

# Competition for pollinator visitation between deceptive and rewarding artificial inflorescences: an experimental test of the effects of floral colour similarity and spatial mingling

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## Summary

1. While many plant species offer rewards (e.g. nectar) to pollinators, some species, particularly in orchids, do not provide rewards. Ecological factors, such as interactions with rewarding co-flowering species may affect pollinator visitation rates to such deceptive species by influencing pollinator ability to learn to avoid deceptive plants (avoidance learning).
2. We tested the effect of flower colour similarity (similar *vs* dissimilar) and fine-scale spatial mingling (monospecific *vs* heterospecific patches) of rewarding and deceptive artificial plants on pollinator visitation in a fully crossed design. We also examined the effect of these factors on learning of initially naïve bumblebees.
3. Over time, bumblebees increasingly avoided the deceptive plants, but at a significantly faster rate when deceptive and rewarding plants had dissimilar flower colours than when they were similar.
4. Deceptive plants received more visits when mingled in heterospecific patches with rewarding plants of similar flower colour than when mingled with dissimilar ones. This difference was not significant when rewarding and deceptive plants were spatially separated in monospecific patches.
5. In conclusion, both spatial mingling and flower colour similarity affected pollinator visitation to and avoidance learning of deceptive plants. This proves the validity of artificial experimental systems to study the isolated and joint effect of plant traits, and ecological factors that are crucial for the maintenance of deceptive species in natural populations.

*Key-words:* bumblebee, foraging, learning abilities, Orchidaceae, visitation rate

## Introduction

Most Angiosperms reward their pollinators with nectar, pollen or both, to establish a faithful relationship and incite them to visit nearby relatives. This ensues from generalized pollinator evolution to associate floral cues (flower colour, scent, shape or inflorescence architecture) to the presence of nectar and pollen (associative learning, Dukas & Real 1993a; Gumbert 2000). Paradoxically, some entomophilous plants do not offer any rewards to pollinators. Although rare among flowering plants, food-deception is particularly widespread within the Orchidaceae (Dafni 1984; Nilsson 1992), in which about one-third of the known species are food-

deceptive (Van der Pijl & Dodson 1966; Ackerman 1986). To limit the costs of visiting deceptive plants, pollinators may learn to discriminate deceptive from rewarding flowers (Smithson & Macnair 1997) and avoid deceptive ones to optimize their foraging efficiency (avoidance learning, Ollason & Ren 2002). Consequently, deceptive orchids usually show a relatively low reproductive success compared to their rewarding counterparts (Neiland & Wilcock 1998). If pollinators have the cognitive abilities to learn to avoid deceptive plants, how can food-deception have been maintained in such a considerable number of orchid species? The local ecological conditions (i.e. the biotic characteristics of a plant community) in which a deceptive species flowers may be of fundamental importance to its reproductive success, in as much that such characteristics are likely to modify pollinator associative and avoidance learning.

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Prior to any foraging experience, naïve generalist pollinators usually prefer flowers according to their innate preferences (Lunau & Maier 1995). After visiting rewarding flowers they usually learn to associate floral cues to the presence of reward (Dukas & Real 1993a). This learning process leads to a learned preference that dominates over innate preferences (Gumbert 2000). However, naïve pollinators may learn to discriminate rewarding from deceptive flowers at different rates depending on the local ecological conditions in which the deceptive plants flower.

Among the floral characteristics used by pollinators to discriminate flowers, corolla colour seems to be of primary importance for flower recognition (Menzel & Shmida 1993) and may be crucial for pollinator learning processes (Heinrich, Mudge & Deringis 1977). Consequently, for deceptive plants, co-occurring with a rewarding species with similar flower colour may be a key ecological condition to enhance pollinator visitation rate (Gumbert & Kunze 2001; Gigord *et al.* 2002; Johnson *et al.* 2003) by slowing down pollinator avoidance learning (Dafni 1984; Ackerman 1986; Nilsson 1992). Also, even highly experienced pollinators may tend to visit more deceptive plants when they are of similar corolla colour to those having previously rewarded them (Gigord *et al.* 2002). In two recent experiments using flowers of corolla colours that were distinguishable by pollinators, the higher the colour similarity between rewarding and deceptive flowers, the slower the rate of pollinator avoidance learning, which resulted in increased pollinator visits to deceptive flowers (Dyer & Chittka 2004b, 2004c). Therefore, colour similarity is likely to be a fundamental ecological condition that may influence animal-mediated reproductive success of deceptive plants by modifying the rate of avoidance learning.

The relative spatial distributions of rewarding and deceptive plants may also be crucial ecological conditions for the reproductive success of deceptive plants (Johnson *et al.* 2003; Internicola *et al.* 2006), potentially affecting pollinator avoidance learning. For instance, pollinator learning rate may be increased by successive and shortly interspersed encounters of both species (Dukas & Real 1993a, 1993b; Internicola *et al.* 2006). Consequently, the degree of mingling of rewarding and deceptive plants (as in monospecific vs heterospecific patches) may affect pollinator learning rate. When foraging within monospecific deceptive patches, naïve pollinators may learn to avoid deceptive plants more slowly than within heterospecific patches where they are often likely to encounter an alternative rewarding species. Indeed, being spatially isolated from rewarding co-flowering species appears to be beneficial to deceptive plants by resulting for instance in increased fruit set (Lammi & Kuitunen 1995). More recently, a study showed that deceptive artificial flowers were more often visited when arranged in distinct patches than when randomly mingled with rewarding flowers (Keasar 2000).

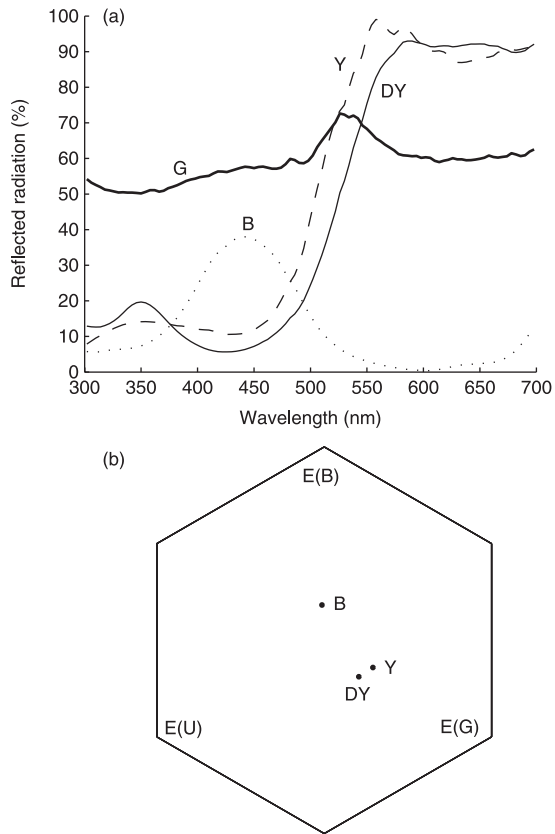
Interestingly, in natural populations flower colour similarity and mingling of a deceptive and a rewarding species are two ecological conditions that are likely to interact. For instance, a deceptive species with a similar corolla colour to the rewarding species may be more often visited by pollinators when it occurs in heterospecific patches compared to monospecific ones, because colour similarity may reduce pollinator avoidance learning (Dyer & Chittka 2004b) and increase naïve or mistake pollination in addition to the increased time spent by pollinators within a patch that on average provides more rewards (Thomson 1978; Lavery 1992). Conversely, a deceptive species of dissimilar flower colour to the rewarding one may exhibit a higher visitation rate in monospecific patches compared to heterospecific ones, because spatial isolation from the rewarding species may decrease inter-specific competition for access to pollinators and slow down avoidance learning (Keasar 2000). Furthermore, deceptive plants may receive more visits when pollinators are still inexperienced. Although recent studies have investigated the effect of mingling or colour similarity as discussed above, to our knowledge, no experimental approach has simultaneously tested their joint effect on pollinator avoidance learning and visitation rate to deceptive plants.

To investigate at fine spatial and temporal scales the optimal combination of these ecological conditions for pollinator visitation rate to a deceptive species, we used a fully crossed, two-factor design that mimicked a natural field situation with deceptive and rewarding orchid-looking artificial inflorescences in a patchy distribution. Inflorescences were either similar (yellow and dark yellow) or dissimilar (yellow and blue) for corolla colour and displayed either in monospecific patches (M) or in heterospecific patches (H). We used initially naïve pollinators and monitored their learning rate and flower visitation behaviour. We hypothesized that these ecological conditions should interact, leading to a slower pollinator learning rate when rewarding and deceptive species of similar corolla colour co-occur in heterospecific patches compared to when they are spatially separated in monospecific patches. Also, we predicted a slower pollinator learning rate when the two species had dissimilar corolla colour and occurred in monospecific patches than in heterospecific patches.

## Methods

### EXPERIMENTAL SYSTEM

We built 120 artificial inflorescences (see Smithson & Gigord 2003) each consisting of a hollow leaf-green stem (a plastic tube of height 28 cm and Ø 1.2 cm) balanced by a wooden cubic leaf-green stand (5.7 cm edge) at its base. Each stem had 10 holes (Ø 0.2 cm), perforated every 1.5 cm vertically starting from the top, in a spiral along the tube. An orchid-looking zygomorphic paper flower (1.2 cm width and 2.2 cm height) was glued on every hole. Through the holes, bumblebees had access



**Fig. 1.** (a) Spectral reflectance functions of the green background (G) and of yellow (Y), dark yellow (DY) and blue (B) flowers. (b) Hexagonal representation of the trichromatic colour vision (photoreceptor excitation) of *Bombus terrestris* in ultraviolet (E(U)), blue (E(B)) and green (E(G)). Points show the relative position of yellow (Y), dark yellow (DY) and blue (B) colours as used in the experiment, i.e. under indirect natural sunlight within a greenhouse and assuming adaptation of the visual system of bumblebees to the green background. Distances between points thus represent similarity between colours.

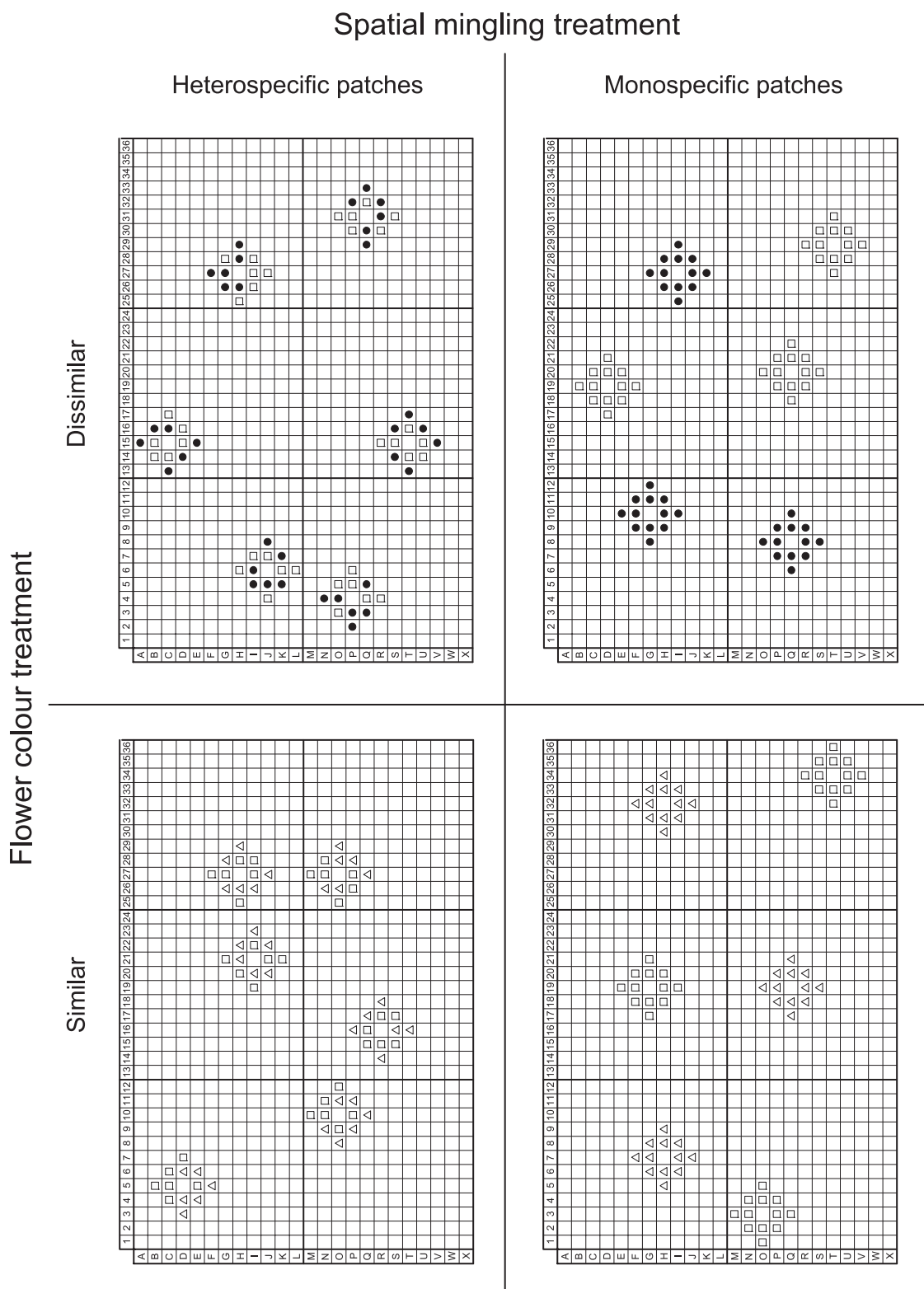
to wells supplemented with 3  $\mu\text{L}$  of liquid, either 30% sucrose solution (rewarding flower) or water (deceptive flower). Wells were held on a plastic rod placed inside the stem, and were 4 mm deep from the flower surface. The inner rod could be removed from the stem to clean and fill the wells. Thus each inflorescence consisted of 10 flowers, each of them providing either nectar or only water. Flowers on inflorescences were either yellow (Y,  $n = 40$  inflorescences), dark yellow (DY,  $n = 40$  inflorescences) or blue (B,  $n = 40$  inflorescences). Colour traits were assessed by spectrophotometric analysis (High Sensitivity Spectrophotometer S2000, New Electro-Optical Concepts). The colour distances were calculated in a hexagon colour space (Chittka 1992) considering the spectral sensitivity functions of *Bombus terrestris* photoreceptors (Peitsch *et al.* 1992), the spectral reflectance functions of the flower colours (Fig. 1) and the spectral distribution of the illumination of the experimental environment. We assumed that the bee's visual system was adapted to the painted green background. Pairwise colour similarity was defined through colour distances in the hexagon colour space (Chittka 1992). Colours separated by a distance of 0.062 hexagon units can be

discriminated by bumblebees that experienced differential conditioning, that is, bumblebees that learned rewarding flowers in the presence of deceptive flowers (Dyer & Chittka 2004b; Dyer 2006). The blue tone was clearly distinct (Y/B distance = 0.418 hexagon units; DY/B distance = 0.417 hexagon units). Yellow and dark yellow were distinguishable for a bumblebee according to colour distance in the hexagon colour space (Y/DY distance = 0.087 hexagon units) and a preliminary set of tests. Ten inflorescences of each colour type were available for bumblebees to forage on. Four naïve bumblebees were tested one by one. Two of them faced with yellow deceptive inflorescences and dark yellow rewarding inflorescences, whereas the two others were confronted with the reverse treatment. The results show that bumblebees unambiguously discriminate between the two colours, rewarding inflorescences being more often visited regardless of their colour ( $\chi^2 = 74.94$ ;  $df = 3$ ;  $P$ -value < 0.001).

Inflorescences were placed in a green flight cage with an area of 8.64 m<sup>2</sup> (2.4  $\times$  3.6 m) and 1.5 m high. The base bore a 24  $\times$  36 grid square system, with grid size 9  $\times$  9 cm, identifying 864 positions to place inflorescences into the cage. We used two *B. terrestris* (L.) hives (NATUPOL©, KOPPERT B.V. Netherlands) connected to the cage by means of a transparent plastic tube, which allowed to see the bumblebees. All bees used for the experiment had hatched in captivity and were totally naïve. Bees were fed *ad libitum* with sucrose syrup and pollen until the hives were shipped to our laboratory, after what we gradually removed the syrup feeder for up to 23 h per day, to encourage bees to forage for nectar in the cage. Before the experiments, bees were allowed to enter the cage, where we randomly placed 10 flowerless leaf-green stems with 6  $\mu\text{L}$  of nectar per well on the grid for 1–4 h per day, to habituate them to the experimental setup. A bee that explored a hole of a stem was followed visually until she had consecutively probed a minimum of five wells, then was caught, marked and released back into the cage to continue its foraging activity. In experiments, we used only marked bees.

#### EXPERIMENTAL DESIGN

We investigated the effect of two fully crossed factors on the foraging behaviour of *B. terrestris* on patchily-distributed rewarding and deceptive inflorescences: (i) colour similarity between deceptive and rewarding flowers (similar: Y/DY vs dissimilar: Y/B), the yellow inflorescences always being deceptive; and (ii) mingling of deceptive and rewarding inflorescences (monospecific patches (M) with only one type of inflorescence per patch vs heterospecific patches (H), with a balanced mix of both types of inflorescences). Thus, there were four treatment combinations (Fig. 2). Each bee was confronted to only one treatment combination. For each treatment combination, we tested a minimum of 20 bees.



**Fig. 2.** Experimental design for the analysis of bumblebee visitation patterns to rewarding and deceptive artificial inflorescences. The scheme shows the experimental cage grid and the four treatment combinations with varying flower colour similarity of rewarding and deceptive inflorescences (similar S vs dissimilar D) and their spatial mingling (monospecific patches M vs heterospecific patches H). Open squares: yellow deceptive; open triangles: dark yellow rewarding; filled circles: blue rewarding.

In each trial, we individually followed bees moving on a display of 72 artificial inflorescences, 36 of each colour, allocated into six identically shaped patches (12 inflorescences per patch). Patches were at least 9 cm apart and randomly placed within the grid at each

trial. Within heterospecific patches we randomized the position of rewarding and deceptive inflorescences.

We refer to each sequence of visits to the artificial inflorescences by a single trained bumblebee, from the moment the bee left the hive until she returned to it, or

until she stopped foraging, as to an experimental bout. After each bout, we randomly re-allocated inflorescences to patches and refilled flowers with either water or nectar. We only included in the analysis bumblebees with at least two experimental bouts in a single day, to avoid possible confounding by over-night memory decay (Keasar *et al.* 1996).

#### DATA COLLECTION

For each experimental bout, one bumblebee was followed by two observers who recorded the spatial sequence of its visits to the artificial inflorescences and flowers. During the first experimental bout, bees experienced for the first time the rewarding and deceptive flowers. As both rewarding and deceptive plants were simultaneously present during bumblebee learning, the sequence of visits recorded here corresponds to naïve bumblebees learning under differential conditioning. A visit was defined as the bumblebee landing on the flower and probing the well. This data provided us with the sequence, patch and inflorescence position, and total number of visits to rewarding and deceptive inflorescences, and flowers for each bout and bee. Because bees learn to discriminate colours separated by 0.102 hexagon units at greater than 70% frequency of correct choice after 30 visits (Dyer & Chittka 2004b, 2004c), we recorded the sequence of visits of each bumblebee up to a minimum of 50 plant visits. The experiment was repeated as two time blocks: (i) from May 24 to July 13 2005; and (ii) from October 9 to November 25 2005. We accounted for the block effect in the analysis. Experiments were run in a greenhouse between 08.30 and 18.30 h, under indirect natural sunlight and temperatures varying between 23 °C and 28 °C.

#### DATA ANALYSIS

Differences in pollinator visitation and learning rate among treatments were analyzed as follows. To enable comparisons between treatments, all sequences of visits to the inflorescences were truncated at 50 visits by keeping for each bee the 50 first visits only, independently of the total number of bouts a bumblebee had achieved and the total number of inflorescences visited. To analyze learning over time, we divided the sequences of 50 visits into five clusters of 10 consecutive visits. Within each cluster, we calculated the following parameters for each bumblebee, leading to a sequence of five values, one per cluster, for each variable and each bumblebee:

1. Number of deceptive plants visited.
2. Number of switch events – i.e. when a bumblebee switched from a plant phenotype to the other. This parameter was used to estimate the bumblebees' avoidance learning. Prior to any learning and in ecological conditions that do not allow discrimination against deceptive plants, bumblebees are expected

to visit rewarding and deceptive plants at random. By contrast, when foraging in ecological conditions that allow bumblebee recognition and discrimination of deceptive plants, bumblebees that learn to discriminate against deceptive plants (i.e. under differential conditioning) should exhibit a lower number of switches from a plant phenotype to the other, restricting their visit to a small number of plant species (Heinrich *et al.* 1977). This so-called 'flower constancy' reflects avoidance learning of deceptive plants and leads to a lower number of switches.

Differences in the number of visits to deceptive plants and in the number of switches within clusters among colour treatments (Y/DY or Y/B), mingling treatments (H or M), sequence and block were analyzed using a mixed ANOVA. To avoid pseudo-replication, we accounted for the effect of individual bumblebees by using the individual bumblebee as the error stratum. Since residuals violated ANOVA assumptions of normality and homoscedasticity, ANOVA were performed with permutation tests on the mean squares (Manly 1997). Effects of colour treatment, mingling treatment and block were estimated by permuting the levels of these factors in the data set separately, by imposing the same level values of the permuted factor within each bumblebee (i.e. for the five sequential values). The effect of sequence was estimated by permuting the levels of this factor within each bumblebee. Pairwise interactions were tested by simultaneously permuting the two interacting factors. We proceeded similarly for three- and four-way interactions. *P*-values were calculated for each factor as the proportion of permuted mean-square estimates larger than or equal to the observed mean-square over 1000 permutations. All statistical analyses were conducted with R 2.2.1 software (R development Core Team 2005). Results are given as estimated mean values  $\pm$  standard errors ( $\bar{x} \pm SE$ ).

#### Results

The number of visits to deceptive plants was significantly influenced by the sequence (i.e. time) and marginally by block (Table 1). The significant sequence effect indicates that over time, bumblebees increasingly avoided the deceptive plants. Interestingly, the interaction between colour similarity and sequence effects was significant. This indicates that the rate at which bumblebees learned to avoid deceptive plants over time was higher when deceptive and rewarding plants differed in colour compared to when they were similar (Fig. 3).

We further investigated the number of switches – i.e. the number of times consecutive visits by the same bumblebee involved a change of 'species', as measured over 50 visits. We found that over time bumblebees switched significantly less often, implying increasing flower constancy with increasing experience. Also, bumblebees switched significantly more often between rewarding

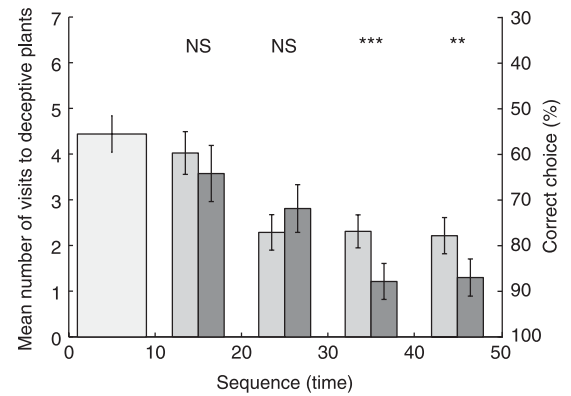
**Table 1.** ANOVA table for the effects of flower colour similarity, spatial mingling, sequence of visits, block and their interactions on the number of deceptive artificial plants visited per cluster of 10 visits. The effect of individual bumblebee was taken into account in the model. Colour similarity, spatial mingling and block have only one level per bee, so that these factors and their interactions are grouped in the first part of the table (Error: Between bees). As sequence is the only factor that has different levels within each bee, the effect of this factor and its interactions are shown in the second part of the table (Error: Within bees)

Error: Between bees			
Source of variation	Df	MS	<i>P</i>
Colour	1	0.0013	0.993
Mingling	1	23.3	0.355
Block	1	86.1	0.079
Colour × Mingling	1	16.9	0.428
Colour × Block	1	14.5	0.480
Mingling × Block	1	27.8	0.347
Colour × Mingling × Block	1	11.1	0.535
Residuals	81	28.1	—
Error: Within bees			
Sequence	4	133.3	<0.001
Colour × Sequence	4	35.2	0.026
Mingling × Sequence	4	7.2	0.318
Sequence × Block	4	1.6	0.660
Colour × Mingling × Sequence	4	17.5	0.112
Colour × Sequence × Block	4	1.9	0.628
Mingling × Sequence × Block	4	5.8	0.362
Colour × Mingling × Sequence × Block	4	9.9	0.236
Residuals	324	5.2	—

**Table 2.** ANOVA table for the effects of flower colour similarity, spatial mingling, sequence of visits, block and their interactions on the number of switches per cluster of 10 visits. The number of switches is defined as the number of times consecutive visits by the same bumblebee involved a change between rewarding and deceptive ‘species’. The effect of individual bumblebee was taken into account in the model. Colour similarity, spatial mingling and block have only one level per bee, so that these factors and their interactions are grouped in the first part of the table (Error: Between bees). As sequence is the only factor that has different levels within each bee, the effect of this factor and its interactions are shown in the second part of the table (Error: Within bees)

Error: Between bees			
Source of variation	Df	MS	<i>P</i>
Colour	1	247.5	<0.001
Mingling	1	347.42	<0.001
Block	1	33.44	0.009
Colour × Mingling	1	130.8	<0.001
Colour × Block	1	3.5	0.507
Mingling × Block	1	23.13	0.096
Colour × Mingling × Block	1	2.6	0.630
Residuals	81	2.6	—
Error: Within bees			
Sequence	4	8.6	0.041
Colour × Sequence	4	4.6	0.124
Mingling × Sequence	4	12.3	0.012
Sequence × Block	4	0.4	0.652
Colour × Mingling × Sequence	4	3.5	0.177
Colour × Sequence × Block	4	1.2	0.425
Mingling × Sequence × Block	4	1.3	0.390
Colour × Mingling × Sequence × Block	4	1.9	0.299
Residuals	324	1.7	—

and deceptive plants when these were of similar (rather than dissimilar) colour, and when these were mingled in heterospecific patches (rather than in separate monospecific patches; Table 2). The interaction between sequence and mingling treatment on the number of

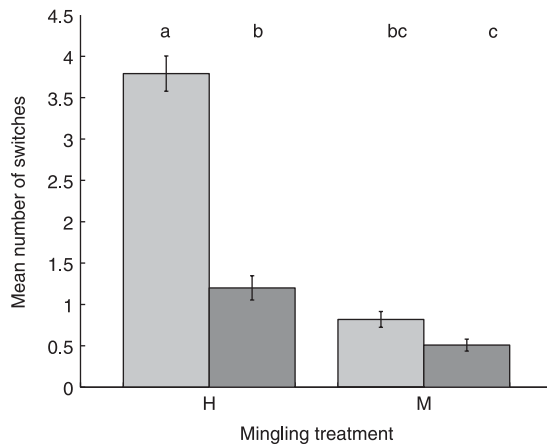


**Fig. 3.** Mean number of bumblebee visits to deceptive artificial plants and correct choice (i.e. percentage of rewarding plants visited) per cluster of 10 sequential visits, illustrating avoidance learning rate. We pooled the data for similar and dissimilar pair species in the first cluster of 10 visits to provide a relevant baseline data point to compare bumblebee avoidance learning of similar and dissimilar deceptive and rewarding plants (white bar). Light grey bars = deceptive and rewarding plants were of similar colour (Y/YD); dark grey bars = deceptive and rewarding plants were of dissimilar colour (Y/B). NS:  $P > 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ .

switches was significant. Over time, bumblebees switched less often between rewarding and deceptive plants when these were in separate monospecific patches, and more often when they were presented in heterospecific patches. Moreover, there was a highly significant interaction between colour and mingling treatments. Bumblebees were significantly more likely to switch between rewarding and deceptive plants when these were of similar colour and co-occurred in heterospecific patches (SH treatment), compared to the other three treatments. Also, bumblebees switched significantly less often when dissimilarly-coloured rewarding and deceptive plants were in monospecific rather than in heterospecific patches (Fig. 4). The number of switches observed varied significantly between the two blocks but there was no significant interaction between this effect and the experimental treatments sequence, colour and mingling.

## Discussion

In a controlled manipulative experiment using artificial inflorescences in a patchy distribution, we tested for the joint effect of flower colour similarity and spatial mingling of deceptive and rewarding plants on the number of visits to the deceptive plants and the number of switches between phenotypes during consecutive visits. Over time, naïve bumblebees visited fewer deceptive plants and switched less often between phenotypes, indicating that bumblebees progressively learned to avoid deceptive plants with increasing foraging experience. This pattern was true regardless of the degree of colour similarity (Dyer & Chittka 2004a), probably because bumblebees learned under differential conditioning (i.e. when both rewarding and deceptive plants were



**Fig. 4.** Mean number of switches per cluster of 10 visits depending on flower colour similarity and spatial mingling of rewarding and deceptive artificial plants. Light grey bars = deceptive and rewarding plants were of similar colour (Y/DY); dark grey bars = deceptive and rewarding plants were of dissimilar colour (Y/B); M = deceptive and rewarding plants occurred in separate monospecific patches; H = deceptive and rewarding plants co-occurred within heterospecific patches. The letters show significant ( $P < 0.05$ ) differences between treatment groups (Tukey post-hoc comparison test).

simultaneously available), which is crucial for discrimination of very similar flower colours (Dyer & Chittka 2004c; Dyer 2006). However, bumblebees learned more slowly to avoid deceptive plants when rewarding and deceptive plants were of similar flower colour than when dissimilar. This result is in accordance with those of Dyer & Chittka (2004b, 2004c). In particular, bumblebee learning rates when rewarding and deceptive plants were of similar flower colour (0.087 hexagon units) are consistent with those found by Dyer & Chittka (2004a, 2004b, 2004c) for colour distances that were larger (0.102 hexagon units) as well as smaller (0.062 hexagon units). Contrastingly, for dissimilar pair species separated by 0.418 hexagon units, bumblebee learning rate was slower in our experiment than in that of Dyer & Chittka (2004b, 2004c). We tested pollinators that were totally naïve (i.e. bumblebees never experienced neither the rewarding nor the deceptive plants before the experiment) under differential conditioning, whereas Dyer & Chittka (2004b, 2004c) tested bumblebees after training bouts during which they learned the rewarding colour. In addition, when bumblebees were experienced, they made on average more correct choices when the two plant species were of dissimilar colour than when similar, which is in accordance with Dyer & Chittka (2004b). Two non-exclusive mechanisms may have led to slower learning rate with increased corolla colour similarity of rewarding and deceptive plants. First, corolla colour similarity may have decreased pollinator discrimination between rewarding and deceptive plants (Dyer & Chittka 2004b), thus decreasing bumblebee learning rate. Second, bees mark rewarding (Giurfa, Núñez & Backhaus 1994; Stout, Goulson & Allen 1998) as well as deceptive (Free & Williams 1983;

Giurfa & Núñez 1993) flowers they recently visited with repellent scents. As a result, scent-marking activity may have increased bumblebee avoidance of recently visited rewarding and deceptive flowers, decreasing bumblebee encounters of both species. Because bumblebee learning rate may strongly depend on bumblebee foraging experience through encounters of both species (Dukas & Real 1993a, 1993b; Internicola *et al.* 2006), this scent-marking activity may slow down bumblebee learning rate, especially when rewarding and deceptive flowers are similar for corolla colour. Indeed, bees show increased scent-marking activity with increasing colour similarity between rewarding and deceptive flowers (Giurfa *et al.* 1994). In natural populations, whatever the mechanism that slowed pollinator learning down, deceptive species should be advantaged when co-flowering with a rewarding species that is similar for corolla colour.

As predicted, both flower colour similarity and spatial mingling jointly influenced avoidance learning. Bumblebees switched more between rewarding and deceptive plants of similar flower colour (compared to dissimilar ones), and between mingled rewarding and deceptive plants within heterospecific patches (compared to rewarding and deceptive plants in separate monospecific patches). A high number of switches may result from pollinator confusion between flower colours that are more difficult to distinguish, potentially leading to slower avoidance learning and increased mistake visits to deceptive plants (Dyer & Chittka 2004b). Alternatively, an increased use of repellent scent-marking when rewarding and deceptive plants are of similar corolla colour may also lead to such a pattern. Also, pollinators switched less often in monospecific than in heterospecific patches, where rewarding and deceptive plants co-occurred close-by. In order to minimize flight costs, pollinators may visit preferentially plants within the same patch, especially after a rewarded visit (Keasar, Shmida & Motro 1996) and when they are still inexperienced. As a consequence, the higher number of switches in heterospecific patches is likely due to switches from a plant phenotype to the other within a patch, so that in natural plant communities, a deceptive species should benefit from being spatially closely mingled with a rewarding species.

In accordance with our prediction, there was a significant interaction between flower colour similarity and spatial mingling on the number of switches, defined as the number of times the same bumblebee went from a plant phenotype to the other in consecutive visits. Indeed, in heterospecific patches, bumblebees switched significantly more often when the rewarding and deceptive plants were of similar flower colour than when they were dissimilar. This suggests that deceptive plants suffered (in terms of increased efficiency of avoidance learning) when co-occurring with rewarding plants of dissimilar colour and even more so when pollinators gained foraging experience. This is consistent with our *a priori* prediction, that deceptive plants

benefit of being similar for flower colour to the rewarding plants and when spatially mingled in heterospecific patches. A possible mechanism of this is the faster avoidance learning when the pollinator encounters both deceptive and rewarding plants successively at short temporal intervals (Dukas & Real 1993a, 1993b; Internicola *et al.* 2006), combined with a weaker ability to learn to avoid deceptive plants, the more similar they are in colour to rewarding plants (Dyer & Chittka 2004b). By contrast, when rewarding and deceptive plants were spatially separated in monospecific patches, the difference was not significant (albeit in the same direction). This is likely due to pollinator behaviour. Pollinators have shown to rapidly leave patches offering little or no reward, increasing both their flight distances to the next flower (Dukas & Real 1993b; Smithson & Gigord 2003) and the probability of switching to another phenotype (Smithson & Macnair 1997). Consequently, flower colour similarity was less likely to influence pollinator behaviour when rewarding and deceptive plants occurred in separate monospecific patches. Thus, the reproductive success of deceptive species in natural populations should be higher when they are mingled with rewarding plants that are of similar flower colour.

The effects of flower colour similarity, spatial mingling and of the interaction between these two factors were significant for the number of switches, but not for the number of visits to deceptive plants. The lack of significant effect on the number of visits to deceptive plants does not fully support our predictions. This indicates that learning avoidance occurred in a manner that influenced the number of switches, but not the number of deceptive plants visited, possibly due to the temporal and spatial scales used in this study. The scales at which avoidance learning was explored may be of great importance for detecting the effect of flower colour similarity, spatial mingling and their interaction. As a result, the choice of sequence length, cage spatial dimensions and spatial distribution of plant patches may influence the likelihood of detecting these effects.

In conclusion, this experiment shows that being of similar colour to rewarding plants is beneficial to deceptive plants in the long term, when pollinator foraging experience increases. Also, pollinator avoidance learning differed depending on colour treatment in heterospecific, but not in monospecific patches, suggesting that flower colour similarity is particularly beneficial when the deceptive and the rewarding plants co-occur on a very local scale. Successfully attracting pollinators to visit an inflorescence relies on a sequence of pollinator behavioural reactions, each of which can be driven by either an innate or learned preference (e.g. in response to colour, or reward quality), and depend on flower colour similarity, spatial mingling and on pollinator foraging experience. The more naïve the pollinators, the more their decisions may be based on innate preferences, like minimizing flight costs or over visiting flowers of the preferred corolla colour when probing several plant species. Thus, even if we clearly

showed that a deceptive species should exhibit a higher reproductive success when spatially closely mingled with a rewarding species of similar corolla colour, the optimal ecological conditions that are favourable for the reproductive success of a deceptive species rely on numerous factors and remain consequently highly complex. The block effect also suggests that the magnitude of the response to such experimental treatments might vary depending on the bumblebee colony (and genetic background) and the environmental conditions in which the experiment is performed. Despite this complexity, our experiment shows that the effects of flower colour similarity and spatial mingling of deceptive and rewarding plants can be detected at fine temporal and spatial scales. These effects can be ascribed to the floral cues and spatial distribution alone, which were the only manipulated traits thanks to the artificial system we used. Importantly, the results obtained with this artificial system generate predictions that can be verified in the field, with the prospect of better understanding which factors enhance the reproductive success and thus ensure the maintenance of food-deceptive plant species.

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## References

- Ackerman, J.D. (1986) Mechanisms and evolution of food-deceptive pollination systems in orchids. *Lindleyana* **1**, 108–113.
- Chittka, L. (1992) The color hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. *Journal of Comparative Physiology A* **170**, 533–543.
- Dafni, A. (1984) Mimicry and deception in pollination. *Annual Review of Ecology and Systematics* **15**, 259–278.
- Dukas, R. & Real, L.A. (1993a) Learning constraints and floral choice behaviour in bumble bees. *Animal Behaviour* **46**, 637–644.
- Dukas, R. & Real, L.A. (1993b) Effects of recent experience on foraging decisions by bumble bees. *Oecologia* **94**, 244–246.
- Dyer, A.G. (2006) Bee discrimination of flower colours in natural settings. *Entomologia Generalis* **28**, 257–268.
- Dyer, A.G. & Chittka, L. (2004a) Fine colour discrimination requires differential conditioning in bumblebees. *Naturwissenschaften* **91**, 224–227.
- Dyer, A.G. & Chittka, L. (2004b) Biological significance of distinguishing between similar colours in spectrally

- variable illumination: bumblebees (*Bombus terrestris*) as a case study. *Journal of Comparative Physiology A* **190**, 105–114.
- Dyer, A.G. & Chittka, L. (2004c) Bumblebees (*Bombus terrestris*) sacrifice foraging speed to solve difficult colour discrimination tasks. *Journal of Comparative Physiology A* **190**, 759–763.
- Free, J.B. & Williams, I.H. (1983) Scent-marking of flowers by honeybees. *Journal of Apicultural Research* **22**, 86–90.
- Gigord, L.D.B., Macnair, M.R., Stritesky, M. & Smithson, A. (2002) The potential for floral mimicry in a rewardless orchid: an experimental study. *Proceedings of the Royal Society of London series B* **269**, 1389–1395.
- Giurfa, M. & Núñez, J. (1993) Efficient floret inspection by honeybees in capitula of *Carduus acanthoides*. *Ecological Entomology* **18**, 116–122.
- Giurfa, M., Núñez, J. & Backhaus, W. (1994) Odour and colour information in the foraging choice behaviour of the honeybee. *Journal of Comparative Physiology A* **175**, 773–779.
- Gumbert, A. (2000) Color choices by bumble bees (*Bombus terrestris*): innate preferences and generalization after learning. *Behavioral Ecology and Sociobiology* **48**, 36–43.
- Gumbert, A. & Kunze, L. (2001) Colour similarity to rewarding model plants affects pollination in a food deceptive orchid, *Orchis boryi*. *Biological Journal of the Linnean Society* **72**, 419–433.
- Heinrich, B., Mudge, P.R. & Deringis, P.G. (1977) Laboratory analysis of flower constancy in foraging bumblebees: *Bombus ternarius* and *B. terricola*. *Behavioral Ecology and Sociobiology* **2**, 247–265.
- Internicola, A.I., Juillet, N., Smithson, A. & Gigord, L.D.B. (2006) Experimental investigation of the effect of spatial aggregation on reproductive success in a rewardless orchid. *Oecologia* **150**, 435–441.
- Johnson, S.D., Peter, C.I., Nilsson, L.A. & Ågren, J. (2003) Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology* **84**, 2919–2927.
- Keasar, T. (2000) The spatial distribution of nonrewarding artificial flowers affects pollinator attraction. *Animal Behaviour* **60**, 639–646.
- Keasar, T., Motro, U., Shur, U. & Shmida, A. (1996) Overnight memory retention of foraging skills by bumblebees is imperfect. *Animal Behavior* **52**, 95–104.
- Keasar, T., Shmida, A. & Motro, U. (1996) Innate movement rules in foraging bees: flight distances are affected by recent rewards and are correlated with choice of flower type. *Behavioral Ecology and Sociobiology* **39**, 381–388.
- Lammi, A. & Kuitunen, M. (1995) Deceptive pollination of *Dactylorhiza incarnata*: an experimental test of the magnet species hypothesis. *Oecologia* **101**, 500–503.
- Laverty, T.M. (1992) Plant interactions for pollinator visits: a test of the magnet species effect. *Oecologia* **89**, 502–508.
- Lunau, K. & Maier, E.J. (1995) Innate colour preferences of flower visitors. *Journal of Comparative Physiology A* **177**, 1–19.
- Manly, B.F.J. (1997) *Randomization, Bootstrap and Monte Carlo Methods in Biology*. Chapman and Hall, London.
- Menzel, R. & Shmida, A. (1993) The ecology of flower colours and the natural colour vision of insect pollinators: the Israeli flora as a study case. *Biological Reviews* **68**, 81–120.
- Neiland, M.R.M. & Wilcock, C.C. (1998) Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany* **85**, 1657–1671.
- Nilsson, L.A. (1992) Orchid pollination biology. *Trends in Ecology and Evolution* **7**, 255–259.
- Ollason, J.G. & Ren, N. (2002) Taking the rough with the smooth: foraging for particulate food in continuous time. *Theoretical Population Biology* **62**, 313–327.
- Peitsch, D., Fietz, A., Hertel, H., de Souza, J., Ventura, D.F. & Menzel, R. (1992) The spectral input system of hymenopteran insects and their receptor based colour vision. *Journal of Comparative Physiology A* **170**, 23–40.
- R development Core Team (2005) *r: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-00-3. URL: <http://www.R-project.org>.
- Smithson, A. & Gigord, L.D.B. (2003) The evolution of empty flowers revisited. *American Naturalist* **161**, 537–552.
- Smithson, A. & Macnair, M.R. (1997) Negative frequency-dependent selection by pollinators on artificial flowers without rewards. *Evolution* **51**, 715–723.
- Stout, J.C., Goulson, D. & Allen, J.A. (1998) Repellent scent-marking of flowers by a guild of foraging bumblebees (*Bombus* spp.). *Behavioral Ecology and Sociobiology* **43**, 317–326.
- Thomson, J.D. (1978) Effect of stand composition on insect visitation in two-species mixtures of *Hieracium*. *American Midland Naturalist* **100**, 431–440.
- Van der Pijl, L. & Dodson, C.H. (1966) *Orchid Flowers: Their Pollination and Evolution*. University of Miami Press, Coral Gables.