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**Multiple search flights of the dancer's followers: result of information or energy deficiency**

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**ABSTRACT**

Waggle-dance communication in honeybees is widely accepted as a method of information transfer of profitable food sites in honeybee colony. It has been shown that bees which approach the dancer on the comb and follow it during wagging actually find the food location. Many research reports as well our own observations showed that the follower usually flies several times out of the hive and follows for several times the dancers inside of the hive before actually reaching the experimental feeding site. If we understand honeybees as machine automata then bees do repetitions of following to obtain more accurate information about food location. Multiple following and multiple searching flights were two of the strongest arguments advanced by scientists who pointed out the possibility of finding a food site without location information from dance pattern, but rather by searching for olfactory cues outside the hive. Regardless of information transfer, there exists another important limitation for recruitment success of the follower. The follower needs energy to reach potential food location. It is possible that follower is not supplied with enough honey in the honey stomach or its metabolism is not adapted to flight demands. Based on literature reports and our recent findings we favored more energy than information deficiency as a cause of multiple search flights of followers.

**Keywords:** *recruitment, dance language, metabolism*

**INTRODUCTION**

It is well known that foraging honeybees perform a waggle dance upon entering the hive (von Frisch 1965). Stereotypic behavior patterns performed by waggle dancers contain information associated with a foraging location. Various theories have been proposed to account for the transfer of information between dancer and forager. These theories include the acoustical theory (Esch 1961, Wenner 1962, Michelsen et al 1987, Kirchner et al 1988), olfactory theory (Wells and Wenner 1973, Wenner 1974), and tactile theory (von Frisch 1923, Božič and Valentinčič 1991, Rohrseitz and Tautz 1999).

The acoustical and tactile theories propose that bees can use specific information from the waggle dance about direction and distance of food location. The olfactory theory proposes an alternative view that chemical signals from the food are sufficient for successful recruitment. Most of the above studies related to the recruitment success tested hypothesis of successful information transfer and its accuracy.

Regardless of hypothesis, potential recruit, which has followed the dancer, should fly direct to the communicated food site, if information transfer was correct. It has long been notice that

forager bees do not reach the food site during the first flight after following the dancer inside of the hive (Esch and Bastian 1970, Mautz 1971). Do bees need to repeat following to obtain dance information and find site location? Are there other possible explanations for repeated search flights of potential recruits?

The purpose of our report is to postulate new hypothesis, which can explain delayed recruitment without difficulties of dance information transfer or way of information transfer. We performed recruitment experiments with individually marked bees. We compare our results with previous data (Esch and Bastian 1970) and other experiments in which we researched physiological changes in the body (Božič and Woodring 1997, 2000) and brain (Božič and Woodring 1998) of potential recruits.

## **MATERIAL AND METHODS**

Bees were kept in an observation hive with two Langstroth's frames of comb. Recruitment experiments were performed with the observation hive positioned in the middle of an open space in the forest by Ljubljana ZOO in July and August 1991. Two days before the experiment, 600 bees were marked with spots of colored lacquer. First foragers were attracted to the feeding plate at the hive entrance. They were replaced to feeding site 150m to the south of the hive with the step by step method (von Frisch 1965). The feeding site contained a 50% sugar solution scented with the odor of orange tea.

### **FIRST RECRUITMENT EXPERIMENT**

The behavior of the foragers returning from the experimental station to the observation hive was recorded with a VHS camcorder (macro setting). The recorder was turned on when the forager began dancing. The behavior of the dancing bee was recorded as well as the behavior of bees around the dancer. If there was a marked bee in contact with the dancer it was recorded until it left the hive or patrolled for more then two minutes after the last contact with the dancer. Observers monitored arrival of marked bees to the feeding station. One control station was established with the same food as experimental but was located 60° to the west.

The behavior of bees recorded in the hive was analyzed during slow or frame by frame replay of video-recordings. Time series of behavior events were recorded in computer during video replay. In our experiments we counted the number of attended or followed waggle runs, patrolling on the comb and food exchange with bees on the comb before leaving the hive or for two minutes after having a contact with the dancer. Attending was recognized as behavior when the bee didn't move along with the dancer and has a contact with her or was directed to her without any other bee between them. If bees keep contact with the dancer during whole waggle run then that was counted as following the dancer (see also Božič and Valentinčič 1991). Frequencies of observed behaviors were compared with the recruitment success as observed at the feeding place. Frequencies of observed behaviors were tested against recruitment success with logistic regression analysis using Proc Probit (SAS Institute Inc. 1988a, 1988b).

We used the Proc Probit analysis to reanalyze the data of Esch and Bastian (1970). We selected this experiment because the procedure was similar to ours with the exception that they captured all arriving bees at the feeding station with the exception of the dancing foragers.

We performed a second recruitment experiment in August 1991. The purpose of the experiment 2 was to control all potential recruits that come in contact with oriented dances. For that purpose we lied the observation hive horizontally. Forager bees are disoriented on

horizontal surface if they can't get orientation of the sun through observing the sky (see von Frisch 1965). Only 6 by 8cm area was exposed to direct sky light (omitting direct sun due to overheating), to limit possible area with oriented dances. That area was continuously recorded with VHS camcorder. This enabled us to record all marked bees which had contact with oriented dances on the comb. All other procedures were the same as in Experiment 1.

In both experiments, forage site visits were analyzed according to the previous contacts with the observed dancers in the hive and the previous visit to the feeding place. Dependent variables were: 1) the number of visits of first time recruits, 2) repeated visits of recruits, 3) first time visits of marked bees not observed near the dancer (first time foragers) and, 4) repeated visits of marked foragers.

## RESULTS

### A) RECRUITMENT SUCCESS OF THE BEES OBSERVED AROUND THE DANCER

Only one third of the bees, which were observed to have contacts with the dancer in the hive, actually arrived at the feeding site on the same day (15 of 45, Table 1A). All of the recruited bees except one performed what we have defined as following behavior. More than half the bees were observed to leave the hive after following the dancer. Among recruited bees, only following bees had a significant positive contribution to the recruitment success (Table 1A). Increased frequency of following of waggle runs for one increased recruitment rate 1.47 times and for two for 2.16 times (odds ratio for recruitment success). Attending the dancer had no significant effect on the recruitment success. On the other hand, occurrence of the patrolling bees negatively affected recruitment (Table 1A).

We observed a significant intercept in the logistic regression model for recruitment success in our experimental situation (Table 1A), but it was not significantly different from zero in the experiment of Esch and Bastian (1970) (Table 1B). We calculated higher and also significant parameters for following behavior in their experiment. Increasing frequency of following for one resulted in increased recruitment rate for 4.65 times. Number of contacts with the dancers also had negative effect on the recruitment as in our first experiment.

In our first recruitment experiment we had 172 marked bees at the feeder and we were able to record only 8.7% of them (15) in the hive around the dancers (Fig. 1A, recruits). In our second experiment with the horizontal hive we obtained 50% (13 recruits of 26 at the feeder) of marked bees at the feeder, which we observed inside the observation hive (Fig. 1B).

### B) DYNAMICS OF RECRUITMENT

Half of the recruited bees were observed at the feeding place more than 20 minutes after leaving the hive. The fastest arrival was 8 minutes after exiting the hive. First recruits arrived just few minutes after establishing the feeding site (Fig. 1), but they were not observed at the dancers in the hive during our recording. During the first 30 minutes, there were no bees which had previously contact with the dancers in both experiments. In the second experiment (Fig. 1B) we controlled all potential recruits that attended or followed oriented dances (see Methods). The first marked bee was observed 10 minutes after the start of the experiment and there were 5 more in the next 5 minutes (Fig. 1B, Experiment 2). All first 6 bees didn't attend or follow oriented dances in the hive. There were 9 more new marked foragers and all together 27 visits of marked foragers before the first recruit from the dance arrived at the feeder. The first recruit was observed 30 minutes after the beginning of the experiment 2. In the next 25 minutes we observed only 5 new recruits that made a total of 7 visits at the feeding site. At the same time we observed only 2 new marked foragers but also 38 visits of

previous foragers. In the next hour we observed 8 new recruits which visited 27 times the feeder together with previous recruits. There was very similar frequency of other marked foragers' visits (25) with new 7 marked foragers in the same observation time (Fig. 1B).

## DISCUSSION

The recruitment dynamics in our experiments is similar to previous recruitment reports (Esch and Bastian 1970, Mautz 1971). We have shown that recruitment success of the bees, which have a contact with the dancer, is depended on following the waggle run of the oriented dances. On the other hand, there were bees which successfully reach the feeder without attending or following the oriented dances inside the hive. Such recruitment is very fast and dominates the first 30 minutes. In the next 30 minutes recruitment from oriented dances dominates, but there were still more visits of previously recruited foragers. After the first hour, visits of recruits from the dancer and other marked bees were similar.

There are several possibilities to explain this variation in recruitment dynamic. Recruitment of bees which did not follow oriented dances can be explained with the olfactory hypothesis (Wells and Wenner 1973, Wenner 1974), when foraging, motivated bees received olfactory signals through throphallaxis or antennation from the returning foragers. The stimulation provided by the returning foragers elicited searching behavior for food sites outside the hive.

In addition to the smell of the food site, foragers could also be guided by pheromones (no bees at the control feeder) (Free 1987). Moreover, bees which were recruited after following the oriented dances might also use chemical signals to search the food site. These bees also had the opportunity to use dance information during search of food site.

The purpose of our experiments was not to determine if our recruited bees really used dance information. That possibility has already been studied in the experiments of Gould (Gould 1975). Gould did not use a proper control station to exclude the possibility of pheromone marking of the test stations. Nevertheless, the data distributions in their reports favor the use of dance information rather than just olfactory cues.

Our second experiment showed that bees which are able to make immediate forage trips can find the food location without dance information. Repeated following and exiting of the observation hive before recruitment suggest that potential recruits which follow the dance are not able to reach food site. Many experimenters have observed similar behavior (Esch and Bastian 1970 Mautz 1971). Failure to find the food site is not necessarily due to lack of information or improper information transfer during following of the dance inside the hive. We propose a new possibility for delayed recruitment. Bees which follow the dancer are most likely not prepared physiologically for sufficient foraging flight. They have to established proper metabolic pathways to have enough of a high conversion of glucose to trehalose, which is necessary to perform foraging flight as it was shown during extreme condition of swarming (Božič and Woodring 1997).

It is also possible that potential recruits perform several search flight because of low motivation for foraging and a low arousal for flying. Internal stimulation by hormones (e.g. juvenile hormone, (Robinson 1987, Božič and Woodring 2000) could be one possible way of increasing motivation for following and search flights outside of the hive. Mechanical and chemical stimulation during following can trigger exiting of the hive and searching of a food location. Previously (Božič and Woodring 1998) we have proposed that dopamine system is involved in regulation of recruitment behavior. Future studies of recruitment behavior should consider the regulation of energy requirements, maturation, and arousal of the central nervous system to perform the complex tasks of exploring new feeding sites.

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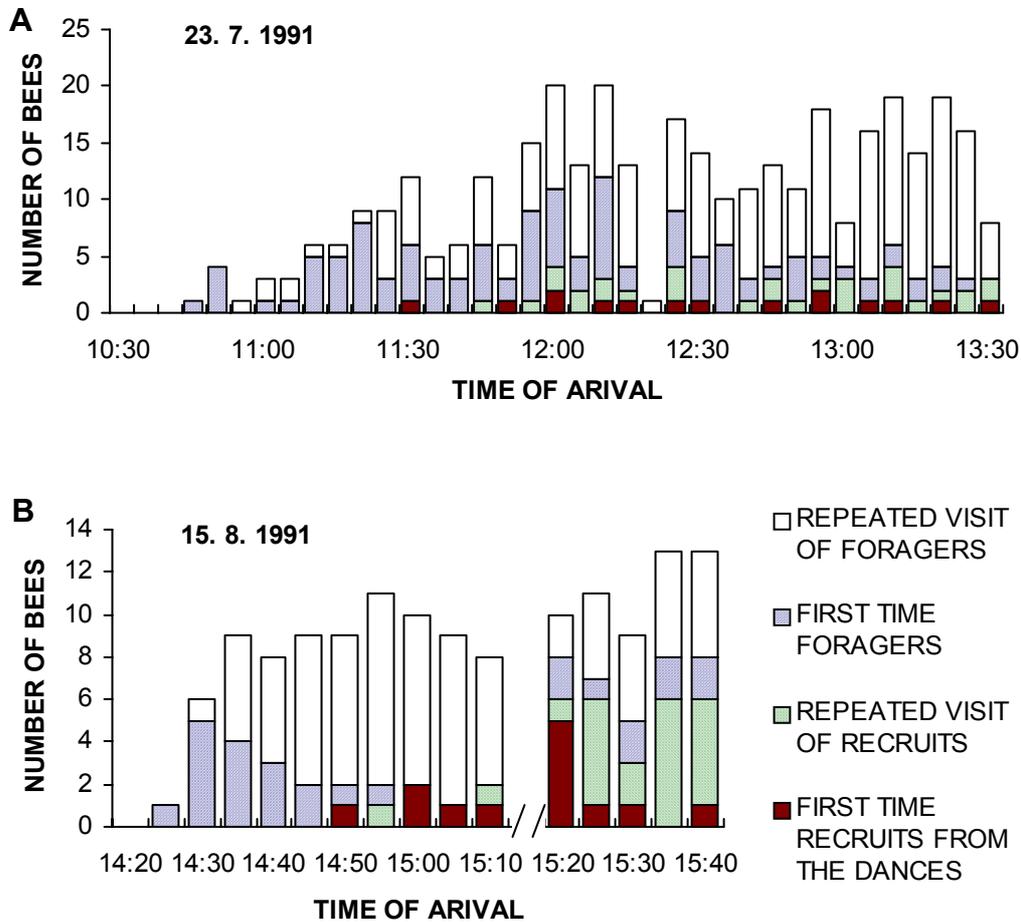
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**Figure 1:** Dynamic of recruitment in two experiments. First recruits from the dance came at the feeding site at least 30 minutes after start of the experiment. In the first experiment (A) we were not able to record possible simultaneous dances (see Method), therefore some recruits from the dances might be included among first time foragers. In Experiment 2 all oriented dances were recorded (see Method). Broken x-axis indicates 5 minutes of record-tape change in camcorder.

**Table 1:** Logistic regression analysis of recruitment success of bees observed in the dancers' surrounding. Bees were more likely recruited if they followed the dancer more frequently and they were less likely recruited if they were more frequently engaged in the activities not directly related to the dance communication (e.g. Patrolling or Contacts). SE - standard error of the estimate,  $\chi^2$  - Chi-Square value,  $p > \chi^2$  - probability for greater Chi-Square then calculated,  $\theta$  - estimated Odds ratio for the difference in frequency for one (dx=1) or two (dx=2).

### A) Bees from two experimental days

Variable	Estimate	SE	$\chi^2$	$p > \chi^2$	$\theta$ (dx=1)	$\theta$ (dx=2)
Intercept	2,136	0,446	22,88	0,0001		
<b>Patrolling</b>	<b>-0,295</b>	<b>0,107</b>	<b>7,61</b>	<b>0,0058</b>	<b>0,74</b>	<b>0,55</b>
Attending	-0,007	0,153	0,00	0,964	0,99	0,99
<b>Following</b>	<b>0,151</b>	<b>0,056</b>	<b>7,19</b>	<b>0,0073</b>	<b>1,16</b>	<b>1,35</b>
Food exchange	0,195	0,196	0,99	0,3197	1,22	1,48

Log Likelihood= -51,3

N of bees=107, N of recruits=25

### B) Bees from the first experimental day

Variable	Estimate	SE	$\chi^2$	$p > \chi^2$	$\theta$ (dx=1)	$\theta$ (dx=2)
Intercept	1,187	0,814	2,13	0,1446		
<b>Patrolling</b>	<b>-0,626</b>	<b>0,246</b>	<b>6,50</b>	<b>0,0108</b>	<b>0,53</b>	<b>0,29</b>
Attending	0,324	0,282	1,32	0,2508	1,38	1,91
<b>Following</b>	<b>0,386</b>	<b>0,145</b>	<b>7,08</b>	<b>0,0078</b>	<b>1,47</b>	<b>2,16</b>
Food exchange	0,511	0,384	1,77	0,183	1,67	2,78

Log Likelihood= -20,6

N of bees=45, N of recruits=15

### C) Bees from the Esch and Bastian (1970) experiment

Variable	Estimate	SE	$\chi^2$	$p > \chi^2$	$\theta$ (dx=1)	$\theta$ (dx=2)
Intercept	-1,372	1,066	1,66	0,1979		
<b>Contacts</b>	<b>-0,525</b>	<b>0,317</b>	<b>2,75</b>	<b>0,0974</b>	<b>0,59</b>	<b>0,35</b>
Feeding	-0,409	0,620	0,43	0,5097	0,66	0,44
<b>Following</b>	<b>1,537</b>	<b>0,659</b>	<b>5,44</b>	<b>0,0197</b>	<b>4,65</b>	<b>21,61</b>
Dances	0,020	0,078	0,07	0,7931	1,02	1,04

Log Likelihood= -14,75

N of bees=32, N of recruits=13