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Carry-over effects of bumblebee associative learning in changing plant communities leads to increased costs of foraging

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Abstract Flower visitors learn to avoid food-deceptive plants and to prefer rewarding ones by associating floral cues to rewards. As co-occurring plant species have different phenologies, cue-reward associations vary over time. It is not known how these variations affect flower visitors' foraging costs and learning. We trained bumblebees of two colonies to forage in a community of deceptive and rewarding artificial inflorescences whose flower colours were either similar or dissimilar. We then modified the community composition by turning the rewarding inflorescences into unrewarding and adding rewarding inflorescences of a novel flower colour. In the short term, bees trained to similar rather than dissimilar inflorescences experienced higher costs of foraging (decreased foraging speed and accuracy) in the novel community. The colonies differed in their speed-accuracy trade-off. In the longer term, bees adapted their foraging behaviour to the novel community composition by increasingly visiting the novel rewarding inflorescences.

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Introduction

Generalist flower visitors such as bees must continually forage for nectar and pollen to satisfy their nutritional needs and those of their progeny (Kearns and Inouye 1997). Their survival directly relies on their effectiveness in choosing flowers which offer sufficient amounts of food to balance the costs of foraging (Heinrich 1976; Wolf and Schmid-Hempel 1989). For such flower visitors, visiting food-deceptive plants, which do not offer rewards, is costly. Therefore, while initially naïve bees may choose flowers according to innate preferences (Briscoe and Chittka 2001; Chittka et al. 2004; Raine et al. 2006a), they rapidly learn to discriminate deceptive from rewarding flowers (Smithson and Macnair 1997) and to avoid deceptive plants (avoidance learning, Ollason and Ren 2002) when acquiring foraging experience. Also, by visiting rewarding flowers, naïve bees usually learn to associate floral cues to the presence of reward (associative learning, Dukas and Real 1993), which leads to a learned preference toward rewarding plants. Such learned preference dominates over innate preferences as long as the floral cues the bee has to choose among remain unchanged (Gumbert 2000). These learning abilities allow bees to focus their visits to a restricted number of rewarding species (flower constancy) that they remember how to handle (Chittka et al. 1999), thus optimizing their foraging output. However, bees' avoidance learning depends on the traits of deceptive and rewarding sympatric plants. In particular, flower colour similarity between rewarding and deceptive plants slows down avoidance learning (Dyer and

Chittka 2004a; Internicola et al. 2007). As a result, bees usually visit more deceptive plants when they co-occur with rewarding species of similar rather than dissimilar flower colour (Gumbert and Kunze 2001; Gigord et al. 2002; Johnson et al. 2003; Internicola et al. 2007). This suggests that the species composition of a plant community, and especially how rewards associate with floral cues, can affect bees' foraging choices and efficiency.

An important and possibly overlooked aspect is that the flowering species composition of natural plant communities changes over time within a season, because plant species often have different flowering schedules (Elzinga et al. 2007) that can additionally vary depending on climatic conditions (Sparks et al. 2000). Plant species often differ in flower traits (e.g. corolla colour, Gumbert et al. 1999; or nectar content, Heinrich 1976; Raine and Chittka 2007a), and therefore the composition of a plant community in terms of rewards and their association to floral cues may change within a season. Moreover, there is a fine-scale phenology of nectar production (e.g. some plant species produce nectar at specific times of the day, Comba et al. 1999; Trevelyan 1995; Corbet et al. 2001; Gottlieb et al. 2005), and variation in air temperature may modify nectar content through evaporation, so that even rewarding species might temporarily lack rewards. Such seasonal and daily fluctuations may affect bees' foraging efficiency. In particular, if the plant species preferentially visited by bees was to lack rewards, experienced bees may continue to behave accordingly to previously learnt associations (i.e. before the nectar depletion). For instance, bees may preferentially switch to flowers of similar rather than dissimilar colour to that of the known rewarding species or, if no similar flowers are available, they may visit flowers according to their innate preferences (Gumbert 2000). For a co-flowering deceptive species of similar flower colour to the rewarding species in which nectar is depleted, such carry-over effects of associative learning may result in increased exploratory visits. However, when bees are trained to two similar flower colours, one being associated with nectar while the other is penalised with NaCl solution, they visit a novel flower colour in preference to the trained rewarding colour, shifting their peak of response in the direction away from the penalising colour. This phenomenon, known as peak shift, is emphasised either by increasing the risk of choosing the penalising flower colour, or by decreasing the quality of the reward offered by flowers during training (Lynn et al. 2005). As visiting deceptive plants is penalising in terms of time and energy waste, the carry-over effect of associative learning may be reduced if a rewarding plant species which bees can easily identify starts flowering. On the whole, even if such changes in bees' foraging behaviour may happen only in the short term, they may suffice to affect bees' foraging efficiency and the reproductive success of a deceptive species. To our knowledge, no experimental study investigated how the nectar depletion of bees' preferred food source affects foraging efficiency and visitation rate to a co-flowering deceptive species.

In this study, we trained bumblebees to rewarding and deceptive plants, whose flower colours were either similar or dissimilar. Then, we simulated a nectar depletion of the rewarding plants and simultaneously introduced rewarding plants of a novel flower colour, which was, respectively, either dissimilar or similar to the colour of the deceptive flowers. We experimentally addressed the following questions: (1) Are the foraging behaviour, the foraging costs and the learning of experienced bees affected by the nectar depletion of their preferred food source? (2) How does such a change affect the visitation rate to a deceptive plant species? Based on Gumbert (2000), we expect that bees' learning should carry-over after the nectar depletion of the rewarding plants. Thus, we predict that experienced bees should visit more often the deceptive plants when they are of similar flower colour to the depleted rewarding plants than when dissimilar, hence suffering from higher costs linked to exploratory visits. However, according to the peak shift phenomenon, bees may switch more rapidly to the novel rewarding plants when these are dissimilar rather than similar to the deceptive plants, hence reducing the number of exploratory visits to the deceptive plants and the costs associated to them. Also, we predict that bees should learn to prefer the novel rewarding plants, hence increasingly visiting the deceptive plants when these are similar rather than dissimilar to the novel rewarding plants.

Methods

Experimental system

We used artificial inflorescences consisting of a 28 cm hollow leaf-green plastic tube (stem) of 1.2 cm diameter. A wooden cubic leaf-green stand of 5.7 cm edge balanced each stem at its base. Each stem had 10 holes (Ø 0.2 cm) perforated every 1.5 cm vertically starting from the top and separated by an angle of 90°, in a spiral along the tube. On every hole, we glued a zygomorphic paper flower with the proportions of a typical orchid flower (1.2 cm width and 2.2 cm height) perforated in its centre. Through the holes, bumblebees had access to wells supplemented with 3 μl of liquid, either 30% sucrose solution (rewarding flower) or water (deceptive flower). Wells held on a plastic rod placed inside the stem and were 0.4 cm 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 providing either nectar (rewarding) or water (deceptive). Flowers on inflorescences were either all yellow (*Y*), dark yellow (*DY*) or blue (*B*). The three colours were distinguishable from each other for a bumblebee according to colour distance in the hexagon colour space (Chittka 1992). The blue colour was clearly distinct from both yellow colours (*Y*/*B* distance = 0.418 hexagon units, *DY*/*B* distance = 0.417 hexagon units), whereas, yellow and dark yellow were more similar (*Y*/*DY* distance = 0.087 hexagon units).

As flower visitors, we used *Bombus terrestris* (L.) (Natupol©, Koppert B. V., Netherlands). All bees had hatched in captivity and were naïve, i.e. they never visited any natural or artificial flower prior to the experiment. We could thus control for the uniformity of bumblebee foraging experience and learned preferences. We connected the bee hive to a flight cage (area = 8.64 m^2 (2.4 m × 3.6 m) and height = 1.5 m) with a transparent plastic tube. We placed the inflorescences in the flight cage on a 24 × 36 grid square system, with grid size 9 × 9 cm, identifying 864 potential positions.

Experimental design

To investigate bumblebee incorrect choices (i.e. unrewarded visits) and learning after a change in cue-reward associations, we ran the experiment in two phases: a training phase and a test phase. During the training phase, we offered to bumblebees two inflorescence types of different flower colours, one deceptive and one rewarding. The deceptive and rewarding flowers were either similar (*Y*/*DY*) or dissimilar (*Y*/*B*) for corolla colour, the yellow inflorescences (*Y*) being deceptive. Also, the deceptive and rewarding inflorescences, allocated into patches, were either mingled within each patch (with a balanced mix of both types of inflorescences) or spatially separated in different patches (one type of inflorescence per patch). Thus, there were four treatment combinations, which we preserved in the test phase (Fig. 1). At the beginning of the test phase, we simulated a cessation in nectar availability of the rewarding inflorescences by turning them into deceptive inflorescences. Simultaneously, we introduced a third coloured type of inflorescence to simulate the onset of flowering of a novel rewarding species. The inflorescences that were deceptive in the training phase remained deceptive in both the training and the test phases.

We thus used three types of inflorescences:

(1) the *deceptive* inflorescences, which were deceptive in both the training and the test phases
(2) the *unrewarding* inflorescences, which were rewarding in the training phase and deceptive in the test phase
(3) the *rewarding* inflorescences, which were absent in the training phase and rewarding in the test phase

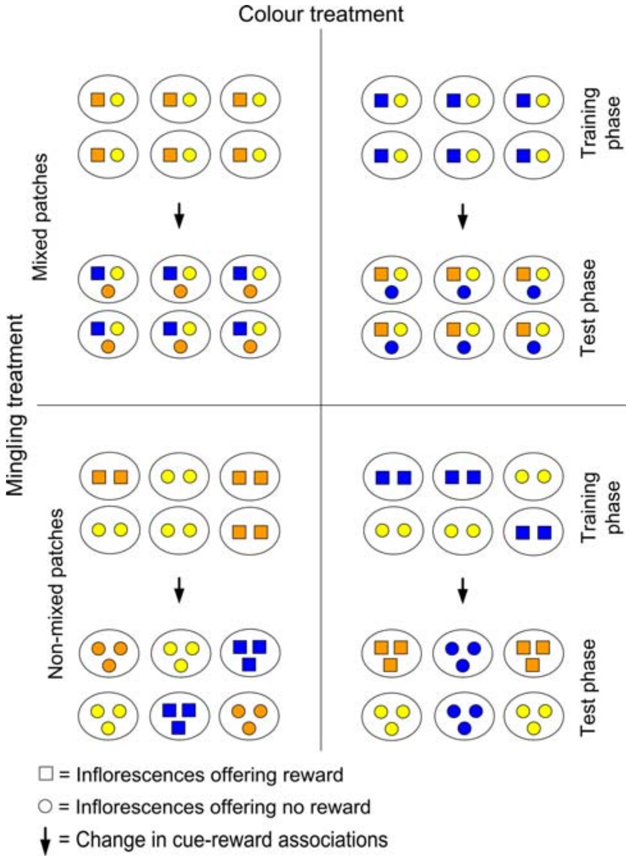


Fig. 1 Experimental design simulating a change in cue-reward associations within a plant community. The ovals represent patches within communities for the four treatment combinations of colour and mingling. Each patch contained 12 inflorescences. In each treatment combination, a representative sample of the inflorescence composition of each patch is represented for both the learning and the test phases. In the training phase, the community included only two types of inflorescences, one deceptive and one rewarding. In the test phase, the community contained three types of inflorescences, one *deceptive*, one *unrewarding* (in which nectar supplementation ceased) and one *rewarding*. The arrows represent the change in cue-reward associations within the community that occurred between the learning and the test phases. The colour of the shapes corresponds to the flower colour of the inflorescences within the patch. Circles = *deceptive* and *unrewarding* inflorescences. Squares = *rewarding* inflorescences

The colour of the *rewarding* inflorescences was either similar (*DY*) or dissimilar (*B*) to the colour of the *deceptive* inflorescences (*Y*), whereas the *unrewarding* inflorescences were, respectively, either dissimilar (*B*) or similar (*DY*) to the *deceptive* inflorescences (Fig. 1).

Each bee was tested for only one treatment combination in both the training and the test phases.

We allowed bees to individually forage on a display of 72 artificial inflorescences—36 of each colour in the training phase and 24 of each of the three colours in the test phase—allocated into six diamond-shaped patches (12 inflorescences/patch). Patches were at least 9 cm apart and randomly placed within the grid at each trial. We randomized the position of the two (training phase) or three types (test phase) of inflorescences within mixed patches. When each bee returned to the hive—at the end of a trial—we removed, cleaned and refilled the inner rod of each inflorescence with either water or nectar and randomly re-allocated inflorescences to patches. We only included in the analysis bumblebees which performed at least 50 plant visits and two trials within each phase in a single day, to avoid possible confounding by over-night memory decay (Keasar et al. 1996).

Behavioural observations

For each trial, two observers followed one bumblebee and recorded the identity of each visited inflorescence, the chronological sequence of the visits and the time spent foraging between each flower visit. We recorded a visit when a bumblebee probed a well. These data provided us with the sequence and total number of visits to *rewarding*, *unrewarding* and *deceptive* inflorescences for each trial and bee. We carried out the experiment in two time blocks, using two different colonies, one from May 24th to July 13th 2005, and the other from October 9th to November 25th 2005. We tested 21–22 (min–max) bumblebees for each treatment combination for a total of 85 bees tested and included in the analysis. To prevent an effect of colony membership on the results, we proportionally balanced group assignment across the two colonies. We ran the experiments in a greenhouse at the University of Lausanne between 08h30 and 18h30, under indirect natural sunlight and temperatures varying between 23°C and 28°C.

Statistical analysis

To ensure that bumblebee foraging efficiency resulting from the associative learning did not differ amongst the four treatment combinations, we counted the number of rewarded visits during the last 10 visits recorded in the training phase. We tested for the effect of colour, spatial mingling and block on the number of rewarded visits by using a mixed-model ANOVA. Since residuals deviated from normality and homoscedasticity, we performed the ANOVA with permutation tests on the mean squares (Manly 1997). We estimated the effects of colour, spatial mingling and block by permuting the levels of these factors in the data set separately. We tested pairwise and three-way interactions by simultaneously permuting the two or three interacting factors. We calculated P-values for each factor as the proportion of permuted mean-square estimates larger than or equal to the observed mean-square over 1000 permutations (Manly 1997). Also, to test whether bumblebee associated a specific flower colour with reward at the end of the training phase (i.e. whether bumblebee visitation pattern differed from random), we performed a one-sample Wilcoxon rank sum test.

To analyse bumblebee visitation and learning rate within the test phase, we used the *deceptive* inflorescences as focal plants. Bumblebees are able to learn to discriminate colours separated by only 0.045 hexagon units within 50 visits (Dyer and Chittka 2004c). As in our study the two more similar colours were separated by 0.087 hexagon units, we only considered the 50 first visits to the inflorescences for each bumblebee. To analyse how fast experienced bees switched to the *rewarding* inflorescences after nectar depletion of their learned food source (*unrewarding* inflorescences), we counted for each bee the number of unrewarded visits (to both the *deceptive* and *unrewarding* inflorescences) before the first visit to the *rewarding* inflorescences (i.e. latency to the first rewarded visit). We also summed the time that each bee spent foraging before the first visit to the *rewarding* inflorescence. These two variables measure the costs associated with unrewarded exploratory visits that bees required before switching to the *rewarding* inflorescences. We also counted the number of visits to the *deceptive* inflorescences before the first rewarded visit. This variable measures the benefit that *deceptive* inflorescences obtain from bumblebee exploratory visits and incorrect choices after a change in cue-reward associations. We tested for the effect of colour, spatial mingling and block on the number of unrewarded exploratory visits, on the time spent before the first rewarded visit and on the number of visits to the *deceptive* inflorescences by using three similar mixed-model ANOVAs. As above, we ran the ANOVAs with permutation tests (Manly 1997).

To analyse learning over time within the test phase, we divided the sequences of 50 visits recorded in this phase into five clusters of 10 consecutive visits. For each bumblebee, we calculated separately the number of *deceptive*, of *unrewarding* and of *rewarding* inflorescences visited within each cluster. We analysed differences in the number of visits to the three types of inflorescences within clusters amongst colour, spatial mingling, sequence and block by using three similar mixed-model ANOVAs, one per type of inflorescence. As above, we ran the ANOVAs with permutation tests. We estimated the effects of colour, spatial mingling and block by permuting the levels of these factors in the data set separately and by imposing the same level values of the permutated factor within each bumblebee (i.e. for the five sequential values). We tested the effect of sequence by permuting the levels of this factor within each bumblebee. We tested pairwise, three- and four-way interactions by simultaneously permuting the interacting factors.

We conducted all statistical analyses with R 2.2.1 software (R development Core Team 2005). Results are given as estimated mean values ± standard errors.

Results

We found no effect of colour, spatial mingling, block and their interactions on the number of rewarded visits in the last 10 visits during the training phase, indicating that bumblebee foraging efficiency did not differ amongst the four treatment combinations at the end of the training phase (Table 1). In each treatment combination, bumblebees learned to associate a specific corolla colour with reward at the end of the training phase ($W = 3214.5$, P-value < 0.001).

Experienced bumblebees required most exploratory visits (to both the *deceptive* and the *unrewarding* inflorescences and to the *deceptive* inflorescences alone) and foraged for a longer time before the first rewarded visit when the *deceptive* inflorescences co-occurred with dissimilar *rewarding* and similar *unrewarding* inflorescences. This was significantly different from the reverse colour treatment (i.e. similar *rewarding* and dissimilar *unrewarding* inflorescences; Table 2; Fig. 2). The second colony required more exploratory visits (both to the *deceptive* and *unrewarding* inflorescences and to the *deceptive* inflorescences alone) before the first rewarded visit than the first colony (Table 2; Fig. 2). By contrast, the first colony spent more time foraging before the first rewarded visit than the second colony.

Table 1 ANOVA table showing the effects of flower colour similarity, spatial mingling, block and their interactions on the number of rewarded visits in the last 10 visits during the training phase

Source of variation	Df	MS	*P*
Colour	1	0.131	0.866
Mingling	1	0.472	0.797
Block	1	3.513	0.438
Colour × Mingling	1	2.350	0.508
Colour × Block	1	0.354	0.816
Mingling × Block	1	0.048	0.924
Colour × Mingling × Block	1	14.620	0.119
Residuals	77	5.656	

Experienced bumblebees increasingly visited the *rewarding* inflorescences, indicating that they learned to associate them with reward (Table 3; Fig. 3), and decreasingly visited the *unrewarding* inflorescences (Table 3; Fig. 3), indicating that they learned to avoid them. The significant interaction between colour and sequence (Table 3) shows that, bumblebees increasingly visited the *deceptive* inflorescences when they resembled the *rewarding* inflorescences, whereas they visited an equal number of *deceptive* inflorescences over time when they co-occurred with dissimilar *rewarding* inflorescences (Fig. 3). This suggests that, colour similarity slows down the learning process. We found a significant interaction between colour and spatial mingling (Table 3). In mixed patches, bumblebees visited the *deceptive* inflorescences more often (1.75 ± 0.17 plants visited per cluster) when they co-occurred with similar *rewarding* and dissimilar *unrewarding* inflorescences. This was significantly higher than the number of visits to the *deceptive* plants within mixed patches containing dissimilar *rewarding* and similar *unrewarding* inflorescences (0.77 ± 0.10 plants visited per cluster). In monospecific patches, bumblebees visited the *deceptive* inflorescences regardless of the colour treatment (similar *rewarding* and dissimilar *unrewarding* inflorescences: 0.99 ± 0.18 plants visited per cluster; dissimilar *rewarding* and similar *unrewarding* inflorescences: 1.38 ± 0.22 plants visited per cluster).

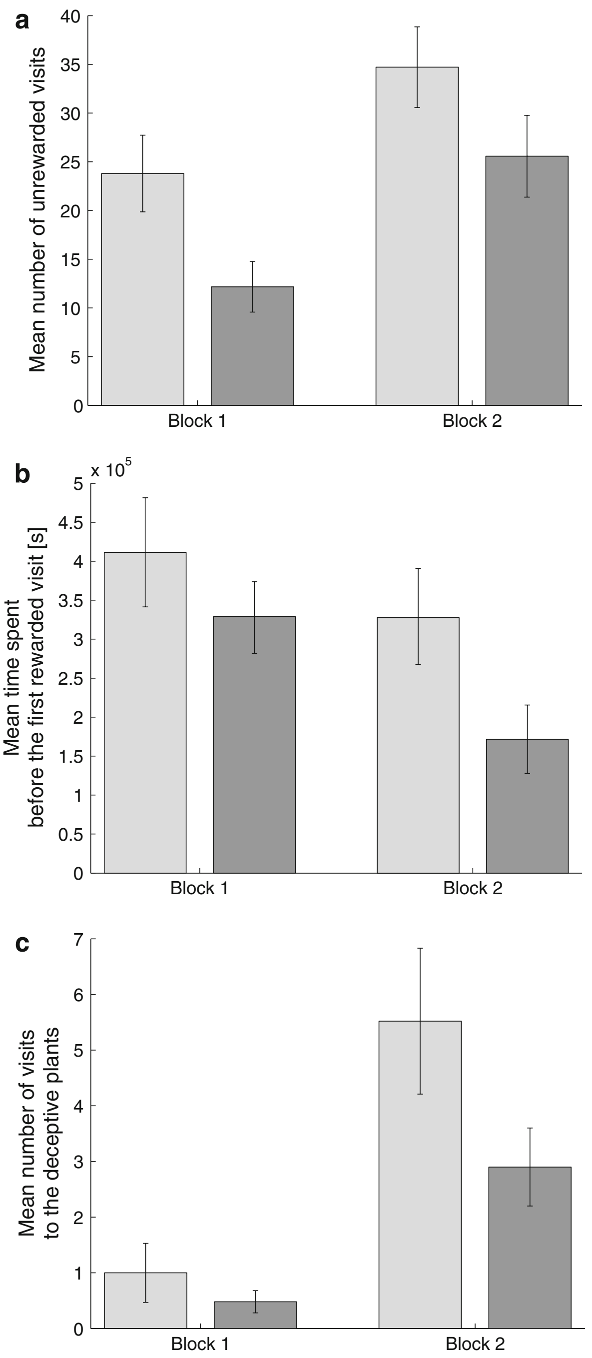
Discussion

While the survival of many generalist flower visitors relies on their effectiveness in choosing flowers based on previous foraging experience, it remains unsolved how they deal

Table 2 ANOVA table showing the effects of flower colour similarity, spatial mingling, block and their interactions on the number of unrewarded visits (to the *deceptive* and *unrewarding* inflorescences), on the time spent foraging and on the number of visits to the *deceptive* inflorescences before the first rewarded visit

		Unrewarded visits		Time spent foraging		Deceptive	
Source of variation	Df	MS	*P*	MS	*P*	MS	*P*
Colour	1	3352.6	0.001**	329891.9	0.020*	259.3	<0.001***
Mingling	1	67.2	0.640	68287	0.310	41.4	0.076[a]
Block	1	2301.3	0.009**	313925.5	0.019*	50.5	0.042*
Colour × Mingling	1	76.6	0.644	1494.8	0.882	28.4	0.179
Colour × Block	1	28.1	0.787	25587.4	0.559	20.6	0.251
Mingling × Block	1	42.7	0.731	281.9	0.954	12.4	0.343
Colour × Mingling × Block	1	321.8	0.338	172.4	0.963	0.01	0.977
Residuals	77	288.9		66331.3		11.9	

[a] $P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$



◀ **Fig. 2** **a** Mean number of unrewarded visits (to *deceptive* and *unrewarding* inflorescences) before the first rewarded visit within the test phase according to block and colour treatment. This variable measures the costs associated with unrewarded exploratory visits that bees required before switching to the *rewarding* inflorescences. **b** Mean time spent before the first rewarded visit within the test phase according to block and colour treatment. This variable measures the costs in terms of time associated with unrewarded exploratory visits that bees required before switching to the *rewarding* inflorescences. **c** Mean number of *deceptive* plants visited before the first rewarded visit within the test phase according to block and colour treatment. This variable measures the benefit that *deceptive* inflorescences obtain from pollinator exploratory visits and incorrect choices after a change in cue-reward associations. Light grey bars = Yellow *deceptive*, dark yellow *unrewarding* and blue *rewarding* inflorescences. Dark grey bars = Yellow *deceptive*, blue *unrewarding* and dark yellow *rewarding* inflorescences

with changes in the plant community composition to ensure sufficient food collection. In a controlled experiment, we trained bumblebees to forage in a community including two types of inflorescences, one rewarding one deceptive. We then tested how a nectar depletion of the initially rewarding inflorescences and the introduction of an alternative type of rewarding inflorescence affected bumblebee learning, foraging efficiency and costs, and visitation to *deceptive* inflorescences.

Effect of the change in plant community composition on bee behaviour

Out of the last 10 visits in the training phase, bumblebees made on average 8.55 rewarded visits, which significantly differed from a random visitation pattern. Thus, bumblebees associated a specific corolla colour to reward. This associative learning carried over at the beginning of the test phase (i.e. in the first cluster of 10 visits) where over 70% of the visits were to the *unrewarding* inflorescences in which nectar was depleted. This suggests that, bumblebees may suffer costs in terms of decreased foraging efficiency after nectar depletion of their learned food source, due to carry-over effects of associative learning. In particular, bees that visited only yellow flowers (i.e. yellow and dark yellow) during the training phase required more exploratory visits (to both *deceptive* and *unrewarding* inflorescences) and more time to switch to the blue *rewarding* inflorescences than bees that experienced yellow and blue flowers in the training phase to switch to the dark yellow *rewarding* inflorescences. This result is surprising since naive *Bombus terrestris* show an innate preference for violet and blue colours (Briscoe and Chittka 2001; Chittka et al. 2001; Chittka et al. 2004; Raine et al. 2006a; Raine and Chittka 2007b) and this preference is maintained even after associative learning with other colours takes place (Gumbert 2000). However, associative learning can inhibit this innate preference as long as one of the colours that the bee has to choose amongst is similar to the colour it previously associated with a reward (Gumbert 2000). In accordance with this, the learned preference for dark yellow flowers acquired during the training phase may have inhibited bumblebee innate preference for blue colour. Moreover, bumblebees did not exhibit peak shift as expected. The peak shift phenomenon was observed in bees trained to rewarding and penalising flowers and was emphasised either by increasing the risk of choosing the penalising flower colour, or by decreasing the quality of the

Table 3 ANOVA table for the effects of flower colour similarity, spatial mingling, sequence of visits, block and their interactions on the number of *deceptive*, of *unrewarding* and of *rewarding* inflorescences visited per cluster of 10 visits

		Deceptive		Unrewarding		Rewarding	
Source of variation	Df	MS	*P*	MS	*P*	MS	*P*
Error: Between bees							
Colour	1	8.726	0.200	98.318	0.143	52.600	0.249
Mingling	1	0.747	0.702	60.269	0.247	74.376	0.168
Block	1	0.912	0.694	92.566	0.147	111.643	0.086
Colour × Mingling	1	49.500	<0.001***	85.698	0.163	3.738	0.760
Colour × Block	1	10.741	0.160	9.653	0.646	37.195	0.306
Mingling × Block	1	5.425	0.289	9.177	0.638	0.523	0.923
Colour × Mingling × Block	1	0.002	0.982	7.312	0.661	9.204	0.656
Residuals	77	4.700		42.596		38.875	
Error: Within bees							
Sequence	4	4.208	0.223	235.526	<0.001 ***	190.933	<0.001 ***
Colour × Sequence	4	11.526	0.038*	7.820	0.372	1.612	0.674
Mingling × Sequence	4	2.076	0.378	3.749	0.517	3.856	0.519
Block × Sequence	4	7.097	0.120	3.485	0.541	3.598	0.532
Colour × Mingling × Sequence	4	0.960	0.555	3.353	0.558	2.782	0.578
Colour × Sequence × Block	4	3.158	0.285	25.934	0.089	18.934	0.172
Mingling × Sequence × Block	4	2.409	0.381	13.099	0.224	6.952	0.379
Colour × Mingling × Sequence x Block	4	3.055	0.308	14.744	0.194	9.763	0.309
Residuals	308	2.600		6.421		7.062	

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)

*$P < 0.05$, ***$P < 0.001$

reward offered by flowers during training (Lynn et al. 2005). In our training phase, using deceptive flowers, instead of penalising flowers, at equal frequencies with respect to rewarding flowers may thus have reduced the chances of observing peak shift. If both innate preferences and peak shift are unsuitable to explain our result, two mechanisms may potentially lead to the pattern observed. First, bees may switch more rapidly when the novel rewarding flowers are similar rather than dissimilar to those they previously encountered (Gumbert 2000). Second, increasing the difficulty of the discrimination task may allow faster bee learning than a difficult discrimination task followed by an easier one (Dyer and Chittka 2004b). Whatever the mechanism by which colour similarity affected bumblebee foraging choices in the test phase, a change in cue-reward associations within the community, as may happen when co-occurring species have different flowering schedules, may result in temporary costs to bees, arising from unrewarded exploratory visits.

The two colonies used in our experiment differed in the number of exploratory visits and in the time spent foraging before the first rewarded visit in the test phase. This suggests innate variation in bumblebee learning abilities according to colony membership (Raine et al. 2006b; Raine and Chittka 2008) or variations in environmental conditions. Interestingly, while bumblebees from the first colony required less exploratory visits compared to those from the second colony, they spent more time before the first rewarded visit than those from the second colony. Trade-off between foraging speed and accuracy was found amongst individual *Bombus terrestris* within a colony when solving colour-based discrimination tasks (Chittka et al. 2003; Dyer and Chittka 2004b). However, our result suggests that, such trade-off may have a genetic background, some colonies favouring rapid choices at the expense of precision while others make accurate choices at the expense of time decision. Whatever the speed-accuracy trade-off, both colonies experienced increased costs associated with unrewarded visits when confronted to *deceptive* inflorescences similar to *unrewarding* inflorescences and dissimilar to *rewarding* inflorescences.

Potential consequences for the pollination of deceptive plant species

In accordance with this, the *deceptive* inflorescences benefitted from more bumblebee exploratory visits when they were similar rather than dissimilar to the *unrewarding*

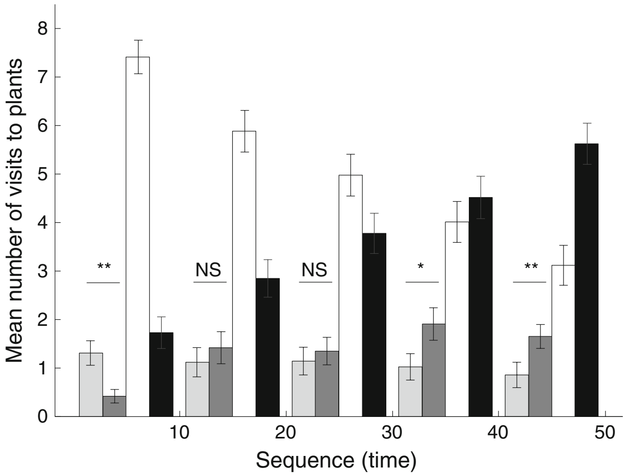


Fig. 3 Mean number of bumblebee visits to the three types of artificial inflorescences per cluster of ten sequential visits within the test phase, illustrating avoidance and associative learning over time. Light grey bars = mean number of visits to the *deceptive* inflorescences when yellow *deceptive* and dark yellow *unrewarding* inflorescences co-occurred with blue *rewarding* inflorescences; dark grey bars = mean number of visits to the *deceptive* inflorescences when yellow *deceptive* and blue *unrewarding* inflorescences co-occurred with dark yellow *rewarding* inflorescences; white bars = mean number of visits to the *unrewarding* inflorescences (in which nectar supplementation ceased in the test phase); black bars = mean number of visits to the *rewarding* inflorescences. NS: $P > 0.05$, *: $P < 0.05$, **: $P < 0.01$

inflorescences. This suggests that, the colour similarity between a deceptive and a rewarding species may increase the reproductive success of the deceptive species (Gumbert and Kunze 2001), even if the nectar in the rewarding species is depleted, at least in the short term. This may provide an explanation to the observation of Johnson et al. (2003) that some experienced bumblebee queens were more prone to visit a deceptive orchid when they already carried pollinaria of this species (i.e. when they already visited it previously) than when they did not.

Experienced bumblebees learned novel information and progressively adapted their behaviour to the novel community composition, as indicated by the increasing number of visits to the *rewarding* inflorescences throughout the test phase. However, when the *rewarding* inflorescences resembled the *deceptive* inflorescences, bumblebees increasingly visited the *deceptive* inflorescences (Gumbert and Kunze 2001; Gigord et al. 2002; Johnson et al. 2003), despite the avoidance learning acquired during the training phase (see also Internicola et al. 2007). By contrast, we found no significant difference in the number of *deceptive* inflorescences visited throughout the test phase when the *rewarding* inflorescences were of dissimilar colour (blue) to the *deceptive* inflorescences (yellow). According to these results, bumblebees visited the *deceptive* inflorescences late in the test phase more (two last clusters of 10 visits) when these resembled the *rewarding* inflorescences (dark yellow) than when they differed in corolla colour. This suggests that similarity in floral cues affects how experienced bumblebees learn novel information and adapt their behaviour to the changing characteristics of plant communities. Thus, a deceptive species may benefit from changes in cue-reward associations in the long term, if floral cues similarity between a rewarding and the deceptive species increases.

A particularly interesting situation is given when the deceptive species exhibits a flower colour polymorphism, such as, e.g. in *Dactylorhiza sambucina* and *D. romana* (Delforge 2005). Such colour-polymorphic species may benefit from increased visitation rate in both the short and the long term, because each colour morph may resemble different rewarding species that have different flowering schedules. By increasing the pollination success of different colour morphs at different times (i.e. fluctuating selection), bees' behaviour may favour the maintenance of colour polymorphism and explain the wide geographical variation of relative morph frequency in such deceptive species (Pellegrino et al. in press). In addition to flower colour, other floral cues that pollinators may associate with reward (e.g. flower odour) vary within deceptive species (Little 1983; Salzmann and Schiestl 2007). Thus, the carry-over effect of colour similarity on pollinator foraging behaviour found in the present study may also extend to or interact with other floral cues.

In conclusion, bees can adapt their foraging behaviour to the changing characteristics of natural plant communities, but learning shows carry-over effects and is modified by cue similarity between co-occurring species on multiple time scales (contemporary coexistence and in the recent past). However, such carry-over effects of memory are limited in time, since bees learn the novel cue-reward associations. Therefore, the costs associated with incorrect choices arising from temporal variation of the plant community composition may occur only in the short term, only slightly reducing the foraging efficiency of bee colonies. While the costs associated with incorrect choice may not be sufficient to affect the survival of bee colonies, the reproductive success of deceptive species may strongly increase if it is similar to a rewarding species that flowered previously. Under field conditions, the reproductive success and maintenance of deceptive species may be affected by temporal variation of the plant community composition, with respect to similarity in floral cues to rewarding sympatric species and pollinator foraging experience. In particular, a deceptive species may benefit from being polymorphic for floral cues, because different morphs may be favoured at different times. Measures of reproductive success of deceptive plant species within natural plant communities, coupled with bumblebee foraging behaviour observations, would be necessary to fully validate this hypothesis under natural conditions.

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