

Learning by Observation Emerges from Simple Associations in an Insect Model

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Summary

Recent debate has questioned whether animal social learning truly deserves the label “social” [1]. Solitary animals can sometimes learn from conspecifics [2, 3], and social learning abilities often correlate with individual learning abilities [4–6], so there may be little reason to view the underlying learning processes as adaptively specialized. Here, we demonstrate how learning by observation, an ability common to primates, birds, rodents, and insects [7–9], may arise through a simple Pavlovian ability to integrate two learned associations. Bumblebees are known to learn how to recognize rewarding flower colors by watching conspecifics from behind a screen [9], and we found that previous associations between conspecifics and reward are critical to this process. Bees that have previously been rewarded for joining conspecifics copy color preferences, but bees that lack such experience do not, and those that associate conspecifics with bitter substances actively avoid those flower colors where others have been seen. Our findings place a seemingly complex social learning phenomenon within a simple associative framework that is common to social and solitary species alike.

Results and Discussion

Second-order conditioning is a two-step associative learning process that was first demonstrated by Pavlov [10], who trained dogs to associate the sound of a metronome (a conditioned stimulus; CS1) with the presentation of food (an unconditioned stimulus; US). He then paired the presentation of a black square (CS2) with the metronome (CS1) in the absence of food, finding that the dogs would subsequently salivate when confronted with the black square (CS2) alone. Thus, the CS2 became associated with the US, even though the two had never been experienced together (Figure 1A). Second-order conditioning has since been demonstrated in a wide range of species including humans, rats, fish, bees, fruit flies, and sea slugs [11–19]. Its ecological relevance has rarely been considered, but we have previously suggested that it might provide a powerful explanation for some forms of observational learning ([20]; see also [21, 22]).

Bumblebees are known to remember the flower color preferences of foragers that they have been allowed to observe through a Perspex screen and to “copy” these preferences

when foraging alone [9]. Here, we tested whether this behavior derives from second-order conditioning and thus critically depends on a first-order association between conspecific visual cues and food reward. Following our hypothesis, bees copy the preferences of demonstrators because their foraging experience has led them to associate the visual appearance of conspecifics (CS1) with food (US) [23]; conspecifics only tend to linger on rewarding inflorescences. When conspecifics (CS1) are subsequently seen on a particular flower color (CS2), observer bees form a secondary association between that flower color (CS2) and a food reward (US; Figure 1B). Two direct predictions are that bees lacking such experience should not copy and that bees that associate conspecifics with an alternative, bitter substance should actively avoid those colors where others have been seen to forage.

We trained individual bees ($n = 158$; 11 colonies) that had no previous experience of foraging to visit a vertical array of six small transparent feeding platforms in a flight arena. Three platforms were occupied by dead or model conspecific “demonstrators” (three per platform; nine in total), and the remaining three platforms were unoccupied. For subjects in the “sucrose” group, the demonstrators could only be found on platforms that contained sucrose solution; unoccupied platforms contained aversive quinine solution [24]. For those in the “quinine” group, the occupied platforms contained quinine solution, and the unoccupied alternatives contained sucrose solution. Bees in both groups were successful in learning this first-order task (cf. [23]), choosing the rewarded alternative significantly more often than expected by chance by the final training bout (chance expectations: 50%; mean performance \pm SD: quinine group, $93 \pm 10\%$; sucrose group, $89 \pm 13\%$; one-sample t tests: $t = 4.01$, $df = 49$, $p < 0.001$. and $t = 3.57$, $df = 52$, $p < 0.001$, respectively). A third, “naive” group underwent the same training, foraging from three platforms containing sucrose solution and three containing quinine solution but in the absence of demonstrators.

Immediately after this first-order training phase, we allowed each bee to view the foraging arena from behind a Perspex screen for 10 min. Inside the arena, the subject could now see an array of six colored flowers, of two types—either three orange and three green or three blue and three yellow, all on a brown background. In every case, all flowers of one of the color alternatives were occupied by demonstrators. After this observation period, we exchanged the flowers for clean, unoccupied, empty replacements of the same colors, and altered the spatial arrangement of the colors. The subject was then released into the arena and the number of landings on each flower color recorded.

As expected under our hypothesis, copying behavior reflected the prior associations that bees had developed between conspecifics and aversive or appetitive unconditioned stimuli (Figure 2). Bees that associated conspecifics with sucrose were more likely to visit a flower color if they had seen demonstrators there than if demonstrators had been seen on the alternative color ($F = 5.27$, $df = 1$, $p = 0.02$); the reverse was true for bees that associated conspecifics with quinine ($F = 4.23$, $df = 1$, $p = 0.04$). Bees in the naive group showed no significant preference in either direction ($F = 1.21$,

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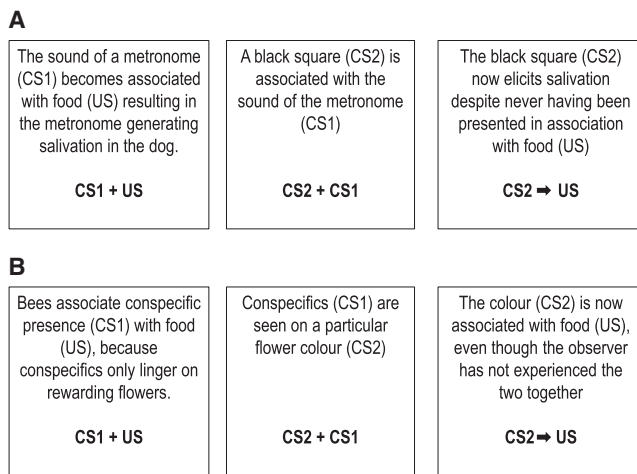


Figure 1. Second-Order Conditioning

(A) Pavlov's dogs learned to salivate on presentation of a black square that had never been paired directly with food.

(B) The same mechanism is suggested to underlie observational learning in bumblebees.

df = 1, p = 0.28). Data for the orange/green and blue/yellow choice tests were pooled because we found no significant effect of choice test on the data (F < 1.5, df = 1, p > 0.2 in each case).

Social learning about food typically involves attraction to feeding sites or food types. However, previous authors have pointed out that if social information use is a form of associative learning, animals should be able to learn negative associations as well as positive ones [1, 25, 26]. In other words, if the presence of conspecifics is inversely correlated with food quality or availability, animals should avoid popular resources. Our data provide empirical evidence that negative relationships can indeed lead to avoidance behavior. Furthermore, it is not unrealistic to expect that wild foraging bees might sometimes learn to negatively associate conspecifics with food [27], because conspecifics drain flowers. We extrapolate our finding with caution, because an actively aversive stimulus such as quinine may be more effective in eliciting avoidance behavior than simply a lack of food. Nonetheless, the possibility that wild bees may be more likely to avoid flower species where conspecifics have been seen, rather than prefer them, deserves further study.

In traditional social learning terminology, color copying in bumblebees might be termed "stimulus enhancement" [28], whereby the presence of demonstrator bees exposes the observer to the flower color, perhaps by drawing attention to it. Alternatively, if the presence of the demonstrator exposes the observers to the association between that flower color and reward, the term "observational conditioning" [7] would be used. Although both mechanisms can be encompassed within an individual learning framework (single-stimulus learning or stimulus-stimulus learning, respectively [24]), these explanations do not address the question of why the demonstrator's behavior brings about such exposure. The assumption is that selection has led social animals to treat conspecific behavior as a biologically important stimulus. Second-order conditioning presupposes no such preprogrammed response to social cues [24].

Our findings do not imply that color-preference copying in bumblebees involves no adaptive specialization. Individuals

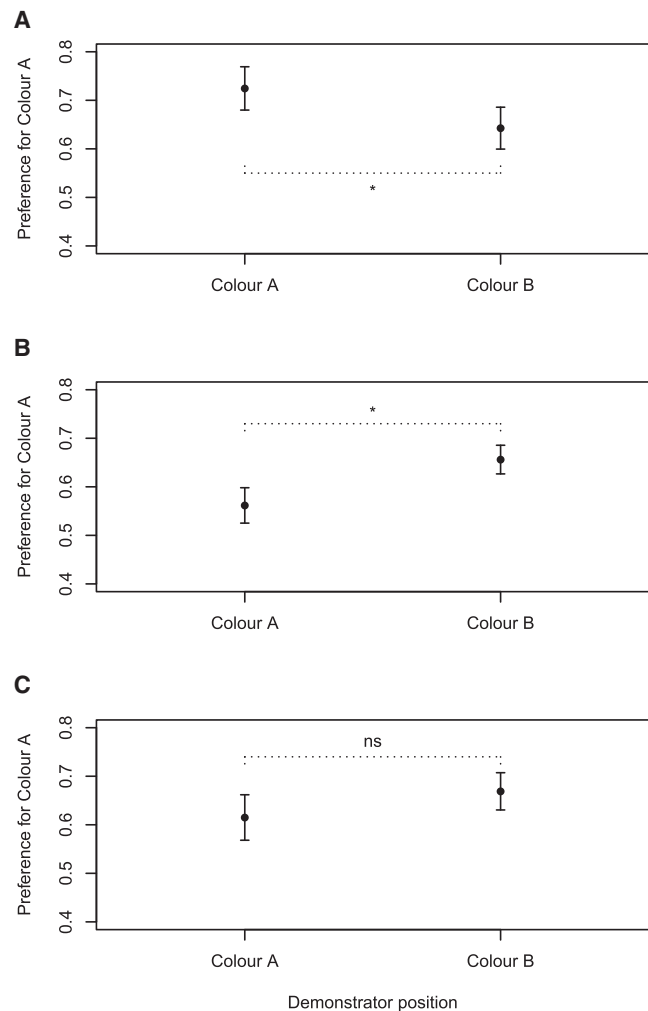


Figure 2. Flower Color Preferences according to the Presence of Demonstrators

(A–C) Sucrose group (A), quinine group (B), and naive group (C). Data for orange/green and blue/yellow choice tests were pooled because no significant differences were found between the two experiments. Thus, "color A" represents orange or blue, respectively, and "color B" represents green or yellow. Preferences are the proportion of all landings made during the test that were directed to color A for each bee (n=158 bees). Note that preferences for color B are the inverse of preferences for color A and so were not analyzed separately. Bars represent SE. * denotes p < 0.05; ns denotes not statistically significant (p > 0.05).

may be more likely to learn first-order associations involving conspecifics if they have visual systems that are adapted to perceive social cues, perceptual systems that prioritize them, or motivational systems that encourage proximity [1, 29, 30], although there is limited evidence that this is the case in bumblebees [31]. In other words, input mechanisms (cf. "data acquisition mechanisms" [32]) may well be adapted to preferentially process social information [1]. Rather, the novelty of our findings lies in showing that, although selection might favor animals that prioritize social stimuli, associative learning can explain why these stimuli acquire relevance. Thus, any animal that is capable of second-order conditioning should also be capable of this form of observational learning in the right circumstances.

We have shown that learning by observation can develop if animals learn to associate food, a biologically important

stimulus, with the visual presence of conspecifics. When these visual cues are then observed in the context of another biological stimulus, that second stimulus also becomes associated with food through a simple Pavlovian process. Importantly, there is no reason that first-order conditioned stimuli should be generated by conspecifics exclusively. If heterospecific behavior can be used to predict reward, second-order conditioning provides a means by which animals can capitalize upon such information, and there is growing evidence to suggest that social learning indeed traverses species boundaries [31, 33–36].

Experimental Procedures

Test Subjects and Arena

All experimental procedures were approved by the Zoological Society of London Ethics Committee. Eleven bumblebee (*Bombus terrestris*) colonies (provided courtesy of Syngenta Bioline Bees, Weert, the Netherlands) were each housed in wooden nest boxes (28 × 16 × 11 cm) that were connected to a flight arena (117 × 72 × 30 cm) by a Plexiglas tube. Prior to experimentation, bees were fed via gravity feeders within the nestbox and individually marked with numbered tags. Because our bumblebee nestboxes are kept dark, individuals had no prior opportunity to associate the visual presence of conspecifics with food.

Phase One: Training

We trained over 250 bees, and 158 completed the test without losing motivation to forage ($n = 54, 50,$ and 54 in sucrose, naive, and quinine groups, respectively). Subjects were trained alone. Each was permitted to visit an array of six transparent platform feeders (1.5 × 1 × 1.5 cm; two vertical rows of three platforms each, mounted on a cardboard background). Nine demonstrators (see below) were mounted around three platforms (three demonstrators per platform, two pinned and one dangling by thread from a pin to allow movement when a fan was turned on); the remaining three were unoccupied.

Model bees, or dead unrelated worker bees killed by freezing on the day prior to experimentation, served as demonstrators. Models were made from oven-baked clay (Fimo soft, Staedtler) and painted to reflect natural *Bombus terrestris* color properties according to bumblebee color space [37, 38]. We used models to avoid killing excessive numbers of bees; previous work has found that models, dead bees, and live bees all elicit equivalent copying behavior [9, 39]. Models do not produce scent, but our test phase (see below) involved viewing from behind a screen, so only visual cues were available in both cases.

In the sucrose treatment, the three occupied platforms contained 20 μ l of 2 M sucrose solution, and the unoccupied platforms contained 20 μ l of saturated quinine hemisulphate solution. In the quinine group, occupied platforms contained quinine and unoccupied platforms contained sucrose solution. Bees in the naive treatment visited the same array, where three platforms contained sucrose solution and three quinine solution, but with no demonstrators present. A small hand fan (“The Cooler” by Design Go; 8.5 × 4.5 × 4 cm) was turned on during the training phase to create the appearance of movement by the demonstrator bees (following [9]).

When bees have collected a full crop of sucrose solution, they deposit it in the nest before returning to forage again. The test bee was permitted to return eight times during the training phase; each time the position of the occupied and unoccupied platforms was changed to avoid subjects solely learning the position of rewarding flowers. Platforms were replenished every time they were depleted and the number of landings on occupied and unoccupied platforms was recorded.

Phase Two: Observation

Our observation phase was designed to replicate that used by Worden and Papaj [9]. On returning to the arena after depositing the final load of sucrose collected during training, each subject was confined to an observation box attached to the side of the arena containing a UV-transparent Perspex screen (21 × 22 cm), through which the feeding array could be viewed from a distance of 11 cm. The training feeding array was replaced with a similar array, where each feeding platform was marked by a colored cardboard “flower” (57 mm diameter) against a brown cardboard background. The six flowers comprised either three orange and three green flowers or

three blue and three yellow flowers. Within the choice set, colors were randomly allocated to platforms. The color contrast between each color and the background, calculated in a color space for bees [37, 38], was slightly higher for blue (0.21) than for yellow (0.18) and moderately higher for orange (0.2) than for green (0.14), where the maximum theoretical contrast between any color and its background is 1. Each feeding platform was filled with 20 μ l of water.

Three bee demonstrators were attached to flowers of one color, in the same positions as described in the training phase setup. Once again, a fan was used to move the hanging demonstrators. Test subjects were allowed to view the floral array, with demonstrators, for a period of 10 min. The vertical arrangement of the array meant that all flowers could be viewed equally from behind the screen. Halfway through this observation period, the position of the orange and green or the blue and yellow flowers was reversed, to minimize the likelihood that bees associated demonstrators with locations rather than colors.

Phase Three: Testing

Immediately after the observation period, the floral array was replaced with an identical floral array, save that the position of each flower color was randomly reallocated, and, importantly, no demonstrators were present. All feeding platforms were filled with 20 μ l of water. The test subject was released into the arena, and the landing choices of the subject were recorded for a 5 min period following (and including) the first landing on a flower. If no landings were made within 10 min of the subject being released, the test was stopped.

Color Preference Test

Our experimental design incorporated two types of choice test: orange/green and blue/yellow, and our response variables were therefore “preference for orange” or “preference for blue” respectively. To compare innate preferences for these colors, we tested 30 entirely naive bees that had never previously seen any colored stimuli on each pair of colors. Orange is moderately preferred over green, and blue over yellow (means \pm SE: 0.64 ± 0.04 and 0.60 ± 0.05 , respectively), as one might predict from differences in color contrast to the background (see “Phase Two: Observation” section above). These preferences for orange and blue do not differ significantly from one another ($F < 0.01$, $df = 1$, $p = 0.95$), and correspondingly, data were pooled for the two choice tests, with “choice test” included as a predictor in each model.

Statistical Tests

Our hypothesis predicts that preferences for any flower color should be relatively greater when sucrose-associated demonstrators are seen on that color than when they are seen on the alternative choice. The reverse is predicted for quinine-associated demonstrators, whereas bees in the naive group should be indifferent to demonstrator presence. Thus, for each of the three treatment groups, we modeled the response variable “preference for color A,” where color A was orange or blue, respectively. We nominated orange/blue as “color A” because innate preferences for the two colors do not differ significantly (see “Color Preference Test” section above). Note that preferences for color B are the exact inverse of color A, so modeling these would produce identical results.

For each group, we modeled the proportion of each subject’s flower visits that were directed to color A, using generalized linear models with a quasibinomial error structure [39]. Position of demonstrators (color A or color B), choice test (orange/green or blue/yellow), and proportion of correct choices in the final training bout were fitted as predictors. Nonsignificant terms were dropped sequentially until further simplification significantly decreased the explanatory power of the model. p values represent the effect of removing significant terms from the minimal model, assessed using F tests [40].

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