TECHNICAL PAPER
How to Train a Honey Bee

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In the early twentieth century, Karl von Frisch performed seminal work on the organization of social behavior of honey bees. Much of his work involved training individual foragers to distant artificial feeders. Similar training methods have been used in research laboratories for the better part of a century, and these methods lend themselves well to advanced undergraduate biology classes in animal behavior. In recent years, students have used these methods in group projects to study color preference and time-memory. In this Technical Paper, we describe the basic steps of training honey bees to a distant feeder. We also provide alternative methods for answering specific types of questions that students in animal behavior classes might wish to address.

Key words: animal behavior; student projects; Apis mellifera; field training; learning and memory

In 1973, Nikolaas Tinbergen, Konrad Lorenz, and Karl von Frisch shared the Nobel Prize in Physiology or Medicine for their work "concerning the organization and elicitation of individual and social behaviour patterns". This was the first time the award had been given for studies in this relatively new field of study called "ethology" (Marler and Griffin, 1973). Tinbergen was recognized for work in innate behavior, Lorenz was recognized for work in imprinting, and von Frisch was recognized for various sensory and social discoveries in honey bees, and in particular for deciphering the honey bee dance language. The work of these three pioneers continues to guide us today, but here we will focus on the training methods developed by von Frisch.

Von Frisch's body of work taught us a great deal about the social organization of a cosmopolitan eusocial insect, the European honey bee (*Apis mellifera*), and much of that work involved training foragers to artificial feeders attended by human observers (von Frisch, 1967). The basic task entails leading a small number of foragers to the training station and then allowing those foragers to recruit the experimental subjects via the "waggle dance" (Figure 1). After returning to the hive, a successful forager wishing to communicate the location of a valuable food source to fellow foragers will do so by performing this dance. The forager traverses the comb in a straight line while wagging her abdomen. She then turns back to her starting point without wagging and repeats the waggle run. Each time she turns around, she alternates direction so that the dance resembles a figure eight. The angle of the waggle run from vertical on the comb represents the angle of the food source from the sun's azimuth. The duration of the waggle run in time is proportional to the distance of the source from the hive (1 s ≈ 1 km). The waggle dance, then, communicates a vector: angle and distance. It is the only known symbolic language outside of the primates (Preece and Beekman, 2014). The dancer also carries the scent of the source for recruits to sample.

Von Frisch's training methods have been adapted and modernized and are still used in research to this day (e.g.,

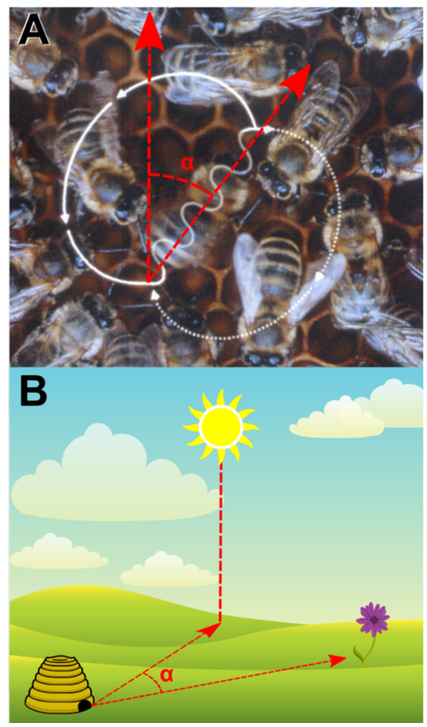

Figure 1. The honey bee waggle dance. *(A)* On the "dance floor" (a region of comb inside the hive near the entrance), a returning forager wishing to advertise her source performs the waggle dance. A forager who perceives her source to be highly valuable will dance vigorously and make many waggle runs in order to attract the attention of potential foragers. If she perceives her source to be less valuable, she will make fewer waggle runs and will do so less vigorously. If she perceives her source to be poor, she will not dance at all even if she continues foraging from it herself. *(B)* The waggle dance communicates a vector. The angle of the waggle run from vertical (α) represents the angle of the source from the azimuth of the sun at that moment. The duration in time of each waggle run is proportional to the distance to the source. [*(A)* Modified from Wikipedia, released under Creative Commons Attribution 2.5 Generic; modifications in red. *(B)* Modified from Pixabay, released under Creative Commons CC0.]

Moore and Rankin, 1983; Moore et al., 1989, 2011; Moore and Doherty, 2009; Naeger et al., 2011; Al Toufailia et al., 2013; Wagner et al., 2013; Van Nest et al., 2017). This Technical Paper will provide basic instructions for training bees to an artificial feeder along with some optional variations for answering different types of ethological questions suitable for undergraduate students in animal behavior classes or independent research projects. In recent years, student groups have used these methods to examine two questions: (1) Do honey bee foragers preferentially collect nectar from artificial flowers of certain colors? (2) Does artificial flower color affect honey bee time memory?

Honey bees are renowned for their ability to perform complicated tasks and learn complex associations. Their ability to be trained quickly both in indoor laboratory settings and in outdoor field experiments makes them valuable research subjects. These abilities allow for experiments ranging from simply measuring color or odor preference to more complicated problems like appetitive or aversive associative learning experiments (using color, pattern, odor, etc.) to the much more complicated tasks of reversal learning, time-memory, or learning abstract concepts such as “above” versus “below”. Few other invertebrates can perform these tasks, and rodents are expensive, slow to train, and require time-consuming institutional oversight.

Here we provide detailed instructions for the basic steps of training forager honey bees to visit a distant feeder. This includes initially luring bees from their hive and then getting them to reliably and continually visit the feeder while recruiting their hivemates. At this point, the bees are primed to learn, and students can proceed with experiments of their own design to answer questions of their own choosing. While students may need assistance in the early stages of training, once the bees are reliably flying to the training table, many experiments can easily be performed by undergraduates with little or no supervision. After the basic instructions, we provide several suggestions for how to get started with different types of experiments (e.g., preference testing, association learning, time-memory, etc.). Through these experiments, students can learn interesting aspects of innate animal behavior and learning and memory; these activities can complement lecture classes in animal behavior, sensory neurobiology, neuroethology and learning and memory.

MATERIALS AND STUDY SITES

This protocol is intended for training forager honey bees (*Apis mellifera*) and can be used with any type of beehive. If a research or educational apiary is not available, local beekeepers might volunteer access to their hives. Beekeepers often enjoy working with the public and showing off their trade or hobby. Most counties have beekeeping organizations that can easily be reached via a web search. If the colony is moved into a new hive, or if the hive is moved to a new location, it is best to let the colony acclimate several days prior to training. If possible, choose a colony that is known to be relatively gentle. Beekeepers tend to be familiar with the temperament of their hives.

Timing is also important but will vary by climate and geographic region. In regions that typically freeze, training in the winter is of course impossible. In such regions, training bees in the spring can still be difficult. Competing with natural foraging opportunities can make the initial steps of training (Initiation and Orientation) take a week or more. Training between midsummer and early fall tends to be much easier. Having little else to forage from, bees at this time are eager to exploit any food provided, and Initiation and Orientation can sometimes be completed within an hour. Training in late fall can be challenging, however, as the foragers become desperate and begin acting erratically. Projects undertaken in these regions should be done either in summer terms or as early as possible in fall terms. For independent student research, students may have longer periods of time available to hash out experiments; they will not have the same time constraints as class projects would require. In warmer climates, season may be less of an issue, and training may go quickly most months of the year.

Ideally, the location of the training station should be at least 100 m from the hive. When foragers advertise a valuable source that is near the hive, the short waggle runs reduce the waggle dance to a “round dance” (von Frisch, 1967), which conveys little or no directional information. Recruits appear to glean directional information better from longer waggle runs. Should such distance not be available, training just 40 or 60 m will still suffice.

A training kit should be assembled ahead of time, the contents of which depend on the question being addressed (see Training below). Suggested items for the training kit are provided in Table 1. Obtain chairs for observers and a table for the feeder that are weather proof and light enough to move periodically. At least during Initiation, the table should be roughly the same height as the hive entrance.

METHODS

For simplicity of explanation, we are dividing the training process into four phases. (1) Initiation consists of getting bees to collect sucrose solution from a feeder at the hive. (2) Orientation consists of moving the feeder to the final location of the training station and leading the bees there. (3) Training consists of recruiting experimental subjects to the feeder at the training station and training them to perform some kind of task or to learn some association. And finally, (4) Testing consists of observing the bees’ behavior after some period of training, usually without a sucrose reward. Not all experiments will utilize the Testing phase.

Most experiments involve adding scent to the feeder. This serves two purposes: (1) It makes for a more salient learning stimulus. Employing several sensory modalities and stimulus features (color, pattern, odor, etc.) provides an enriched *Gestalt* and likely enhances learning (Bogdany, 1978). For this reason, we also typically paint the bottom of the transparent feeder a bright color. (2) The waggle dance conveys direction and distance, but it does not describe the source further. If the dancer carries the scent of the source, the recruits can search for it once in the location directed by the dance. If the experimental question being studied involves scent in some way, then scent should only be applied during the Training and Test phases as per the experimental protocol. Otherwise, applying scent uniformly throughout the entire experiment usually speeds up the process of recruitment at the feeder.

Initiation

There are two general methods for the Initiation phase. Method 1 tends to be much faster than Method 2 but is notably more hazardous, as it requires working for extended periods of time immediately in front of the hive entrance where the guard bees are stationed. Only attempt Method 1 if you have experience working with bees and know that the colony is gentle enough to proceed.

Method 1

At the beginning of Initiation, avoid sitting or standing in front of the hive, if possible, to minimize obstructing and confusing returning foragers and to be of less concern to the guard bees.

Figure 2 illustrates the process of Method 1. Place the table directly in front of the hive entrance and place the feeder (see Table 1) filled with 2-M sucrose on the table as near as possible to the entrance (just 10–20 cm, if possible). Do not apply scent yet. Soak a few strips of filter paper in 2-M sucrose and place them partially inside the hive entrance. Once a strip has several bees collecting from it, carefully lift it and set it on top of the feeder on the table. As the bees continue to collect, add more sucrose-soaked strips to the hive entrance so that a few are continuously present. After the bees on the feeder fill their crops, they will fly back to the hive. Reuse those strips once all bees are clear of them.

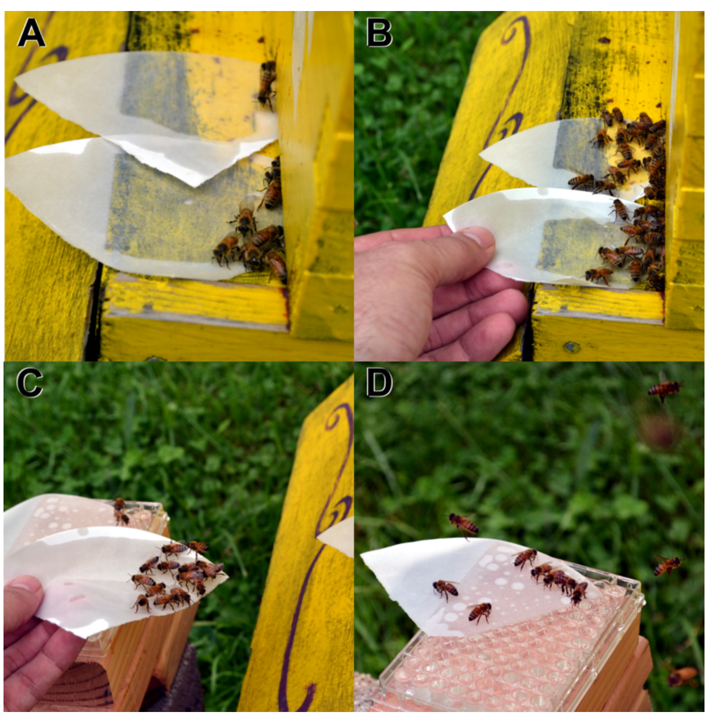

Figure 2. Initiation. *(A)* Sucrose-laden strips of filter paper are placed in the hive entrance. *(B)* Once several bees are collecting sucrose from a strip of paper, transfer it to the feeder directly in front of the hive. *(C)* If movements are gentle, most bees will remain on the strip of paper. *(D)* Once bees fill their crops, they will fly back to the hive. Continue transferring bees in this manner until they begin flying to the feeder on their own.

Once bees begin flying to the feeder on their own, stop transferring bees on paper strips. Let the bees work the feeder undisturbed for a few minutes and then move the table and feeder away from the hive a short distance (20–30 cm). If lifted gently, most bees will stay on the feeder and continue collecting. If flights to the feeder stop, resume transferring bees to the new location as before while watching for flights. Once bees are regularly flying to the feeder, and when at least 6 bees are actively collecting from the feeder, move the feeder 1 m further. Allow the foragers to make flights to this new location for at least a few minutes. Then, when at least 6 bees are on the feeder, move it 1 m again. Repeat this process until the feeder is 3 m from the hive entrance. Then set the feeder on top of a sheet of filter paper and apply 4 drops of scent to the filter paper (see Table 1). The sudden appearance of scent might confuse the bees and slow their rates of return for a few minutes. Let them acclimate and continue collecting undisturbed for several minutes before proceeding. This allows many of the foragers to experience the scent before the feeder is moved again and ensures those foragers can easily find the feeder again after it has moved.

Method 2

Method 2 is easier and safer to perform than Method 1, but it can take considerably more time. Unless we (the instructors) are performing the Initiation phase for our students, we require our undergraduate students to utilize Method 2.

Place the table directly in front of the hive entrance, 3 m away. Place the feeder (see Table 1) on a sheet of filter paper. Apply 4 drops of scent to the filter paper and fill the feeder with 2-M sucrose. Foragers will eventually discover the feeder on their own and begin collecting from it and recruiting other foragers. Honey bees exhibit a phenomenon called "flower constancy" (Free, 1963) in which foragers will typically bypass profitable food sources on their way to the source they were already exploiting. Method 2 is slow because usually only scout bees, not already working some source, will discover it. Most forager traffic will simply pass by. On the other hand, because the feeder is highly rewarding (2-M sucrose) and substantially scented, some foragers already engaged in sources elsewhere may begin working the feeder too (see Wagner et al., 2013).

Orientation

It is important to differentiate the orientation bees from the experimental subjects recruited later. The orientation bees will have the very unusual experience of chasing a feeder across a field. As this is not natural behavior, such individuals should not be used in behavioral experiments. Their experience at the feeder will be very different than that of the experimental subjects. Furthermore, the quantity of experience the orientation bees accrue may not be known accurately. Throughout Orientation (and Training later), keep the feeder filled. When it gets empty, experienced foragers start getting jumpy and erratic. Note that it may be necessary to lower the sucrose concentration to limit recruitment to rates such that it is not too difficult to paint

Item	Notes	Price[1]
Box	To hold the contents of the kit. Should be closeable and reasonably rainproof (e.g., Centrex Plastics, 19-qt clear tote with lid)	8.00
Water jug	For cleaning (any large closeable bottle)	
Water squirt bottle	For cleaning (e.g., Fisherbrand 500mL Bottles (#02-897-11)	8.00
Sucrose squirt bottle	For filling the feeder (e.g., Fisherbrand 500mL Bottles (#02-897-11)	8.00
Paper towels	For cleaning	
Paints	For marking bees (e.g., ¼ oz jar Testors Enamel, #1197TT)[2]; See Table 2.	2.00 each
Paint box	To hold the paint jars (e.g., a used micropipette tips box)	
Applicator sticks	To apply paint to the bees (e.g., Chef Craft Thin Bamboo Skewers, 6-inch, 300 count). Sharpen the applicator sticks if necessary.	6.00
96-well plate	To use as a feeder (any type will do)[3]	
Filter paper	15-cm disc (any type). The feeder is placed on the paper, and the paper is scented	
Weights	For keeping the filter paper on the table. Four large metal washers work well.	
Scent vial	Essential oil or alcohol-based extract[4]	
Transfer pipette	For applying scent to the filter paper (any type)	
Scent box	Any tightly closeable glass box or jar to hold the scent vial and transfer pipette to help contain the scent	
Large forceps	For removing bees from the feeder when necessary	
Two-way radios	Helpful for two-station experiments (e.g., Floureon brand)	18.00/pair
Clock/thermometer/ hygrometer	Helpful if interested in time-training and/or environmental conditions (e.g., Fisherbrand 06-662-4)	62.00
Notebook	For notetaking	
Large zipper bags	To protect notebook and electronics in case of rain	
Extra pens	Paper Mate InkJoy brand tend to work well in damp conditions	

Table 1. Suggested items for the training kit.

1 Approximate price in U.S. dollars at common stores in the United States, Canada, and online (e.g., Amazon.com, Michaels, Fisher Scientific, Home Depot).
2 Alternatively, Craft Smart brand paint pens work well (US$3.00 each).
3 Alternatively, a 9-cm Petri dish cut to a height of approximately 4 mm (or any shallow dish) will work. Bees tend to land in such dishes, however, and will then waste time grooming. These also spill more easily than 96-well plates.
4 Extracts are less expensive than essential oils but evaporate quickly and must be applied more frequently.

the newly arriving bees.

As each bee is collecting sucrose at the feeder, use a wooden applicator to apply one small dot of some pre-determined orientation color (e.g., gold) to the dorsal thorax (see Figure 3A and Tables 1 and 2). If done gently, the bees will remain still and continue collecting. For a measure of progress, record how many bees are painted with the orientation color. For the remainder of the Orientation phase and throughout the Training phase, do not allow any bee to leave the feeder without having an identification mark. In order to have a complete record of behavior for each bee, her identity must be known at all times. As you approach the final destination (and throughout the Training phase), it might be necessary to use large forceps to remove and kill a bee if she is about to leave without being marked.

Continue moving the feeder incrementally to the pre-selected training site. Wait at least 5 min between moves to ensure several bees get rewards at the current location and wait until at least 6 bees are on the feeder before moving. Early moves should be no more than 2–3 m, but eventually large jumps (as far as 10 or 20 m) can be made provided the new location is within view of the old location. Bees excel at memorizing visual landmarks, and those returning to the old location will have memorized the sight of a feeding table, chairs, and humans, as well as the applied scent, and will be able to relocate to the new location within a few minutes. If the final destination cannot be reached on the first day, remove the scented filter paper and discard it offsite. Wash the feeder and tabletop with water, and leave the empty feeder, table, and chairs at the current location overnight. Resume at the current location the following day at the same starting time as before with new scented filter paper. Due to the bees' strong time-memory, they will show up again on subsequent days at the appropriate time (Moore, 2001).

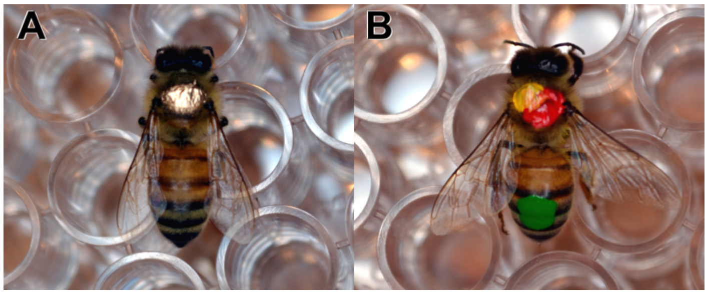

Figure 3. Labeled honey bee foragers. *(A)* A forager with a single-dot code. This bee would be identified in the notes as “O”. *(B)* A forager with a three-dot code. This bee would be identified in the notes as “YRG”. See Table 2.

Once the final destination is reached, let the bees continue foraging from the feeder 5–10 min more to ensure that they learn where the training site is, and continue painting any new recruits with the orientation color. Training may commence at this time (and this should be the start time for all subsequent days), or Training may begin the following day. If Training is to start the following day, wash the feeder and tabletop with water, and remove the scented filter paper and discard it offsite. For the remainder of the experiment, the feeder, table, and chairs should remain at this location.

Training

From this point forward, students will execute their planned experimental protocol. Unless the experimental question requires it, the appearance and scent of the training station should not change throughout Training and Testing. The nature of the Training phase will depend on the experimental question being asked. Students might continue using the same feeder as in Initiation and Orientation, or they may construct artificial flowers. They may use geometric or colorful patterns on the table or feeders. They may have multiple feeders on the table that differ in some way. They may alter scent or combinations of scents. See for example Van Nest et al. (2017), in which bees had to choose among multiple artificial flowers of different colors.

The length of the Training phase and the amount of training individual bees receive will also depend on the experimental question. In some types of experiments, individual foragers are trained for several days, during which different cohorts will form, each with differing amounts of experience. Then students might ask how experience influences some behavior. For example, Moore et al. (2011) looked at how experience affects memory extinction. In other types of experiments, individual bees’ behaviors need only be monitored for a short period of time. Van Nest et al. (2017) looked at how long it took for bees to learn which color flower contained a reward. Each bee was monitored for only 12 visits on one day. For very simple color preference experiments (e.g., what color flower does a bee choose when first arriving?), each bee would be observed just once and would then be painted a single dot to indicate she has already been observed.

Other aspects of Training will also depend on the experimental question. For instance, for some types of experiments, the sucrose concentration needs to remain constant throughout Training. For example, in Moore and Doherty (2009), cohorts of bees were trained over several days to examine how experience affects the accuracy of time-memory; all other factors needed to remain constant. For others (e.g., Van Nest et al., 2017), the actual concentration is immaterial, but it needs to be adjusted continually to carefully control the rate of forager recruitment.

Testing

Depending on the experimental question being asked, a Testing phase may or may not be necessary. A Testing phase is typically done for learning and memory experiments, where bees received sucrose rewards as they learned some task or association during the Training phase. The foragers are then tested on one or more test days. Typically on test days, everything at the training station remains exactly as it was throughout Training with the exception that the feeder remains empty. Scent may or may not be applied depending on the question being addressed. The observers record the behavior of all the experimental subjects arriving at the training table without offering any reward.

Variations and Suggestions

Many questions can be asked using the basic training methods above. Below are some variations that may help students develop new questions along with brief descriptions of what different types of experiments entail. Students and instructors are also encouraged to review the references in this paper for additional insight, especially von Frisch (1967). Another rich source of interesting honey bee training experiments is Seeley (1995).

Studies in Foraging Preference

Experiments asking if foragers prefer different features upon initial arrival at a training station are among the easiest for undergraduate students to perform. These could simply be a comparison in the number of arrivals at a blue feeder versus a yellow feeder or one odor versus a second odor on the training table. Students may develop much more creative questions. Such studies might require only a single training day and no test days.

Studies in Feature-Learning

Students may design associative learning experiments by training bees to associate the presence of a sucrose reward with some particular feature of a specific feeder. For instance, the training table might contain two feeders (or many feeders) of different colors, patterns, or other kind of varying features, where one type contains a sucrose reward, and the other remains empty. Bees may be allowed to receive some number of rewards to determine how quickly they learn the association. Keep in mind that the feeders may need to be moved periodically. Foragers tend to remember the location of a feeder more readily than other features (see Van Nest et al., 2017).

Studies in Time-Memory

Honey bees have a strong time-memory (Moore, 2001);

often the German term *Zeitgedächtnis* is used. It is postulated that honey bees utilize a continuously consulted circadian oscillator (Pittendrigh, 1958; Moore, 2001). This clock allows bees to keep track of the position of the sun throughout the day for both the purposes of navigation by sun-compass and communication of food sources via the waggle dance. The clock also allows bees to memorize the time a profitable food source was found so that they can begin exploiting it at the appropriate time of day on subsequent days without having to expend excess energy rediscovering it each day (see Moore and Doherty, 2009).

When performing time-memory experiments, the start time and end time of sucrose availability at the feeder must be the same each training day. Experimenters must carefully record each arrival time each bee makes throughout Training and Testing. Comparisons of arrival times on test days can be made between experimental groups (e.g., varying feeder color, sucrose concentration, scent, etc.).

Multi-Color Paint Codes

In typical research projects (e.g., Wagner et al., 2013), it is necessary to keep track of a large number of individually identifiable foragers. On training days, each new forager arriving at the feeder needs to be assigned a unique identification code. We usually do this with a combination of paint dots. For quick notetaking, we use a special alphabet (Table 2). Figures 3 and 4 show examples of paint codes. It is helpful to have a preprinted list of all possible codes, which can be checked off as they are painted during Training (see Appendix 1). Such shortcuts are helpful during chaotic training days. In other experiments (e.g., Van Nest et al., 2017), the ages of the foragers also need to be known. This is done by tagging a large number of newly emerged bees with paint dots and/or queen number tags (e.g., from Betterbee, Greenwich, New York, USA) prior to being placed in the experimental hive. Colonies generally accept newly emerged foragers regardless of relatedness. After pre-tagged bees begin foraging in 2–3 weeks, they can be recruited to the training station for experimentation.

Using newly emerged bees also allows for more advanced experiments. For instance, newly emerged adults from different donor hives (*i.e.*, from different, unrelated queens) could be deposited into one experimental hive to look for differences in foraging preference or learning ability between different genetic sources. Or, newly emerged adults can be treated pharmacologically before being deposited into the experimental hive. For example, the mushroom bodies (MB) are insect brain regions associated with learning and memory, and the projection neurons into the MB from the primary sensory neuropils are cholinergic (Fahrbach, 2006). Newly emerged adult bees fed pilocarpine (a muscarinic-type cholinergic agonist) increased dendritic complexity within the MB, and scopolamine (an antagonist) inhibited dendritic growth (Dobrin et al., 2011). Similar treatments can be performed, and then behavioral differences can be examined. For the typical undergraduate class project, however, methods using multi-color paint codes or number tags are usually very difficult to perform successfully and too labor-intensive. Often a single paint dot given at the training station is sufficient. It is important that students understand, however, that it is impossible to differentiate 100 foragers visiting 10 times each from 10 foragers visiting 100 times each when they are marked the same. For any given experimental question, the necessity to differentiate each forager will need to be weighed against the difficulty of managing complex paint codes. Single- or two-dot codes with many different colors might be a reasonable compromise.

Color	Code	Color	Code
Blue[2]	B	Gray[3]	A
Green[2]	G	Orange[3]	N
Pink[2]	P	Purple[3]	U
Red[2]	R	Copper[4]	C
White[2]	W	Gold[4]	O
Yellow[2]	Y	Silver[4]	S

Table 2. The honey bee training alphabet used for identification codes[1]. See Figs. 3 and 4.

[1] When it is necessary to record an unmarked bee in the notes, we use the code “X”.

[2] The standard 6 colors that are easily distinguishable in the field.

[3] Additional colors for larger numbers of bees.

[4] Colors typically used for Orientation.

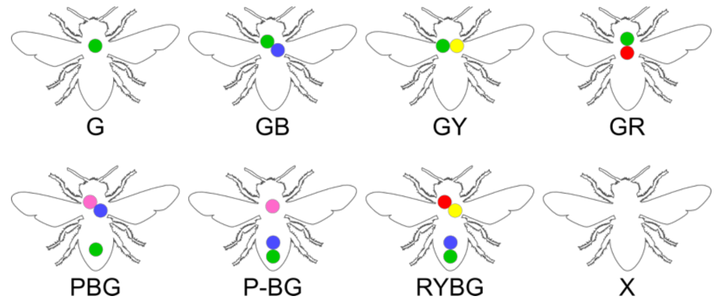

Figure 4. Paint codes. Generally, codes are read from top-left toward the bottom (when viewing the dorsal side with the head upwards). One-dot codes are as shown above (*G*) and in Figure 3A. Ideally, two-dot codes (and the first two dots in multi-dot codes) are painted diagonally (e.g., *GB*). If the dots are not painted diagonally, then they are read left-to-right (e.g., *GY*) or top-to-bottom (e.g., *GR*). Typically, three-dot codes are as shown above (*PBG*) and in Figure 3B. When a larger number of paint codes is required, additional colors can be added to the alphabet, or other dot arrangements can be added (e.g., *P-BG* and *RYBG*). Unmarked bees are identified by *X*. See Table 2. [Honey bee outline from Pixabay, released under Creative Commons CC0.]

Two-Station Experiments

It is often useful to train bees to multiple feeders in different locations simultaneously. The feeders can then act as controls for one another. These are very labor-intensive experiments and should perhaps be reserved for advanced students doing independent projects. If this is to be attempted, a separate training kit should be assembled for each training station (Table 1). Also, ensure that the two sites are at least 60° apart (e.g., one north of the hive and one east of the hive).

After completing Initiation, but before beginning Orientation, set up two feeders on two tables side-by-side. Once bees are reliably visiting both feeders, begin moving the tables apart very gradually. Once the two feeders are a few meters from one another, Orientation can begin. Begin applying scent and painting orientation codes. Unless the experimental question demands otherwise, the two feeders should be different colors and scented differently (e.g., peppermint and lilac). Additionally, the orientation bees associated with the different feeders should be painted different colors (e.g., gold and gray).

During Training, the two feeders can differ in whatever way the experimental question dictates (different feeder colors, patterns, scents, sucrose concentrations, etc.). It is important that none of the experimental bees from one feeder gets a reward from the other feeder. This is best avoided by continuing to paint the bees from the different feeders differently. If the wrong bee arrives at a feeder, shoo her away. If she successfully lands and receives a reward, she will need to be removed and killed or at least excluded from analysis later.

Two stations can also be set up at different times of day (whether at the same or different locations; e.g., Naeger et al., 2011). Standard Initiation and Orientation phases are employed at the relevant times. Again, the feeders should be scented differently (as the scent brought into the hive by foragers may activate the other foraging group; Reinhard et al., 2004), and the bees associated with the different feeders should be painted differently and kept from collecting from the wrong feeder.

Other Tips and Tricks

The following are a set of additional suggestions that may be helpful for many types of experiments:

- When painting multi-color paint codes on bees, it is usually easier to paint the dorsal thorax than the dorsal abdomen. Even though the abdomen is larger, the wings often get in the way. We usually paint one- and two-dot codes on the thorax only. Longer codes (for larger numbers of bees) have dots on both the thorax and the abdomen. See Figure 4.
- When using a preprinted list of multi-color paint codes, the codes should be sorted into different blocks, the first of which require relatively infrequent swapping of paint jars, and the last of which require more frequent swapping of paint jars. During hectic periods, codes in the early blocks should be issued, and during calm periods, codes in the latter blocks should be issued. See Appendix 1.
- When performing two-station experiments, it is helpful to use the first dot in multi-color paint codes to designate training station. For example, bees with codes starting with R, W, and B are trained to Station 1, and bees with codes starting with G, Y, and P are trained to Station 2 (See Table 2 and Appendix 1).
- In experiments requiring frequent changes of sucrose concentration, it is helpful to have an array of concentrations in 50-mL conical centrifuge tubes (e.g., Falcon 352070, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Drill holes in the caps to fit the stems of plastic transfer pipettes (e.g., VWR 16001-180, VWR International, Radnor, Pennsylvania, USA), which fit well into these tubes. The holes will be small enough to prevent bees from entering and to minimize evaporation of sucrose solution. Sort the tubes in a suitable tube rack. A make-shift tube rack can be made by discarding the inner rack from a 1000-µL micropipette tips box and drilling holes in the lid to accommodate the centrifuge tubes.

A Note on Honey Bee Safety

Initiation can be somewhat hazardous; it may be best for this phase to be done only by experienced instructors or beekeepers. Appropriate beekeeping PPE should be worn as needed. Two points should be made, however: (1) It is difficult or impossible to manipulate filter paper while wearing beekeeping gloves. One or two layers of nitrile gloves may be worn as an alternative, as these are known to provide some protection from bee stings. (2) Spending long periods of time under the sun or in warm weather can also be hazardous. Beekeeping clothing resists stingers by being very tightly woven and as such does not breathe well. The risk of heat injury should be weighed against the risk of getting stung. At a minimum, a beekeeping face veil should be worn.

The likelihood of a student getting stung at the feeder once it is far from the hive entrance is relatively minimal. Only guard bees at their hive entrance are aggressive; foragers are never aggressive. It is easy to paint bees and harass them in various ways while they are collecting from the feeder, provided that movements are gentle. Even when a forager feels threatened, she will only act defensively if she cannot escape. The only significant risk of getting stung usually comes from accidentally resting one's arm on the table or arm of the chair where an unseen forager happens to be grooming away from the feeder.

DISCUSSION

The methods described in this Technical Paper are relatively easy to perform. They are also quite inexpensive. If an apiary is already available, the total cost is quite minimal, as many of the materials needed are commonly available in teaching laboratories. However, while the methods are simple, it is critical that they are carried out meticulously. In many types of experiments (in particular time-training experiments), a complete history of each bee's behavior must be known. Thus, it is imperative that every orientation bee is marked as such. Attention to detail in all regards is important. Such details include the number of rewards each bee receives, the number of days of training each bee experiences, the start and end times of sucrose availability, the sucrose concentration, etc. Even recording weather patterns can sometimes reveal interesting behavior (e.g., Moore et al., 2011). Seasonal variation can also be studied by perhaps having different student groups repeating experiments at different times of the year.

Honey bee behavior can be astonishingly sophisticated. Bees can count landmarks (Chittka and Geiger, 1995), learn complicated geometric patterns (Greenspan, 2007; Giurfa, 2012), and learn abstract concepts such as "same" versus "different" and "above" versus "below" (Giurfa et al., 2001; Avarguès-Weber et al., 2011). However, while basic preference, decision making, and learning and memory experiments can be performed with single-color, population paint codes, much more intricate behavior can be observed when each individual bee is uniquely labeled (e.g., Figure 3B). As stated previously, these types of experiments are labor intensive. It is helpful to have one person dedicated to notetaking, while one or more people paint bees, observe and refill the feeder, and dictate activities to the notetaker. More challenging or long-term experiments might be better suited to independent research projects (e.g., capstone projects or undergraduate thesis projects) than to class projects. It might also be interesting to perform field-training experiments as complements to laboratory experiments (e.g., the proboscis extension response, PER; Van Nest, 2018). This might enable students to examine contextual differences such as free-flying versus harnessed. Students might even be able to perform field and lab tests on the same individuals by capturing bees at the feeder after Training and Testing and bringing them into the lab for PER training. Different types of learning may be inhibited or enhanced by the situation (e.g., are color preferences context-dependent?)

We have had students use the basic training methods in group projects in animal behavior classes a number of times. Sometimes the students piggybacked their project with ongoing research and collaborated with graduate students. Some of these students continued participating in the research and got published (e.g., Wagner et al., 2013; Van Nest et al., 2016). Other students performed their own independent research for their class project. With instructor guidance, they formulated their own research questions and designed their own experiments. They studied a particular research literature and learned to update and refine their questions. One group simply tested for color preference in foraging by counting visits at feeders of different colors. Another group tested if feeder color affected the time-accuracy of the foragers' time-place memory. Instructors helped with Initiation and Orientation, but the students performed the Testing and Training unsupervised. These students had the opportunity to examine fascinating, innate animal behavior, learn new areas of neuroscience (e.g., chronobiology), and learn some basic statistics to fully understand what their subjects were doing. As is always the case, students got as much out of these projects as they were willing to put into them. However, these training methods are a powerful tool, and motivated students can use them to study virtually any type of learning and memory question.

We believe that more field studies are needed in science and in science education. The study of natural behavior is especially important in light of today's changing climate, habitat loss, urbanization, and light pollution. This bee training tool can be adapted by students to determine the potential influences of a wide variety of different stimuli on a wide variety of behavioral responses. For example, one such line of study could test the influence of abnormal environmental conditions on the temporal accuracy of time-place learning. Some easily testable environmental factors might include the presence versus the absence of artificial light on the bee colony at night, urban versus natural habitats, the presence versus the absence of low levels of common pesticides in the sucrose solution provided at the training station, and comparing the performance of similar colonies kept at stressful versus moderate temperatures.

Students in animal behavior and neuroscience classes are increasingly relying on invertebrate species. They are typically less expensive to maintain than vertebrates, and experiments using them generally require no institutional review. Invertebrates are also capable of surprisingly complex behavior and are therefore valuable options in the classroom (Abramson, 1986).

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APPENDIX 1 – CODE LISTS FOR TWO-STATION EXPERIMENT

Codes are sorted into blocks. The smallest blocks (just two codes each) are used when training is slow and calm. The largest blocks are used when training is hectic. All codes within each block can be painted without swapping any paint jars. Each code is checked off when painted and then again each day that that bee is seen.

STATION ONE
All Station One bee codes start with Red, White, or Blue. Orientation color is Gold.

		Dates:							
Needed	Num	Code	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Red, White, Blue	1	RWB							
	2	RBW							
	3	RWR							
	4	RWW							
	5	RBR							
	6	RBB							
	7	WRR							
	8	WRW							
	9	WRB							
	10	WBR							
	11	WBW							
	12	WBB							
	13	BRR							
	14	BRW							
	15	BRB							
	16	BWR							
	17	BWW							
	18	BWB							
	19	RRR							
	20	WWW							
	21	BBB							
	22	RRW							
	23	WWR							
	24	RRB							
	25	BBR							
	26	WWB							
	27	BBW							
Red, White, Green	28	RWG							
	29	RGW							
	30	RGG							
	31	RGR							
	32	WRG							
	33	WGR							
	34	WGW							
	35	WGG							
	36	RRG							
	37	WWG							
Red, Blue, Green	38	RBG							
	39	RGB							
	40	BRG							
	41	BGR							
	42	BGB							
	43	BGG							
	44	BBG							
White, Blue, Green	45	WBG							
	46	WGB							
	47	BWG							
	48	BGW							
Red, White, Yellow	49	RWY							
	50	RYW							
	51	RYY							
	52	RYR							
	53	WRY							
	54	WYR							
	55	WYW							
	56	WYY							
	57	RRY							
	58	WWY							
Red, Blue, Yellow	59	RBY							
	60	RYB							
	61	BRY							
	62	BYR							
	63	BYB							
	64	BYY							
	65	BBY							
Red, Blue, Pink	66	RBP							
	67	RPB							
	68	BRP							
	69	BPR							
	70	BPB							
	71	BPP							
	72	BBP							

		Dates:							
Needed	Num	Code	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
White, Blue, Yellow	73	WBY							
	74	WYB							
	75	BWY							
	76	BYW							
Red, White, Pink	77	RWP							
	78	RPW							
	79	RPP							
	80	RPR							
	81	WRP							
	82	WPR							
	83	WPW							
	84	WPP							
	85	RRP							
	86	WWP							
White, Blue, Pink	87	WBP							
	88	WPB							
	89	BWP							
	90	BPW							
R, G, Y	91	RGY							
	92	RYG							
W, G, Y	93	WGY							
	94	WYG							
B, G, Y	95	BGY							
	96	BYG							
R, G, P	97	RGP							
	98	RPG							
W, G, P	99	WGP							
	100	WPG							
B, G, P	101	BGP							
	102	BPG							
R, Y, P	103	RYP							
	104	RPY							
W, Y, P	105	WYP							
	106	WPY							
B, Y, P	107	BYP							
	108	BPY							
Red, White, Blue	109	R-WW							
	110	R-WR							
	111	R-RW							
	112	R-RR							
	113	R-WB							
	114	R-BW							
	115	R-BB							
	116	R-RB							
	117	R-BR							
Red, White, Green	118	R-WG							
	119	R-GW							
	120	R-GG							
	121	R-GR							
	122	R-RG							
Red, White, Yellow	123	R-WY							
	124	R-YW							
	125	R-YY							
	126	R-YR							
	127	R-RY							
Blue, Green, Yellow	128	R-YG							
	129	R-GY							
	130	R-YB							
	131	R-BY							
Red, White, Pink	132	R-WP							
	133	R-PW							
	134	R-PP							
	135	R-PR							
	136	R-RP							
Blue, Green, Pink	137	R-PB							
	138	R-BP							
	139	R-PG							
	140	R-GP							
Y, P	141	R-PY							
	142	R-YP							
B, G	143	R-GB							
	144	R-BG							

STATION TWO
All Station Two bee codes start with Green, Yellow, or Pink. Orientation color is Gray.

		Dates:							
Needed	Num	Code	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Green, Yellow, Pink	1	GYP							
	2	GPY							
	3	GYG							
	4	GYY							
	5	GPG							
	6	GPP							
	7	YGG							
	8	YGY							
	9	YGP							
	10	YPG							
	11	YPY							
	12	YPP							
	13	PGG							
	14	PGY							
	15	PGP							
	16	PYG							
	17	PYY							
	18	PYP							
	19	GGG							
	20	YYY							
	21	PPP							
	22	GGY							
	23	YYG							
	24	GGP							
	25	PPG							
	26	YYP							
	27	PPY							
Green, Yellow, Red	28	GYR							
	29	GRY							
	30	GRR							
	31	GRG							
	32	YGR							
	33	YRG							
	34	YRY							
	35	YRR							
	36	GGR							
	37	YYR							
Green, Pink, Red	38	GPR							
	39	GRP							
	40	PGR							
	41	PRG							
	42	PRP							
	43	PRR							
	44	PPR							
Yellow, Pink, Red	45	YPR							
	46	YRP							
	47	PYR							
	48	PRY							
Yellow, Green, White	49	GYW							
	50	GWY							
	51	GWW							
	52	GWG							
	53	YGW							
	54	YWG							
	55	YWY							
	56	YWW							
	57	GGW							
	58	YYW							
Green, Pink, White	59	GPW							
	60	GWP							
	61	PGW							
	62	PWG							
	63	PWP							
	64	PWW							
	65	PPW							
Green, Pink, Blue	66	GPB							
	67	GBP							
	68	PGB							
	69	PBG							
	70	PBP							
	71	PBB							
	72	PPB							

		Dates:							
Needed	Num	Code	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Yellow, Pink, White	73	YPW							
	74	YWP							
	75	PYW							
	76	PWY							
Green, Yellow, Blue	77	GYB							
	78	GBY							
	79	GBB							
	80	GBG							
	81	YGB							
	82	YBG							
	83	YBY							
	84	YBB							
	85	GGB							
	86	YYB							
Yellow, Pink, Blue	87	YPB							
	88	YBP							
	89	PYB							
	90	PBY							
G, R, W	91	GRW							
	92	GWR							
Y, R, W	93	YRW							
	94	YWR							
P, R, W	95	PRW							
	96	PWR							
G, R, B	97	GRB							
	98	GBR							
Y, R, B	99	YRB							
	100	YBR							
P, R, B	101	PRB							
	102	PBR							
G, W, B	103	GWB							
	104	GBW							
Y, W, B	105	YWB							
	106	YBW							
P, W, B	107	PWB							
	108	PBW							
Green, Yellow, Pink	109	G-GG							
	110	G-GY							
	111	G-YG							
	112	G-YY							
	113	G-GP							
	114	G-PG							
	115	G-PP							
	116	G-YP							
	117	G-PY							
Green, Yellow, Red	118	G-YR							
	119	G-RY							
	120	G-RR							
	121	G-GR							
	122	G-RG							
Green, Yellow, White	123	G-YW							
	124	G-WY							
	125	G-WW							
	126	G-WG							
	127	G-GW							
Pink, Red, White	128	G-WR							
	129	G-RW							
	130	G-WP							
	131	G-PW							
Green, Yellow, Blue	132	G-YB							
	133	G-BY							
	134	G-BB							
	135	G-BG							
	136	G-GB							
Pink, Red, Blue	137	G-BP							
	138	G-PB							
	139	G-BR							
	140	G-RB							
W, B	141	G-BW							
	142	G-WB							
P, R	143	G-PR							
	144	G-RP							

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