nature and originates from structures with periodicity at wavelength scale. Iridescence produces some of the most saturated and colourful displays in nature, but also very weak colorations. In plants, iridescence is present in leaves [2], fruits [3] and flowers [4], and is produced by multi-layer interference or by diffraction gratings [5]. The presence of iridescence in flowers has been suggested based on the regular striations on the epidermis surface [5] of the flowers in at least 12 different families, spanning from Liliaceae to Asteraceae or Solanaceae [4–7]. While iridescence may protect against destructive UV radiation in some species [7], it may also serve plant–pollinator communication. Among floral displays facilitating flower detection by pollinators, corolla pigment displays operate at large [8,9] or short distances (i.e. at large visual angles [10–12]). They have been shown to improve pollinator orientation and reward finding (references in [9]). Recent studies have shown that bumblebees are able to perceive floral or artificial iridescence irrespective of floral corolla coloration, and to use it to find food rewards [6,13]. Questions
regarding the frequency of floral iridescence, its visibility under artificial or natural conditions, and its potential role in plant–pollinator communication [14–18] are still unexplored or lively debated, calling for more experimental research [17].

Efficient pollination requires that pollinators exhibit flower constancy (i.e. the tendency to restrict foraging bouts to one or few species or morphs with a known reward). Flower constancy limits pollen loss and ensures intraspecies fertility [19]. Constancy is particularly important when pollinators are generalists, like hymenopterans [20]. More specifically, constancy requires that pollinators learn and memorize associations between floral cues and reward (nectar or pollen) [21]. As flowers slightly vary in visual appearance (depending on orientation in space, illumination, developmental stage, etc.), constancy requires that pollinators generalize floral choice to novel situations, while preserving the learned cues [22,23]. Hence, any floral feature that would make the signal–reward association more easily learned, generalized or retained in long-term memory would be favoured by selection. If iridescence-related cues are used for foraging, what form of iridescent signals would be selected, especially given that it would interplay and potentially interfere with other flower displays like corolla coloration? Rare recent studies have brought some elements to that question: floral iridescence should be strong enough to provide visually exploitable cues [15], but not too strong, as this would corrupt flower identity and decrease constancy [13].

Here, we experimentally explored how floral iridescence and corolla pigment coloration interact in the context of pollinators’ foraging choices. We trained bumblebees to discriminate between punishing non-iridescent targets and rewarding iridescent targets displaying a specific iridescence relative to pigment optical effect [6,13], which could be either high or low. We predicted that increasing the iridescence relative to pigment optical effect would enhance the detectability of rewarding targets, and thus accelerate and/or improve the efficiency of learning an iridescence–reward association, irrespective of flower corolla coloration. Furthermore, we posited that a higher iridescence relative to pigment optical effect would facilitate generalization to novel objects and retention in memory. Beyond documenting the potential communicative value of flower iridescence, studying an iridescence sender–receiver system in controlled conditions helps to understand pollinator–plant relationships and the evolutionary issues related to iridescent signals generated by diffraction gratings, which are widespread in nature [1].

2. Material and methods

(a) Animals and housing conditions

Purchased bumblebee (Bombus terrestris) hives (Biobest) were divided into two compartments, one light-safe with the entire colony and one transparent to allow only specific individuals (selected by the experimenter) entering the experimental arena. Hives were fed with pollen and maintained at 30°C throughout experiments (20 days). The testing room was kept at the same temperature. Age has no great impact on learning and memorization in bumblebee workers (at least for in the olfactory domain [24]). Every day, we collected randomly individuals inside the nesting box and marked them individually using paints (Email Color, Revell GmbH). Selected individuals were naïve both with respect to natural flowers and to the experimental set-up. They were starved individually during 48 h before experiments to increase their foraging motivation.

(b) Testing arena

A foraging flight cage 170 × 120 × 200 cm (L × M × H) was connected to the hive by a transparent PVC pipe through which the experimenter controlled a bee’s entrance via two shutters. In the flight cage, a circular arena was presented. The arena was painted with a mixture of white and black acrylic paints (70% black and 30% titanium white, Prismo, Dalbe) to achieve a dark grey background that was achromatic for the bees' sensitivity [11]. Twenty-four translucent, 12 cm-high pedestals 4 cm apart from each other were arranged on the arena to evenly fill a circular area (electronic supplementary material, figure S1). This arena was used to train bees and to test their memory retention. For generalization tests, we used a circular arena with 12 pedestals.

We placed artificial flower targets (3 cm diameter resin targets; see below for preparation) at random on pedestals (electronic supplementary material, figure S1). All flower targets were covered with mylar film to prevent bumblebees from using polarization signals (electronic supplementary material) [6]. The artificial flower field had the same number of targets of each colour, and the same number of rewarding and non-rewarding targets. The foraging flight cage was illuminated by two cool-white LED bulbs (7.5 W, 40°, 4000 k; Sylvania) and an ultraviolet (UV) multiple LED bulb (7.5 W, 15°, 370 nm; VioLED) centrally located 1 m above the foraging arena. We measured the emission spectrum of the two LED sources with a spectroradiometer (spectros 1020 UV, JETI), and adjusted the ratio between the two light sources so that it would represent the ratio found in sunny open habitat light (UV corresponding to approximately 12% of the total; irradiance spectrum presented in electronic supplementary material, figure S2). Thus, the illumination provided included the wavelengths to which bumblebees are sensitive (300–650 nm) [25] and constituted a satisfying approximation to daylight conditions.

(c) Visual stimuli, goniospectrometric measurements and analysis

We created iridescent artificial blue, yellow, red and violet artificial iridescent and non-iridescent targets by casting a UV-transparent resin impregnated with pigments on the grated side and the smooth side of compact discs (CDs), respectively, as in [6] (details in electronic supplementary material). Target coloration results from diffraction (iridescent part depending on surface structure only, on CD characteristics) and reflection from pigmented resin (non-iridescent part, flower corolla coloration depending on resin pigment concentration). We created two treatments by manipulating not iridescence itself but the pigment part, hence the iridescent-to-pigment ratio (hereafter called IRP; e.g. figure 1e,f). We used a pigment concentration of 1.6 mg l⁻¹ for the high IRP ratio (figure 1e) and a pigment concentration of 40 mg l⁻¹ for the low IRP ratio (figure 1f). For each treatment, both iridescent and non-iridescent targets had the same pigment concentration. Increasing pigment concentration decreased the IRP ratio, hence iridescence detectability.

We measured targets in goniospectrometry to explore angle-dependent optical properties over the range of bumblebee visual sensitivity (300–650 nm). We set the incident light at 0° as in the arena. For iridescent targets, we rotated the target until finding the highest diffraction peak (defining the ϕ = 0° orientation). For non-iridescent targets, we chose the ϕ = 0° orientation at random. We varied light collection angle θ. Unless otherwise stated, we measured one iridescent and one non-iridescent disc per colour and IRP level, and measured one reflectance spectrum per configuration (ϕ,θ) chosen:

- Fixed target orientation ϕ = 0°, varying collection angle from θ = 26° to θ = 70° to illustrate iridescence by diffraction. θ range allowed to detect all second-order diffraction peaks.
We illustrated how targets were perceived by bumblebees in their colour space (figure 1c,d; electronic supplementary material, figure S6a,b). In that space, we computed the colour volume (minimal volume encompassing a set of points) for iridescent targets of a given pigment concentration, and predicted that it would be larger for high than for low IRP ratio according to the notion that iridescent-related cues would be more detectable when pigmentation was low.

Varying target orientation $\psi$, fixed collection angle $\theta = 38^\circ$. At $\theta = 38^\circ$, we explored all possible target orientations $\psi$ (360

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**Figure 1.** (a,b) Reflectance spectra obtained in spectrometry with an integrating sphere. Field background (dashed line) and flower targets of (a) low (high pigment concentration) and (b) high (low pigment concentration) level of iridescence. (c,d) Chromatic location of colours in the triangle colour space for targets with (c) low (high pigment concentration) and (d) high (low pigment concentration) level of iridescence detectability (shown in colour in figure S6a,b). The triangle is not entirely presented for clarity reasons. Spectra were measured in goniospectrometry every 2\'\', between $\theta = 26^\circ$ and $\theta = 70^\circ$, between 300 and 650 nm (details in electronic supplementary material). (e,f) Examples of spectra acquired in goniospectrometry for yellow targets of (e) low (high pigment concentration) and (f) high (low pigment concentration) level of iridescence. The peak of the diffraction ray is shifted towards longer wavelengths (darker lines) as the angle of light collection departs from the normal to target surface, as expected in a linear diffraction grating. For the sake of clarity, the intensity of diffracted rays was set to a maximum of 200%. Target colours were violet (triangle), blue (circle), yellow (square) and red (diamond) for high (empty symbols) or low (grey symbols) detectability of iridescence, and for iridescent (large symbols) and non-iridescent (small symbols) targets. Note that diffraction intensity decreases with an increasing collection angle, a fact that is characteristic of blazed gratings (explanation in electronic supplementary material).
possible orientations) by rotating the target on itself. We tested whether targets displayed recurrent static chromatic and/or achromatic cues at a given viewing angle and at orientations where diffraction emerged. Such cues could be learned by bumblebees instead of iridescence-related cues (see electronic supplementary material for details).

— Fixed target orientation \( \psi = 30^\circ \), fixed collection angle \( \theta = 38^\circ \). We measured three non-iridescent disks and three iridescent disks per colour and IRP level. Each disc was measured in four randomly chosen locations, each at \( \psi = 30^\circ \). We tested whether iridescent targets differed from non-iridescent targets by their resin pigmentation, at orientations where diffraction did not emerge. Such cues could be learned by bumblebees instead of iridescence-related cues (see electronic supplementary material for details).

In all cases, spectra were analysed to extract the dominant wavelength of the diffraction peak and analyse its variations between and within targets (electronic supplementary material, figure S5). We quantified the chromatic contrast between a target and the arena background using the RNQ model, which provides an appropriate representation of bee colour vision (details in the electronic supplementary material). We also quantified relevant visual achromatic parameters such as S (short wave), M (mid wave) and L (long wave) receptor-specific contrasts, defined as a receptor’s response to the target divided by its response to the background [11]. From these receptor-specific contrasts, L contrast mediates achromatic detection of visual targets at small subtended angles in bees and other visually driven performances [11,22], while the subtractive contributions of S, M and L contribute to the perception of chromatic contrast via opponent processing.

(d) Conditioning and testing protocol

We used differential conditioning to train two groups of 10 bumblebees to discriminate between iridescent and non-iridescent targets. Targets presented three different colour pigments; all targets had the same IRP level. One group of 10 bumblebees was rewarded with sucrose solution on iridescent targets of high IRP level, and punished with quinine solution on non-iridescent targets of high IRP level. Another group of 10 bumblebees was rewarded with sucrose solution on iridescent targets of low IRP level, and punished with quinine solution on non-iridescent targets of low IRP level. An individual was neither presented with targets with an IRP level different from that experienced during training, nor given the choice between targets of both IRP levels. It only had to choose between iridescent and non-iridescent targets displaying the pigment concentration used during training.

Fifteen microlitres of 50% sucrose solution were used to reward iridescent targets. Fifteen microlitres of a 0.02% quinine hemisulfate solution were used as negative reinforcement. Quinine improves visual discrimination learning in free-flying bees [26,27] and cannot be detected via olfaction at a concentration of 0.02% used in visual conditioning experiments (e.g. [27]). Here, we chose a lower concentration as higher ones tended to decrease foraging motivation (G.d.P. 2013, personal observations).

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To analyse the learning phase, we performed a binomial test on a moving window of 15 consecutive visits and we considered that learning was completed when the proportion of correct choices was statistically significant (12/15 correct choices, \( p \)-value = 0.03). Since the test concerned the last 15 consecutive visits performed by an individual at any moment, the learning phase stopped at exactly 12 correct choices for all individuals (80% correct choices). The learning phase lasted 45 min on average (range: 18–77 min), depending on bumblebee motivation and performance. Once learning was completed, we immediately changed the arena to perform the generalization test and allowed the focal individual to perform 15 additional visits to targets presenting a novel colour. After 24 h, we tested the same individual for memory retention test, and allowed it to perform 15 visits.

(e) Statistical analyses

All details of statistical analyses are in the electronic supplementary material.

3. Results

(a) Target optical properties

We could not find any statistically significant systematic achromatic or chromatic difference in pigmentation between iridescent and non-iridescent targets that could have been learned by bumblebees (electronic supplementary material, table S1). Likewise, we found that iridescent targets had
unstable (thus unpredictable) chromatic and achromatic appearance, hampering any learning and generalization based on such cues (electronic supplementary material, table S2). As expected from targets of various colours with linear gratings, achromatic S, M and L contrasts varied with target colour and faded when target orientation departed from the orientation at which diffraction was highest, both around 0° and around 180°. More interestingly, we revealed that static cues delivered at a given viewing angle showed unpredictable variations between and within targets. (i) The intensity of the diffraction peak varied within a target, depending on whether the target was seen at the 0° or around the 180° orientation. (ii) How quickly the intensity of the diffraction peak faded when the target was turned on itself starting from the peak depended both on the orientation at which the target was seen (0° or approximately 180°) and on target colour (electronic supplementary material, table S2, and figure S5). (iii) The dominant wavelength of the peak diffracted was different at the 0° or at the approximately 180° orientation (electronic supplementary material, figure S5d). Hence, bumblebees were very unlikely to have learned a static information per angle but probably relied on dynamic information of iridescence itself, like the change in colour/ intensity with the viewing angle.

We can note that iridescent targets were much more largely dispersed in the colour space than non-iridescent targets, illustrating the potential contribution of flower identity (figure 1c,d; electronic supplementary material, figure S6a,b). Spectra documented iridescence overshadowing by the non-iridescent part of the overall colour signal, illustrating IRP level (figure 1c,f for low and high IRP resp.). Note that within the same order of diffraction, peaks showed a decreased intensity with increasing viewing angle (increasing wavelength), a typical optical feature of blazed (irregular) diffraction gratings. This reinforced the overshadowing effect of iridescent component at long wavelengths (i.e. for yellow and red targets). High IRP targets (with low pigment concentration) occupied in general a larger portion of the colour space than low IRP targets (with high pigment concentration; respectively 0.30/0.23 for blue, 0.32/0.30 for violet, 0.36/0.35 for red but 0.39/0.44 for yellow targets), suggesting a higher detectability for low pigment concentration, except for yellow.

(b) Influence of IRP effect on learning, generalization and memory retention

All bumblebees were able to learn the discrimination between iridescent and non-iridescent targets (electronic supplementary material, figure S8). The best model accounting for the bees’ performance (among those including factors without interactions) retained the visit rank as a significant factor, thus suggesting that the probability of visiting a rewarded target increased with visit rank (0.019 ± 0.003; p < 0.0001). Irrespective of the IRP level used for training (low-level or high-level), all bumblebees reached 80% of correct choices upon completion of the learning phase (p > 0.19 for IRP level in a model retaining both visit rank and IRP level). The number of correct choices increased from the first 10 to the last 10 visits of the learning phase, independently of the IRP level (U-test, p < 0.001 both for the high and the low IRP treatment). Individuals marginally required fewer visits to achieve learning when iridescent signals were more detectable (low IRP treatment: mean ± s.e.: 68.7 ± 5.06 visits, high IRP treatment: 54.7 ± 4.70 visits; t-test, p = 0.057; figure 2a). Yet no significant difference was found for learning duration (high IRP treatment: 41 ± 4.70 min, low IRP treatment: 49.1 ± 4.97 min; t-test, p = 0.25; figure 2b) or learning speed (number of visits/duration; t-test, p = 0.84). Moreover, the date and the period had no influence on the total number of visits (t-test, p-value = 0.55 and 0.81, respectively) nor the time required (t-test, p-value = 0.48 and 0.92, respectively) to complete the learning phase. Overall, individuals trained with high and low IRP iridescent targets did not differ in the time necessary to reach the learning criterion but those trained with high IRP needed fewer visits to reach that criterion.

During the learning phase, individuals rewarded on high or low IRP targets did not differ in their foraging patterns (except for the number of flower visits), the number of bouts required to complete learning (t-test, p-value = 0.13), nor the duration of foraging bouts (linear mixed model, p-value = 0.51). The total number of iridescent targets visited was higher when IRP was low (t-test, p-value = 0.037), confirming that errors occurred in the sequence of visits. Neither the date nor the period of the day (morning/afternoon) affected the total number of visits or the time required to complete the learning phase (t-test, p-values > 0.48).

In the generalization test, bees of the high and low IRP groups did not differ statistically in terms of the errors made when foraging on a novel colour. (M–W test, p = 0.47; figure 2c). Yet they took less time to complete the 15 visits of this test when the IRP level was high (M–W test, p-value = 0.048; figure 2d). In this case, generalization to a novel colour of the learned rule was faster.

In the retention test performed 24 h after the completion of the learning phase, individuals of the high IRP treatment made fewer errors than those of the low IRP treatment (M–W test, p-value = 0.027; figure 2e). Yet no differences between both group were found in the time necessary to complete the 15 visits (t-test, p = 0.23; figure 2f).

(c) Influence of target colour on learning, generalization and memory retention

During the learning phase, bees visited significantly more violet and blue targets than yellow or red targets, regardless of the IRP level trained (linear mixed-effects model, colour effect, p = 0.016). Individuals did not show a significant change in their colour preferences between the first 10 and the last 10 visits of the learning phase (factor not retained in the best model), thus suggesting that colour preferences were unaffected by the on-going learning of iridescence-related cues. Irrespective of the colour trio on which bumblebees were trained, they did not differ statistically in the number of choices to complete the learning criterion (one-way ANOVA, p = 0.55). Yet individuals took marginally less time to complete learning when trained on violet–blue–red or violet–yellow–red trios than on violet–blue–yellow or blue–yellow–red trios (one-way ANOVA, p = 0.011).

In the generalization test, individuals made significantly fewer errors when the novel colour was violet rather than blue (linear mixed-effects model, p = 0.05) and marginally fewer errors when the novel colour was violet compared with red (p = 0.09). The time needed to complete the generalization test did not relate to receptor-specific contrasts (S-receptor contrast, p = 0.89; M-receptor contrast, p = 0.84; L-receptor contrast, p = 0.95; electronic supplementary
material, figure S9a), achromatic contrast (L-contrast, \( p = 0.95 \)) or chromatic contrast (RNQ colour contrast, \( p = 0.55 \); electronic supplementary material, figure S9b). The duration of the generalization test tended to be longer for targets with longer dominant wavelengths (\( p = 0.057 \); electronic supplementary material, figure S9c). The number of correct choices was not related to any physical or biological descriptor of target colour (\( p > 0.13 \)). Likewise, the duration of the retention test and the proportion of correct choices were not linked to the category of the novel colour of the generalization test (ANOVA, \( p > 0.42 \)), nor its chromatic or achromatic features (\( p > 0.14 \)).

4. Discussion

(a) IRP level affects learning, generalization and memory retention

Our results confirm that bumblebees are able to learn cues associated to iridescent targets for discriminating rewarding from non-rewarding targets, as shown in previous studies [6,13]. Learning performances were similar to those reported in previous bumblebee studies using non-iridescent targets, both for learning rate and variability between individuals [29]. Interestingly, although bees learned both high and low IRP levels, a higher iridescent-relative-to-pigment optical effect improved their cognitive performances. In this case, individuals required fewer visits (but the same amount of time) to learn the visual discrimination between iridescent and non-iridescent targets, generalized faster their response to a novel object with a different colour but displaying the IRP level previously learned, and exhibited better retention in an early long-term memory test performed 24 h after training. Our results thus show that a higher IRP level promotes detectability and better cognitive performances in pollinators in a foraging context.

Which cues are used by bumblebees? This subject has been much debated [6,18,30]. While some studies argued that bees use dynamic cues provided by iridescent targets [6,13], other opinions maintained that bees exploit static cues that appear in a consistent way from target to target at specific viewing angles [30]. Our measurements rule out that bumblebees would discriminate iridescent from non-iridescent targets based on polarization cues (excluded by mylar films that covered all targets) or pigment cues (as we could not detect any systematic achromatic or chromatic difference in reflectance signals coming from pigments of iridescent and non-iridescent targets). Bumblebees had to use signals related to diffraction gratings, and only at orientations where diffraction emerges. There were three possibilities. First, if iridescent targets delivered a static chromatic or achromatic cue constant at a given viewing angle, bumblebees could learn that cue (e.g. if at orientations where diffraction emerged, iridescent targets all

Figure 2. Effect of the level of iridescence detectability on the duration (a–c), number of visits (d) and proportion of correct choices (e,f) during learning (a,d), generalization (b,e) and memory retention test (c,f).
concentrations were used (e.g. 60 mM in [26,34]). In the training phase, bees trained on low IRP could be due to a punishment intensity that the bees can potentially collect nectar more efficiently than slow, inaccurate decisions at the cost of visiting non or poorly rewarding flowers [32]. With a higher IRP level, bumblebees gained speed in the generalization test without sacrificing the 80% accuracy that they inherited from the learning phase, and gained accuracy without sacrificing speed in the retention test. During learning, the IRP level affected the total number of visits but not the time needed to complete learning. Bumblebees of the high IRP group had a slower foraging speed, probably because they took more time to make foraging decisions and/or fed longer before switching to another target. Bumblebees of the low IRP group visited more targets in general, including more rewarding ones, during a similar overall time, which suggests that fast, inaccurate bees can potentially collect nectar more efficiently than slow, accurate bees, as shown in other studies [31]. This behaviour of bees trained on low IRP could be due to a punishment intensity that was not aversive enough to limit incorrect choices. Indeed, in experiments in which quinine was used to improve discrimination via a reduction of foraging speed [26,27,33], higher concentrations were used (e.g. 60 mM in [26,34]).

(b) Target coloration, iridescence and communication efficiency

Target colour affected bumblebees’ performances in the generalization test: generalization of the learned rule was faster for a novel violet colour than for blue and red, a result that is congruent with the reduced travel time of bees on iridescent blue than on iridescent red disks [13]. During the learning phase (but not during retention), individuals globally visited more violet and blue iridescent targets (reflecting over 373–442 nm) than yellow or red iridescent ones (reflecting over 532–630 nm). This bias can be related to bees’ innate preferences for natural colours maximally reflecting in the short wavelength range [35–37]. Previous studies have shown that generalization is more efficient when novel and learned colours are similar in dominant wavelength [35] and colour contrast [38–40], a fact that could explain why generalization was faster for violet and blue compared with yellow and red, but not why it was faster in violet than blue. We suggest that violet is the least chromatic colour used (electronic supplementary material, figure S4b,c), presenting a higher relative contribution of iridescence to the overall signal, thus facilitating the extraction of iridescence-related cues. An alternative interpretation could be that violet has a higher innate appeal than blue for bumblebees [35,41]. Finally, iridescent targets exhibited irregular (blazed) gratings in which the intensity of diffraction peaks decreased at longer wavelengths. Such gratings overshadowed iridescence at longer wavelengths like yellow or red, thus reducing the range of wavelengths at which iridescence-related cues would be easy to extract. Thus, the preference for colours reflective in shorter wave-lengths can be accounted for by the fact that bumblebees searching for iridescence-related cues detect it more easily in such a wavelength range.

In our protocol of iridescence manipulation, with identical diffraction gratings, bumblebees performed worse when targets were more pigmented, hence more detectable in chromatic terms against the background, a fact that may be seen as contradictory of what is known in the literature. At large distances (visual angles smaller than 15°), visual detection is mediated by the achromatic L-receptor-based contrast [14], and higher contrast increases flower attractiveness [10,42]; at closer distances (visual angles larger than 15°), detection is mediated by chromatic contrast [11], and attractiveness is maximal for a centripetally increasing chromatic contrast between background, flower corolla and nectar guides [38,40]. In our experiments, performances were improved when targets offered a low chromatic contrast against the background, and when dominant wavelengths were in the short range of the bees’ visual spectrum, in particular in the case of a dark violet coloration. This can result from two mutually non-exclusive processes: a competition process in which iridescence dominates over chromatic contrast if the latter is decreased, and an innate appeal to violet facilitating generalization to that colour. The existence of a trade-off between flower colour and iridescence is supported by the recent finding that discrimination of colour similar in dominant wavelength is impaired when bees exploit iridescence-related cues [13]. Moreover, in our experiments, targets of distinct colours converged to the same loci in the colour space, suggesting a stronger corruption of flower identity. While this can hamper performances when flower constancy is required [13], blurring flower identity may have helped bumblebees to learn faster iridescence-related cues and to generalize it to novel targets.

Several non-mutually exclusive display strategies may allow solving the flower colour–flower iridescence trade-off. (i) Flower patterns with highly chromatic petals (visible from large distance) and diffraction gratings against a dark violet area (maintaining the detectability of iridescence), as in Hibiscus trionum [5,17], Ixia viridiflora [6] or in Tulipa spp. [4,6]. Yet many flowers do not follow this pattern. (ii) Imperfect gratings, which may combine high iridescence but no
corruption of flower identity [13]. This strategy is restricted to short wavelengths for which it is easier to extract iridescence-related cues, a fact that may explain the interest of blue and violet colours. Yet the fading of intensity of diffraction peaks at longer wavelengths may help maintaining flower identity for colours like red or yellow. (iii) Gratings with a reduced path length, which reduces angle dispersion, concentrates light (see electronic supplementary material) and shifts accessible wavelengths towards UV. This solution may find a limit given the reduced spatial acuity of bees, which would result in not as many wavelengths being exploitable in shorter-path gratings compared to longer-path gratings.

As a conclusion, we show that pollinators can exploit flower iridescence-related cues predicting appetitive reinforcement and for the first time that increasing the relative contribution of iridescence to that of pigments facilitates learning, generalization and 24 h retention of the learned iridescence cues in bumblebees. A thorough goniospectrometric investigation of target optical properties excluded the use of differences in pigmentation or angle-specific static cues. They confirmed that bumblebees had to rely on iridescence-related cues generated by diffraction gratings, either iridescence by-products (enhanced intensity and/or altered colour at orientations where iridescence emerges) or iridescence per se (the presence of a dynamic change in intensity and/or colour, or its quantification). Flower corolla coloration affects the bees’ ability to extract iridescence information, suggesting a potential competition between both kinds of signals in evolution. Further research is needed to characterize the structural diversity of natural flower gratings, and to test experimentally their efficiency at attracting pollinators. Beyond pollinator-plant communication, these results highlight that exploiting iridescence may occur to the detriment of static colour signals, a fact that should be considered when studying iridescence in animals or plants.

**Data accessibility.** Data are accessible on Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.dj15b [43].


**Competing interests.** We declare we have no competing interests.

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**References**

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Figure S1: Flight cage, with the tube (green) connecting the hive to the experimental field. Here, the field is set for learning, for a colour trio of blue, yellow and red, and for a low IRP level (iridescence supposed to be poorly detectable). The field comprises 24 artificial targets, half of which is iridescent and rewarding, the other half being non iridescent and aversive. Photo © G. de Premorel.
Figure S2: Irradiance spectrum of the light source used in behavioural experiments, expressed in watt.m^{-2}. The light was composed of white and UV LEDs. We adjusted in intensity the UV light (by masking some of the multiple LEDs assembled in the bulb) in order to obtain a proportion of UV close to that of natural daylight.
Diffraction gratings, physics recall

Figure S3. Schematic representation of a perfect diffraction grating (figured with squared battlements), and illumination (a) at any incidence angle, with the convention of positive angles left to the normal, negative on the right. (b) At normal incidence, the incidence which was used in our measurements and in the setup. (c) Diffraeted rays at normal incidence: on the left, the incident beam is represented, and on the right (for clarity reasons, but it occurs at every path) the diffraeted rays, at different diffraction orders. In the general case (a), wavelengths are diffracted according to the relation \( m \lambda = d (\sin \theta_r + \sin \theta_i) \), where \( m = \pm 0, 1, \ldots, k \) is the diffraction order (explanation in [1]). In our experimental design and measurement configuration, light comes from above. In (b) the relation becomes \( m \lambda = d \sin \theta_r \). (c) Wavelengths gathered at specular angle (0°) are more dispersed for higher orders, and longer wavelengths are dispersed at higher angles, sometimes two can be present at the same angle. Decreasing \( d \) or decreasing \( m \) results in a lower angular dispersion of wavelengths (difference in the angles diffracting any two given wavelengths). For clarity reasons, incident and diffracted beams are figured at distinct paths. Within the second order, we found that intensity of the diffracted beam was maximal for a given wavelength and decreased away from that wavelength (Fig 1e or 1f), which is typical of blazed gratings [2]. A blazed grating occurs when the top of the squared battlements is slightly titled or when triangular battlements are unequal, which is due to target casting.
Target casting

We casted 1.25ml of coloured resin (Specifix-40 resin Struers, chosen for its high UV transmission) onto the diffraction grating of disassembled CDs (CD-R A2O 700MB, Verbatim; with 1.45 ± 0.05 µm path length, Fig S3) following Whitney et al. [3]. The smooth side of CDs was used to create non-iridescent artificial targets. We checked for the presence of gratings in microscopy and scatterometry (EZ-Contrast 80M, Eldim SA). We selected four distinct colours (violet, blue, yellow, and red) for flower corolla colouration. These colours are frequently found in floral structures which produce iridescence in nature [3–5] and have already been included in experiments testing the function of flower iridescence [3]. Pigments were manganese violet (Manganviolett, Kremer), cobalt blue (n°307 Sennelier), chrome yellow (n°549 Sennelier), and cinnabar red (Zinnober SHINSYA Nr. 14, Kremer). We covered all targets with a depolarizing mylar layer (Grafix Clear 0.003, Dura-Lar) which removes polarization signals – that bees detect and use [6]– leaving colouration intact [3,7].

Target colour measurements in goniospectrometry

To investigate target colour characteristics, angle-dependent optical properties and to illustrate how target appearance changed with the viewing angle and/or orientation, we measured targets with a goniospectrometer. We used a 75W xenon light source (emission between 200 and 1100 nm), collimated fibres with UV-transparent lenses at 17 cm from the sample, and a spot diameter of 2.5 mm. The target was placed on a sample holder equipped with a rotating platen which allowed to precisely control the orientation of the disk (called ϕ, 1° precision, Fig S5c), allowing to measure reflectance at the same point on the disk and in all directions. We set the incident light to be at 0°, as in the arena. We moved the collecting fibre and sample holder to change θ and ϕ. The collected light was transferred to an Avantes spectrometer (Avaspec 2048L) connected to a computer and displayed using Avasoft 8 software. We measured reflectance between 300 and 650 nm, for targets of different colours (blue, violet, yellow, and red), IRP level (high and low) and iridescence level (iridescent and non-iridescent). Preliminary surveys showed that targets exhibited their second order of diffraction between 26° and 70° so we restricted our prospection to that range of collection angles. In a randomly chosen disk area, we rotated the platen (ϕ in Fig S5c) until diffraction emerged and was highest (in that orientation, the grating was locally perpendicular to the plane formed by the illuminating fibre and the collecting fibre). We defined this orientation as the 0° orientation (ϕ in Fig S5c).

a) Fixed collection angle θ and orientation ϕ, to test whether iridescent and non-iridescent targets differed by their pigmentation

We measured three targets per colour, IRP level and iridescence level. We set the collection angle to θ=38° and disk orientations to ϕ =30° (0° being identified as the orientation where diffraction emerged most strongly). We measured each target in four different randomly chosen points on the disk. This configuration tested whether, despite of precautions taken during casting, iridescent targets may be systematically more or less pigmented than non-iridescent targets of the same colour and IRP level. In case we failed at revealing any statistically significant difference between iridescent and non-iridescent targets, this would mean that bumblebees could very likely not make any difference between iridescent and non-iridescent targets based on their pigmentation.
b) Changing collection angle θ and orientation φ, to illustrate iridescence at the orientation where diffraction was maximal

We measured one target per colour, IRP level and iridescence level. In a randomly chosen disk area, we rotated the platen (φ in Fig S5c) until diffraction emerged and was highest (in that orientation, the grating was locally perpendicular to the plane formed by the illuminating fibre and the collecting fibre). We defined this orientation as the 0° orientation (Fig. S5c). At this orientation, we measured reflectance spectra every 2 degrees from θ=26° to θ=70°. We then changed disk orientation φ to 30°, 60°, 90°, 120°, and 150°. For each orientation, we measured reflectance spectra every 4 degrees from θ=26° to θ=70°. We used the second-order diffraction spectra taken in the 0° orientation to compute coordinates in bumblebee triangular color space and to illustrate iridescent targets. In general, diffracted rays were about 2000% reflectance but slightly varied according to position on the target or disk. We normalized the peaks of diffracted rays of the second order to a maximal response of 2000% before analysing them in bumblebee colour space (Fig 1c and 1d), in order to facilitate comparison.

c) Changing collection angle θ, fixed orientation φ, to test whether bees could use static chromatic or achromatic cues

We measured one iridescent and one non-iridescent target per colour and IRP level. We set the collection angle to θ=38° and we measured spectra at various disk orientations φ. We measured a spectrum every degree from -10° and +10° around the 0° orientation. We measured a spectrum at φ =30°, 60°, 90°, 120°, and 150°. We rotated the target until finding the other orientation at which diffraction emerged. A perfect linear grating generates diffraction at φ=0° and φ=180° only (angular separation of 180° between the two orientations). A curved grating (like ours, cast on CDs) shows an angular separation lower than 180° which value depends on grating curvature (how close to the CD centre the disk was cast). We defined the ~180° orientation the orientation at which diffraction emerged and was highest. We took a spectrum every degree from -10° and +10° around that orientation.

Colour analysis

We analysed spectra to extract physically and biologically relevant information. First, we computed dominant wavelength [8], as the wavelength of maximal reflectance (for bell-shaped spectra) or maximal change in reflectance (for S-shaped spectra) to characterize this aspect in physics. Second, we computed various biologically-relevant variables using visual modelling. We used the irradiance spectrum of the ambient light used in our experiments (Fig. S2), the reflectance spectrum of the target colours, the reflectance spectrum of the background (Fig 1a, Fig 1b), and bumblebee photoreceptor absorbance curves [9]. With visual modelling, we computed S, M, and L receptor-specific contrasts. Each receptor-specific contrasts was computed as the ratio of the response of the receptor to the target, divided by the response of the receptor to the background, as in [10]. In bees, chromatic detection is based on S, M, and L receptors whereas achromatic detection is mediated by the L receptor and used in many vital tasks such as navigation or foraging (review in [11]). Hence, L receptor specific contrast is used to quantify achromatic differences between targets while S, M, and L contrasts are used to explore chromatic differences between targets. We computed the RNQ Colour contrast as computed from Vorobyev and Osorio’s Receptor Noise-Limited discrimination Model [12] in its log form [13], with quantum noise (RNQ). This latter model states that discrimination is limited by receptor
noise, which is inversely proportional to quantum catches, thereby following von Kries’ law. This model predicts dim stimuli are more difficult to detect, which is particularly suited to account for the lab light conditions used in our case. More specifically, we used the same $\omega$-values of 0.13, 0.06 and 0.12 for S, M, and L receptors respectively, and the same equations as in [14]. We also computed Endler & Mielke’s model with not log-corrected quantum catches[15], to illustrate the position of targets in bumblebee triangle colour space (Fig. 1c and S4a, 1d and S4b).

**Statistical analyses**

a) Target optical properties

Based on measurements performed with the goniometer, we tested whether iridescent and non-iridescent targets exhibited recurrent chromatic (S-, M-, L- receptor contrast, RNQ contrast) or achromatic differences that could be used by bumblebees to discriminate them: (i) in pigment concentration, away from the orientation at which diffraction emerged (in linear mixed models). We used iridescence (present/absent), IRP level (high, low) and their interaction as fixed effects and the target as a random factor. We predicted that bumblebees could learn differences in pigment concentration if this signal varied systematically between iridescent and non-iridescent targets. (ii) in static cues experienced at a given viewing angle (in linear models). We used target colour (B, Y, R, V), coarse orientation (around 0°, around ~180°), fine orientation (in degrees away from the peak) and their interactions as fixed effects. We predicted that bumblebees could learn a specific chromatic or achromatic signal at a given viewing angle only if this signal did not vary between nor within targets. We selected the best model by minimizing the AIC criterion.

b) Influence of IRP effect on learning, generalisation and memory retention

High IRP level should facilitate learning, generalisation and retention. It should reduce the time needed to complete a foraging bout and improve its efficiency by increasing the proportion of correct choices. Both variables (time and proportion of correct choices) were available for all three phases (learning, generalisation, and retention); in the case of the learning phase, we used 80% of correct choices as a criterion for completion of learning. We used complementary classic and mixed-model approaches. Mixed models - with the probability of visiting a rewarding target as a variable, the individual as a random factor, a binomial error distribution, and visit rank, iridescence, IRP level, and target colour as fixed effects – only converged when no interaction was considered, a fact that limited their relevance as interaction effects were the most interesting for our hypotheses. We thus used them only to check whether individuals learned the trained visual discrimination. We used classic parametric tests (one-way Anovas with Tukey’s test and student t-tests) for normally distributed variables and non-parametric tests (Mann-Whitney U tests and Kruskal-Wallis) otherwise. We tested if bumblebees learned the visual discrimination by computing the number of correct choices over the first ten and the last ten visits of the learning phase and examined learning speed using the total number of visits, learning duration and learning rate, parameters commonly used in conditioning experiments [16]. Discrepancies between visits and duration may reveal potential differences between the time taken to reach decisions and the number of decisions reached, consistent with potential speed-accuracy trade-offs. IRP level (high or low) may affect the number of rewarding flowers visited, and thus foraging activity patterns. We thus examined the total number of foraging bouts during a phase, their duration, and the number of rewarding flowers visited per bout or per learning phase. We also tested whether day period (morning/afternoon) affected our results. In generalisation and retention tests, we...
computed the number of correct choices performed by individuals as well as the time needed to complete the test.

c) Influence of target corolla colouration on learning, generalisation and memory retention
We used complementary classic and mixed-model approaches. For the learning phase, we tested bumblebee colour preferences and whether preferences changed with the IRP level using mixed models. We included the total number of flowers of a specific colour visited during the first and last ten visits of the learning phase as the response variable, the individual as the random effect and a normal error distribution. We included the target colour (blue, yellow, red, and violet), the colour trio used during training, the sequence (first ten or last ten), the IRP level (high or low) and their interactions as potential fixed effects. We also used classic statistics and computed the total number of visits during the learning phase and the learning duration; we tested the influence of colour trio using one-way ANOVAs.

For the generalisation test, we computed the proportion of errors made by individuals and analysed this proportion using linear mixed models, with the individual as a random effect. We tested the influence of the novel colour and of the IRP level presented in this phase on behavioural choices. Using linear regressions, we also analysed the correlation between the duration of the generalisation test and the targets’ dominant wavelength, the bumblebee receptor-specific contrasts, and the chromatic contrast derived from the RNQ models.

For the retention test, we computed the proportion of correct choices (i.e. the proportion of choices of iridescent targets irrespectively of their colour) and analysed this proportion using linear mixed models with the individual as random effect. We also analysed the effect of the novel colour on the proportion of correct choices. All analyses were performed using R packages [17].
<table>
<thead>
<tr>
<th>Variable</th>
<th>Presence of iridescence effect</th>
<th>IRP effect</th>
<th>IRP x Presence effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant wavelength</td>
<td>4.84 (±9.39) 0.52</td>
<td>-6.17 (±9.39) -0.66</td>
<td>3.75 (±9.39) 0.40</td>
</tr>
<tr>
<td>S receptor contrast</td>
<td>-0.02 (±0.04) -0.44</td>
<td>-0.23 (±0.04) -6.06***</td>
<td>-0.05 (±0.04) 1.47</td>
</tr>
<tr>
<td>M receptor contrast</td>
<td>0.01 (±0.07) 0.23</td>
<td>-0.25 (±0.07) -3.84***</td>
<td>-0.02 (±0.07) 0.36</td>
</tr>
<tr>
<td>L receptor contrast</td>
<td>0.12 (±0.25) 0.45</td>
<td>-0.74 (±0.25) -2.93**</td>
<td>-0.07 (±0.25) -0.26</td>
</tr>
<tr>
<td>RNQ colour contrast</td>
<td>0.002 (±0.005) 0.36</td>
<td>-0.02 (±0.005) -3.79**</td>
<td>-0.003 (±0.005) -0.46</td>
</tr>
</tbody>
</table>

Table S1: Differences in visual characteristics between iridescent and non-iridescent targets, for high and low IRP (iridescent relative to pigment ratio) level, as estimated from linear mixed models. We computed the physical parameter dominant wavelength, and biological parameters. L receptor contrast described brightness differences while all other parameters described chromatic differences between targets. Measurements in goniospectrometry were done at an orientation of 30°, and a collection angle of 38°, in four randomly chosen points on three disks of each colour, iridescence (non-iridescent, iridescent) and IRP level. We provide coefficient estimated values ±standard errors, t-values with a symbol representing the associated p-value (no symbol p>0.10,* p<0.05, ** p<0.01, ***p<0.001).
Figure S4: (a) L-receptor-specific contrasts of the different targets. L-receptors mediate achromatic detection in bees. (b) S-receptor specific contrasts of the different targets, and (c) colour contrast obtained from RNQ model. Targets were measured in spectrometry with an integrated sphere. They are identified by their colour (circle for blue, square for yellow, diamond for red, triangle for violet), their pigment concentration (empty for low, grey for high concentration), and the presence of iridescence (large symbols for present, small symbols for absence). Tick labels indicate the colour (B for blue, Y for yellow, R for red, and V for violet), the IRP level (H for high, and L for low) and the presence of iridescence (I for iridescent, and N for non iridescent).
Variations in $\Delta S$ are held constant at a maximum of 2000 for all measurements, including all orientations and collection angles. We then selected the spectra with the collection angle of 38°.

We provide the following analysis of goniospectrometric measurements performed on iridescent targets with incident light at 0° and collected light at 38°. Variations in $S$-contrast, M-contrast and L-contrast between targets, and variations in $\Delta S$ colour contrast computed with the RNQ model, as explained by their colour (B=blue, Y=yellow, R=red, V=violet), their orientation (around 0° or around “180°”), and their orientation (away from the diffraction peak in degrees) and the interaction between these factors explored using linear models. The best model was selected by minimizing the AIC criterion and was identical in all cases. We provide coefficient estimated values (standard errors, $t$-values with a symbol representing the associated $p$-value (no symbol $p>0.10$, ~$p<0.07$, * $p<0.05$, ** $p<0.01$, ***$p<0.001$). For a given target, we scaled diffraction peak intensity to a maximum of 2000 for all measurements, including all orientations and collection angles. We then selected the spectra with the collection angle of 38°. For both orientations (0° and “180°), we retained the spectra from the peak to 5 degrees away in each direction to include all possible intensities spanning from the maximum to the minimum (see Fig. S5).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Lcontrast</th>
<th>Mcontrast</th>
<th>Scontrast</th>
<th>$\Delta S$ colour contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour. (B&gt;V)</td>
<td>$-0.02(\pm0.22) -0.11$</td>
<td>$0.19(\pm1.17) 0.16$</td>
<td>$-0.25(\pm0.07) -3.70^{***}$</td>
<td>$0.02(\pm0.04) 0.41$</td>
</tr>
<tr>
<td>Colour. (Y&gt;R)</td>
<td>$-0.19(\pm0.22) -0.86$</td>
<td>$-6.25(\pm1.16) -5.40^{***}$</td>
<td>$-0.69(\pm0.07) -10.39^{***}$</td>
<td>$-0.08(\pm0.04) -2.35^*$</td>
</tr>
<tr>
<td>Colour. (BV&gt;YR)</td>
<td>$-0.80(\pm0.16) -5.16^{***}$</td>
<td>$-1.65(\pm0.82) -2.00^*$</td>
<td>$0.03(\pm0.05) 0.52$</td>
<td>$-0.03(\pm0.03) -1.28$</td>
</tr>
<tr>
<td>Orientation (° away from peak)</td>
<td>$-0.35(\pm0.05) -7.15^{**}$</td>
<td>$-2.25(\pm0.26) -8.62^{***}$</td>
<td>$-0.06(\pm0.02) -3.80^{***}$</td>
<td>$-0.05(\pm0.01) -5.98^{***}$</td>
</tr>
<tr>
<td>Orientation. (0°&gt;~180°)</td>
<td>$0.39(\pm0.16) 2.50^*$</td>
<td>$2.79(\pm0.82) 3.40^{***}$</td>
<td>$-0.15(\pm0.05) -3.24^{***}$</td>
<td>$0.09(\pm0.03) 3.30^{**}$</td>
</tr>
<tr>
<td>Colour. (B&gt;V) x Orientation (° away from peak)</td>
<td>$-0.002(\pm0.07) -0.02$</td>
<td>$-0.18(\pm0.38) -0.47$</td>
<td>$-0.15(\pm0.05) -3.24^{**}$</td>
<td>$-0.004(\pm0.01) -0.36$</td>
</tr>
<tr>
<td>Colour. (Y&gt;R) x Orientation (° away from peak)</td>
<td>$0.19(\pm0.07) 2.73^{**}$</td>
<td>$1.42(\pm0.36) 3.93^{***}$</td>
<td>$0.02(\pm0.02) 0.96$</td>
<td>$0.05(\pm0.01) 4.10^{***}$</td>
</tr>
<tr>
<td>Colour. (BV&gt;YR) x Orientation (° away from peak)</td>
<td>$0.10(\pm0.05) 2.02^*$</td>
<td>$0.56(\pm0.26) 2.15^*$</td>
<td>$0.07(\pm0.02) 3.21^{**}$</td>
<td>$-0.002(\pm0.008) -0.19$</td>
</tr>
<tr>
<td>Colour. (B&gt;V) x Orientation. (0°&gt;~180°)</td>
<td>$-0.17(\pm0.22) -0.75$</td>
<td>$-2.88(\pm1.17) -2.41^*$</td>
<td>$0.02(\pm0.02) 1.32$</td>
<td>$-0.13(\pm0.04) -3.34^{**}$</td>
</tr>
<tr>
<td>Colour. (Y&gt;R) x Orientation. (0°&gt;~180°)</td>
<td>$0.12(\pm0.21) 0.55$</td>
<td>$2.17(\pm1.16) 1.88\sim$</td>
<td>$0.18(\pm0.07) 2.64^{**}$</td>
<td>$0.02(\pm0.04) 0.59$</td>
</tr>
<tr>
<td>Colour. (BV&gt;YR) x Orientation. (0°&gt;~180°)</td>
<td>$0.48(\pm0.16) 3.04^{**}$</td>
<td>$3.31(\pm0.82) 4.03^{***}$</td>
<td>$0.08(\pm0.07) 1.70\sim$</td>
<td>$0.06(\pm0.03) 2.31^*$</td>
</tr>
<tr>
<td>Or (° away from peak) x Or (0°&gt;~180°)</td>
<td>$-0.15(\pm0.04) -3.04^{**}$</td>
<td>$-0.85(\pm0.26) -3.26^{**}$</td>
<td>$0.03(\pm0.02) 1.78\sim$</td>
<td>$-0.02(\pm0.01) -2.73^{**}$</td>
</tr>
<tr>
<td>Colour. (B&gt;V) x Or (° away from peak) x Or (0°&gt;~180°)</td>
<td>$0.03(\pm0.07) 0.48$</td>
<td>$0.88(\pm0.37) 2.36^*$</td>
<td>$-0.007(\pm0.02) -0.37$</td>
<td>$0.04(\pm0.01) 3.00^{**}$</td>
</tr>
<tr>
<td>Colour. (Y&gt;R) x Or (° away from peak) x Or (0°&gt;~180°)</td>
<td>$0.03(\pm0.07) 0.43$</td>
<td>$-0.47(\pm0.36) -1.3$</td>
<td>$-0.04(\pm0.02) -1.92\sim$</td>
<td>$0.002(\pm0.01) 0.88$</td>
</tr>
<tr>
<td>Colour. (BV&gt;YR) x Or (° away from peak) x Or (0°&gt;~180°)</td>
<td>$-0.15(\pm0.05) -3.12^{**}$</td>
<td>$-0.97(\pm0.26) -3.75^{***}$</td>
<td>$-0.03(\pm0.01) -1.73\sim$</td>
<td>$-0.02(\pm0.008) -2.23^*$</td>
</tr>
</tbody>
</table>

Table S2. Analysis of goniospectrometric measurements performed on iridescent targets with incident light at 0° and collected light at 38°. Variations in $S$-contrast, M-contrast and L-contrast between targets, and variations in $\Delta S$ colour contrast computed with the RNQ model, as explained by their colour (B=blue, Y=yellow, R=red, V=violet), their orientation (around 0° or around “180°”), and their orientation (away from the diffraction peak in degrees) and the interaction between these factors explored using linear models. The best model was selected by minimizing the AIC criterion and was identical in all cases. We provide coefficient estimated values (standard errors, $t$-values with a symbol representing the associated $p$-value (no symbol $p>0.10$, ~$p<0.07$, * $p<0.05$, ** $p<0.01$, ***$p<0.001$). For a given target, we scaled diffraction peak intensity to a maximum of 2000 for all measurements, including all orientations and collection angles. We then selected the spectra with the collection angle of 38°. For both orientations (0° and “180°), we retained the spectra from the peak to 5 degrees away in each direction to include all possible intensities spanning from the maximum to the minimum (see Fig. S5).
Figure S5. Variations in reflectance of the peak diffracted at a collected angle of $\theta=38^\circ$, for (a) high IRP and (b) low IRP targets. (c) Scheme of goniometric measurements, with light incident at 0°, collected at an angle $\theta=38^\circ$, in various disk orientations $\phi$ (red dots). A perfect linear grating generates diffraction at $\phi=0^\circ$ and $\phi=180^\circ$ only (angular separation of 180° between the two orientations). A curved grating (like ours, cast on CDs) shows an angular separation lower than 180° which value depends on grating curvature (how close to the CD centre the disk was cast). We defined as the 0° and the ~180° orientations the orientations at which diffraction emerged and was highest. We took a spectrum every degree from -10° and +10° around these two orientations, and a spectrum at $\phi =30^\circ$, 60°, 90°, 120°, and 150°. In (a) and (b), we extracted the dominant diffracted wavelength and scaled the peak reflectance relative to its minimal and maximal values (over all possible $\phi$ values) so that all targets could be compared. In (d), we presented the dominant wavelength of the reflectance peak diffracted at 38° for the orientations around 0° and “180” for all iridescent targets. Although cast on identical CDs, iridescent disks show a large variation in their dominant wavelength at a given viewing angle: between targets, and within targets according to the orientation (around 0° and around “180”) from which a spot is seen. While the between-target variation may be due to the fact that targets are cast at different distances from the CD centre, the within-target variation shows that diffraction gratings are blazed (irregular), probably due to casting process and resin polymerisation. Iridescent targets are violet (triangle), blue (circle), yellow (square) and red (diamond) for high IRP (empty symbols) or low IRP effect (grey symbols), as in Fig. 1.
Figure S6. (a,b) Chromatic location of colours in the triangle colour space for targets with (a) low and (b) high IRP level (respectively high and low pigment concentration). The chromatic space was based on Endler and Mielke’s colour space for trichromatic vision, with not log-corrected quantum catches [15]. The triangle colour space is not entirely represented for clarity reasons. Spectra were measured in goniospectrometry with target orientation $\phi=0^\circ$, light incident at $0^\circ$, and light collected every $2^\circ$ between $\theta=26^\circ$ and $\theta=70^\circ$, between 300 and 650 nm (details in ESM). For iridescent targets, diffraction peaks were scaled to a common maximum of 2000% as this value was commonly found. Symbols are the same as in Figure 1. Target colours are violet triangles, blue circles, yellow squares, and red diamonds, for non-iridescent (small symbols) and iridescent (large symbols) targets, of low IRP (filled symbols) or high IRP (empty symbols) of iridescence. In (b), all points are empty but some are very close to each other, and appear as if filled.
Figure S7: Individual bumblebee feeding on an iridescent rewarding violet target. The individual is involved in the treatment of high IRP treatment. Photo © G. de Premorel.
Figure S8. Variation of the proportion of correct choices during the learning phase both for low (grey symbol) and high (empty symbol) IRP level. The proportion of correct choices was computed based on a series of 10 visits; the last series could be therefore incomplete. It was presented if at least 6 visits could be recorded, which led to a proportion of correct choices higher than 80%. The series for low IRP was slightly offset in y coordinate for clarity reasons.
Figure S9. Duration of the generalisation test according to the (a) L-receptor specific contrast mediating brightness detection, (b) colour contrast as computed with RNQ model, and (d) dominant wavelength of the novel colour. Flower colours are violet (triangle), blue (circle), yellow (square) and red (diamond) for high IRP (empty symbols) or low IRP effect (grey symbols), as in Fig. 1. Three points were systematically slightly offset in duration not to overlap, for clarity reasons.
REFERENCES


