

## REGULATED NECTAR HARVESTING BY THE HONEYBEE

THOMAS E. RINDERER

Bee Breeding and Stock Center Laboratory, Agricultural Research Service, Science and Education,  
USDA, Baton Rouge, LA 70808, USA

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### Summary

Empty storage combs in honeybee nests regulated the nectar-harvesting activities of occupant bees. Increased honey storage stimulated by the presence of more empty comb was associated with more intense nectar-harvesting behaviour both in and away from the hive. Foragers from hives with more comb were less likely to collect food of lower sugar concentration; however, when they did collect it, they were more likely to perform recruitment dances upon their return to the hive. Colonies with more empty comb stored more honey during major nectar flows but less during minor flows. Among colonies supplied with similar amounts of comb, those that stored more honey during major nectar flows stored less during minor ones.

### Introduction

The nests of social insects are usually considered in the context of products, rather than causes, of behaviour (see e.g. Wilson, 1971). Less frequently, nests have been studied as behavioural stimuli. As stimuli, nests are known to provide a proper context for nest-cleaning behaviour (Rothenbuhler, 1964), to aid the return of nest inhabitants (Butler, 1966; Butler et al., 1969; Hangartner et al., 1970), and to impart chemical cues to inhabitants that cause them to be recognized as colony members (Kalmus & Ribbands, 1952).

Substantially increased amounts of empty comb in the nests of colonies of honeybees (*Apis mellifera*) during periods of abundant nectar secretion and availability (major nectar flows) significantly increased the amount of honey they stored (Rinderer & Baxter, 1978). Also, increased amounts of empty comb resulted in increased rates of sucrose-solution hoarding (Free & Williams, 1972; Kulinčević & Rothenbuhler, 1973) by caged bees in laboratory experiments (Rinderer & Baxter, 1979; Rinderer & Baxter, 1980). Volatiles collected from empty comb also increased hoarding by caged bees, indicating that the stimuli provided by comb are chemical (Rinderer, 1981). These experiments showed that empty comb in a honeybee nest plays a major role in influencing nectar-harvesting (collection and related activities) by honeybees. The experiments reported here examine certain behavioural and seasonal effects of empty comb on open-mated European honeybees.

### Experiment 1: Effects of empty comb on dance behaviour and flight activity

Observation hives with differing amounts of empty comb were established, and observations were made on: (1) numbers of nectar-collectors dancing, (2) numbers of recruit bees following dancers, (3) duration of dances, (4) time spent by foragers in the field, (5) numbers of foragers in the field, (6) amount of honey stored.

### Methods

Twenty honeybee colonies were established in glass-walled observation hives. Each hive contained a laying queen from a small colony, about 0.25 kg (2500) of adult worker bees, and one brood comb (16 × 45 cm) containing worker brood (about half sealed and half unsealed), with a small amount of honey and pollen. Each hive entrance was fitted with an 8-cm length of clear plastic tubing (16mm internal diameter); this permitted the observation of incoming and outgoing bees. The colonies were left undisturbed for 4 days after establishment, to allow queens to begin laying eggs and field bees to adjust to their new location. On the day before the experiment began, 10 randomly chosen observation hives were given 1 additional empty comb above the brood comb, and the remaining 10 were given 3 additional combs.

This experiment was conducted during the major nectar-flow season; the primary nectar source was white clover (*Trifolium repens*) (Oertel et al., 1980).

Each colony was observed for 6 days. During the entire experiment, bees were seen only on the bottom two combs of the hives, in areas being immediately used for storage or brood

rearing. Also, in no instance were eggs laid in storage combs. Observations were made from 9.00 to 10.00 and from 13.00 to 14.00 Standard Time. During each of these 1-h observations, the number of nectar foragers that began dancing in each hive during a 2-min period was recorded. These observation periods were short enough, and the numbers of dancing bees were few enough, for two observers to count numbers of initiated dancers accurately, although the bees were not marked. The maximum number of bees that followed each dancer during a 10-s period was recorded, as was the number of bees entering each hive during a 2-min period. The entire durations of the dances of 5 nectar foragers in each hive were also recorded. To obtain measurements of dance durations, colonies were observed at random between 9.00 and 14.00 Standard Time on the 8th and 9th days of the experiment. At the beginning of the experiment each hive was weighed; 10 days later the hive was again weighed, and the change in weight was recorded. Most of this change in weight represented stored honey and nectar; a much smaller part resulted from a marginal expansion of the brood nest, in both treatment groups. The data on honey storage and dance duration were analysed by *t*-tests; other data by analyses of variance and orthogonal comparisons. All data met the assumptions of parametric analysis.

## Results

The number of dancing nectar foragers was strongly influenced by the number of storage combs in the hive (Table 1). In the morning, the average number of dancing nectar foragers was significantly higher ( $P < 0.01$ ) in hives with 3 storage combs (4.0) than in hives with only 1 (2.5). By the afternoon, the average number of dancers in hives with 3 storage combs dropped significantly ( $P < 0.05$ ) to 1.7. This number was significantly lower ( $P < 0.05$ ) than the afternoon mean for hives with 1 storage comb (2.4).

In the morning the mean number of recruit bees following each dancing forager (Table 1) was significantly higher ( $P < 0.01$ ) in hives with 3 storage combs (3.7) than in hives with only 1 (3.0). By the afternoon, numbers of recruits following each dancing forager in both types of hives became similar (3 combs, 2.5; 1 comb, 2.6). Colonies in hives with 3 combs had significantly fewer ( $P < 0.01$ ) dance-followers per dancing bee in the afternoon than they had in the morning.

The mean duration of dances (Table 1) was significantly higher ( $P < 0.01$ ) in hives with 3 storage combs (107 s) than in hives with 1 storage comb (75 s).

The number of bees returning to hives increased significantly ( $P < 0.01$ ) from the morning to the afternoon, both in hives with 3 combs and in hives with 1 comb: 24 to 32, and 28 to 38, respectively (Table 1). In both morning and afternoon, the number of bees returning to the hives with 1 comb was significantly greater ( $P < 0.05$ ) than the number returning to hives with 3 combs. Numerical data were not obtained, but visual inspection of the colonies revealed that during the day there were substantially fewer bees in hives with 3 additional combs than in hives with 1. Similar inspections at night revealed approximately equal numbers of bees in all hives. Consequently, since foragers from colonies with 3 combs returned to the hive less frequently than foragers from colonies with 1 comb (Table 1), foraging bees from colonies with 3 combs spent more time on each foraging trip.

Average honey storage (Table 1) was significantly higher ( $P < 0.01$ ) in hives with 3 storage combs (0.5 kg) than in hives with only 1 comb (0.06 kg). Limitations in availability of storage space were not a factor, since a single comb could hold 2.2 kg of honey.

Generally, bees in hives with more empty storage comb engaged in significantly stronger recruitment activity when the nectar flow began each morning. Recruitment activity decreased as the available nectar-gathering bees were directed towards the exploitation of nectar sources. This exploitation, characterized by foraging flights of longer duration and more foragers, resulted in more stored honey.

## Experiment 2: Effect of comb on food acceptability

Experiment 1 showed that additional empty comb in a hive modified several aspects of nectar-harvesting behaviour. Such far-ranging modification supports the hypothesis that additional empty comb in a hive intensifies nectar-harvesting motivation. Differences in food acceptability as measured both by foraging and dancing after foraging, might be predicted. Experiment 2 was conducted to test that prediction.

TABLE 1. Analysis of variance, means, and standard errors for 3 measurements of nectar-forager recruitment dances, numbers of returning foragers, and honey storage, for colonies housed in observation hives with either 1 or 3 storage combs.

\*, \*\* Significant at  $P < 0.05$  and  $P < 0.01$ , respectively; when following means they denote a significantly higher mean for storage-comb comparisons in the same observation period.

$\Delta$ ,  $\Delta\Delta$  Significantly higher mean for the same number of storage combs in comparisons between morning (AM) and afternoon (PM) observations. Degrees of freedom for factorial effects are given in parentheses following  $F$ . Tests for  $t$  are 2-tailed.

No. storage combs	No. dancing nectar foragers		No. recruit bees following each dancing bee		Duration of dancing (s)	No. bees returning to colonies		Honey stored (kg)
	AM	PM	AM	PM		AM	PM	
3	3.98 ± 0.31**	$\Delta\Delta$ 1.68 ± 0.25	3.68 ± 0.10**	$\Delta\Delta$ 2.55 ± 0.14	106.76 ± 5.68**	23.75 ± 0.65	32.03 ± 0.85 $\Delta\Delta$	0.50 ± 0.08**
1	2.47 ± 0.22	2.35 ± 0.24*	2.96 ± 0.12	2.56 ± 0.10	74.52 ± 5.58	27.52 ± 0.71*	37.76 ± 0.90*, $\Delta\Delta$	0.06 ± 0.09
No. observations per mean	60	60	238 (3-comb) 148 (1-comb)	101 (3-comb) 141 (1-comb)	50	60	60	10
Factorial effects	Treatment (T) Days (D) T × D	F(3,216) = 15.06** F(5,216) = 3.47** F(15,216) = 1.10	Treatments Days T × D	F(3,604) = 26.30** F(5,604) = 2.95* F(5,604) = 0.93	t = 3.96 P < 0.01	Treatments Days T × D	F(3,216) = 6.94** F(5,216) = 6.09** F(5,216) = 1.58	t = 3.79 P < 0.01

## Methods

This experiment was conducted in late fall when there was no natural nectar flow. On each of 3 occasions, 2 colonies of bees similar in composition to the colonies used in Experiment 1 were established in observation hives.

In preparation for the experiment, bees from each colony were trained (von Frisch, 1967) to their own feeding station, *c.* 50 m from the hive entrance. Hive entrances were *c.* 4 m apart, and sites of final feeding stations *c.* 80 m apart. Orange-scented 1·8-M sucrose solution was used during training, and the 20-cm petri dish holding the solution was placed on a distinctive coloured card to aid orientation. Early in the training period when the food dish was very near the hive entrance, the bees from each colony were marked with paint to indicate their colony membership. Thereafter, during both training and experiments, only appropriately marked bees were permitted to forage at the feeding station; all others arriving at the station were killed. The results of the training efforts were two groups of marked bees, each from a different colony and each collecting food from their own separate feeding station. After training, 1 of the observation hives was given 1 empty comb above the brood nest and the other was given 3. In all cases empty combs remained empty throughout the experiment; eggs were not laid in them and too little food was given to the colony to result in food being stored.

Experiments began the day after the hives were supplied with additional comb. Foraging at the feeding stations was initiated by the marked bees after food scent was placed at each of the hive entrances. The food dishes were filled with 1·8-M sucrose solution. After the bees had been foraging on this solution for *c.* 30 min, a graded series of sucrose solutions (2-M, 1-M, 0·5-M and 0·25-M) was made available at each of the two stations. The solutions were presented in sequence from the most to the least concentrated, each being offered for 30 min. The number of marked bees collecting food at each feeding station was recorded for the six 5-min intervals during which each sucrose solution was available. Intervals of 5 min were chosen because marked bees were found to take *c.* 5 min for a single foraging trip during training.

While food collection at the feeding stations was being observed, the observation hives were inspected for marked bees initiating and performing recruitment dances appropriate for the distance and direction to the stations (von Frisch, 1967). The number of these dances in each colony was recorded for six 2-min observations taken at equal time intervals for each concentration of sucrose. The choice of 2-min observation periods was based on the average dance time during training.

This experiment was performed once with the first pair of colonies and 3 times each with the second and third pairs. In all cases, data from the initial 5-min observation (or 2-min observation of dancing) were omitted from calculations; errors resulting from the mechanical disruptions associated with changing the sucrose solutions were thereby eliminated. This precaution also eliminated errors that otherwise would have resulted from including counts of bees foraging on the previous solution and then dancing shortly after the solutions were changed. In each test the remaining 5 observations in a hive-concentration category were pooled to produce a 25-min score for collecting or a 10-min score for dancing. Data for the numbers of collection events and dances were transformed to  $\log_e (X + 1)$  for statistical analysis.

After the transformed data had been found to meet the assumptions required for parametric analysis, they were analysed by analysis of variance and Duncan's new multiple range test for differences among replications, comb number, concentrations of sucrose, and comb number — concentration interactions. The analyses were performed on both numbers of dances and numbers of food collections.

## Results

Empty comb in the observation hive nests influenced the acceptability of the sucrose solutions to foraging bees (Table 2). The comb numbers — sucrose concentration term in the analysis was highly significant ( $P < 0\cdot001$ ). Bees in the two comb-number groups made very similar numbers of foraging trips on 2-M solutions (average of 84 bee-trips for 3 combs and 83 for 1) and 1-M solutions (average of 94 for 3 combs and 91 for 1), and then decreasing numbers as the concentration of the food was lowered. However, the bees from hives with 3 combs reduced their number of foraging trips more precipitously (42 at 0·5-M and 7 at 0·25-M) than bees from hives with 1 empty comb (64 and 27 respectively, Table 2). The analysis also revealed no

TABLE 2. Analysis of variance, means, and standard errors for numbers of trained bees from hives with 1 or 3 storage combs foraging and dancing after foraging on 4 sucrose solutions. Means and standard errors of data transformed to  $\log_e(X + 1)$  are given in parentheses. Means not followed by the same letter are significantly different ( $P < 0.05$ ) as determined by Duncan's multiple-range test.

No. storage combs	Concentration of sucrose solution				Analysis of variance			
	2M	1M	0.5M	0.25M	Source of variation	df	F	P
3	83.7 ± 14.4 (4.3 ± 0.08) <sup>a</sup>	94.4 ± 17.2 (4.4 ± 0.09) <sup>a</sup>	42.4 ± 7.09 (3.7 ± 0.08) <sup>b</sup>	6.8 ± 2.6 (1.7 ± 0.06) <sup>d</sup>	Replicate	6	1.28	0.275
1	82.9 ± 13.3 (4.3 ± 0.06) <sup>a</sup>	91.3 ± 9.2 (4.4 ± 0.05) <sup>a</sup>	63.9 ± 9.6 (4.1 ± 0.07) <sup>ab</sup>	27.0 ± 9.4 (3.0 ± 0.08) <sup>c</sup>	Number of combs (N) Concentration (C) N × C	1 3 3	16.00 68.94 7.16	0.001 0.001 0.001
3	12.0 ± 0.9 (2.5 ± 0.03) <sup>a</sup>	10.6 ± 1.3 (2.4 ± 0.05) <sup>a</sup>	Foraging		Replicate	6	0.78	0.567
1	12.2 ± 1.2 (2.5 ± 0.04) <sup>a</sup>	2.7 ± 0.4 (1.2 ± 0.05) <sup>b</sup>	0.0 ± 0.0 (0.0 ± 0.0) <sup>d</sup>	0.0 ± 0.0 (0.0 ± 0.0) <sup>d</sup>	Number of combs (N) Concentration (C) N × C	1 3 3	31.98 259.24 13.24	0.001 0.001 0.001
					Dancing after foraging			
					1.1 ± 0.6 (0.6 ± 0.07) <sup>c</sup>			

significant difference among experiments, a significant difference ( $P < 0.001$ ) between comb-number treatments, and significant differences ( $P < 0.001$ ) among sucrose concentrations (Table 2).

The pattern of dancing response was quite dissimilar to the pattern of food collection response. Bees in hives with 3 empty combs danced more frequently for the feeding station when the 1-M solution was being fed than bees in hives with 1 comb (averaging 11 and 3 respectively, Table 2). Occasionally bees in hives with 3 additional combs were observed dancing at the feeding station when the 0.5-M and 0.25-M solutions were being fed (8 and 1 total dances, respectively), whereas no bees in hives with 1 additional comb were observed to dance during the feeding of these solutions. Analysis terms evaluating comb-number and the comb number — concentration interaction were both significant ( $P < 0.001$ ). Differences among experiments were not significant, but differences among concentrations were ( $P < 0.001$ ).

Overall, the data show that bees from hives with more comb were less likely to forage on less concentrated food, but when they did forage on it, they were more likely to perform recruitment dances upon their return to the hive. In contrast, bees from hives with less comb were more likely to forage on less concentrated food and less likely to perform recruitment dances. Thus, empty comb affected the acceptance of nectar sources by bees.

### **Experiment 3: Effects of comb on the seasonal collection of nectar**

The results of Experiment 2 suggested the hypothesis that changing amounts of empty storage comb in a honeybee nest throughout the year results in a regulation of the nectar foraging of resident bees to seasonally appropriate levels. Also, the results of Experiments 1 and 2 suggested the hypothesis that nectar load characteristics would differ between bees supplied with different amounts of storage comb. Experiment 3 was designed to explore these hypotheses.

#### **Methods**

Two apiaries of 6 colonies each were established in late April. All colonies had a young mated queen and similar bee populations, brood nests, and stored honey and pollen in the brood nest area. Half the hives in each apiary were equipped with empty storage comb with a total surface area of 4m<sup>2</sup> and the rest with empty storage comb with a surface area of 2m<sup>2</sup>. At the end of each month from May to September, storage comb containing honey or nectar was removed and replaced with equal areas of empty comb. At the same time the colonies were inspected to ensure that they were similar with respect to numbers of bees, brood nests, and stored honey and pollen in the brood nest area.

Each month, various features of nectar foraging and honey storage by bees from these hives were measured. At the end of the month the storage combs were removed from the hives, the honey was extracted and weighed, and the amount stored by each colony recorded. During the second week of each month 10 nectar-gathering bees (bees returning to the hive without pollen) were collected on each of 4 days for each colony. Nectar loads were expelled from the bees (Gary & Lorenzen, 1976), and volumes and concentrations of dissolved solids were measured and recorded. Volumes were measured in micropipettes and concentrations with a refractometer. These measurements permitted calculation of the percentage of successful nectar-foraging bees, the average caloric content of the loads collected by successful nectar foragers, and the average caloric content of the loads collected by all nectar foragers.

Data for most types of measurement met the assumptions for parametric analysis and were analysed by a 3-way analysis of variance; counts of successful nectar foragers were analyzed by  $\chi^2$ . The analyses, which included data for all 5 months of observation, indicated the desirability of a closer inspection of results, by means of 2 further sets of analyses: for data from the first 2 months, and for data from the last 3 months.

#### **Results**

Major differences occurred in the weight of honey stored by different colonies (Table 3). A greater amount of empty comb resulted in a significantly greater ( $P < 0.01$ ) storage of honey. Storage comb area was always well in excess of the amount used for storage. Differences in weight of honey between the 2 apiaries and differences between months, were both significant

( $P < 0.001$ ). Apiary location interacted with amount of storage comb ( $P < 0.05$ ) and with months ( $P < 0.005$ ). Most important was a highly significant ( $P < 0.005$ ) interaction between amount of storage comb and months.

The nature of the interaction between amount of storage comb and months is apparent from an inspection of Fig. 1. In May and June, colonies with  $4\text{m}^2$  of storage comb stored more honey than colonies with  $2\text{m}^2$ . In July, August and September the colonies with  $4\text{m}^2$  of comb stored less honey than colonies with  $2\text{m}^2$ . Further analysis showed the difference in May and June to be significant ( $P < 0.005$ ) (Table 4). While the change in relative production between the 2 treatments was strong enough to result in a highly significant interaction term in the 5-month analysis, the size of the difference in July, August and September, although consistent, was not large enough to be significant in the 3-month analysis (Table 5).

Nectar load data for each of the 5 measurements followed the same trends as the honey storage data, although the differences tended to be less strong. For data from the entire season (Table 3), all 5 measurements were numerically greater for bees from colonies with  $4\text{m}^2$  of storage comb than for bees from colonies with  $2\text{m}^2$ . All 5 measurements were also significantly greater ( $P < 0.05$  to  $0.001$ ) for nectar loads of bees from Apiary 2 than for loads of bees from Apiary 1. Differences between months were significant for all 5 nectar load measurements. Data for volumes, concentrations, and both estimates of colonies per forager also exhibited weak interactions ( $P < 0.20$ ) with amount of storage comb and months.

The analyses of the data collected in May and June indicate that all 5 measurements of nectar loads were significantly greater ( $P < 0.05$  to  $< 0.001$ ) for bees from hives with  $4\text{m}^2$  to storage comb than for bees from hives with  $2\text{m}^2$ . Bees in Apiary 2 carried nectar loads with significantly greater values ( $P < 0.001$ ) for 4 measurements than those in Apiary 1. The fifth measurement, percentage success, was numerically greater for bees from Apiary 2 (Table 4).

The analysis of the data collected in July, August and September showed that each of the 5 measurements or calculations of nectar loads was numerically larger for bees from colonies with  $2\text{m}^2$  of storage comb than for those with  $4\text{m}^2$  of comb. Nectar loads of bees from Apiary 2 had higher values for all 5 measurements than loads of bees from Apiary 1. The difference between nectar volumes was significant ( $P < 0.05$ ) (Table 5).

Thus, storage comb in honeybee nests interacted with seasonal nectar-flow conditions. In the major nectar flow in early summer colonies with large amounts of empty storage comb stored more honey than those with small amounts; in the poorer late summer-fall nectar flow colonies with large amounts of empty storage comb stored less honey.

Moreover, greater honey storage from a given nectar flow was accompanied by a larger percentage of successful foragers. As these foragers collected larger volumes of nectar of high sugar concentration, their loads had a greater caloric value.

#### **Experiment 4: Seasonal honey storage patterns of bees with equal amounts of storage comb**

Experiment 3 provided evidence that bees given large amounts of empty comb stored more honey during periods of major nectar flows, whereas bees with smaller amounts stored more honey during minor flows. Colonies with equal amounts of storage comb may have different basic levels of nectar-harvesting intensity, and accept different nectar sources as suitable for foraging because of long-term conditions. The long-term conditions might be genotypic differences or very local, stable differences in environment such as exposure to sunlight. If differences in level of nectar-harvesting intensity and nectar source selection affect the amount of honey stored during different nectar flows, patterns of honey storage should differ predictably among colonies.

#### **Methods**

Early in the history of our laboratory, an apiary was maintained with 4 to 8 colonies in hives on individual balance-beam scales. Each month for 25 years (1942-1967) changes in hive weight were recorded. Records were also kept of the occurrence of queenlessness, queen supersedure, disease, and other events that would be expected to affect nectar gathering adversely. The colonies were maintained as field honey-production units. Swarm prevention measures were taken routinely, and honey was removed from the hives after the major nectar flow. Empty

TABLE 3. Analysis of variance, means and standard errors of measurements of honey storage, nectar loads, and numbers of successful foragers from colonies with 2 amounts of storage comb in 2 apiaries for a 5-month period.

Values of  $F$  and  $P$  from the analysis of variance are given in parentheses (NS = non-significant). Degrees of freedom for  $F$  ratios involving months = 4, 40 and for others = 1, 40. Numbers of successful foragers were analysed by  $\chi^2$ ; values for  $\chi^2$  and  $P$  are given in parentheses; degrees of freedom for months = 4 and for other comparisons = 1.

Source of variation	Honey per month (kg)	Vol. nectar loads ( $\mu$ l)	Nectar concn (%)	Calories collected by:		Successful nectar foragers
				All nectar foragers	Successful nectar foragers	
Area of storage comb (SC)	4m <sup>2</sup>	24.0 $\pm$ 1.1	35.5 $\pm$ 0.9	26.6 $\pm$ 1.8	36.9 $\pm$ 2.0	No. 827 68.9
	2m <sup>2</sup>	22.8 $\pm$ 1.1 (1.8; 0.16)	34.9 $\pm$ 1.1 (0.7; NS)	25.2 $\pm$ 2.3 (0.7; NS)	34.8 $\pm$ 2.4 (1.2; NS)	785 (3.33; NS) 65.4
Apiaries (A)	4.5 $\pm$ 0.9	21.2 $\pm$ 1.1	34.0 $\pm$ 1.0	21.9 $\pm$ 2.1	31.4 $\pm$ 2.5	786 65.5
	9.3 $\pm$ 1.9 (47.5; 0.001)	25.6 $\pm$ 1.0 (22.5; 0.001)	36.5 $\pm$ 1.0 (10.3; 0.005)	29.9 $\pm$ 1.6 (20.7; 0.001)	40.4 $\pm$ 1.6 (21.8; 0.002)	844 (6.4; 0.05)
SC $\times$ A	(6.4; 0.05)	(2.1; 0.15)	(0.0; NS)	(0.1; NS)	(0.0; NS)	
Months (M)	7.7 0.9	26.5 2.0	32.6 1.3	25.1 3.4	33.0 3.4	331 69.0
	19.3 $\pm$ 2.9	18.3 $\pm$ 0.9	42.0 $\pm$ 0.7	21.2 $\pm$ 1.6	32.0 $\pm$ 1.8	306 63.8
	5.7 $\pm$ 1.1	29.2 $\pm$ 1.1	35.6 $\pm$ 1.3	36.7 $\pm$ 2.8	47.7 $\pm$ 2.7	352 73.3
	1.0 $\pm$ 0.4	24.6 $\pm$ 1.1	36.0 $\pm$ 1.1	29.5 $\pm$ 2.4	41.5 $\pm$ 3.0	349 72.9
	0.7 $\pm$ 0.2 (93.2; 0.001)	19.2 $\pm$ 1.2 (20.2; 0.001)	29.8 $\pm$ 1.0 (27.5; 0.001)	16.8 $\pm$ 2.4 (15.2; 0.001)	25.1 $\pm$ 2.4 (16.7; 0.001)	291 (27.3; 0.001)
SC $\times$ M	(51.8; 0.005)	(2.1; 0.17)	(1.8; 0.16)	(1.7; 0.17)	(1.8; 0.16)	
A $\times$ M	(12.3; 0.005)	(3.7; 0.05)	(5.4; 0.05)	(6.1; 0.005)	(3.8; 0.01)	



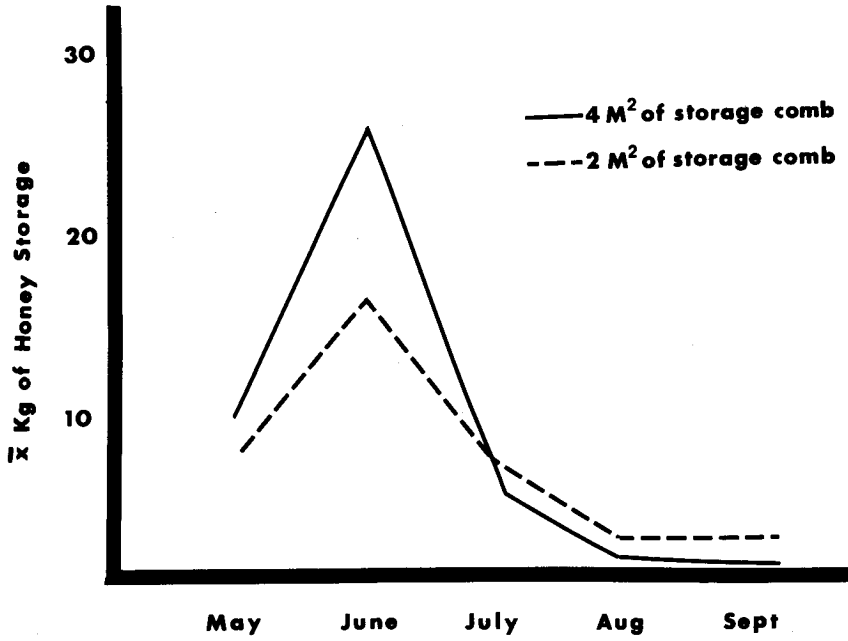


Fig. 1. Average monthly weight of honey (kg) stored by colonies with 4m<sup>2</sup> or 2m<sup>2</sup> of storage comb.

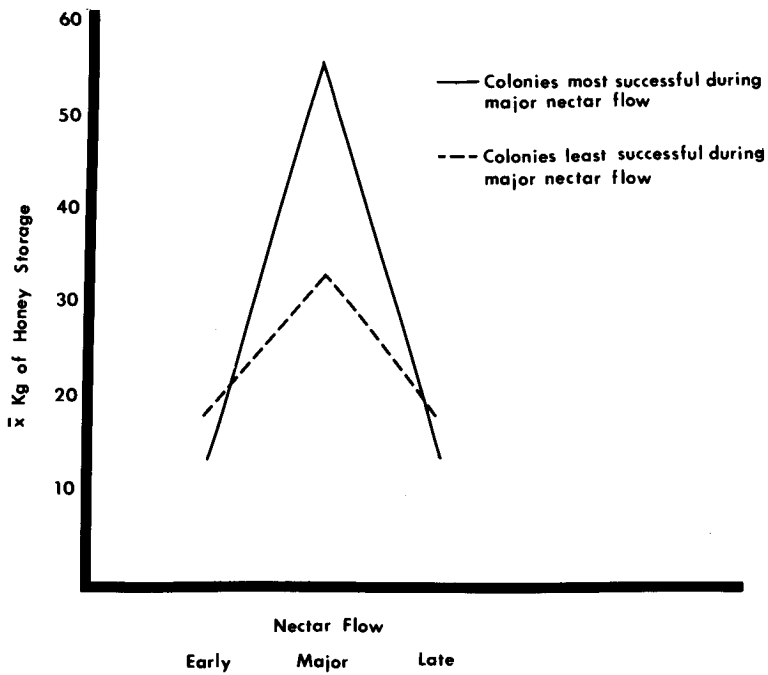


Fig. 2. Average seasonal weight of honey (kg) stored by colonies storing the most and least honey, respectively, during the major nectar flow in each of 25 years.

TABLE 4. Analysis of variance, means, and standard errors of measurements of honey storage, nectar loads, and the number of successful foragers from colonies with 2 amounts of storage comb in 2 apiaries for May and June.

Means and standard errors, are for months appearing in Table 3; values of  $F$ ,  $P$ , and  $\chi^2$  are presented as in Table 3; df for  $F$  ratios = 1, 16; df for  $\chi^2$  values = 1.

Source of variation	Honey per month (kg)	Vol nectar loads ( $\mu$ l)	Nectar concn (%)	Calories collected by:			Successful nectar foragers
				All nectar foragers	Successful nectar foragers	No.	
Area of storage comb (SC)	4m <sup>2</sup>	16.4±3.1	23.9±1.9	38.5±1.5	26.2±2.5	36.3±2.7	336
	2m <sup>2</sup>	10.6±1.9 (15.1; 0.005)	20.1±1.7 (7.7; 0.05)	36.2±2.0 (6.7; 0.05)	20.1±2.7 (15.8; 0.005)	28.7±2.1 (36.4; 0.001)	301 (5.7; 0.05)
Apiaries (A)	1	9.1±1.2	18.8±1.2	34.8±1.8	17.3±1.5	25.5±1.6	305
	2	17.9±2.2 (34.2; 0.001)	25.2±2.0 (22.0; 0.001)	39.9±1.4 (30.5; 0.001)	29.0±2.5 (59.9; 0.001)	39.5±1.9 (122.7; 0.001)	332 (3.4; NS)
C × A	(4.1; NS)	(0.2; NS)	(1.1; NS)	(0.6; NS)	(1.3; NS)		
Months (M)	(59.5; 0.001)	(29.2; 0.001)	(105.8; 0.001)	(6.9; 0.05)	(0.8; NS)		(2.92; NS)
C × M	(4.4; NS)	(0.7; NS)	(1.3; NS)	(0.6; NS)	(0.6; NS)		
I × M	(12.4; 0.005)	(8.2; 0.05)	(1.4; NS)	(41.6; 0.001)	(30.3; 0.001)		

TABLE 5. Analysis of variance, means, and standard errors of measurements of honey storage, nectar loads, and the numbers of successful foragers from colonies with 2 amounts of storage combs in 2 apiaries for July, August, and September.

Means and standard errors are for months appearing in Table 3; values of  $F$ ,  $P$ , and  $\chi^2$  are presented as in Table 3; df for  $F$  ratios involving months = 2, 24, and for others = 1, 24; df for  $\chi^2$  analysis = 2 for months and = 1 for other variables.

Source of variation	Honey per month (kg)	Vol nectar loads ( $\mu$ l)	Nectar concn (%)	Calories collected by:		Successful nectar foragers	No.	%
				All nectar foragers	Successful nectar foragers			
Area of storage comb (SC)	4m <sup>2</sup>	2.2±0.8	24.0±1.4	33.6±1.0	26.9±2.6	37.4±3.0	491	68.2
	2m <sup>2</sup>	2.7±0.7	24.6±1.3	34.0±1.4	28.4±3.1	38.8±3.4	502	69.7
Apiaries (A)	1	1.4±0.5	22.9±1.2	33.4±1.3	24.9±3.3	35.2±3.7	481	66.8
	2	3.6±0.9	25.8±1.1	34.2±1.0	30.5±2.2	41.0±2.3	511	71.1
SC × A		(13.3; 0.005)	(5.7; 0.05)	(0.5; NS)	(3.4; NS)	(3.4; NS)		(3.06; NS)
Months (M)		(2.3; NS)	(2.3; NS)	(0.5; NS)	(0.2; NS)	(0.2; NS)		
SC × M		(29.3; 0.001)	(22.5; 0.001)	(12.4; 0.001)	(14.2; 0.001)	(18.8; 0.001)		(23.0; 0.001)
A × M		(0.13; NS)	(0.4; NS)	(1.2; NS)	(3.8; 0.05)	(0.5; NS)		
		(5.47; 0.05)	(1.8; NS)	(5.3; 0.05)	(7.4; 0.01)	(1.9; NS)		

combs were returned to hives after removal of the honey, and all colonies in sound condition had hives with 6m<sup>2</sup> of storage comb. From the records of each year we identified, from among the sound colonies, those that stored the most and the least honey during the month in which the most honey was stored in the apiary. Data on honey storage by this pair of colonies in the first and last months with a nectar flow in each year were then identified. Binomial tests of sign relationships for the 25 pairs of colonies were performed on these 2 sets of data.

## Results

The colonies that stored the least honey during the major nectar flow consistently stored the most during both the early ( $P < 0.01$ ) and the late minor flows ( $P < 0.001$ ) (Fig. 2). These patterns of honey storage conformed to predictions, and supported the hypothesis that more intense nectar harvesting and the acceptance of primarily highly rewarding nectar sources result in more honey storage during major nectar flows, while less intense harvesting and the acceptance of lower-quality nectar sources result in greater storage during minor flows.

## Discussion

The presence of different amounts of empty comb in their nests resulted in differential responses by honeybees across a wide range of nectar-harvesting activities. Because of the diversity of the activities and because several of them are performed away from the nest, it is probable that empty comb changed the physiology of bees so as to alter both the intensity of their nectar-harvesting activities and the level at which nectar sources were accepted as sufficiently rewarding.

Predictable seasonal trends in nectar production commonly occur in temperate climates (Crane, 1975; Oertel et al., 1980). In many areas, early spring nectar sources tend to be both few and poor. This situation changes rapidly, and by late spring to early summer nectar sources tend to be both varied and rich. A decline in variety and abundance follows, until by late summer only a few poor-quality nectar sources usually exist.

The amount of empty storage comb in a feral honeybee nest also varies with season. If the nest is already filled with comb, then in early spring intensified brood rearing and consequently greater numbers of bees result in a rapid reduction in the amount of stored honey and a concomitant increase in empty comb. During the major nectar flow, the empty comb is utilized for honey storage. Because of this, empty comb becomes increasingly less available, as the year progresses. The colony uses its stored honey primarily in the maintenance of overwintering adult bees through fall and winter, which again empties storage comb. These changes in the availability of empty storage comb in a honeybee nest coincide temporally, and vary directly, with changes in the availability of nectar in the field. Availability of empty comb also influences nectar-harvesting. High levels of nectar-harvesting intensity and the acceptance of primarily highly-rewarding nectar sources result in more stored honey during the major nectar flow, while lower levels of intensity and the acceptance of lower quality nectar sources result in more stored honey both earlier and later. Therefore, the amount of empty comb in the nest regulates the intensity of nectar foraging by resident bees, and also regulates their selection of nectar sources in ways that result in the maximum storage of honey throughout the nectar season.

The adaptive value for honeybees of intense nectar-harvesting from highly rewarding nectar sources during a major flow, and less intense nectar-harvesting from available sources during a minor flow, are clear. In specific instances, nectar-harvesting intensity and nectar-source selection could conceivably be inappropriate to flow conditions. For example, a colony with storage combs filled with honey part way through an unusually strong and prolonged nectar flow would have a lower nectar harvesting intensity than appropriate. This situation would only serve to increase the number and the probability of the survival of swarms issued by the colony. The parent colony would not suffer a disadvantage, since colonies establish themselves in selected cavity volumes (Seeley, 1977; Seeley & Morse, 1976) that are presumably large enough to hold food reserves capable of supporting the survival of the colony through the season of dearth. Adversity (unusual weather, disease, or predation) may result in a colony with large amounts of empty comb (and low food reserves) at a time before or after the main nectar-flow period. Such a colony is in danger of starvation, and a high level of

nectar-harvesting (and acceptance of only high-quality nectar sources) are not inappropriate. Maximum foraging on poor-quality nectar sources would not provide sufficient food reserves for survival. However, finding and intensely exploiting a good quality nectar source (perhaps the honey reserves of a nearby colony), even though unlikely, would have a greater chance of ensuring the colony's survival than foraging on poor quality nectar sources.

Different amounts of empty comb appear to lead to differences in foraging strategy. Data from Experiments 2 and 3 indicate that bees strongly stimulated by empty comb tended to accept only the more rewarding food resources. At the same time, their threshold for recruitment dancing was such that they tended to dance for all sources meeting their minimal requirements for nectar-gathering. Generally, these two tendencies resulted in the selection of only highly rewarding nectar sources, and through dance communication in exploiting those resources with group-foraging behaviour. Alternatively, less stimulated bees were more willing to accept less rewarding food sources. At the same time, their threshold for recruitment dancing was such that they tended to dance only for sources better than their minimum requirements for gathering. These two tendencies resulted in less stimulated bees accepting less rewarding nectar sources, and foraging as solitary individuals still capable of group exploitation through dance communication of better sources if they were found.

Johnson and Hubbell (1975) also advanced the argument that group-foraging behaviour is better suited to exploiting highly rewarding nectar sources, while solitary foraging is better suited to exploiting less rewarding sources. They found two species of stingless bees (*Trigona*) foraging on the same plant species. One *Trigona*, using group foraging, collected nectar from large dense growths of the nectar-plant species; the other *Trigona*, using solitary foraging, collected nectar from much more scattered growth of the same plant species. Through regulation by empty comb, honeybee colonies use a seasonally appropriate combination of both foraging strategies.

The regulatory function of empty comb is facilitated by the effect of temperature on the release of comb volatiles. Nectar flows tend to be stronger when ambient temperatures are high (Shuel, 1975). While the brood area is maintained at *c.* 35°C, the remainder of the nest is more influenced by ambient temperatures (Farrar, 1963). Since the vapour pressure of comb volatiles is greater at higher temperatures (Rinderer, 1981), ambient temperatures contribute to the regulatory function of empty comb by causing comb to release greater or less amounts of volatiles as appropriate. Furthermore, during cold periods when no nectar is available, the vapour pressure of volatiles is low. This would extend the period of usefulness of comb in a nest.

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