

Current Biology

Flower Iridescence Increases Object Detection in the Insect Visual System without Compromising Object Identity

Highlights

- Iridescence can increase object detectability
- Iridescence can increase the disruption of accurate color identification
- Detectability and disruption are influenced by variation in iridescent structures
- Floral iridescence is an optimal trade-off between detectability and identification

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In Brief

Some animal-pollinated flowers are iridescent, but this iridescence is subtle when compared to bolder animal iridescence. Whitney et al. show that iridescence could make flowers more detectable to bees. However, subtlety may be beneficial to flowers as bolder iridescence also disrupts the ability of bees to identify colored targets.



Flower Iridescence Increases Object Detection in the Insect Visual System without Compromising Object Identity

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SUMMARY

Iridescence is a form of structural coloration, produced by a range of structures, in which hue is dependent on viewing angle [1–4]. One of these structures, the diffraction grating, is found both in animals (for example, beetles [2]) and in plants (on the petals of some animal pollinated flowers [5]). The behavioral impacts of floral iridescence and its potential ecological significance are unknown [6–9]. Animal-pollinated flowers are described as “sensory billboards” [10], with many floral features contributing to a conspicuous display that filters prospective pollinators. Yet floral iridescence is more subtle to the human eye than that of many animal displays because the floral diffraction grating is not perfectly regular [5–9]. This presents a puzzle: if the function of petals is to attract pollinators, then flowers might be expected to optimize iridescence to increase showiness. On the other hand, pollinators memorize floral colors as consistent advertisements of reward quality, and iridescence might corrupt flower color identity. Here we tested the trade-off between flower detectability and recognition, requiring bumblebees (*Bombus terrestris*) to identify artificial flowers that varied in pigmentation and degree of iridescence. We find that iridescence does increase target detectability but that “perfect” iridescence (produced by an artificial diffraction grating) corrupts target identity and bees make many mistakes. However, “imperfect” floral iridescence does not lead to mistaken target identity, while still benefitting flower detectability. We hypothesize that similar trade-offs might be found in the many naturally “imperfect” iridescence-producing structures found in animal-animal, as well as other plant-animal, interactions.

RESULTS AND DISCUSSION

Modeling the Impact of Iridescence on Bee Color Perception

Iridescence can produce some of the most saturated and colorful displays in nature. Biological nanostructures can induce the perception of the entire range of a rainbow’s color, but often only a reduced range of colors is produced in biological iridescence; there are often sharp discontinuities between target areas that display iridescence and those that do not, and perceived colors can change dramatically with viewing angle (not just gradually along a rainbow). Diffraction gratings (where light is diffracted by a periodic structure, usually of ridges) are one way in which biological iridescence can be produced.

Differences in the degree of “perfection” in diffraction gratings that generate iridescence might impact the visual system of insect pollinators. For definition of the optical effects of different surfaces, the spectral reflection of the range of hues produced by target epoxy disks (varying in iridescence production and pigment) was measured using optical spectroscopy in the 300-to-700-nm wavelength range (near-UV to infrared). Light from a xenon light source was reflected off the target disks at a 45° incidence angle, and the reflected and scattered light was analyzed at angles varying from 45° to 90° in 5° steps [11]. This was repeated in 10° steps from a position of 90° to one of 0° across the diffraction grating. The spectral reflectance curves measured at each angle can be used to determine the relative excitation values in each of the bees’ color receptors (UV, blue, and green), and, using the methods specified by Chittka [12], these excitation values were used to calculate hexagon color loci under lab conditions (as detailed in [13]) for all target flowers used in this study (Figure 1).

Figure 1A shows the color loci of two disks that contain the same blue pigment but have different types of diffraction grating, calculated in bee hexagon color space [12]. Artificial gratings were cast from a commercial diffraction grating film, whereas floral diffraction gratings were cast from the iridescent tepal of the Queen of the Night tulip cultivar. Both the two different types of diffraction grating (artificial “perfect” and floral “imperfect”) produce scattering within bee color space;

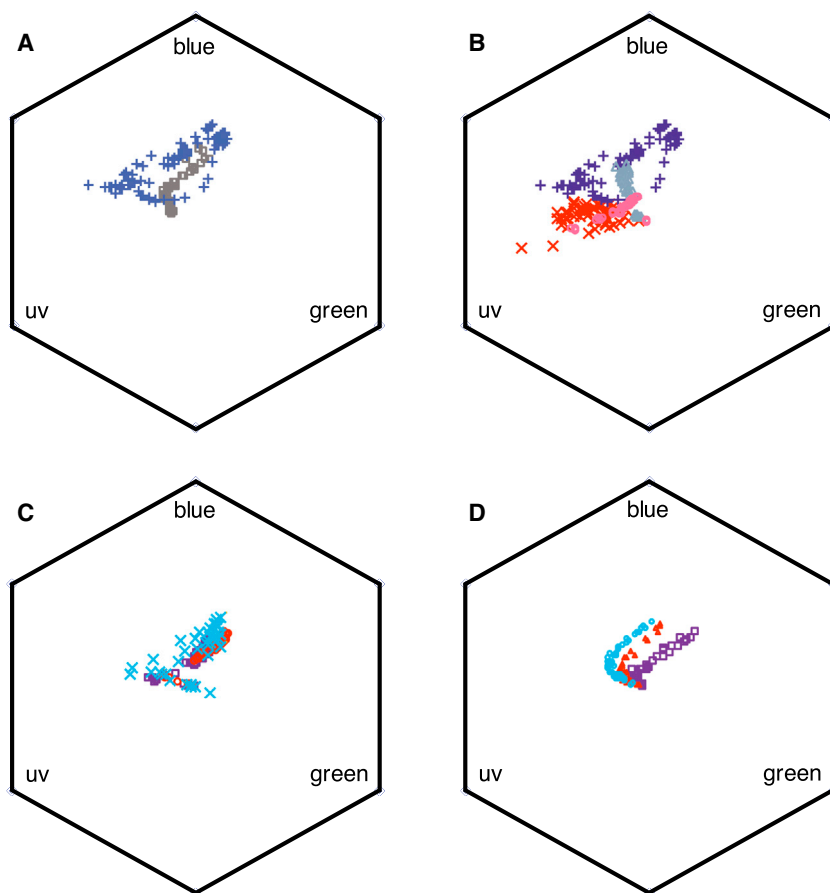


Figure 1. Color Hexagons Showing the Scatter of Loci in Bee Color Space Measured from Disks Used

Color loci in this color space are determined by the relative photoreceptor signals of the bees' UV, blue, and green receptors in response to viewing a particular target. The angular position (as measured from the center) of a color point indicates its bee-subjective hue, so that color loci on the top region will appear as "bee blue" (generated by objects that predominantly stimulate the bees' blue receptors), loci in the bottom-right corner will be perceived as "bee green," etc. The distance between two color points indicates their similarity within bee visual color space.

(A) Blue disks with overlying artificial (blue crosses) or floral (green-gray squares) iridescence.

(B) Red (red crosses) and blue (blue crosses) disks with overlying artificial iridescence and from the equivalent blue (pale blue-gray triangles) and red (pink circles) non-iridescent disks.

(C) Purple (purple squares), blue-purple (blue crosses), and red-purple (red circles) disks with overlying artificial iridescence.

(D) Purple (purple squares), blue-purple (blue circles), and red-purple (red triangles) disks with overlying floral iridescence.

See also [Figures S1–S3](#) and [Tables S1](#) and [S2](#).

An explanation for this is suggested by [Figure 1B](#), showing a spread of color loci in the non-iridescent disks, due to specular reflection or "gloss" (gloss measurements, as measured in [\[16\]](#), are included in [Figure S2](#)). So that the impact of iridescence on flower

however, the pattern of this scattering differs between the two types of grating. The distribution of colors produced by the "perfect" grating is considerably wider than that produced by the "imperfect" floral grating. The underlying pigment also impacts the perceived color, with two disks with identical "perfect" diffraction gratings but different underlying pigment displaying discrete scattering in color space ([Figure 1B](#)). Further analysis of the individual receptor signals suggests that the interaction between underlying pigment and overlying iridescence may have a range of effects; for example, the stimuli whose color loci are displayed in [Figure 1B](#), when analyzed for individual receptor contrast, show a much wider spread in the UV channel for the blue pigment disk than for the red pigment disk ([Figure S1](#) and [Tables S1](#) and [S2](#)).

These findings support the hypothesis that iridescence could facilitate the detectability of an object. Iridescent objects show a broader spread of loci within bee color space, and detectability depends in part on the amount of contrast that is presented to a visual system over time [\[14, 15\]](#). However, the color loci analysis also suggests that there could be additional effects. The analysis of the individual receptor signals ([Supplemental Information](#)) shows that there is a diversity in the median and range of signals shown by different disk types. Although blue artificial iridescent disks show the greatest range of UV receptor signals, it is actually non-iridescent disks that show the highest green receptor signal (which is of interest because this signals determines target detectability, in addition to color contrast [\[15\]](#)).

detection and identification by bees could be determined, direct behavioral experiments were therefore performed.

Iridescence Increases Detectability and Can Perturb Object Identity

It has previously been shown that the color contrast of an object with its background can impact its salience (detectability) [\[17\]](#). However, all investigations into this topic have used pigment colors. Iridescence, including that generated by plant structures, can be extremely colorful and produce highly contrasting bands of distinct hues [\[18\]](#). This could provide a high contrast with any surrounding background, as well as between the different hues produced by the iridescence, and make the object highly detectable, which would be a distinct advantage for any floral "billboard." Additionally, the color-shifting qualities of iridescence itself could also increase flower detectability as an animal moves toward it. Target detectability depends in part on the amount of contrast that is presented to a visual system over time [\[14, 15\]](#). With an iridescent target, the hue of color produced is dependent on the angle from which it is observed. For a mobile forager searching for suitable targets, an iridescent object could therefore produce more visual change per unit time than one where the color is produced by pigment and is therefore homogenous. This would increase detectability and could have significant effects on the behavioral ecology of foragers, to the advantage of both flower and visitor. Although studies have explored the effectiveness of iridescence as a sexual signal [\[19, 20\]](#), none

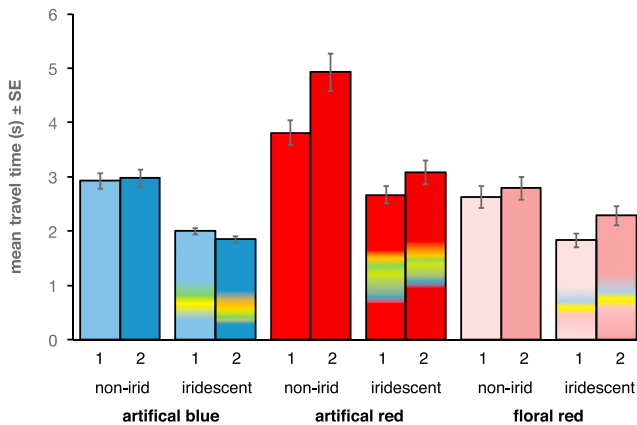


Figure 2. Bee Travel Times

Travel time (in seconds; mean \pm SEM) between first and second (1) and between second and third (2) disk choice for the blue disks with either no iridescence or overlying artificial iridescence, red disks with either no iridescence or overlying artificial iridescence, and red disks with either a non-iridescent floral surface or overlying floral iridescence. The differences between visits are described in the [Supplemental Experimental Procedures](#).

has yet analyzed the detectability of iridescent targets compared to conventional visual cues that do not change appearance with viewing perspective.

Using the method described in Spaethe et al. [17], we determined the extent to which the addition of diffraction-grating generated iridescence (either “perfect” iridescence derived from an artificial grating or “imperfect” iridescence derived from a flower) to an object influenced its detectability. The contrast of the chemical (pigment) color of an object with that of the background has been shown to determine its detectability [17], and in both plant and animal systems structural color is usually combined with pigments [3]. In this study, targets with different pigment colors (ultramarine blue and quinacridone red) were used in combination with “perfect” iridescent, “imperfect” floral-derived iridescent, or non-iridescent disks (equivalent, but lacking diffraction grating, artificial or floral surfaces were used to make non-iridescent disks) [5]. Bees were pre-trained to locate 3-cm disks of a single color randomly located in a flight arena at set distances from each other. During the test phase, the time taken by an individual bee to locate 1-cm disks of the same color was recorded (disk 1 to disk 2 and disk 2 to disk 3). This was repeated with an independent set of ten bees for both red and blue disks with the different iridescence options.

We find that the presence of iridescence reduced the travel time between disks ($F_{1,88} = 33.349$, $p < 0.001$; [Figure 2](#)). Both the underlying pigment color and the type of overlying iridescence affected the travel time ($F_{2,88} = 13.325$, $p < 0.001$). Bees take longer to find red “perfect” disks than they do to find the equivalent blue disks, suggesting that when overlying iridescence is equal, the underlying pigment color contributed to disk detectability in a similar way to that previously described in the literature (Tukey’s honest significant difference [HSD] between red “perfect” and blue, $p < 0.001$; [Figure 2](#)) [17]. Differences in overlying iridescence also impact disk detectability. Although bees took longer to detect red “perfect” iridescent

disks than blue “perfect” iridescent disks, they were faster to detect floral red disks compared to “perfect” red ones (Tukey’s HSD, $p < 0.001$; [Figure 2](#)) and were equally fast between blue disks with “perfect” iridescence and red disks with “imperfect” floral iridescence (Tukey’s HSD, $p = 0.780$; [Figure 2](#)).

For flowers, where a high level of detectability is important, producing pigment colors with strong contrast to the background increases detectability [17]. Flowers that produced both pigment colors with a high degree of contrast to the background and iridescence would have the greatest color-dependent salience. Furthermore, this increase in detectability is achieved to an equivalent or even higher degree by “imperfect” floral-derived iridescence compared to “perfect” iridescence. Our findings show that red targets with floral iridescence are located as quickly by bees as are blue flowers with “perfect” iridescence. Producing iridescence could therefore be of benefit in situations, including flowering, where a high level of detectability is important. This might apply to plants that flower in relative isolation and would benefit from a greater likelihood of being spotted by a pollinator, to flowers in highly competitive situations where any increase in detectability would be an advantage, and to plants with very short-lived flowers that depend on high detectability in a narrow time window. On this point, it is interesting to note that the iridescent flower of *Hibiscus trionum* only blooms for 2–3 hr before wilting. A more detectable flower will have the potential to attract pollinators from a greater distance and would also benefit foraging insects because flowers that require minimal search time will minimize time and energy expended on searching. Optimal foraging theory predicts that search time is a key variable in determining the value of a target food source [21, 22]. So the more detectable a flower, the more valuable it becomes as a resource. This would promote flower constancy in visiting pollinators and thus the effective transfer of pollen between members of the same plant species.

These observations lead to the quandary as to why so many animals subject to high levels of predation, such as beetles, have non-sexually selected iridescence if it increases their detectability by visual predators [2, 23]. The answer to this may lie in aposematism [23, 24], but the visual effect resulting from the shift in hues might come with an additional effect—it may also obscure object identity.

Our data therefore raise a similar question regarding the impact of floral iridescence. Most flowers are not iridescent, and those that are do not display the bright, intense iridescence that, for example, fruits are capable of producing [5, 6, 18]. There may be a good reason why floral iridescence is “imperfect”—the detectability arising from “perfect” iridescence might come with a cost. This cost may arise if iridescence has the potential to corrupt object identity. In certain situations, where camouflage is important, this corruption of identity by disruptive coloration could be a significant advantage [4, 25]. However, to a foraging pollinator, disruption of color identity would weaken search image formation and compromise the pollinator’s ability to identify rewarding flowers.

Previous work has shown that foraging bumblebees can see iridescence independently of other visual cues, including underlying pigment color [5]. Although this is important in determining that iridescence can act as a cue in itself, iridescence does not

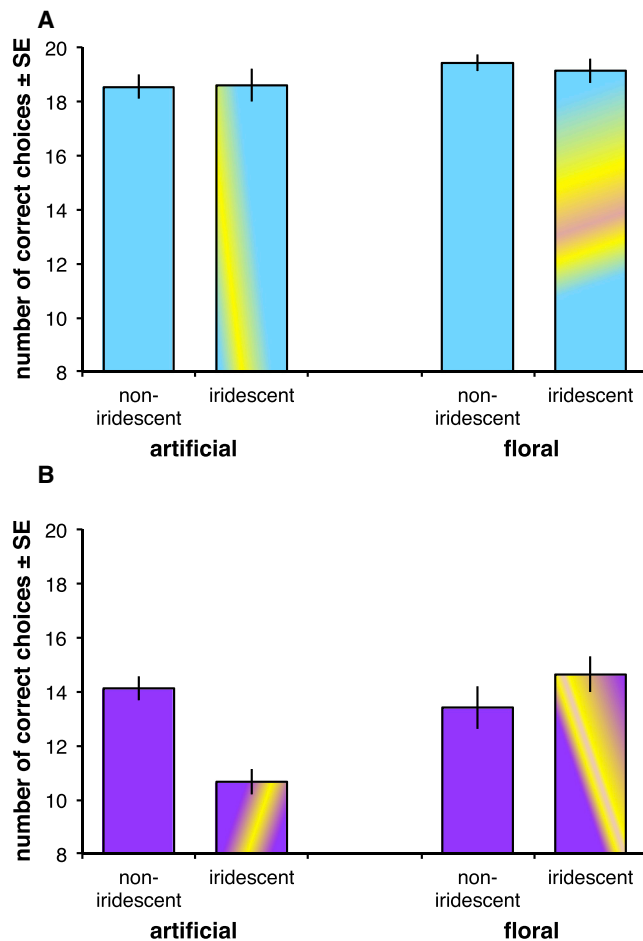


Figure 3. Bee Preferences in Color Discrimination Tests

(A) Mean preference (\pm SEM; out of 20 choices) of bees for blue disks when trained to blue and presented with both blue disks and red distracter disks, comparing floral and artificial surfaces (iridescent or non-iridescent).

(B) Mean preference (\pm SEM; out of 20 choices) of bees for purple disks when trained to purple and presented with equal numbers of purple target and blue-purple and red-purple distracter disks, comparing floral and artificial surfaces (iridescent or non-iridescent).

usually occur independently in most natural systems, with both animal and plant iridescence frequently being enhanced by chemical color or requiring an underlying pigment [3]. In flowers, the iridescence overlies the well-characterized pigment color signal. We hypothesize that in the visual perception of an insect, iridescence could interact with pigment color and thus produce disruption of color identity?

To test whether this hypothesis could be correct when flowers have strongly distinct pigment colors, we trained bees with absolute conditioning [26] to blue disk targets (“perfect” iridescent, “imperfect” floral-derived iridescent, or non-iridescent disks). Individual bees were then tested for 20 visits with both blue target disks and an equal number of red “distractor” disks.

In this initial experiment, we find that “perfect” iridescence does not disrupt color identity when targets are perceptually

far apart in color space (median correct visits with “perfect” iridescent disks = 19.5 [range = 14–20, $n = 10$], non-iridescent disks = 18 [range = 16–20, $n = 11$]; $W = 59.5$, $p = 0.764$; Figure 3A). These data are supported by the optical analysis shown in Figure 1B, which shows that although both the red and blue iridescent disks display a wide scatter of perceived hues in bee color space, there is no overlap in bee color space of the perceptual color distances between the range of colors produced by the targets. Therefore, we conclude that iridescence does not always perturb color identity.

When this experiment was repeated with “imperfect” floral-derived iridescence, again the presence of iridescence did not disrupt color identity (median correct visits with floral iridescent disks = 20 [range = 17–20, $n = 7$], non-iridescent disks = 20 [range = 18–20, $n = 7$]; $W = 22.5$, $p = 0.831$; Figure 3A).

However, flowers within a bee’s flight range are often much closer in pigment color than the distinct red and blue used here. For assessment of whether iridescence disrupts identity when targets are more similar in pigment hue, the experiment was repeated using disks that contained pigments much closer in bee color space (purple, purple blue, and purple red). Bees were trained to purple target disks (“perfect” iridescent, floral-derived “imperfect” iridescent, or non-iridescent disks) again using absolute conditioning. Individual bees were then tested with target disks (purple) and an equal number of distractor disks (purple blue and purple red). Using these disks, with the spectral reflectance of the underlying pigments closer in color space, it was found that “perfect” iridescence disrupted target identity significantly (median correct visits with artificial iridescent disks = 10 [range = 9–14, $n = 12$], non-iridescent disks = 14 [range = 13–16, $n = 8$]; $W = 6$, $p = 0.001$; Figure 3B). However, this disruption does not occur if the iridescence is floral derived (mean correct visits with “imperfect” floral iridescent disks = 14.63 ± 0.66 [SE], $n = 11$; non-iridescent = 13.42 ± 0.79 , $n = 12$; $t_{20.66} = 1.18$, $p = 0.252$; Figure 3B). These results are supported by the spectrometry measurements of the disks and the modeling of these data in bee color space (Figures 1C and 1D). Unlike the red and blue disks, the three shades of purple are much closer in bee color space, such that when the disks have iridescence generated by a “perfect” diffraction grating, individual points (representing positions in bee color space) overlay and overlap between the different disks (Figure 1C, with photographs of the disks in Figure S3). However, when the iridescence is generated by an “imperfect” floral diffraction grating, the individual points do not overlap, except in the center of the hexagon, which corresponds to the specular reflection of the white light source from the surface of the disk (Figure 1D). The modeling also confirms that the underlying pigment and overlying iridescence interact, with the floral iridescence generating the same pattern of scatter in all three pigment disks and the contribution of the pigment meaning that the loci are scattered along lines that radiate separately from the center. That this second color-discrimination test was a more difficult perceptual task is also confirmed by the behavioral data for the non-iridescent disks, where the accuracy rate drops from the 90% accuracy shown with the non-iridescent red and blue disks to 72% in the purple disk discrimination. This drop in accuracy for discrimination of similar colors has been shown previously [27].

Conclusions

Iridescence appears to have multiple impacts on an insect visual system both independently and through interaction with any underlying pigmentation. Iridescence can both increase the detectability of an object and also alter the extent to which aspects of the object can be accurately identified.

Our behavioral data are supported by modeling spectral analysis of iridescent targets in bee color space. They confirm that both underlying pigment and overlying iridescence contribute to the hue perceived. They also confirm that iridescence can produce a broader scatter within color space compared to non-iridescent objects and that this scatter has an impact both on how detectable an object is and on the extent to which it disrupts the identity of the underlying pigmentation color. Although the pattern of scatter within color space may be key in determining the extent to which the iridescence impacts detectability and identity, analysis of the contrast of each color receptor showed that there may be multiple factors at work here, with color contrast and achromatic specular contrast contributing to different extents in different iridescence-pigment combinations. Some optical effects can be dismissed, for example, the polarization of reflected light. Although iridescence is known to impact the polarization of light reflected from a target [5], this is not a cue that bees use when identifying iridescent targets, and furthermore it is not a cue that bees can use when targets are presented to them horizontally [5, 13]. More work is needed to determine the interactions of iridescence with other optical effects such as gloss. Further spectral and behavioral studies of iridescence, particularly when compared with an analysis of which floral pigments are found in combination with floral iridescence, will be useful to unravel these factors and determine the mechanisms by which floral iridescence affects flower detection and identification.

The iridescence produced by floral diffraction gratings may appear to the human eye to be subtle (examples of floral iridescence are included in [Figure S3](#)); however, in the production of iridescence, there appear to be two conflicting pressures: vivid iridescence to enhance detectability and reduced iridescence to maintain potential color constancy. The subtle floral iridescence therefore appears to be optically optimal for its role to advertise to pollinators while maintaining their ability to identify rewarding flowers. Floral iridescence increases detectability while minimizing the color disruption that iridescence generated by an artificial diffraction grating produces. However, this is not to say that floral iridescence may not have any costs. The petal epidermal surface is multifunctional, and the shape and structure of this epidermis contributes to many biotic and abiotic interactions [28]. For example, the presence of conical cells (which would preclude the production of floral iridescence by scattering light) might impact the temperature and wettability of the petal surface [28, 29] and would also enhance the ability of a foraging insect pollinator to grip and handle a flower [30, 31]. The flat, striated surface that produces iridescence in flowers has been shown to reduce the ability of insects to grip when it is found on leaves [32]. Therefore, there is a potential trade-off between floral surface structures that provide a secure grip for pollinators and those that enhance floral detectability. This trade-off may be another factor behind the relative rarity of floral iridescence and may also explain why that, when it does occur, it frequently oc-

curs in defined regions of the petal in combination with spatially distinct conical epidermal cells [33].

In all organisms that produce iridescence, there is potential for trade-off between increasing detectability and maintaining signal identity. In the case of floral-generated iridescence, the scatter of the iridescence appears to optimize detectability while minimizing disruption. However, if iridescence is used as camouflage, the reverse could be the ideal, with increased disruption and reduced detectability. Conceivably, either objective could be attained by modifying the structure responsible for the generation of the iridescence or by combining the diverse ways in which iridescence can be produced, each of which gives rise to different optical properties, each of which would differently impact visual detectability and disruption. In the case of floral-derived iridescence, we find that while salience is increased, floral constancy is not sacrificed. The apparently “imperfect” diffraction gratings of flowers generate a signal whose adaptive nature only emerges when one considers the color vision and behavior of the intended signal receivers, the pollinators of the flowers.

EXPERIMENTAL PROCEDURES

Generation of Target Disks

Target disks were generated using methods detailed in [5]. Full details and photographs of the disks are included in the [Supplemental Experimental Procedures](#).

Optical Measurement of Target Disks and Color Analysis

Target disks were measured using a Zeiss MCS 230 diode array photometer, with illumination by a Zeiss CLX 111 Xenon Lamp. The illumination fiber optic was held at 45° to the normal by a Zeiss GK 111 goniometer. The angle of the measurement fiber optic was held at angles ranging from 45° to 90° to the normal, in 5° increments [11]. Spectra were recorded in 1-nm steps from 300 to 700 nm and were recorded relative to a Spectralon 99% white standard. Both this white-standard calibration and a true-dark reference calibration (where light was fully blocked from the detector) were taken immediately prior to measurement of each disk, to minimize measurement error. Each measurement was taken from a 2-mm diameter spot on the disk, randomly chosen from within a uniform region. For each disk, spot measurements were taken at each 5° increment, the disk was then rotated 10°, and measurements repeated from a position of 90° to one of 0° across the diffraction grating.

Bumblebee Experiments

General bumblebee experimental conditions were as described in [13]. Full experimental conditions are also detailed in the [Supplemental Experimental Procedures](#). Flower-naive *Bombus terrestris* colonies were supplied by Syngenta-Bioline.

Measurement of the Impact of Iridescence on Bumblebee Search Time

Experimental design followed the method described in [17]. Each bee was tested on one disk type only (i.e., one combination of iridescence and pigment). Bees were initially trained on the large 3-cm disks of the stated iridescence and pigment type, each of which provided a 30% sucrose solution reward in a cap in the center of the disk. Three disks were positioned in an equilateral triangle pseudo-randomly orientated within the flight arena. A successful trial was one in which the bee found and drank from all three disks. Once the bee had successfully completed 15 bouts of training, the disks were swapped for those of the same type but smaller size (1 cm). The time taken for the bee to locate and land on each disk type was then recorded. This was repeated for five bouts for an individual bee.

The data were analyzed with SPSS 19 using a repeated-measures general linear model. Iridescence (whether the disks presented were iridescent or not), and disk type (whether the disks were blue with an artificial pattern, red with an artificial pattern, or red with a floral pattern) were considered as

between-subject factors. Visit order (the lengths of time to move between the first and second disk and between the second and third disk) and experimental repeat (the five consecutive repetitions on each individual) were considered as within-subject factors. Initial analyses suggested that third- and fourth-order interactions were non-significant; therefore, within the model used, interactions were not considered between the two within-subject factors or between the two between-subject factors. Data were log-transformed to satisfy test assumptions. Post hoc Tukey's HSD tests were conducted to explore differences between disk types.

Measurement of the Impact of Iridescence on Bumblebee Color Discrimination

For determination of whether bees could distinguish between the target color and that of the distractor disks, absolute conditioning was used. In these experiments, a single trial consisted of releasing a marked bee from the colony into a flight arena containing an array of disks of the target color, each of which provided a 30% sucrose solution reward in a cap in the center of the disk. The bee was permitted to forage on disks of the target color until at least 20 visits had been made to different target disks and permitted to return to the colony. The array was then changed such that an equal number of target and distractor disks were presented. The trained bee was released again into the flight arena, and the first 20 disks that the bee landed on recorded.

The correct number of choices made during the first 20 visits was compared between iridescent and non-iridescent pairings using either a Welch's *t* test of the log-transformed data (floral three-color test) or a Wilcoxon rank-sum test with continuity correction, using *R* 2.15.1.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2016.01.026>.

AUTHOR CONTRIBUTIONS

Conceptualization, H.M.W., L.C., and B.J.G.; Formal Analysis, S.A.R.; Investigation, H.M.W. and A.R.; Writing – Original Draft, H.M.W., L.C., and B.J.G.; Writing – Review & Editing, H.M.W., S.A.R., L.C., and B.J.G.; Visualization, H.M.W. and S.A.R.

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