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Low Strength Magnetic Fields Serve as a Cue for Foraging Honey Bees but Prior Experience is More Indicative of Choice

Ana M. Chicas-Mosier,[1] Medhat Radi,[2] Jack Lafferrandre,[3] John F. O'Hara,[4] Hitesh D. Vora,[5] and Charles I. Abramson[1,3]*

[1]Department of Integrative Biology, Oklahoma State University Stillwater, Stillwater, Oklahoma
[2]Department of Pest Physiology, Plant Protection Research Institute, Dokki, Egypt
[3]Department of Psychology, Oklahoma State University Stillwater, Stillwater, Oklahoma
[4]School of Electrical and Computer Engineering, Oklahoma State University, Stillwater, Oklahoma
[5]Department of Mechanical Engineering Technology, Oklahoma State University, Stillwater, Oklahoma

Species of migrating insects use magnetic fields as a navigational tool that is independent of current weather conditions and non-migrating species have been shown to discriminate anomalies in magnetic field from the earth's baseline. Honey bee discrimination of magnetic field has been studied in the context of associative learning, physiology, and whole hive responses. This article uses a combination of free-flight and laboratory studies to determine how small fluctuations from Earth's magnetic field affect honey bee (*Apis mellifera* L.) decision-making. Honey bees were tested in three experiments: (i) recruitment to an aqueous sucrose feeder, (ii) an artificial free-flight flower patch with floral color-dependent magnetic field strength, and (iii) a Y-maze with alternating colors on a stronger magnetic field. In free-flying feeder experiments, magnetic field served as a temporary cue, but when offered an equal caloric alternative with lesser magnetic field, the latter was preferred. Flower patch experiments showed initial color biases that were abandoned as a response to magnetic field induction. In laboratory experiments, bees showed a color-dependent behavioral response to the magnetic field. The results of this study indicate that bees may use small fluctuations in magnetic fields as a cue but that it is likely low-value as compared with other stimuli. Bioelectromagnetics. 2020;41:458–470. © 2020 Bioelectromagnetics Society.

Keywords: ***Apis mellifera*; decision-making; conditioning; magnetic field; associative learning**

INTRODUCTION

The United States has increased energy consumption by 66.62 quadrillion BTU since 1950 [U.S. Energy Information Administration, 2019]. Globally, it is expected that energy usage growth will continue at a rate of 1.8% per year until 2040 [International Energy Agency, 2019]. With this tremendous increase in energy use comes increased electromagnetic and magnetic field (MF) sources. Sources of moving charge that produce MFs such as transformers, power lines, and cell phones are globally ubiquitous in modern society and may alter the behaviors of some organisms [Martin et al., 1989].

Insects and other organisms such as sea turtles and birds notably use MFs to direct their movement and migration patterns [Baker and Mather, 1982;

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*Correspondence to: Charles I. Abramson, Department of Integrative Biology, Oklahoma State University Stillwater, 401 North Murray, Stillwater, OK 74078. E-mail: Charles.Abramson@okstate.edu

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Lohmann, 1991; Srygley et al., 2006; Wiltschko et al., 2011; Gao et al., 2014; Guerra et al., 2014; Xu et al., 2017; Dreyer et al., 2018]. These organisms use the earth's MF to direct their migrations to breeding or wintering grounds in substandard conditions. MFs are not modified by temporary conditions, making them better indicators of positionality than alternatives such as light location [Lohmann, 1991; Wiltschko et al., 2011]. MF can act as a compass that is not hampered by time of year or weather patterns and has been detected in several insects. It is expected that the use of MF is an evolved mechanism to effectively navigate in low light or poor weather conditions [Srygley et al., 2006; Wiltschko et al., 2011; Gao et al., 2014; Dreyer et al., 2018]. As MFs are typically studied in the context of orientation, migratory species have been the modern model organisms for research; however, other species likely use these fields as an environmental cue.

The magnetic field of the earth (electromagnetic field [EMF]) is fairly minute (~5×10^{-5} T) compared with the variety of fields that humans and other organisms encounter daily (up to 0.2 T) [World Health Organization, 2018]. Small increases from the baseline environment may serve as an environmental or orienting cue to other species that rely heavily on accurate orientation. Foraging species that must recall a specific location may be able to use distinct MF changes to their advantage for relocation, similar to long-distance flights in migratory species. Prior work has shown that insects including moths, butterflies, and honey bees can detect these changes and discriminate them for navigation [Walker and Bitterman, 1989a,b; Kirschvink et al., 1997]. In honey bees, this is likely coordinated by magnetite in their abdomens that responds to MF direction [Hsu et al., 2007; Lambinet et al., 2017].

Honey bees (*Apis mellifera* L.) represent a non-migratory, relatively globally ubiquitous species. These insects are experiencing population decline, along with other pollinators as a result of pathogens, habitat destruction, and pesticide application [Goulson et al., 2015; Hallmann et al., 2017]. Honey bees have been used as a model organism to study responses to pesticide exposure, learning, neuroadaptation, and environmental impacts and have been shown to discriminate between MFs [Walker and Bitterman, 1989a,b; Abramson et al., 2012a; Williamson and Wright, 2013; Karahan et al., 2015; Chicas-Mosier et al., 2019]. They also are easy to rear, manage, and allow for large sample sizes with similar environmental background conditions. Prior investigation into honey bee magnetoreception showed that the waggle dance changes with comb direction and MF [Martin and Lindauer, 1977; De Jong, 1982], EMF and induced MFs assist in orienting honey bees toward a compass direction [Collett and Baron, 1994; Válková and Vácha, 2012], and honey bees can discriminate weak anomalies from EMF [Walker and Bitterman, 1985, 1989a,b]. Honey bees can be used to understand how small changes in MF from Earth's baseline may affect non-migratory insects and if changing MF environments present a potential threat to species of concern.

MFs are used for orientation by bees, but this can come at a cost as small anomalies from EMF can result in changes in proteins in the brain and consequentially impede the waggle dance and reduce flight speeds [Martin et al., 1989]. In addition to protein damage, honey bees and other insects may actively avoid anomalies from EMF, and sources such as cell towers are correlated to altered insect communities and reduced population densities [Lázaro et al., 2016; Vanbergen et al., 2019]. Lambinet et al. [2017] and Hsu et al. [2007] have shown that honey bees contain magnetite in their abdomens for navigation and these processes can be disrupted following exposure to MFs [Balmori, 2015]. In addition to navigational disruption, close-contact to mobile phones in the hive was shown to negatively impact communication, activity, and forager orientation [Favre, 2011].

Honey bees are conditioned to stimuli very quickly and this is exploited when testing learning in both field and laboratory conditions (see Abramson et al. [2012a] or Karahan et al. [2015] for examples). These conditioning techniques use the pairing of a stimulus (e.g. color or scent) to a consequence (e.g. sucrose or shock) [Abramson et al., 2012a; Dinges et al., 2013]. As MF anomalies are detected by honey bees, they may be used as a cue for locating food sources [Walker and Bitterman, 1985; Kirschvink et al., 1997; Liang et al., 2016]. The experiments described in this article will test if MF can be paired with a reward in free-flight and laboratory conditions to determine if the observed detrimental effects of MF translate into poor choice-making in honey bees.

Honey bees orient foraging flights and communicate food location through odors and the waggle dance. The waggle dance is hypothesized to be a mechanism of communication using the sun's location as a reference point, taking into account its movement across the sky over the course of the day, regardless of current weather [Von Frisch, 1954, 1967; Von Frisch and Lindauer, 1956]. Given that EMF changes can cause honey bees to realign the directionality of their hive construction to preserve magnetic bearing, can discriminate between MF, and can use MF as a reward cue, it is possible that anomalies will also affect their free-flight foraging decisions [De Jong, 1982; Walker and Bitterman, 1985, 1989a,b].

The authors hypothesize, based on prior literature, that honey bees are using EMF to orient foraging but that weak inhomogeneities in the field caused by environmental changes may interrupt these MF-based cues causing reduced return success and a lower frequency of correct caloric choice. To test this hypothesis, the authors employ three experiments to simulate different scenarios that bees may experience. The first two experiments will investigate how conditional MF cues in honey bees affect their free-flight decisions with and without color stimuli. The third experiment will investigate choice in the laboratory and will be utilized to determine if, when confined, MF can serve as a food source cue or if stronger MFs elicit deterrent/attractant effects in bees. It is expected that if honey bees experience navigation or communication deficits or are deterred from the induced MF that feeder visitation will remain comparatively low, color biases will be abandoned, and avoidance will be observed in the laboratory. The results of these experiments will lend insight into how honey bees utilize MF anomalies in their daily foraging choices.

METHODS

Study Species

Honey bees (*A. mellifera* L.) were from one of four hives outside of Stillwater, OK, USA, maintained by the Comparative Psychology and Behavioral laboratory at Oklahoma State University. All subjects were foragers collected from a ~0.1 M sucrose feeder 20 m from the nearest hive. Only foraging bees were used in an effort to standardize for honey bee age (>21 days) [Huang and Robinson, 1996]. All experiments occurred within the same foraging season during the summer of 2019.

Magnetic Field Production

Magnetic fields for all experiments were produced by a magnetic field generator manufactured following the description in Kirschvink and Kirschvink [1991]. The magnetic field generator consisted of a power supply, an operator's board, and two disks, each with two coils. As in Kirschvink and Kirschvink [1991], electric current could be directed such that different currents flowed through the two coils in each disk, thereby producing different magnetic fields. In the "cancelling" disk, current flowed anti-parallel in two windings on each coil, thereby producing only small, incidental fields. In the "reinforcing" disk, current flowed parallel in the two windings of each coil, such that each coil produced a relatively strong field [Kirschvink, 1992]. However, the coils were also oriented anti-parallel to each other, which further modified the produced magnetic field, as in Kirschvink and Kirschvink [1991]. The produced fields were simulated using COMSOL Multiphysics simulation software (Comsol, Los Angeles, CA) and are shown in Figures 1-3. Magnetic field measurements were taken at the location that a bee would feed from, approximately 2–3 cm above the center of the reinforcing disk (Fig. 2). With this wiring arrangement, both disks experienced exactly the same net magnitude of current flow, consumed the same power, and equivalently produced the same heat. A switch on the operator board enabled the magnetic field reinforcing/cancelling roles of each disk to be swapped. For all experiments, the current was set to 1 A. Before each experiment, the magnitude of magnetic field at the designated feeding location for bees was measured with a gauss meter. Due to imperfections in the windings and geometries, detection of magnetic field occurred on both disks despite the field cancellation attributes of the generator, although generally weaker fields were detected in the cancelling disk. Detected magnetic fields across all experiments ranged from 72–280 μT. This is a much greater range than has been shown to be distinguishable by honey bees and is more similar to the range described as a potential human health risk [Shupak et al., 2004; Bogdan et al., 2014; Balmori, 2015].

Experiment 1: Feeder Choice

Experimental setup

Honey bees found foraging on a ~0.1 M sucrose feeder (henceforth primary feeder) were captured in empty matchboxes and relocated to one of two (eastern and western) 1 M feeders (henceforth secondary feeders). The secondary feeders were 25 m from hives and 35 m from the primary feeder and separated by a building (Fig. 4). Bees were moved from the south side of the building to this north side to limit unrecruited bees from finding the secondary feeders. Bees were not typically found foraging on the north side of the building prior to the experiment; therefore, all bees that were found on secondary feeders were assumed to have been placed on the feeder with a matchbox or recruited by a relocated bee. Upon relocation to one of the secondary feeders (Fig. 4), bees were slowly released by opening the

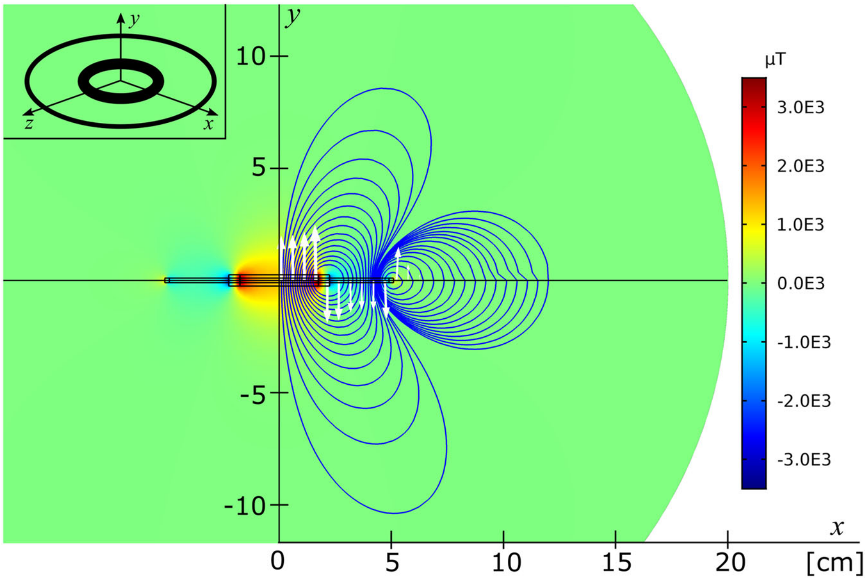

Fig. 1. Plot of the magnetic flux density around the device. The xy-plane colormap shows the amplitude of the *y*-component flux density (*By*) in µT where *y* is the rotation axis of the disk. Streamlines show the directionality of the magnetic fields around the device but do not provide field amplitude information. White arrows give an approximate magnitude and direction of *y*-oriented magnetic flux density along the *x*-axis. Streamlines and arrows are only shown for $x > 0$; however, the rotational symmetry of the disk ensures the fields are unchanged as the view is rotated about the *y*-axis. The inset in the upper-left shows the two coils in perspective to help provide orientation for the flux density plots.

matchboxes at the base to encourage sucrose feeding. The eastern and western secondary feeders (ESF and WSF) were equidistant from the primary feeder and approximately 2 m from each other.

Bees that drank from a secondary feeder upon release were marked with a location-specific Testor's enamel paint on the thorax (white: 1145TT or green: 1124TT; Testor, Vernon Hills, IL). The feeder

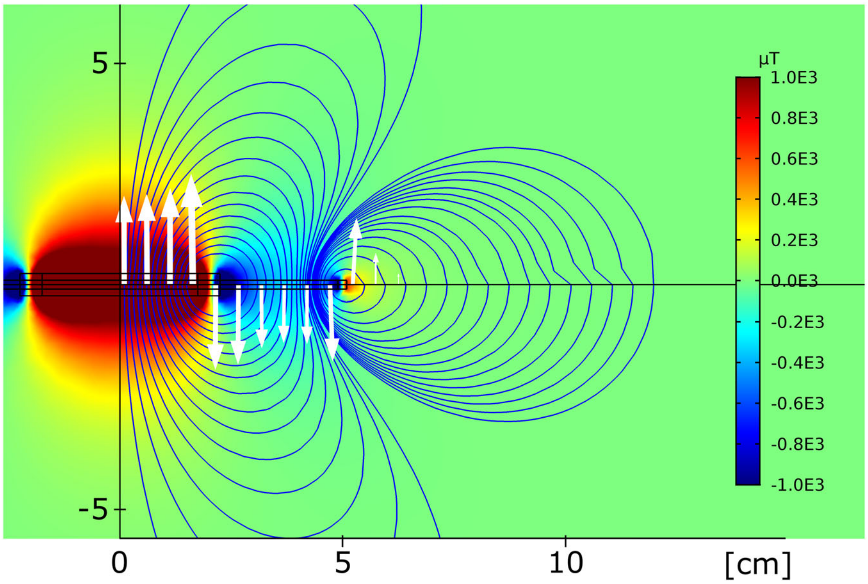

Fig. 2. Zoomed in picture of the magnetic flux density shown in Figure 1 around the coils in the disk. The flux density abruptly changes direction at the inner and outer coil ($x = 2$ and $x = 5$ cm).

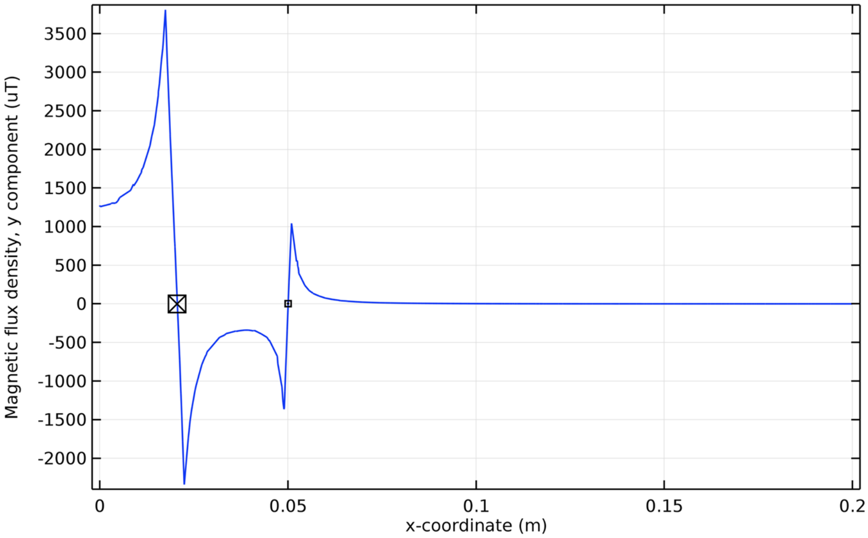

Fig. 3. Plot of the magnetic flux density (y-component) along the x-axis at $y = 0$. The rotational symmetry of the disk means the same plot applies to the simulation of the flux density along any radius from the y-axis. The black box with the "X" inside represents the location and overall size of the inner coil, where the "X" indicates current flow is directed into the page in this view. The smaller black box with the dot represents the thinner outer coil with current flowing out of the page in this view.

experiment was repeated for 3 days, in which Day 1 and Day 3 utilized induced MF. Bees that were marked on Day 1 were observed on Day 3, implying that some bees did participate in all 3 days of experimentation, although some attrition likely occurred.

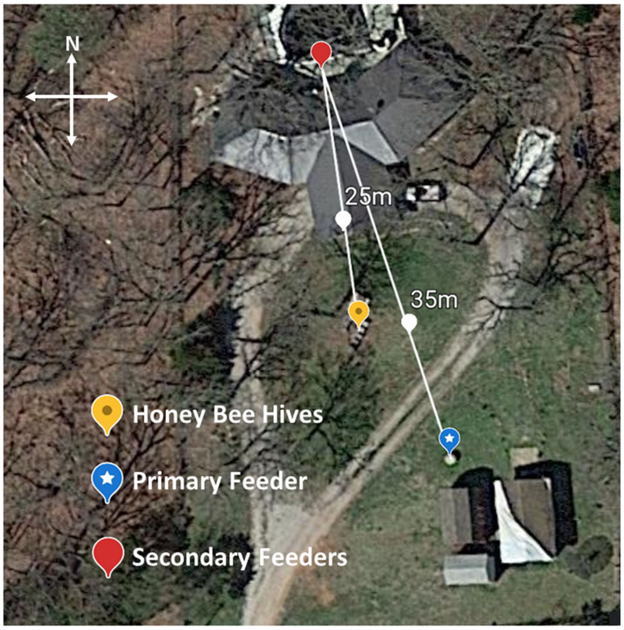

Fig. 4. Diagram of hive locations with reference to feeders, map data ©2020 Google Earth.

Day 1. For Day 1 experiments, the WSF was set on top of the MF generator and the ESF served as the control. Bees were released in pairs so that when a bee was released on WSF, a bee was simultaneously released on ESF to limit side bias at the feeders. Both human releasers were naïve to the practice of honey bee matchbox transfer to control for experience affecting successes (i.e. the number of bees that fed before leaving the feeder).

Images were taken from above each secondary feeder every 5 min following the first bee's introduction. Five minutes was the chosen time period as it was the approximate time needed to collect bees from the primary feeder and relocate them to the secondary feeders. Following the first bee's introduction to the feeder, two to five bees were released on both secondary feeders every subsequent 5-min period for a total of 100 min (20 releases/feeder). Following the honey bee capture and release period, 15 min image frequency was determined to be sufficient to visualize recruitment. All images were taken from a minimum of 1 m away from the feeders, using zoom to add detail, and from above to limit introduction of additional MF or other disturbance of feeding honey bees.

After the first 100 min, the point at which bees were self-recruiting, bees were allowed to continue their own recruitment for an additional 3 h on Day 1 and for the following experimental days described below. The Day 1 experiment occurred between 10:30

and 15:00 h. Images for each experimental day were scored for the number of bees actively feeding at the base of each secondary feeder and cumulative totals were calculated to account for the likelihood that the same bees were counted repeatedly across photos. Secondary feeders were only available to honey bees during described observation periods to eliminate undocumented recruitment.

Day 2. On the second day, the secondary feeders were set out for 2 h (10:30–12:30 h). Images were taken every 15 min and coded as described for Day 1. During Day 2, neither feeder contained an MF. This served as a control day to establish the feeders as a recurring event with a conditional MF cue. If MFs served as a positive locational cue, then Day 1 and Day 2 data are expected to be near 1:1 with minimal additional recruitment. If MF served as a low-value cue and typical recruitment behaviors were equally or more valuable, such as conspecific scent, then additional recruitment on Day 2 is expected.

Day 3. The third day served to counterbalance Day 1. Day 1 contained the MF on the WSF and Day 3 the MF generator was placed under the eastern feeder. Images on Day 3 were taken every 15 min between 10:30 and 15:00 h to match the observed period during Day 1; images were scored for the number of feeding bees.

Data Analysis

χ^2 Tests were used to compare the average daily change in cumulative bees per feeder to estimate the effect of the MF on the feeder visitation. Comparisons were made within WSF and ESF by day as well as between locations.

Experiment 2: Artificial Flower Patch

An artificial flower patch was used to understand how foraging behavior is altered by the presence of MF. The flower patch consisted of four transparent 5 × 5 cm Plexiglas squares, two yellow and two blue, set over a white plastic board. The flower patch design is a common method used to study how foraging behaviors of honey bees are affected by variables such as toxicants [Karahan et al., 2015], or subspecies [Cakmak et al., 2010]. The present study used a scaled-down version from prior literature with larger flowers to control for MF gradient, and a reduction in the number of choices from 36 to 4 because of the number of observers required to conduct a full-scale flower patch [Karahan et al., 2015]. Yellow and blue flower colors were chosen as bees typically have a strong strong preference for one or the other as opposed to a blue-white comparison in which preferences can be manipulated [Chicas-Mosier et al., 2017, 2019]. The white plastic board that supported the flowers was balanced on both MF generator disks. The MF generating disk was under one color flower (e.g. blue) while the field canceling disk was under the adjacent other flower color (e.g. yellow).

Each flower contained a small well (1.5 ml microcentrifuge tube lid) in the center that was filled with 20 µl of 1 M aqueous sucrose and refilled as needed throughout the experiment. The experiment consisted of three experimental subsections; no field, field on color 1, and field on color 2 (Table 1). Before each subsection, the magnitude of the MF directly above the flower well, where a bee would stand while feeding, was measured with a gaussmeter. All flower wells showed some detectable MF during trials; however, per experimental subsection, the observed MF on the focal flower exceeded the magnitude of the MF on the non-focal flowers (Table 1).

To start the experiment, up to six honey bees were marked with Testor's enamel paint (9115×; Testor) for individual identification throughout the experiment. A visit was counted if a bee extended her proboscis or drank from the sucrose well. For each visit, flower color and location were recorded. The three subsections were counterbalanced by color and flower location during the second and third sections and to limit bias caused by the color that contained MF first. Table 1 includes the blue and yellow MF magnitude for both subsections and the values for

TABLE 1. The Recorded Range of the Magnitude of Magnetic Fields in Feeding Wells of the Artificial Flower Patch Experiment, Where Subsection 1 Contains No Induced Field and 2 and 3 Counterbalance Magnetic Field on One of the Two Colors

	2		3	
No field induction	Location	Range	Location	Range
141–207 µT	Blue with MF	130–198 µT	Blue with MF	130–280 µT
	Yellow no MF	140–196 µT	Yellow no MF	150–260 µT
	Yellow with MF	155–240 µT	Yellow with MF	83–273 µT
	Blue no MF	149–175 µT	Blue no MF	137–196 µ

The overlap in magnitude range is across multiple days and experimental subsections; per experimental subsection, observed field on non-focal flowers did not exceed that of the flower above the reinforcing magnetic field disk.

each color during counterbalanced trials. Each subsection was 45 min in length. Some bees were actively foraging after the 45 min expired, in this case, data collection continued until the bee exited the experimental platform area and returned to the hive before the flower patch was prepared for the next subsection.

There was a comparatively high range of observed MF when the inducer was turned off, for this reason; the subsection 1 data were only used to assess initial color bias and deviations from this bias in subsequent subsections.

Data Analysis

χ^2 Analysis was used to test changes in the proportion of blue visitation as compared with the first experimental subsection.

Experiment 3: Y-Maze

A Y-maze was used to estimate avoidance/attraction of food resources near the higher MF. The maze consisted of a three-dimensional (3D) printed white poly (lactic) acid (PLA) polymer Y-maze (Fig. 5, printing details provided as supplementary materials). The Y-maze had a clear Plexiglas cover and base to easily record the honey bee's movement (Fig. 5A). The maze consisted of three arms: a start box with no choice variable (Fig. 5C), and two choice arms that terminated with a removable painted panel (Fig. 5B). Fused deposition model printing technology was used via the commercially available CraftBot 3D printer (CraftUnique, Carrollton, TX) to manufacture the Y-maze, removable color panels, and bee containment box. 3D printing was performed using a 0.4 mm diameter steel nozzle and 1.75 mm filament of PLA, optimal printing parameters for PLA filament materials. Extruder and bed temperatures were 220 °C and 60 °C, respectively. The clear Plexiglas cover was cut to size using a CO_2 laser. Panels were painted with Testor's enamel paint in either yellow (1177TT; Testor) or blue (1111TT; Testor).

Foraging honey bees were captured from a ~0.1 M aqueous sucrose feeder and brought to a nearby indoor laboratory facility, 12.4 km from the apiary. A 1:2 mixture of honey:sucrose was provided to bees after removal from the feeder until transfer to individual bee holding containers (Figure 5C). The honey sucrose blend creates a paste that is easy to transport without drying out and provides more complex nutrition for the bees than sucrose solution alone [Standifer et al. 1978]. Upon arrival at the laboratory, two bees selected for immediate use were transferred to the 3D-printed containment box (Fig. 5C) and held for 10 min. Bee holding in C was to allow for adjustment to the new enclosure and reduce post-handling stress variables. Additional handling following transfer to the holding chamber was avoided. MF disks were attached vertically behind panels B at the end of both choice lanes. Painted sucrose wells were placed at the base of the panels (Fig. 5B). The inside walls of each well were painted with the corresponding color of B, blue or yellow. Both wells contained 200 µl of 1 M sucrose for use throughout the experiment.

MF strength was measured directly above each well, where bees would be standing while feeding, before each experiment (Table 2). The side with MF did not change over the course of the experiment; however, the color of the panel on the field-producing side was alternated for each subsequent trial (five trials of each color on MF and five of each color without field per bee). This design was chosen to determine if color bias or MF was the stronger factor in determining bee side-choice. Alternating which color stimulus occurred with MF also worked to control for scents left by the prior bee. Pheromone stimuli are inherent to honey bee communication and ecology and therefore were not removed between trials; however, the apparatus was washed and rinsed with ethanol between experiments so only the stimuli from the mutually running bees were present. Although washing between trials could have removed some scent between mutually running bees, in honey bee studies including the present work, washing between each trial is not feasible and likely does not entirely remove all detectable odors [Abramson et al., 2012b; Karahan et al., 2015].

Each experiment included two honey bees. Boxes (Fig. 5C) containing each bee were labeled accordingly. To start each trial, a box containing a bee was docked at C, the start gate was opened, and a 5 min timer was started. After 5 min, the experimental bee was coaxed back into C with minimal handling and C was replaced with the other containment box, allowing a 5-min rest period for each bee between

TABLE 2. Y-Maze Range of Magnetic Fields by Side. Magnetic Field was Counterbalanced by Side Between Experiments but Not Within Experiments; Therefore, Field was Paired with Position Rather than Color

	Magnitude of field range	
	Well containing field	Well with cancelled field
Field on left side	90–174 µT	72–93 µT
Field on right side	120–156 µT	76–89 µT

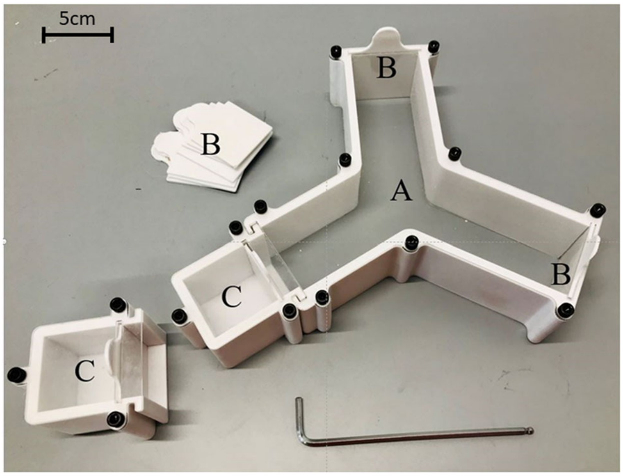

Fig. 5. Diagram of the Y-maze where A is the 3D printed maze, B are removable color panels, and C are bee containment boxes.

trials. Bees were video recorded from above for the entirety of the experiment (10 experimental trials and 10 rest periods per bee). Videos were later coded for total amount of time spent per choice lane and amount of time spent feeding.

Data Analysis

Least square regression was used to model the effect of increasing field on the percent of time spent per arm. The analysis was further divided by MF side (canceling or reinforcing disk) to reduce errors induced by the relatively close magnitudes between the two MF disks.

All analyses for experiments 1–3 were conducted in SAS JMP 13 (2016, Cary, NC).

RESULTS

Experiment 1: Feeder Choice

Comparisons of average cumulative bees were made by feeder location and between feeder locations to determine if recruitment was more closely tied to placement or MF. For the WSF, which held field on Day 1, the cumulative number of bees drinking was highly positively correlated with MF ($\chi^2 = 20.08$, $df = 1$, $P = 0.0002$; Fig. 6). However, for the ESF, which held field on Day 3, MF was negatively correlated with cumulative feeding bees ($\chi^2 = 38.6$, $df = 1$, $P = 0.0014$; Fig. 7). These analyses do not include June 27th, when no MF was used, to directly compare the 2 days when MF was an available cue. When both MF days were compared between location there was no significant effect of MF on cumulative number of bees.

Experiment 2: Artificial Flower Patch

Bees were categorized by their flower selection in the first subsection (Table 1) of the experiment when the MF generator was unplugged. Preference was defined as more than 60% visitation of a single color of flower during subsection 1. Of the 21 bees that completed the experiment, 52.4% preferred blue flowers, 38.1% preferred yellow flowers, and 9.5% held no preference. Of the blue preference bees, 62% only visited blue flowers during the first phase whereas only 18% of yellow preference bees were this loyal.

χ^2 Analysis was conducted to compare visitation in the initial subsection (without MF) and visitation in the subsequent sections (Fig. 8). For the no color bias bees, blue visitation $40\% > \bar{x} < 60\%$; in the first phase, there was no significant change in visitation when blue flowers held the MF but significantly more blue visitation when yellow flowers held the field ($\chi^2 = 9.96$, $df = 1$, $P = 0.0014$). For blue color bias bees (>60% blue visitation in subsection 1), there was a significant reduction in blue visitation when either color contained the MF (blue: $\chi^2 = 43.42$, $df = 1$, $P < 0.0001$, yellow: $\chi^2 = 36.65$, $df = 1$, $P < 0.0001$). Yellow color bias bees did not demonstrate a change in visitation proportion when blue artificial flowers held the MF but increased their blue visitation when yellow flowers held field ($\chi^2 = 4.14$, $df = 1$, $P = 0.048$).

Experiment 3: Y-Maze

Honey bees did not spend a significantly higher percentage of total time in a single-arm regardless of MF presence; 12.36% in blue when blue held field, 12.28% in blue when yellow held field, 11.37% in yellow when blue held field, 9.51% in yellow when yellow held field. Overall, bees spent less than 50% of the 300 s trials in the choice arms (54.7% no response). Distinct from percentage of total time spent per arm was whether MF strength had an effect on the percent of time spent per arm. A least square-fit model demonstrated that the percentage of time spent in the blue arm was not affected by MF strength; however, percent of time spent in the yellow arm significantly increased with increasing field both on the weaker field side (field on blue: $t = 2.18$, $P = 0.032$ and field on yellow: $t = 2.32$, $P = 0.023$) and the stronger field side (field on blue: $t = 2.02$, $P = 0.046$ and field on yellow: $t = 2.46$, $P = 0.016$; Fig. 9).

DISCUSSION

This study further demonstrates that honey bees can use anomalies from Earth's MF to locate food sources but introduces a novel component regarding how they use these cues and particularly how prior experience affects use. The flower patch data suggest that there were changes from baseline color biases following MF induction, especially in no bias and yellow bias bees. The similarities in blue flower visitation when either blue or yellow flowers contained the MF may be a result of similar MFs to baseline when blue flowers contained field; however, honey bees have been shown to discriminate changes in MF as low as 26 nT and the magnitudes compared in this study were generally much greater [Walker and Bitterman, 1989b].

The authors attempted to avoid similarities in MF to baseline when designing the flower patch experiment and to limit confounds by counterbalancing by location, field generating disk, and flower color. Despite these precautions, the results showed minimal differences between treatments (Table 1). Considering yellow flower MF, the outcomes were variable, with a decrease in yellow visitation in no bias and yellow bias bees, and an increase in blue bias bees. This implies that color choice fidelity is flexible but is dependent on environment and color bias. Flexibility and manipulation of color bias have been demonstrated in past experiments and it is expected that experience is also a dominant factor in the present work [Black et al., 2018]. The data also suggest that in a free-flight paradigm with side-by-side choices, MF may serve as a slight deterrent to bees (Figs. 6-8).

For the Y-maze experiments, increasing field acted as an attractant to yellow visitation overall; however, blue wells had higher visitation regardless of MF. This may indicate that in a constrained environment, MF can be paired with a reward stimulus but that the effect is strongest among bees with more flexible color-bias. This slight reward-location pairing is also demonstrated with location bias in the feeder experiment indicating that MF may serve as a navigational tool but that prior experience or other bee recruitment is more important when making foraging decisions.

The cumulative data of these procedures suggest that although bees do respond to MF fluctuations, it is dependent on the conditions in which they encounter the fluctuation, how the MF is presented, and prior experience. Bees that were in free-flight were more likely to use the MF initially but avoid induced MF when given identical food choices with or without MF. Contrastingly, bees that were forced to make decisions in MF without an escape option were more likely to use the cue and even increased their forage of the option with stronger MF if they had flexible color-bias. Given these data, further analysis of impacts of magnetic field should focus on ecologically relevant applications, such as were conducted here, rather than laboratory testing or applied fields that are unlikely for honey bees to naturally encounter.

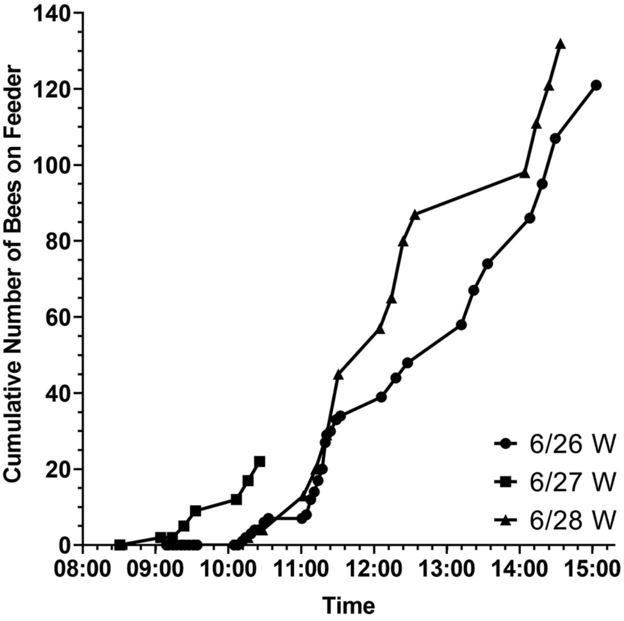

Fig. 6. Cumulative bees on the western secondary feeder by day, where magnetic field induction occurred on 6/26/2019.

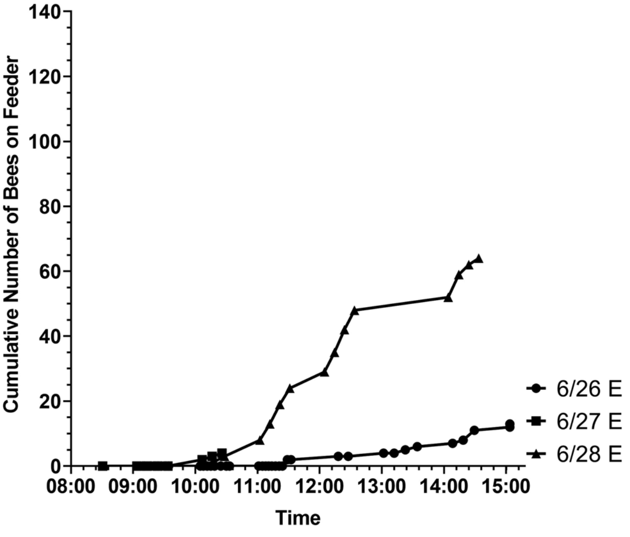

Fig. 7. Cumulative bees on the eastern secondary feeder by day, where magnetic field induction occurred on 6/28/2019.

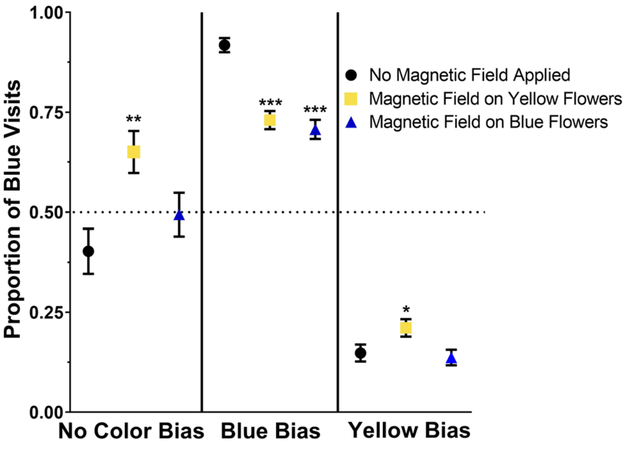

Fig. 8. Proportion of blue artificial flower visits by color bias and subsection ±standard error of the mean.

The results of this study contribute a novel aspect to the current literature on honey bee associative learning and use of MFs. A significant proportion of current work has focused on whole hive, physiological mechanisms, and associative learning [Martin et al., 1989; Walker and Bitterman, 1989a,b; Kirschvink et al., 1997; Hsu et al., 2007; Balmori, 2015; Lambinet et al., 2017]. However, the ecologically relevant outcomes of these data on honey bee day-to-day activity and subsequent foraging success had not been experimentally determined. This study demonstrates, in agreement with Bartzley and Nabity [2018], that the sensitivity of honey bees to MF anomalies may be over-reported because studies have not looked at ecologically relevant behavioral metrics.

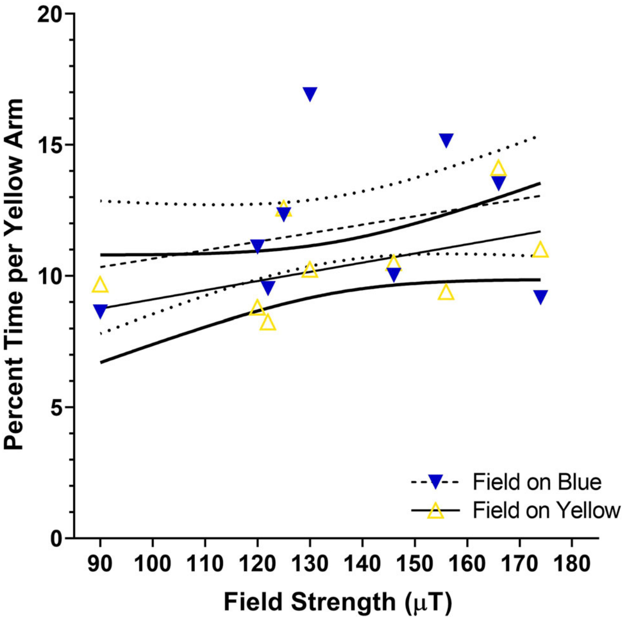

Fig. 9. Percentage of time spent in the yellow arm of the maze by field strength. Dotted lines represent the 95% confidence interval for field on blue (n.s.) and solid are for field on yellow ($P < 0.05$).

The methods described in this paper are modified from established methods in honey bee behavioral literature with the addition of MF cues [Scheiner et al., 2013]. The feeder design has been used to study effects of different variables on recruitment, and flower patch experiments have been used to test work output, toxicant exposure, and subspecies [Von Frisch, 1967; Seeley et al., 1991; Chicas-Mosier et al, 2017]. Y-mazes have been used to study basic choice behaviors and aversion [Carcaud et al., 2009; Nouvian and Galizia, 2019]. Although these methods have been adapted to allow for MF data collection, their use has been standardized [Scheiner et al., 2013]. MF measurements were taken before each experiment was initiated to ensure that comparisons matched prior MF and behavioral literature in honey bees.

Honey bees and other insects can discriminate MFs for navigational purposes [Baker and Mather, 1982; Walker and Bitterman, 1985; Srygley et al., 2006]. Weak MF modifications in Anthropocene environments do not appear to have a negative impact on forage decision making but may offer orientation cues for bees to map their food sources. However, the present study is limited by the strength of the induced field and the similarities between control and treatment. Some bees may avoid higher MF when offered identical food sources in a free-flight environment, indicating that there may be some negative stimulus to increased field. This may be an evolved avoidance considering long-term field exposure may disrupt neural proteins [Martin et al., 1989].

Negative outcomes of MF on insects and other organisms have been reported in prior studies [Favre, 2011; Balmori, 2015; Lázaro et al., 2016]. The results of this study corroborate those findings; however, the present analysis may indicate that although honey bees may be deterred from exposure to MFs, the degree in which these organisms use and are negatively affected by the anomalies from Earth's MF may be over-reported. The results from the present studies show that with small deviations from Earth's MF, but greater than the 26 μT that honey bees can discriminate between, aversion may occur but that it is dependent on prior experience and is not universal [Walker and Bitterman, 1989b].

Honey bees and other insects are experiencing large-scale decline globally [Hallmann et al., 2017]. Understanding how these organisms are affected in changing environments induced by human activity is vital to limiting their decline. It is likely that although decline is attributed to three primary causes (habitat degradation, pesticide application, and pathogens), there are other contributing factors [Goulson et al., 2015]. This article has demonstrated that small fluctuations in MF from Earth can serve as temporary foraging cues for honey bees; however, it is dependent on prior experience and in some cases can be a deterrent.

Larger scale observational experiments with stronger MF near floral resources are necessary to determine how foraging decisions in the presence of different MFs may change, and how initial color preferences affect these decisions. Further study may be able to determine how MFs can be used to encourage or discourage bees to forage in a particular location. The use of MFs in this way may have future applications for deterring honey bees from pesticide-laden fields and attracting them to safer alternatives.

In addition to pesticide avoidance, isolation to a particular foraging location can be useful to beekeepers wanting to isolate their bees to forage on a specific plant species for production of specialty honeys such as Manuka or chestnut. Feeder location is typically established through the use of a scent; however, scent can create challenges in areas with other floral options, and bees can be harmed if the wrong scent it used [Chicas-Mosier et al., 2017]. The authors suggest that with appropriate follow-up study, beekeepers and scientists looking to isolate foraging to a particular location could use small MF fluctuations to attract/deter honey bees. Given that bees were reliably visiting the feeder in less than a day, MFs could be used rather than typical feeder methods to attract honey bees efficiently [Scheiner et al., 2013].

CONCLUSION

This article demonstrates that honey bee responses in free-flight and confined foraging choices in the presence of increasing MF are independent, indicating situational and experiential considerations for MF cues. Additionally, once a food source is located with field, recruitment continues to increase regardless of the continued presence of the MF, indicating that other stimuli serve as superior cues. Future beekeepers for science, hobbyists, or industry could use this information to direct bees to a specific foraging location.

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