The Effects of Reward Frequency Variation on the Foraging Behavior of the European Honeybee, *Apis mellifera* (Hymenoptera: Apidae) in Ankara, Turkey

Daniel S. Song

**Abstract** European honeybee, *Apis mellifera* Linnaeus 1758, foraging behavior has been extensively studied but still some phenomena, such as color constancy, are not well understood. This study compared the foraging behavior of three subspecies, *Apis mellifera carnica*, *Apis mellifera syriaca*, and *Apis mellifera caucasica*, in free flying experiments at sites where specialized predators were absent. Each experiment consisted of a control and three treatments where reward frequency was varied among yellow, blue, and white artificial flowers. The sequence of visits foragers made to the flowers were recorded for each experiment. The mean visitation data were tested to see if foragers responded to varying frequencies of reward between the three colors and if visits to the initial flower color were a predictor of subsequent visits. Foragers from the three subspecies exhibited division of labor; some foragers visited yellow flowers while other hivemates visited blue and white flowers. Foragers did not respond to the varying frequency of reward for any of the flower colors. Additionally, the flower color foragers initially visited was a predictor of subsequent visits during all experiments. The behavior of limiting visits to one color may lead to minimized intra-colonial competition. In an environment free of specialized predators, the behavior is consistent with predation-risk foraging.
Introduction

The European honeybee, *Apis mellifera* Linnaeus 1758, (Hymenoptera: Apidae) is found in tropical forests, savannas, mountains, and on every continent except Antarctica (Ruttner 1988). This ubiquity contributes to the significance of honeybees as crop pollinators. With honeybees as pollinators coffee production increased by 50% (Roubik 2002) but without honeybee pollination, fruit and nut production decreased by as much as 90% (Southwick 1992). The importance of honeybees to agriculture coupled with the mysterious causes of colony collapse disorder (e.g. Stokstad 2007, Oldyord 2007) highlight the need to better understand foraging behavior of honeybees. Forager decision making is complex. Choices that foragers make result in tradeoffs between factors such as handling time, effort, risk, and reward (Harder and Real 1987). One of these factors, “constancy,” was first studied by Aristotle over 2,000 years ago (Grant 1950).

“Constancy” is the behavior where individual foragers will bypass reward and restrict visits to a single plant species (Hill et al. 1997). Honeybee foragers will sample from several flowering plant species but ultimately become “crop attached” to only a few (Ribbands 1954). In some instances, researchers found honeybees to only gather pollen and nectar from a single plant species (Free 1993, Menzel 1990). In addition to preferential visitation to certain flower plants, visual cues, such as color, contribute to foraging decisions (Srinivasan et al. 1999).

Color and honeybee reaction to color have long been studied since the discovery that foragers could be trained to visit certain colors (Frisch 1914) and are able to discriminate different color wavelengths (Daumer 1956). The role of color is important in forager decision making as honeybees can become “constant” to certain colors (Wells and Wells 1983). Using artificial flowers, Wells and Wells (1987) described “individual constancy” as a foraging strategy where individuals visited a single color irrespective of hivemate behavior. This pattern of color constancy may be beneficial to flowering plants by reducing the amount of wasted pollen and preventing stigma blockage by heterospecific pollen (Waser 1983). The benefits and variables driving individual constancy in honeybees are not well understood.

Many of the variables tested thus far have not been found to alter constancy to color. Manipulating reward quality and quantity did not affect color constant behavior (Hill et al. 1997). Also, neither handling time (Sanderson et al. 2006) nor distance (Hill et al. 2001) affected color constant behavior. In other words, foragers took more time, flew longer, and traveled farther to visit a specific color even when presented with an alternate choice of equal, greater, or
closer reward of different color. Moreover, the initial flower color a forager visits may be a predictor of subsequent visits (Hill et al. 1997), even if initially that flower color offered no reward (Ohyama et al. 1995). Hill et al. (1997) reported 99% of visits made by foragers did not deviate from the initial flower color visited. Past research suggests the variables tested thus far do not induce departure from color constant behavior.

One variable not yet extensively tested is the affect of frequency of reward on color constancy. Does variation of reward frequency affect foraging behavior? That is, when presented with three flower color choices, how will changing the probability of receiving a reward from only one of the colors affect foraging behavior? In other words, will foragers deviate from color constant behavior to visit alternate flower colors offering more frequent reward? This paper examined the interaction between color constancy and the probability of receiving reward.

Given the results of previous studies examining effects of reward quantity and quality, I expect varying reward frequency will not affect the foraging behavior of honeybees. To determine whether behavior is modified by varying reward frequency, I conducted free-flying experiments using artificial flowers of three different colors. The artificial flowers allowed control and manipulation of a variable often difficult to replicate in field experiments (Wells and Wells 1983, Wells and Wells 1986).

**Methods**

**Site and Organism Description**
Experiments were carried out at the Middle East Technical University in Ankara, Turkey (Fig. 1). The study employed free flying honeybees (*i.e.* no cage or enclosure/exclosure), *Apis mellifera caucasica* Gorbachev 1916 (Hymenoptera: Apidae), *Apis mellifera carnica* Pollman 1879 (Hymenoptera: Apidae), and *Apis mellifera syriaca* Skorikov 1929 (Hymenoptera: Apidae) obtained from breeders in eastern Turkey near the Republic of Georgia, European Turkey near Bulgaria, and southeastern Turkey near Syria, respectively. DNA restriction polymorphism analysis confirmed the subspecies of all...
colonies used in this study (Wells, H. 2007, unpublished data). Specialized predators were not common and honeybee predators were rarely observed where experiments were carried out.

**Experimental Design** To test whether reward frequency variation affects foraging behavior, visits were recorded under experimental conditions that controlled for flower color and varied reward frequency. Flower color choice has been reported to produce “context specific” foraging behavior (Hill et al. 1997). In response the experiments were performed using blue, white and yellow flowers in equal numbers and randomly arranged with respect to color in each flower patch.

Artificial flower patches (n=12 for each color) were used for experimental control purposes (Wells et al. 1981). Each flower was created using a 6mm thick 28 x 28mm Plexiglas square and contained a 5mm wide and 4mm deep well in the center that served as a receptacle for the sucrose reward. Each flower was mounted on a 90mm pedicel of 5mm wide plastic doweling.

Flower patches (Fig. 2) consisted of the 36 flowers spaced 75mm apart with six rows and six columns in a Cartesian coordinate system on a brown wooden pegboard raised on a stand approximately two feet from the ground.

Flower colors were created by painting the lower surface of each Plexiglas square with blue, yellow, or white enamel paint (Testors™ paint Nos. 1208 blue, 1214 yellow, and 1245 white, Testor Corporation, Rockford, IL, USA). The reflectance spectra for the paints and a color hexagon depicting how they are projected onto the honeybee visual cortex, can be found in Hill et al. (1997). Following each experiment the flowers were washed in unscented detergent, rinsed, and allowed to dry before reuse.

Each experiment was initiated by training a new set of un-caged, naïve, honeybees from an 18 frame hive to fly 50m to a clear petri dish containing lemon-scented 1M sucrose solution (5μL/L lemon oil). After a sufficient number of honeybees visited (~20 individuals), the dish was removed and replaced with an artificial flower patch provisioned with unscented 1M sucrose solution.
rewards. Each bee was allowed to freely choose flowers to visit and each treatment concluded with 80-100 flower visits. Flowers within the patch were randomly rearranged after each experiment.

Bees from six colonies of each subspecies were tested for flower choice in the experiment (n=10 to 12 bees per colony). No more than four bees were followed during any one experiment. Therefore, on average, one or two bees were present on the flower patch at any given time, thus mimicking natural foraging conditions where hive mates would be present. Each individual was followed throughout each experiment of four treatments. Bees used in each experiment were uniquely marked. Superfluous bees were removed from the system. The flower color sequence that each bee visited was recorded during each treatment.

Table 1. Reward volume (μl) comparison of four experimental treatments. Treatment 1 (control) offered equal sucrose for all flower (yellow, blue, and white). Treatments 2-4 offered foragers varying sucrose reward.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yellow</th>
<th>Blue</th>
<th>White</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Control</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2 - Yellow flowers varied</td>
<td>varied*</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3 - Blue flowers varied</td>
<td>2</td>
<td>varied*</td>
<td>2</td>
</tr>
<tr>
<td>4 - White flowers varied</td>
<td>2</td>
<td>2</td>
<td>varied*</td>
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</tbody>
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* varied reward volume: 1/3 flowers (n = 4) offered 6μl while 2/3 (n = 8) offered 0μl.

To assess the effects of varying reward frequency on foraging behavior, each experiment consisted of four treatments (Table 1). The different treatments served to manipulate the reward variance between each of the three colors. The average reward offered by each flower color in each treatment was identical (X̄ =2μ) but the variance in reward volume was rotated among the three flower colors in Treatments 2 (yellow varied), 3 (blue varied) and 4 (white varied). In Treatment 1 (control), all three flower colors contained 4μl of unscented 1M sucrose. Treatment 2 (yellow varied) offered bees 2μl of unscented 1M sucrose in each blue and each white flower, while one-third of the yellow flowers contained 6μl of unscented 1M sucrose and the remaining two-thirds had no reward. The reward frequency variation in Treatments 3 and 4 were the same as Treatment 2 with the exception for the flower color that the frequency was varied (i.e. Treatment 3, blue flowers varied; Treatment 4, white flowers varied).
For each experiment, all four treatments were performed successively without interruption. Treatment 1 (control) initiated each experiment. The order in which bees received Treatments 2, 3 and 4 was random; therefore, some foragers received Treatment 4 before 3, etc. This was done as a control measure to ensure the order in which foragers received varying reward frequency did not affect choice. For the 4µl and 2µl rewards, each flower was replenished with an identical reward when an individual emptied the well. When the 6µl reward in the variably rewarding flower color was consumed a flower without reward of the same color was randomly chosen and filled with 6µl of 1M sucrose. Thus, flower position of the variably rewarding color was not a factor.

Forager-type (i.e. yellow or blue/white) was assigned to individuals based on the favored color in the forager’s first five visits during the control treatment. This grouping was made due to previous reports of “context specific behavior” (Hill et al. 1997) where choices made by Apis mellifera liguistica foragers to limit visits to blue, white, or yellow flowers was dependent on the flower color choices available (Wells and Wells 1983, Wells and Wells 1986). Additionally, foragers were grouped by visits made to blue or white and yellow based on honeybee ability to discern yellow from blue or white. What human’s perceive as blue and white are close to each other in the visual colorspace but separate from yellow (Chittka 1992, Chittka et al. 1994). Analysis of these data will show whether the initial color visited is a predictor of subsequent visits.

**Statistical Analysis** A repeated measures MANOVA (Sall and Lehman 1996) was used to test for subspecies, treatment, forager-type (i.e. yellow honeybee), and interaction effects. The data were tested for the effects of reward variation in the different flower colors and the effect of visiting a flower color first. To meet assumptions of normality for MANOVA, data were transformed via arcsine square root (Sokal and Rolhf 1995, Sall and Lehman 1996).

**Results**

Observations were made on 11,264 flower choices by 32 A. m. carnica foragers from six colonies, 11,036 flower choices by 31 A. m. syriaca foragers from six colonies, and 11,560 flower choices by 34 A. m. caucasica foragers from six colonies. Forager behavior was consistent with yellow and blue or white constancy. There were no significant differences between mean forager visitation proportions to yellow, blue, or white flower colors.
Individuals of all three subspecies visited the initial flower color they foraged from (*Apis mellifera carnica*: F=183.3; df=2.96; p<0.0001; *Apis mellifera syriaca*: F=574.4; df 2.90; p<0.0001; *Apis mellifera caucasica*: F=126.6; df 2.99; p<0.0001). That is, foragers of each subspecies returned to the initial flower color visited (*i.e.* blue/white or yellow). Additionally, there were no significant differences in mean visits to the three colors across the four treatments (no treatment effects; *Apis mellifera carnica*: F=0.018; df=3.94; p=0.997; *Apis mellifera syriaca*: F=0.028; df=3.88; p=0.994; *Apis mellifera caucasica*: F=0.321; df=3.97; p=0.810). That is, foragers of each subspecies did not respond to the varying reward frequency among the different flower colors; instead foragers remained constant to a flower color irrespective of lower probabilities of receiving reward (Fig. 3-5). Moreover, forager type (*i.e.* yellow or blue/white) did not affect whether reward variation was used to make foraging decisions (no interaction effect bee-type x treatments; *Apis mellifera carnica*: F=0.136; df=3.94; p=0.938; *Apis mellifera syriaca*: F=0.009; df=3.88; p=0.999; *Apis mellifera caucasica*: F=0.281; df=3.97; p=0.839).

**Figure 3.** *Apis mellifera carnica* mean percent visitation (±1 SE) by treatment for individuals first selecting blue/white flowers (3a) or yellow flowers (3b). Control treatment did not offer varying in reward frequency. Treatment 2 offered varying reward for yellow flowers, etc. No significant differences between treatments: F=0.018; df=3.94; p=0.997.

**Discussion**

Naïve honeybees, foragers exposed to experiments for the first time, made visitation choices between three flower colors. After visiting the initial flower color, subsequent visits made by foragers of all three subspecies were limited to that initial color (*i.e.* either blue and white or yellow). Foragers from each subspecies did not respond to the varying reward frequency in yellow, blue, or white flower colors. That is, foragers initially visiting yellow flowers...
consistently returned to yellow flowers even if yellow flowers did not offer the most frequent reward available to the forager (i.e. Treatment 2, Fig. 3b, 4b, 5b). This trend was also apparent in foragers initially visiting white and blue flowers (Fig. 3a, 4a, 5a).

Forager type data were recorded due to reports of spontaneous constancy to certain colors depending on the choices available to the forager (Wells and Wells 1986; Hill et al. 1997). The Italian honeybee, *Apis mellifera ligustica*, was observed to become constant to a color depending on the choices available. When given only a choice between blue and white flowers, foragers will visit both, but when also given a choice of yellow some will only visit yellow (Hill

![Figure 4. *Apis mellifera syriaca* mean percent visitation (±1 SE) by treatment for individuals first selecting blue/white flowers (4a) or yellow flowers (4b). Control treatment did not offer varying in reward frequency. Treatment 2 offered varying reward for yellow flowers, etc. No significant differences between treatments: F=0.028; dF=3,88; p=0.994.](image)

![Figure 5. *Apis mellifera caucasica* mean percent visitation (±1 SE) by treatment for foragers first selecting blue and white flowers (5a) or yellow flowers (5b). Control treatment did not offer varying in reward frequency. Treatment 2 offered varying reward for yellow flowers, etc. No significant differences between treatments: F=0.321; dF=3,97; p=0.810.](image)
et al. 1997). This current study combined yellow, blue, and white flower colors to ensure the experimental apparatus would not mask constancy behavior.

These data support the hypothesis that variation of reward frequency would not affect foraging behavior of honeybees. Prior studies report foragers did not respond when reward frequency was varied in blue and white flowers (Wells and Wells 1983, Wells and Wells 1986). These results also corroborate previous observations of individual constancy in honeybees (e.g. Marden and Waddington 1981, Cakmak and Wells 1995, Ohyama et al. 1995, Gegear and Laverty 2004). Also, A. m. ligustica foragers were observed visiting either blue or yellow flower color morphs irrespective of decreased or increased handling time (Sanderson et al. 2006). Furthermore, the non-responsiveness of A. m. caucasica to varying reward frequency among flower colors is consistent with results from experiments conducted in eastern Turkey where no Apis spp. predators were observed (Cakmak and Wells 2001).

Predation-risk models may shed light on the lack of response to reward frequency variation. Where specialized predators are present, honeybees should minimize risk and maximize harvest (Gilliam and Fraser 1987); predation pressures result in high mortality of Apis mellifera (Sharma and Raj 1988). The lack of forager response to varying reward frequencies is consistent with predation-risk foraging decisions in an environment where specialized predator were rare. The predation risk model states that foragers will minimize the predation to foraging-rate ratio (u/f); accordingly, foragers would be expected visit the flower offering the most frequent reward. In this study, predation (u) is zero since specialized predators were rare (and u/f=0 for all f>0), therefore one would not expect foragers to necessarily have to visit flower colors offering more frequent reward (Gilliam and Fraser 1987, Brown 1992). Predation exerts strong influence over foraging decisions, but it is not the only factor that may explain the behavior observed in this study.

In addition to absence of specialized predators, intra-colonial competition could also explain the pattern of foragers of the same hive visiting different flower colors. The strategy of hivemates visiting and becoming individually constant to different colors may decrease intra-colonial competition. As reported in ants, another social species, individuals will gather different resources at various times, reducing intra-colonial competition (Rissing 1981, Schatz et al. 1995). This pattern has been suggested to reduce interference (Wells and Rathore 1994) through
the division of labor among foragers (Heinrich 1976). This behavior may indicate the greater importance of intra-colonial competition over energy maximization.

Studying flower constancy is an experimental challenge. Foraging periods throughout each day differ between each colony and is temporally dependent, such as time of day and season (Dimou and Thrasyvoulou 2007). Experimentation for each subspecies was conducted in random order; the design did not take into account daily temperature differences and time of day. Also, the context specific constancy of the Italian honeybee highlights another challenge in experimental design where the limited color choices and their combinations are inherently restraining choice (Hill et al. 1997). The limited range of colors tested in this study may not show the full complexity of decision-making in *Apis mellifera*.

Future studies on honeybee foraging behavior should attempt to examine the effects of color combinations on foraging decisions and explore other colors in the honeybee’s visual space. Reward is only one of many factors, such as flight time and handling time (Sanderson 2006), involved in decision making. To be sure, the general model of individual constancy does not apply to all pollinators (i.e. bumble bees) or to different honeybee subspecies as it applies to the honeybees tested in this study (e.g. Greggers and Menzel 1993, Keasar et al. 1996, Stach and Guirfa 2001). The literature on foraging behavior includes vastly different designs, with each experimental apparatus potentially eliciting different behaviors (Hill et al. 1997). Experimental design may mask individual constancy and behavior may be dependent on the color choices available (Wells and Wells 1983, Wells and Rathore 1994, Hill et al. 1997, 2001).

This study found varying reward frequency was not enough to affect honeybee color constancy; in itself, manipulating the probability of finding reward did not elicit changes in foraging behavior. Additionally, the initial color foragers visited was a significant predictor of subsequent visits and these behaviors may be consequences of honeybee foraging strategies. Much work remains to uncover the causes of constancy. The difficulty is finding the right environmental cues that ultimately lead honeybees and other pollinators to their floral choices.

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