

RESEARCH ARTICLE

Persistence, reticence and the management of multiple time memories by forager honey bees

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SUMMARY

Honey bee foragers form time memories that enable them to match their foraging activity to the time of day when a particular food source is most productive. Persistent foragers show food-anticipatory activity by making reconnaissance flights to the previously productive food source and may continue to inspect it for several days. In contrast, reticent foragers do not investigate the source but wait for confirmation from returning persistent foragers. To determine how persistent and reticent foragers might contribute to the colony's ability to rapidly reallocate foragers among sources, we trained foragers to collect sucrose from a feeder at a restricted time of day for several days and then observed their behavior for three consecutive days during which the feeder was empty. In two separate trials, video monitoring of the hive entrance during unrewarded test days in parallel with observing reconnaissance visits to the feeder revealed a high level of activity, in both persistent and reticent foragers, thought to be directed at other food sources. This 'extracurricular' activity showed a high degree of temporal overlap with reconnaissance visits to the feeder. In some cases, inspection flights to the unrewarded feeder were made within the same trip to an extracurricular source, indicating that honey bees have the ability to manage at least two different time memories despite coincidence with respect to time of day. The results have major implications for understanding flower fidelity throughout the day, flower constancy within individual foraging excursions, and the sophisticated cognitive management of spatiotemporal memories in honey bees.

Key words: *Apis mellifera*, foraging behavior, flower constancy, food-anticipatory activity, time memory.

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INTRODUCTION

A major determinant of animal behavior is control arising from the internal circadian clock. For example, in most animals, the daily rhythm of sleep and wakefulness is driven by the underlying circadian system. Other physiological and behavioral functions under circadian control include eating and drinking behavior, hormonal secretions, temperature regulation, locomotor activity, antipredator behavior, cognitive performance and reproductive behavior (Moore-Ede et al., 1982; Dunlap et al., 2004). To date, most of our understanding of the circadian control of behavior has come from experiments performed under laboratory conditions. Although it is assumed that possession of a circadian clock is adaptive, enabling the organism to schedule different behaviors at the most appropriate time of day, only a few studies (DeCoursey et al., 1997; DeCoursey et al., 2000) have been conducted on any animals under natural conditions to test this assumption.

Historically, perhaps the first convincing suggestion that circadian clock systems do, in fact, contribute adaptively significant function came from observations of the honey bee time memory (Moore-Ede et al., 1982). A honey bee forager will remember the time of day it exploited a profitable food source and will return to that source at approximately the same time on the following day (von Buttel-Reepen, 1900; Beling, 1929; Wahl, 1932; Wahl, 1933; Renner, 1955; Renner, 1957; Beier, 1968; Beier and Lindauer, 1970; Frisch and Aschoff, 1987; Moore and Rankin, 1983; Moore et al., 1989). This time memory enables bees to match their foraging efforts with nectar

secretion rhythms of flowers by cuing on either the time of highest nectar concentration (Butler, 1945; Corbet and Delfosse, 1984; Kleber, 1935) or highest total sugar (Giurfa and Núñez, 1992; Rabinowitch et al., 1993; Edge et al., 2012). This means that many foragers do not start their day as novices: the time memory eliminates the need to expend excess energy required to rediscover the same food sources each day.

Of course, numerous other inputs besides signals from the circadian clock influence behavior. A largely unexplored area of research concerns how control from the circadian clock is integrated with other influences both internal and external to the animal. Some progress has been made in the case of the honey bee. For example, young adult honey bees typically do not express daily rhythms in behavioral activity in the hive until about 2 weeks of age (Moore et al., 1998; Shemesh et al., 2007) as they begin to make the transition from in-hive duties (performed around-the-clock) to foraging behavior. The onset of behavioral rhythmicity may be delayed, accelerated and even reversed depending on colony demographic influences that also regulate division of labor (Bloch and Robinson, 2001; Bloch et al., 2001). In the case of another clock-driven function, the time memory, the first collecting visit of the day to a particular source appears to establish a temporal link between the circadian clock and the food source, whereas the number of successful collecting visits determines whether the forager will exhibit food-anticipatory flights and, if so, how many inspection flights it will make (Moore and Doherty, 2009). The number of

days a forager is rewarded at a particular food source influences the number of days it will continue to visit that source in the absence of further food rewards (Moore et al., 2011).

At any given time during the flowering season, there may be several different foraging groups within the hive, each group containing individuals that exploit a particular floral source (Wahl, 1933; Kleber, 1935; von Frisch, 1967; Visscher and Seeley, 1982). Recent work has shown that not all foragers trained to collect food from productive feeders during restricted times of the day make inspection flights. Rather, the trained foraging group comprises two classes: persistent bees and reticent bees. Persistent bees leave the hive to reconnoiter their food source in anticipation of the previously rewarding time of day, whereas reticent bees will not visit the source until they receive information from another bee confirming source availability (Moore et al., 2011). On each day following removal of the food source, both classes cluster on the dance floor in anticipation of the training time (B.N.V.N., A.E.W., C.N.H. and D.M., unpublished). Despite the ability of foragers to reactivate other foragers rapidly *via* the waggle dance (Körner, 1940; Seeley, 1995; von Frisch, 1967), a surprisingly high proportion of foragers are persistent – on average 40%, 60% or 80% of foragers with 1, 2 or 3 days of experience at a food source, respectively (Moore et al., 2011). A recent, agent-based foraging simulation model (incorporating time memory-driven anticipatory flights to investigate previously productive food sources) showed that such high levels of persistence are energetically favorable, allowing the foraging group to efficiently exploit food sources under a wide variety of ecological conditions (Van Nest and Moore, 2012).

Despite an abundance of information concerning factors that contribute to efficient foraging by honey bees (including dance recruitment, time memory, the existence of persistent and reticent foragers, etc.), many details of the day-to-day foraging enterprise remain unknown. This is especially true at the level of the individual forager. It is thought that, on a typical day, most members of a foraging group have adjusted the timing of their flight behavior to coincide with the time window during which food at the source is available or at its most profitable (Wahl, 1932; Kleber, 1935). Foragers gather at the dance floor as this optimal time of day approaches (Körner, 1940; von Frisch, 1940; Moore et al., 1989) and apparently station themselves there, either to launch reconnaissance flights to the source or to be alerted by waggle dances performed by a successful group member returning from the source. However, during the food source's time window, when foragers are positioned on the dance floor, they appear to be resistant to recruitment to alternative sources (Kleber, 1935; Moore et al., 1989; Seeley and Towne, 1992). Outside of this time window, foragers withdraw from the activity of the dance floor, apparently to rest (Körner, 1940; von Frisch, 1940; Moore et al., 1989) and are, therefore, unavailable for recruitment. According to von Frisch, most foragers adhere to this scenario (specializing on a single food source) but some may collect food from a second source, if its optimal time does not overlap with that of the first source (von Frisch, 1967). Forager honey bees certainly can be trained to collect sucrose from the same location at multiple times of day (Beling, 1929; Wahl, 1932; Koltermann, 1974) as well as from different locations, each productive at a different time of day (Wahl, 1932; Finke, 1958). However, the recently discovered genotypic differences in individual honey bee preferences for 'early' and 'late' foraging shift work (Kraus et al., 2011) would seem to contribute to flower fidelity by temporally restricting the forager's presence on the dance floor. In nature, food sources for honey bee colonies are ephemeral. Honey bee colonies are well adapted to this dynamic environment

by constantly exploiting profitable new sources and abandoning poor ones as conditions change (Butler, 1945; Visscher and Seeley, 1982). Such reallocation of foragers among food patches is a decentralized process, involving decisions by individual foragers to either abandon or continue foraging on a particular food patch. Each bee independently evaluates its source with respect to distance from the hive and nectar concentration and by interpreting feedback from the food receiver bees in the colony (Lindauer, 1948; Seeley, 1986; Seeley, 1989; Seeley et al., 1991; Seeley et al., 1996). The rates of abandonment of less-profitable sources by individual foragers are presumed to be crucial factors in the colony's foraging success (Seeley, 1995; Cox and Myerscough, 2003; Beekman et al., 2003). However, honey bees are slow to abandon experimental food sources that decline in sucrose concentration through the day (Beekman et al., 2003). It is important to note that foragers do not [as suggested by Tautz (Tautz, 2008)] immediately 'forget' a food source upon finding it empty, never to return to that original source again. Indeed, it is now well demonstrated that many forager bees do, in fact, retain a time-linked memory for the food source over the course of several unrewarded test days (Moore, 2001; Moore and Doherty, 2009; Moore et al., 2011). Furthermore, honey bees can retain the time memory over at least 1 day of inclement weather even when no reconnaissance flights are made; moreover, on unrewarded test days following a day of inclement weather, persistence levels are elevated above those expected for fair-weather days (Moore et al., 2011). Why should honey bees return to a food source that was empty on the previous day? Simply put, it is adaptive for foragers to assume that if nectar or pollen sources are depleted on one day, they may be replenished by the next.

At the level of the individual forager, we have very limited information concerning how foragers switch from one food source to another. How can the tendency of foragers to maintain visitation flights to defunct food sources for several days be reconciled with the ability of the colony to reallocate foragers quickly from poor quality sources to better ones? As a first step in addressing this question, we trained honey bees in two separate trials to forage from an artificial feeder at a fixed time of day for several days. Then, for three consecutive 'test days', during which no food was presented at the feeder, we monitored the departure and arrival times at the hive of all individually marked bees in the training group using cameras fixed to the hive entrance, with particular attention to potential differences in the behavior of persistent and reticent foragers. These data, in combination with parallel records of the arrival times of persistent foragers making reconnaissance flights to the feeder, provided insight into the total foraging activity of all bees in the foraging group.

The primary objective of our study was to examine, over three consecutive days, the flight behavior of individual foragers to a previously productive feeder as well as to alternative food sources. More specifically, we focused on the potential differences between persistent and reticent foragers with respect to the number of days elapsed before the forager would exhibit flight activity to food sources other than the empty experimental feeder. We proposed three hypotheses with respect to the relative roles of persistent and reticent foragers in switching from one food source to another. The first hypothesis (H1) simply asserts that both persistent and reticent foragers tend to remain faithful to the original food source and are slow to find alternative sources. The appropriate prediction for H1 is that neither persistent nor reticent bees will show flight activity to alternative food sources for several days. The second hypothesis (H2) proposes that persistent bees tend to remain faithful to the

original food source but reticent bees do not. A major prediction from this hypothesis is that reticent bees may be recruited to alternative sources on test day 1 after failing to be reactivated to the training station by persistent bees during the previously experienced training time and certainly by test day 2. Persistent bees, in contrast, would not be expected to be recruited to alternative food sources until they stopped making investigative flights to the training station. The third hypothesis (H3) asserts that neither persistent nor reticent bees remain faithful to the original food source. A major prediction from H3 is that both persistent and reticent foragers will be visiting alternative food sources by test day 1 (after determining that the feeder is not productive) or at least by test day 2. The results have implications for understanding how a circadian clock-controlled behavior (food-anticipatory activity) operates within a variable environment.

MATERIALS AND METHODS

Time training

Two trials of a field study were conducted at the former Marine Corps Armory property in Johnson City, TN, USA. This site consisted of wildflower meadows interspersed with clusters of trees. In both trials, forager honey bees (*Apis mellifera* L.) were time trained from a glass-sided observation colony housed in a protective shed. The colony occupied six standard beehive frames (containing about 12,000 bees) in trial 1 and a different colony occupied eight frames (about 16,000 bees) in trial 2. Bee entrances and exits from the colony occurred through the wall of the shed via a 15 cm passageway constructed from clear acrylic sheets. The first trial took place from 25 September to 5 October 2008 and the second trial from 19 to 26 August 2009.

In both trials, foragers were trained to an artificial feeder located 190 m from the hive using a technique described previously (von Frisch, 1967). The feeder, a sucrose-filled 96-well plate positioned over a filter paper disc (15 cm diameter), was placed initially at the hive entrance and then moved away from the colony in incremental steps following discovery by foragers. Once the feeder was several meters from the hive entrance, the filter paper disc was scented with four drops of essential oil of lilac. The training table then was moved gradually until the target distance was reached. All bees recruited during this orientation phase of the experiment were marked on the thorax with silver paint dots (Testors Enamel, The Testor Corporation, Rockford, IL, USA) and excluded from data analyses.

Following orientation, there were eight consecutive training days for trial 1 and five consecutive training days for trial 2, during which observations were made at the feeder for a restricted period (14:00 h to 15:35 h and 13:00 h to 15:00 h, respectively) of food availability and lilac scent presentation. Sucrose concentration was varied from 0.75 to 1.75 mol l⁻¹ in order to maintain a steady rate of recruitment. Foragers naturally recruited hive mates to the feeder via the waggle dance on training days, thus yielding different cohorts of uniquely identifiable bees with differing amounts of experience at the training station. Each new recruit was marked individually on its first arrival to the training station using combinations of colored paint dots (Testors Enamel) applied to the thorax and abdomen (von Frisch, 1967). These color codes allowed observers to record the individual forager's identity and timing of all subsequent rewarded visits. After the time window of sucrose availability ended on each training day, the training table and feeder were thoroughly doused with water, and the filter paper disc was exchanged for a new (unscented) one. For the purposes of the present study, bees that skipped one or more days of training were excluded from all analyses.

Training station observations

Following the training period of each trial, three test days were conducted, during which the feeder remained in place but was not supplied with food and scent. Observers recorded the time and identification of all arrivals by marked bees from 10:00 h to 19:00 h for trial 1 and from 09:00 h to 19:00 h for trial 2. Following the testing period, foragers were classified as persistent or reticent for each day based on training station arrivals. If a forager was seen making a minimum of one reconnaissance flight to the unrewarded training station on a given day, then that forager was classified as persistent for that test day; likewise, a forager that made no reconnaissance flights to the training station on a given day was classified as reticent for that day.

Hive entrance observations

In trial 1, a hive landing platform was constructed to attempt to funnel all entering and exiting bees upright through a single location that could be video recorded from above. The use of a video camera (Sony Handycam DCR-SR65; 30 frames s⁻¹) to monitor the traffic of all marked bees was necessary to determine whether the experimental foragers were exploiting other (natural) food sources. An entryway was constructed from transparent acrylic sheets mounted over a wooden block (20×20×8 cm) that was attached to the flat upper surface of the landing platform proper. To traverse this passageway, bees had to walk up the sheer faces of the block, thus requiring them to pass over the top surface of the block in the upright position. In this orientation, the foragers' paint codes were visible to the camera mounted directly above and focused on the top surface of the block. The top sheet of acrylic was mounted one bee height above the block to prevent bees from climbing over one another and obscuring the identities of the bees below. Unfortunately, a small percentage of foragers (~10%) still were able to cross the platform upside-down. To address this, in trial 2, a new entryway to the hive was constructed with wooden sides and glass slats for the top and bottom to allow recording from both the top and bottom orientations. The entryway height was restricted as before so that bees would be unable to climb over one another. A video frame capture from trial 2 (Fig. 1) illustrates a portion of the entryway with individually marked forager WRB (white-red-blue) and several unmarked foragers.

Following video analysis, the timings of forager entries and exits were compared with training station arrivals to determine which flights were 'extracurricular' (i.e. to sources other than the feeder). Although bees do leave the hive for reasons other than to forage (including the orientation flights of naive bees), such excursions typically are shorter than 5 min (Dukas and Visscher, 1994). Thus, any flight 5 min or greater in duration and not accompanied by a visit to the training station was determined to be a flight to an extracurricular source. Special care was taken to ensure that, for persistent foragers, a flight to the training station would not be misinterpreted as an extracurricular flight: entries and exits from the hive were compared with the timing of arrivals at the training station. Persistent bees' inspections of the feeder on test days rarely lasted more than a few minutes; therefore, pure reconnaissance flights typically lasted only a few minutes. Any flight at least 30 min in duration that was accompanied by a training station visit was assumed to be both reconnaissance and extracurricular.

Census

During the test days of trial 1 and trial 2, hourly hive scans were made from 10:00 h to 19:00 h and from 09:00 h to 19:00 h, respectively, noting the identities and locations (from a grid pattern



Fig. 1. Frame capture showing the entryway (from a camera mounted above it) to the observation hive from trial 2. Foragers were required to transit between two glass panes in order to enter or exit the hive. Seen in this frame is one individually marked forager (lower left, with white, red and blue paint dots; WRB) and several unmarked foragers. The hive entrance is at the top side of the entryway. A horizontal blue strip is positioned on the upper surface of the glass. A second video camera, pointing upwards, is positioned below the glass to record the identities of bees traversing the entryway upside-down.

drawn on the glass sides of the observation hive) of all marked bees within the colony. A hive census was performed for each test day by compiling data from these hive scans, training station observations and videos. If a time-trained (and, therefore, individually marked) forager was observed in any one of these locations on a given test day, it was assumed to be alive through the entirety of that test day as well as on any preceding test days. All analyses in this study were based on data collected from foragers confirmed to be alive on each test day.

Statistical analyses

In cases of relatively small sample sizes, data from training cohorts were pooled. For example, in trial 1, data pertaining to foragers with 6 or 8 days of experience were combined as were the data from foragers with 3 or 4 and 1 or 2 days of experience at the training station. In trial 1, there were no foragers with 7 or 5 days of experience because weather conditions restricted recruitment of new foragers on those particular training days. In trial 2, only data from foragers with 4 or 5 days of experience at the food source were pooled.

The number of extracurricular flights per bee were compared among training cohorts on each test day as well as among test days for each cohort using the Kruskal–Wallis test. The Mann–Whitney *U*-test was used to compare the mean number of extracurricular flights per day per bee as well as extracurricular flight durations between persistent and reticent foragers on each test day.

RESULTS

Extracurricular flights

In addition to reconnaissance flights to the training station, other flights to and from the colony were monitored by one (trial 1) or two (trial 2) video cameras positioned at the hive entrance. Flights of 5 min or more in duration were termed ‘extracurricular’ flights if they did not correspond to a reconnaissance flight to the training station. By definition, all flights of at least 5 min duration made by reticent bees (i.e. those foragers not visiting the training station on any particular test day) were extracurricular. As shown in Table 1, the majority of time-trained foragers (both persistent and reticent) made extracurricular flights on each test day in both trials. Only 10% of all time-trained foragers in trial 1 and 18% in trial 2 failed to make flights to another source on at least one occasion throughout the three consecutive test days.

Of all the foraging flights made by the time-trained bees in this study, a surprisingly large proportion was devoted to food sources other than the training station (Fig. 2). On each test day, in both trials, there was substantial temporal overlap between these extracurricular flights and those flights directed to the training station (Fig. 2). In both trials, the proportion of total flights devoted to reconnaissance of the training station diminished over the three consecutive test days (Fig. 2). In contrast, the mean number of extracurricular flights per bee showed no significant changes within any training cohort over the three test days (Fig. 3; Kruskal–Wallis, $P > 0.05$ in all cases). There also were no significant differences in mean number of extracurricular flights per bee among training cohorts during any of the test days in either trial (Fig. 3; Kruskal–Wallis, $P > 0.05$ in all cases).

With the exception of activity directed toward the training station, other aspects of foraging behavior between persistent and reticent bees were remarkably similar. For example, there were no significant differences in mean number of extracurricular flights made per bee between persistent and reticent foragers on any test day in either trial (Fig. 4; Mann–Whitney *U*-test, $P > 0.05$ in all cases). Similarities in extracurricular foraging behavior between persistent and reticent bees also extended to the duration of extracurricular flights on each test day (Fig. 5): with one exception (trial 1, test day 2), there were no significant differences (Mann–Whitney *U*-test, $P > 0.05$). Additionally, as shown in Fig. 6 (all three test days for both trials), persistent and reticent bees apparently did not partition their extracurricular flights during different phases of the day. The distributions of extracurricular flights with respect to time of day were closely matched; about 81% of all sampled hours during both trials contained extracurricular flights from both persistent and reticent foragers.

Multiple time memory expression

The finding of considerable temporal overlap between reconnaissance visits to the training station and extracurricular flights (Fig. 2) raises an interesting question concerning the planning of

Table 1. Proportions of persistent and reticent foragers on each test day that made extracurricular flights

	Test day 1		Test day 2		Test day 3	
	Persistent	Reticent	Persistent	Reticent	Persistent	Reticent
Trial 1	0.62 <i>N</i> =82	0.64 <i>N</i> =14	0.78 <i>N</i> =45	0.60 <i>N</i> =35	0.75 <i>N</i> =12	0.65 <i>N</i> =49
Trial 2	0.71 <i>N</i> =145	0.56 <i>N</i> =18	0.71 <i>N</i> =70	0.65 <i>N</i> =83	0.68 <i>N</i> =22	0.76 <i>N</i> =117

N, sample size.

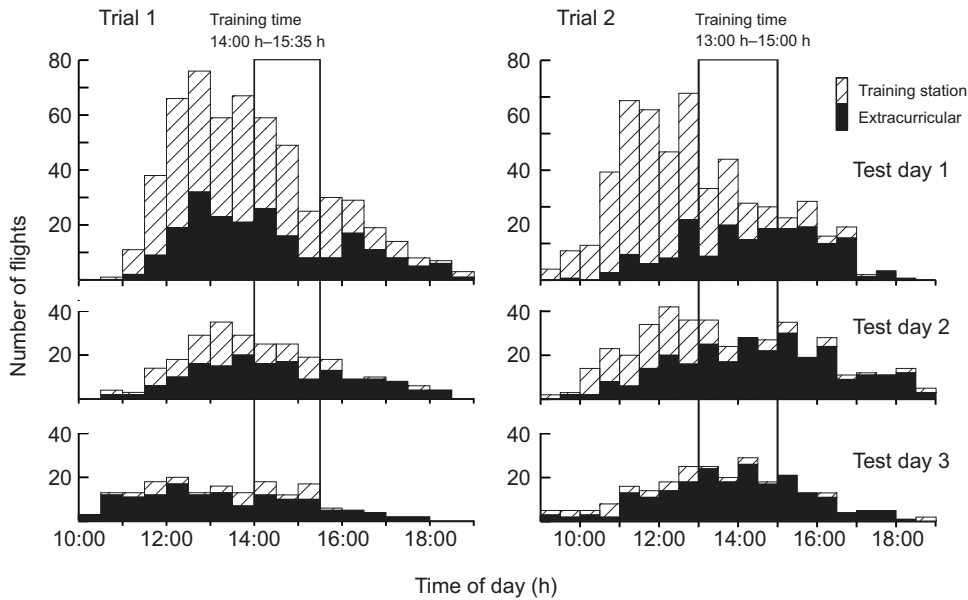


Fig. 2. Total number of extracurricular flights made by all trained foragers stacked with arrivals at the training station by persistent foragers with respect to time of day over all three test days for both trials. Training times are indicated by vertical rectangles. Extracurricular and training station flights overlap nearly completely over the course of the day, suggesting that the two occur concurrently rather than at strictly separate times on test days.

schedules by individual foragers. Are reconnaissance and extracurricular flights always executed as separate foraging excursions or can they be combined during the same trip? The answer is quite clear (Table 2): depending upon the test day, between 11.4% and 22.2% of persistent foragers performing extracurricular flights in trial 1 and between 26% and 40% in trial 2 showed reconnaissance flights overlapping in time with visits to alternative food sources. Using test day 1 of trial 2 as an example, 30 of the 103 persistent foragers exhibiting extracurricular flights incorporated at least one reconnaissance flight within an extracurricular excursion, thereby expressing behavior driven by two different spatiotemporal memories during a single foraging trip. Most of these bees also made pure reconnaissance flights (i.e. flights to the training station that did not overlap with extracurricular sorties). The behavioral profiles of all 30 (which, notably, include members from all of the training cohorts) are illustrated in Fig. 7. In many cases, reconnaissance visits were scheduled at the very beginning of an extracurricular bout (e.g. forager RBY), occasionally at the very end (e.g. forager YWG) or at both the beginning and the end (e.g. forager WWW). Sometimes, reconnaissance visits

occurred more centrally within the extracurricular bout (e.g. forager WGR). In trial 1, for all cases of overlap between training station reconnaissance and extracurricular flights, 48.1% of the reconnaissance visits occurred early (within 5 min of extracurricular bout initiation), 14.8% occurred late (within 5 min of bout termination) and 37.1% occurred at an intermediate time. Similarly, for all cases of overlap in trial 2, 52.8% of the reconnaissance flights occurred early, 7.5% occurred late and 39.6% occurred at an intermediate time within extracurricular bouts.

Persistent foragers exhibited a remarkable degree of variation in the patterning of reconnaissance and extracurricular flights. Some persistent bees failed to show overlap between extracurricular flights and training station visits over all three test days (Fig. 8A). Some foragers showed temporal separation between extracurricular and reconnaissance flights on test day 1 but overlap between the two on test day 2 (Fig. 8B). Others showed overlap on both test day 1 and 2 (Fig. 8C). Both foragers depicted in Fig. 8B,C made reconnaissance flights early during extracurricular foraging bouts and stopped making reconnaissance flights on test day 3. Finally, some foragers scheduled reconnaissance visits repeatedly throughout

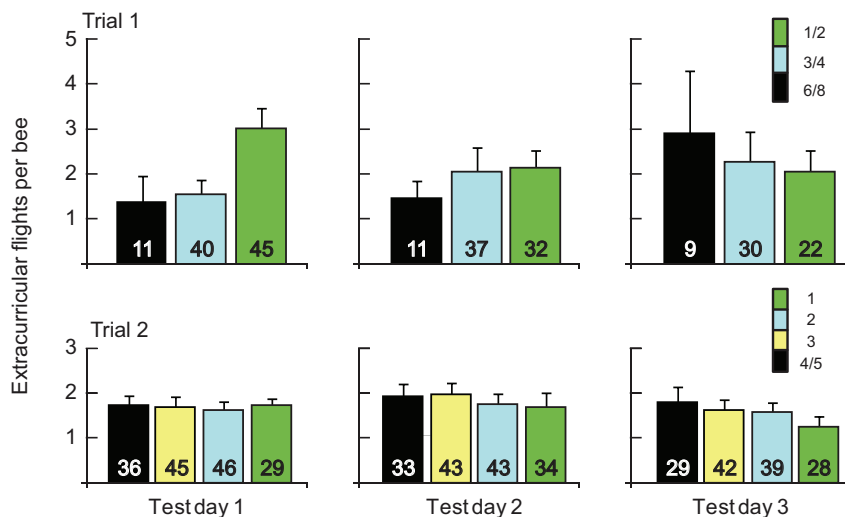


Fig. 3. Mean (and s.e.m.) number of extracurricular flights per bee by training cohort for both trials. Color codes indicate the number days of experience at the training station by cohort. No significant differences were found among any set of cohorts on any test day (Kruskal–Wallis, $P > 0.05$), indicating that the amount of experience a forager received at the training station does not impact the intensity of its activity at other sources. Numbers within bars indicate sample size.

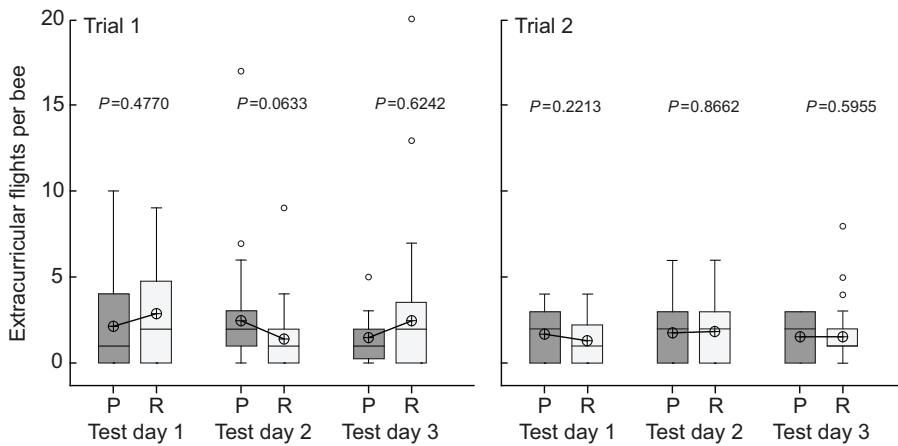


Fig. 4. Number of extracurricular flights per bee by persistent (P) and reticent (R) foragers, compared on each test day for both trials. Box plots depict medians, 25% and 75% quartiles (horizontal lines), lowest and highest values within 1.5 times the interquartile range (whiskers), outliers (circles), and means (crosshairs). Significance values are indicated for each pair: no significant differences were found on any test day (Mann–Whitney *U*-test, $P > 0.05$). The results show that persistent and reticent bees perform a similar number of flights each day directed to sources other than the experimental training station.

extracurricular foraging bouts (Fig. 8D). On two occasions, the extracurricular (natural) food source was positively identified: two persistent foragers from trial 2 on test day 3 were observed foraging on flowers of Spotted Joe-Pye Weed (*Eupatoriadelphus maculatus* L.) in the immediate vicinity of the training station. One of these foragers interrupted its work on Joe-Pye flowers to make two inspections of the training station (not shown) and the other repeatedly alternated between the natural flowers and the unrewarded training station during two separate foraging bouts (Fig. 8D).

DISCUSSION

In both trials conducted for this study, many of the honey bee foragers that collected sucrose during a restricted feeding time for one or more training days returned to the feeder on subsequent unrewarded test days. Those foragers investigating the feeder on any particular test day were characterized as ‘persistent’ foragers for that particular day. Most of the reconnaissance flights performed by persistent foragers (Fig. 2) occurred early with respect to the restricted training time, in agreement with many previous studies concerned with the honey bee time memory (Beling, 1929; Wahl, 1932; Wahl, 1933; Renner, 1957; Beier, 1968; Beier and Lindauer, 1970; Moore and Rankin, 1983; Moore et al., 1989; Moore and Doherty, 2009). Those time-trained foragers not returning to the training station on any particular test day were classified as ‘reticent’ foragers with respect to that food source on that test day.

We compared the flight behavior of persistent and reticent foragers to extracurricular sources over three test days during which

the experimental feeder was not provisioned with food. These experiments were designed to assess how the two behavioral subtypes might contribute to the colony’s ability to rapidly reallocate foragers from poor or defunct sources to productive ones. Previous studies noted that honey bee colonies can shift their foraging force among food sources from one day to the next or even within the same day (Butler, 1945; Visscher and Seeley, 1982; Seeley et al., 1991; Granovskiy et al., 2012). However, the contributions of individual foragers (including those currently or recently engaged with other sources) to such rapid reallocations are largely undocumented. If persistent or reticent foragers can contribute to this rapid reallocation, then it would be expected that at least some of them might start visiting alternative sources after they determine that the training feeder is not productive on test day 1 and certainly by test day 2. Three hypotheses were examined. The first hypothesis (H1: both persistent and reticent foragers are not involved in promptly finding alternative food sources) was not supported: the majority of both persistent and reticent foragers made extracurricular flights (i.e. not directed to the training station) on all three test days (Table 1). As a large proportion of both persistent and reticent foragers were visiting alternative sources on all three test days, the second hypothesis (H2: reticent but not persistent foragers are involved in promptly finding alternative sources) also was rejected. H2 proposed that there were behavioral differences between the foraging subtypes with respect to extracurricular food sources; however, at least three lines of evidence indicate otherwise. First, there were no significant differences between persistent and reticent foragers with respect to the number of extracurricular flights per

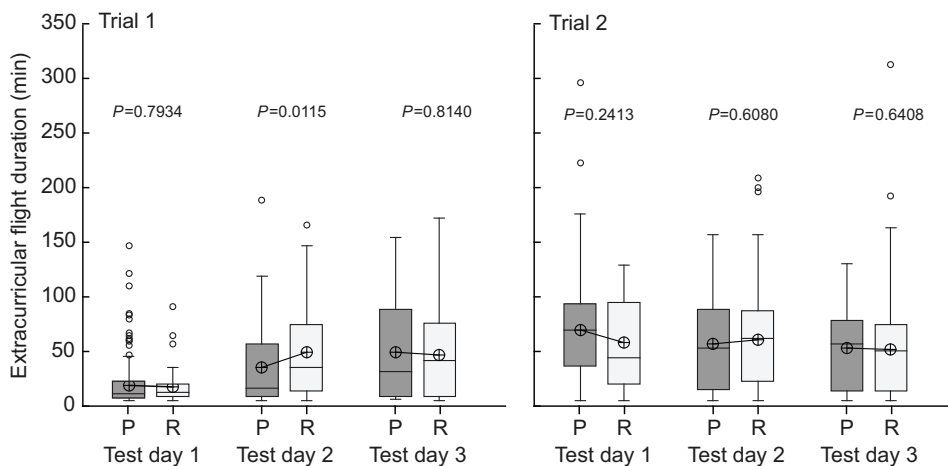


Fig. 5. Extracurricular flight duration by persistent (P) and reticent (R) foragers, compared on each test day for both trials. Significance values are indicated for each pair (Mann–Whitney *U*-tests). Only one pair showed a significant difference (trial 1, test day 2). Box plot characteristics as in Fig. 3. The results illustrate a high level of similarity between persistent and reticent bees in the time invested in foraging behavior to sources other than the experimental training station.

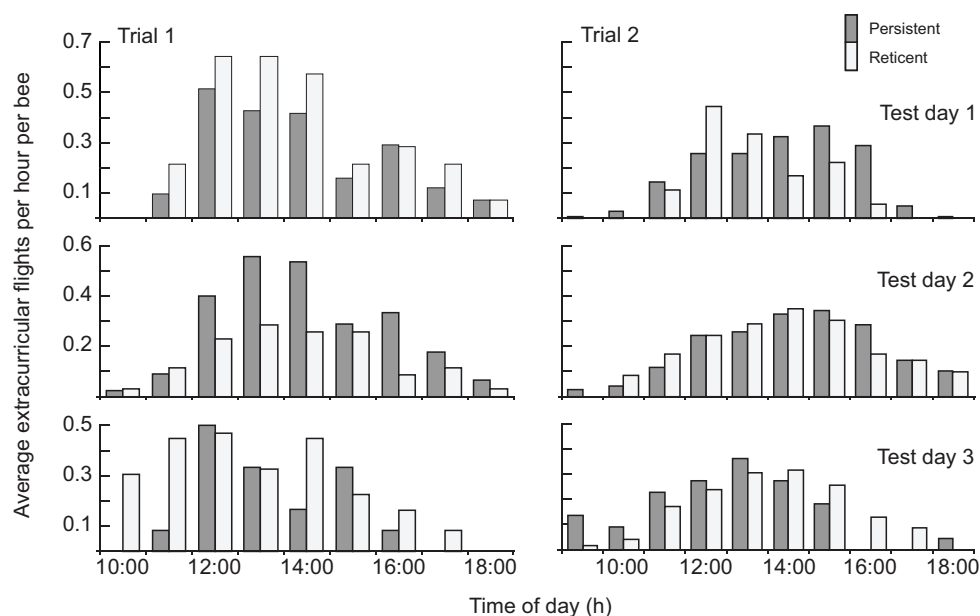


Fig. 6. Average number of extracurricular flights made per hour per bee by persistent and reticent foragers, compared on each test day for both trials. Data were compiled in 1 h bins for all of the observed hours. The results indicate a high level of similarity in the scheduling of flights to sources other than the experimental training station.

bee on any of the three test days in either trial (Fig. 4); second, with one exception (test day 2, trial 1), there were no significant differences in extracurricular flight duration (Fig. 5); and third, there was a great deal of overlap in the timing of their extracurricular flights throughout the day (Fig. 6). Furthermore, because persistent bees with more experience at the training station take more days to abandon the defunct training station than those with less experience (Moore et al., 2011), it might be expected that bees with greater experience at the training station would make fewer flights to alternative sources. However, there were no significant differences in the number of extracurricular flights per bee among any of the training cohorts on any test day in either trial (Fig. 3). These results also suggest that the performance of reconnaissance flights to the training station has no material effect on the performance of extracurricular flights. The fact that both persistent and reticent foragers were making extracurricular flights on all three test days is most consistent with the third hypothesis (H3: both persistent and reticent foragers are involved expeditiously in exploiting alternative food sources). Contrary to the predictions of any of the hypotheses, however, is the finding that both persistent and reticent bees already were foraging on extracurricular sources before the feeder's training time on test day 1 in both trials (Figs 2, 6), indicating that the bees were visiting alternative food sources before they discovered that the training station was not providing food on that day.

As shown in Table 1, many persistent and reticent foragers were performing extracurricular flights on test day 1. The most likely

scenario is that these foragers were working other food sources during training days. The alternative, that they switched to the experimental feeder during training and then switched back to the natural source after training, is unlikely because they were already visiting the extracurricular sources before the feeder's time window (i.e. the training time) on test day 1 (Figs 2, 6), thus precluding their ability to determine whether the feeder was exploitable. These results are contrary to the long-held assumption that most honey bee foragers specialize on a single species each day and that, once the forager has collected food during that source's time window of availability, the forager rests in the hive until the appropriate time on the following day (Körner, 1940; von Frisch, 1940; von Frisch, 1967; Moore et al., 1989; Moore, 2001). According to von Frisch, some foragers may find a second food source, but only during the hours when the first one is not yielding food (von Frisch, 1967). In fact, bees can be trained to collect sucrose from as many as four different places at four different times of day (Finke, 1958). Our data, in contrast, indicate that honey bee foragers manage to attend to two different sources that have overlapping time windows (Figs 2, 6, 7). The conduct of scheduling visits to two different food sources at the same time of day is difficult to reconcile with previous studies showing that foragers do not respond to recruitment dances advertising alternative food sources during the first source's time window of availability (Kleber, 1935; Moore et al., 1989). If foragers were already involved with a profitable food source at a particular time of day, then why would they be susceptible to recruitment to

Table 2. Temporal overlap of extracurricular and reconnaissance flights in persistent foragers

	Test day 1		Test day 2		Test day 3	
	Total	Overlap	Total	Overlap	Total	Overlap
Trial 1	51	7	35	4	9	2
Trial 2	103	30	50	13	15	6

The table shows the total number of persistent foragers on each test day that perform both reconnaissance and extracurricular flights and the number of these foragers that combine these visits within a single excursion (i.e. visits to the unrewarded training station overlap in time with visits to the alternative food source); both trials are shown.

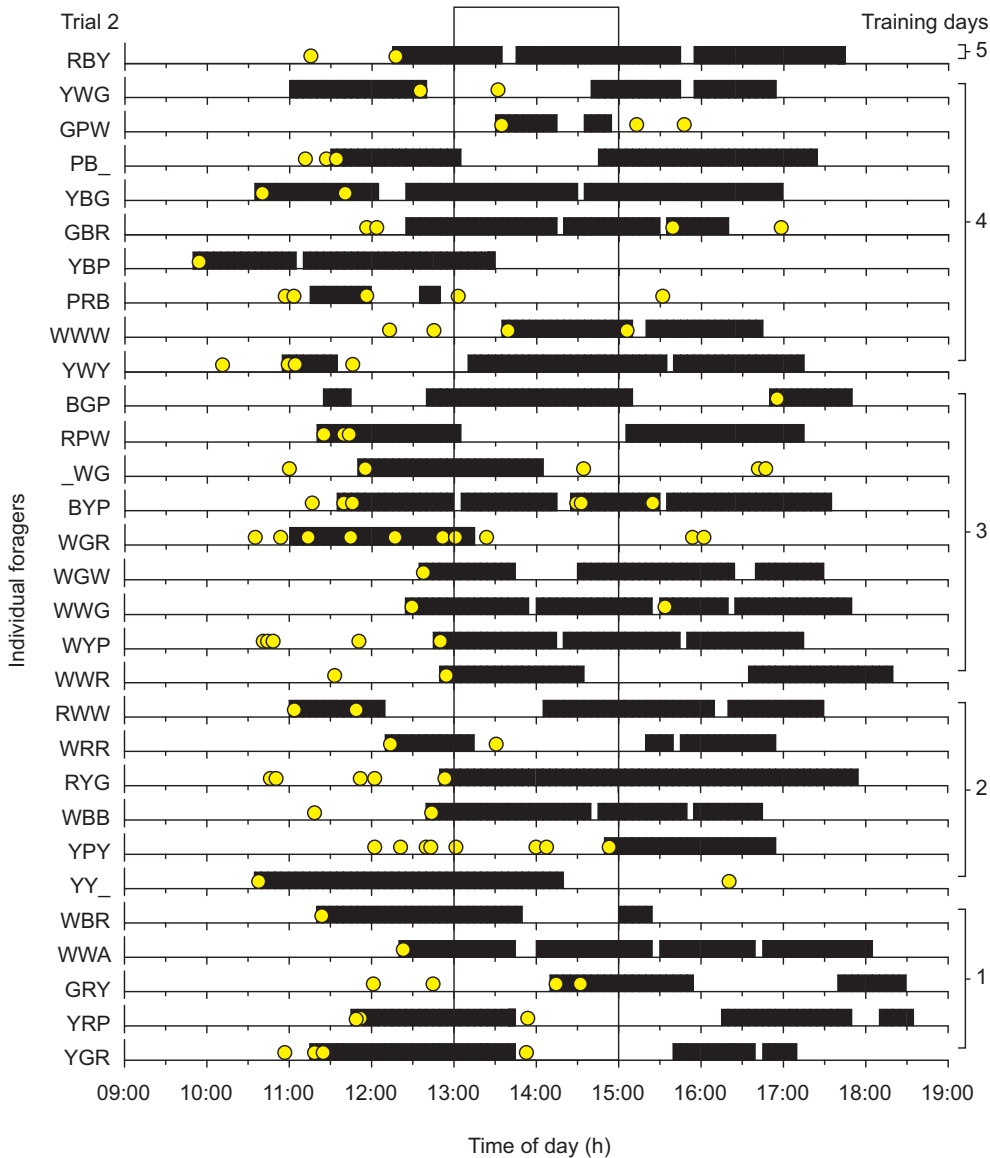


Fig. 7. Time lines showing performance of both reconnaissance visits to the experimental training station (yellow circles) and flight excursions to extracurricular sources (horizontal black bars) by individually marked foragers. Overlap between the two sources indicates that visits to the feeder and to an extracurricular source were made during the same foraging excursion. Data were taken from all 30 foragers showing such overlap on test day 1 of trial 2. Individual bee identities (from paint codes) are indicated to the left of each time line; the number of training days received by each forager is shown on the right. The vertical rectangle indicates when food was presented during training.

yet another source (or begin scouting for other sources) at the same time of day? Given that two different food sources overlap in time, a potential solution to this problem might be found in the behavioral pattern by which the honey bee time memory is expressed. For example, one food source may be productive from 10:00h to 15:00h and a second from 13:00h to 18:00h, providing a 2h period of overlap. It is important to note that foragers anticipate the time of day at which a food source is available by making reconnaissance flights that are earlier than the first successful flight of the previous day (Moore and Doherty, 2009). If the second source is discovered relatively late (say 17:30h), then, on the following day, the forager will schedule its visits to this source somewhat earlier than 17:30h. Over the course of several days, the visits to the second source may encroach into the first source’s productive time window.

Our results indicate that honey bee foraging behavior is much more versatile than previously reported. In a study using individually marked bees (Seeley et al., 1991), it was noted that only 2 of 117 bees attending to two simultaneous feeders switched from the relatively poor feeder to the richer one: reallocation was achieved largely by changes in the recruitment of naive foragers to the two feeders. Similar results were obtained in a more recent study

(Granovskiy et al., 2012) using foragers marked with feeder-specific colors. The increase in the number of foragers to the most profitable feeder among three different feeders (as the highest quality feeder was switched from one to the next throughout the day) was accomplished primarily by an increase in new recruits and visits from foragers previously trained to that feeder. Very few bees previously trained to one feeder switched to feed at a higher quality one. In contrast, in both trials of the present study, the majority of foragers with experience at the artificial feeder apparently also foraged on alternative food sources (Table 1). Because both reticent and persistent foragers have knowledge of at least two sources simultaneously, shifting from one source to another (depending on their relative profitabilities) may be accomplished with ease and with little or no delay. Persistent bees have the ability to shift between sources immediately: if a persistent forager finds the source productive during one of its reconnaissance flights, it may choose to work that particular source until it declines. Reticent bees with prior experience with this particular source may be reactivated to it *via* waggle dances from these persistent bees.

One particularly intriguing set of future studies will be determining exactly when foragers learn of the extracurricular

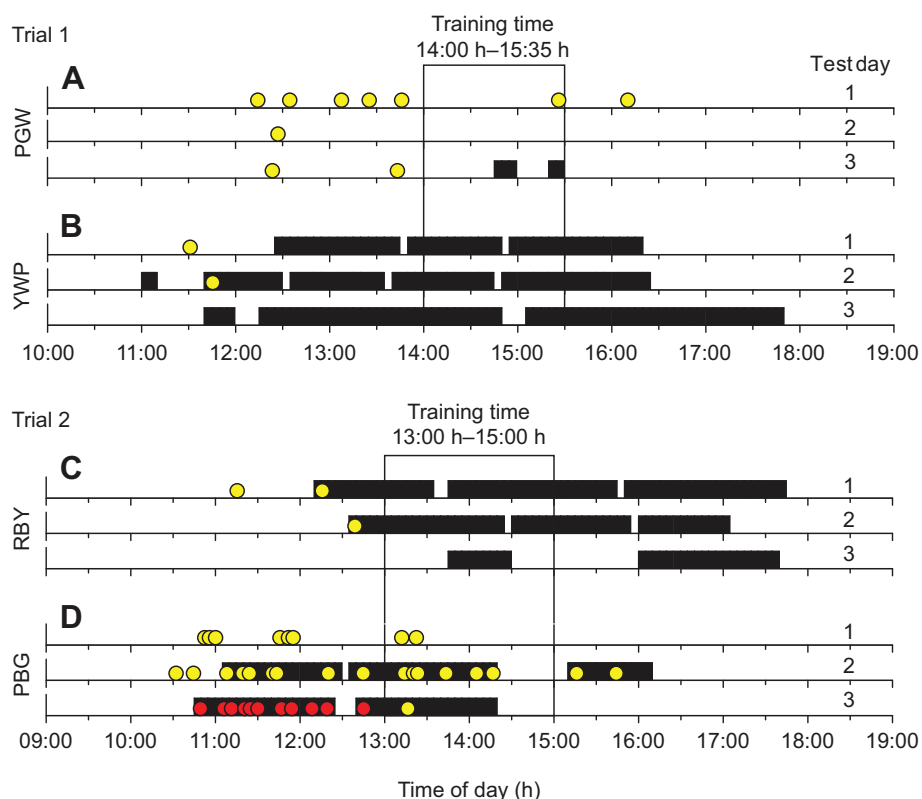


Fig. 8. Time lines showing diversity of foraging behavior by individually marked bees over three consecutive, unrewarded test days (no food presented at the experimental feeder); reconnaissance visits to the experimental training station (yellow circles) and flight excursions to extracurricular sources (horizontal black bars) are illustrated. Vertical rectangles indicate when food was presented during training. Foragers from trial 1 (A,B) and trial 2 (C,D) are represented. Red circles (D) indicate reconnaissance flights that overlap with an identified extracurricular food source. See Results for details.

sources. Previous work (Seeley and Towne, 1992; Seeley, 1995) has shown that foragers show a consistent pattern of behavior following discontinuation of their food source. Bees were monitored in an observation hive during 1 day immediately following 2 days of training at a feeder that provided sucrose from 08:00 h to 17:00 h, a situation similar to test day 1 in the present study (with the exception that our feeder provided food for a relatively short period of time). Typically, foragers did not follow any recruitment dances for several hours but made investigative flights to the feeder. This was followed by a period of ‘cursory dance following’ in which the bee briefly would follow several dancers but would not respond to them. This period also would occupy several hours. Finally, the bee would exhibit ‘thorough dance following’, fixating on a single dancer and would leave the hive in search of the advertised food source. Most of these newly recruited foragers were not successful in locating the source on the first try, but required several dance-guided searches (Seeley and Towne, 1992). These unsuccessful ventures out of the hive averaged about 15 min in duration. The aspect of this scenario most relevant to the present study is that several hours usually transpire between the time when a forager discovers that a food source is not productive on a particular day and when it commences activity directed at finding alternative sources. In both trials of the present study, both persistent and reticent bees were making extracurricular flights well in advance of the training time (Figs 2, 6) and, for a number of persistent foragers, these flights coincided with investigative visits to the training station (Figs 7, 8). Thus, it appears that most of our persistent foragers making both reconnaissance and extracurricular flights already had knowledge of alternative food sources on test day 1 and returned to them before they could determine whether the feeder was productive on that particular day (i.e. they were making extracurricular flights before the onset of the training time and before they discovered that the feeder was empty). An intriguing possibility is that honey bee

foragers may have different strategies in different environments. For example, the differences between our results and the previous work (Seeley and Towne, 1992) possibly could be attributed to differences in the duration of food availability: 1.6–2.0 h for our study compared with 9 h for theirs. Perhaps short time windows of food availability constitute incentives for foragers to seek more foraging opportunities. Other possible explanations are be differences in the number of available alternative food sources (thereby yielding different numbers of foraging groups within the hive) or differences in the manner in which flowers replenish their nectar after it has been collected. These hypotheses can be tested in further field experiments.

Honey bee foragers clearly have the cognitive ability to manage complex information sets and to recall them according to time of day. For example, recent work has shown that honey bee foragers apparently can ‘plan their activities in both time and space’ – they can choose the correct visual pattern within the proper context (associated with either the food source or the hive), and these combinations of factors can change according to time of day (Zhang et al., 2006). Honey bees link a number of cues (circadian time, location, color and visual pattern) into an integrated whole, forming a so-called ‘circadian timed episodic-like memory’ (Pahl et al., 2007). Most recently, Najera and colleagues showed that forager honey bees can switch from one auxiliary feeder to another in an array of feeders depending on the presence or absence of food at a primary feeder and the learned time of day of food availability at three auxiliary feeders, each feeder offering food at a different time of day (Najera et al., 2012). Earlier, Bogdany demonstrated that color, scent and time could be connected, thus forming a learning ‘Gestalt’ (Bogdany, 1978). These studies showed that bees can learn and remember to associate different constellations of cues with different times of day, equivalent to scheduling different appointments at different times. Our work reveals yet another level

of complexity. In both trials of this study, there was substantial temporal overlap between extracurricular flights and reconnaissance flights to the training station on test days (Figs 2, 6). As detailed in Table 1, the majority of both persistent and reticent foragers made extracurricular flights on all test days. Evidence from individual foragers (Figs 7, 8) indicates that reconnaissance visits to the training station often were conducted within longer (extracurricular) flight excursions. In both trials, there were several late-season flowers in bloom during the test days, including tall ironweed (*Vernonia altissima*), New England asters (*Aster novae-angliae*), various goldenrods (*Solidago* spp.) and spotted Joe-Pye weed (*E. maculatus*), thereby providing ample opportunities for extracurricular food collection. Most often, the reconnaissance flights occurred at the beginning of the extracurricular bout and, less often, at the end of the trip or at an intermediate time. Because our field experiments were designed primarily to monitor visits to the training station as well as traffic at the hive entrance, we do not have direct observations of foraging behavior on extracurricular sources by most of our time-trained individuals. However, there were two fortunate cases in which an individually marked forager was observed alternately working a natural flower (Joe-Pye weed) and interrupting its labor to check the training station (Fig. 8D). Also, it is probable that at least some of the extracurricular flights were scouting flights. We also have observed during other field experiments using individually marked foragers (D.M. and B.N.V.N., unpublished) that some persistent bees visit our empty training stations on test days bearing full pollen loads. These results indicate that at least some honey bee foragers possess the ability to monitor one food source while working another. In other words, it is not necessary to have different complex memories parceled into separate time windows (i.e. different phases of the circadian clock). The forager honey bee can schedule two appointments at the same time: it can execute an ongoing bout of foraging activity directed at collecting food from a particular resource (or, alternatively, scouting for new resources) and briefly include within this larger activity one or more investigations of the recently productive food source. Upon finding the resource unproductive, it can resume its primary activity.

Furthermore, the finding that a substantial number of foragers in our study (Table 2, Figs 7, 8) visited the feeder and a natural source during the same foraging flight is contrary to the assumption that honey bees are flower constant (Butler et al., 1943; Butler, 1945; Free, 1963; Waser, 1986; Hill et al., 1997; Chittka et al., 1999). ‘Flower constancy’ is a behavior in which each forager specializes on one particular floral species during any given foraging trip and will bypass other, often equally profitable, food sources while searching for the target species. Presumably, commitment to a single species is adaptive because it eliminates the period of time needed to learn flower handling and nectar extraction when switching to another species (Waser, 1986; Chittka et al., 1999). Honey bees show greater levels of flower constancy in artificial flower arrays when nectar volume, concentration or the number of nectar rewards is increased, providing that these factors are ecologically realistic (Grüter et al., 2011). Another possible explanation for flower constancy is a limited cognitive capacity (Dukas and Real, 1993). This hypothesis gains support from the finding that, in butterflies, the performance of one activity interferes with memory about other activities (Lewis, 1986). In honey bees, color and scent may be linked together with time of day to form an apparent learning Gestalt; however, varying any one of these cues (presumably analogous to encountering a new species of blossom) reduces the orientation to the other two cues (Bogdany, 1978). However, exceptions to flower

constancy have been reported previously but have been widely overlooked. Ribbands clearly showed examples of a small number of honey bees visiting multiple flower species within single foraging flights (Ribbonands, 1949). For example, one bee routinely alternated between meadowfoam (*Limnanthes*) and poppy (*Eschscholzia*) species. The two species were adjacent and intermingled. The meadowfoam offered pollen only, whereas the poppies offered both pollen and nectar. It was assumed that the bee switched from one species to the other according to the relative attractiveness of the flowers throughout the day, based on changes in the amount of time required for the bee to accumulate full loads and return to the hive. How prevalent in nature are honey bees that visit multiple flowers per day or multiple sources within the same foraging trip? Do these behaviors occur only under certain conditions (e.g. relative dearth or abundance of food sources, different seasons, existence of an especially profitable food source, etc.) or are they universal? These questions can be answered only with extensive field studies.

It is well established that forager honey bees are capable of organizing complex sets of information and associating each set with a different time of day (Zhang et al., 2006; Pahl et al., 2007). In other words, foragers ‘plan’ their day according to a sophisticated appointment book. Such appointments form the essence of the honey bee time sense: foragers associate the presence of food with both location and time of day and then schedule anticipatory flights to the appropriate location and time on the following day (reviewed in Moore, 2001). Recently, such spatiotemporal memories have been shown to correspond with distinct neurogenomic signatures, as revealed by microarray analyses on time-trained foragers (Naeger et al., 2011). The results of the present study indicate that a forager’s appointments do not have to occupy different time slots (i.e. circadian phases) but may overlap with one another (as observed earlier by Ribbands, 1949). Recall of one spatiotemporal memory within the temporal confines of another reveals not only a surprising level of cognitive complexity but also an extremely versatile foraging strategy. The dynamics of the interactions among spatiotemporal memories as well as the adaptive significance of such complex behavioral programs have yet to be explored.

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