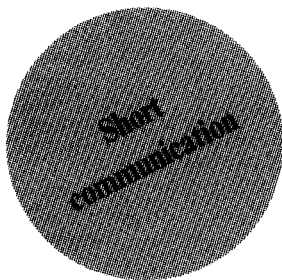


Senescence and learning in honeybee (*Apis mellifera*) workers

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Abstract. Foraging by honeybee workers was investigated from the moment of the first foraging flight until death. To minimise the influence of factors other than senescence the foragers were trained to collect food from an artificial flower close to their hive. During each foraging trip the workers repeatedly visited an artificial flower, collecting one microlitre of 50% sugar solution per visit. During the first 50 flights the mean time taken to collect one portion of food decreased significantly and the number of visits to the artificial flower per flight increased significantly. During flights following the 50th flight, the mean time taken to collect one portion of food increased significantly and the number of visits to the artificial flower per flight decreased significantly. The results confirm earlier observations that the foraging behaviour of honeybee workers is not only influenced by learning, but also by the effects of senescence.

Key words: senescence, learning, foraging, honeybee, *Apis mellifera*

Little is known about senescence in honeybee workers and reports are often contradictory. Some authors suggest that senescence is determined by fixed energetic resources and depends on the amount of time spent in flight (Neukirch 1982, Schmid-Hempel et al. 1985) but others suggest quite the opposite (Guzman-Novoa et al. 1994). Dukas and Visscher (1994) showed that the behaviour of foragers changes near the end of their life. However, in another article the same authors argue that mortality of workers is independent of age, suggesting an absence of senescence (Visscher and Dukas 1997).

The aim of this study was to determine how learning and senescence influence behaviour of honeybee workers, from the moment of their first foraging flight until their death. In the experiment of Dukas and Visscher (1994) only one fourth of foragers attained the age at which signs of senescence become visible. This was caused by mortality independent of senescence and related to unfavourable weather, predators or losing the way to the nest. To exclude this mortality an artificial flower placed near one hive was used.

One colony of about 3,000 workers kept in an observation hive in south Poland was used for the experiment carried out from June to August 1997. The queen (*Apis mellifera carnica*) was instrumentally inseminated with semen from one drone of the same subspecies. Newly emerged workers were marked with individually numbered plastic tags. Before the workers started foraging they were trained to collect food from an artificial flower

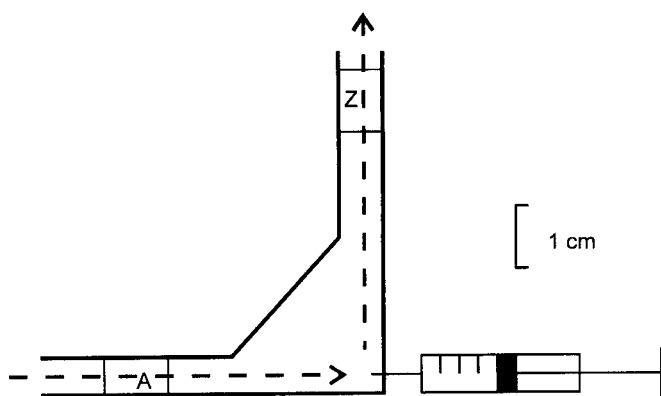


Fig. 1. Schematic of the artificial flower. The dashed line indicates the route of a worker visiting the artificial flower. A forager enters through the barrier at the entrance (A) and collects one portion of sugar solution. To collect the next portion the forager has to go out through the barrier (Z) and re-enter the artificial flower.

placed about 10 m from the hive. The main part of the artificial flower was a tunnel (Fig. 1). When a worker entered the tunnel a syringe pump automatically delivered one microlitre of 50% saccharose solution. Barriers placed at the entrance and exit of the artificial flower allowed movement in only one direction; to receive the next portion of food the workers had to exit the tunnel, take off, fly at least 40 cm and re-enter the artificial flower. Inside the artificial flower the workers could only walk. One of walls of the artificial flower was transparent so that the workers could see the droplet of the solution. During training the workers were taken from the hive and honey solution was offered to them deeper and deeper in the artificial flower. The training was successful only when natural sources of nectar were scarce. Only one worker was trained at any one time. The worker was additionally marked with a piece of aluminium foil, which was detected by an optical sensor. This prevented unmarked workers from entering the artificial flower. The bees typically visited the artificial flower repeatedly before returning to the hive, and were allowed to forage until the end of their life. A computer automatically recorded the time of day when the worker entered the artificial flower. If the time between two successive visits exceeded 100 s it was assumed that the worker had returned to the hive. Six individual workers were studied, but one worker died of causes independent of senescence and was excluded from the analysis. Foragers learn at the beginning of the foraging career and undergo senescence near the end of life. I have arbitrarily assumed that after the 50th flight effects of learning can be neglected. The first 50 flights were used to verify the presence of learning and the later ones were used to verify the presence of the effects of senescence. Spearman's coefficient of rank correlation (Sokal and Rohlf 1981) corrected with Bonferroni procedure (Rice 1989) was used in the statistical analysis. In less than 1% of the cases a worker visited the artificial flower fewer than 5 times per flight. Those cases were excluded.

Workers foraged on average (\pm SE) for 11 ± 1.2 days and performed 319.8 ± 35.7 flights, during which they visited the artificial flower 7838 ± 642.3 times (Table I). During the first 50 flights the mean time to collect one portion of food decreased significantly in three cases and the number of visits to the artificial flower per flight increased significantly in three cases (Table II, Fig. 2). The time required to fly from the artificial flower to the hive and back did not change significantly during this time. During flights following the 50th flight the time necess-

TABLE I

Foraging careers of workers visiting the artificial flower					
Worker No.	Age at start of foraging (days)	Duration of foraging career (days)	Number of flights	Total number of visits	
G28	18	7	273	5893	
B92	18	10	243	7486	
B14	24	12	271	7797	
R47	28	12	414	8110	
R13	18	14	398	9904	

ary to collect one portion of food increased significantly in four cases and the number of visits to the artificial flower per flight decreased significantly in three cases. The time required to fly from the artificial flower to the hive and back increased significantly in four cases (Table III, Fig. 2). All significant relationships remained significant even after application of the sequential Bonferroni procedure.

The number of visits to the artificial flower per flight changed abruptly during the last flights of workers (Fig. 2). On the other hand, the time to collect one portion of food and the time required to fly from the artificial flower to the hive and back, although with some variations, were changing steadily during flights following the 50th flight. This suggests that senescence affects behaviour of workers not only during their last flights but also much earlier.

Honeybee workers start foraging when they are 10-27 days old (Seeley 1982) and in summer they live on average 15-38 days (Winston 1987). This is consistent with my results. The changes in the behaviour of foragers described here are similar to those observed by Dukas and Visscher (1994). In that study both learning and senescence were observed, but the period of learning was shorter in the present study. This probably reflects the difference between foraging in a natural environment and foraging in experimental settings. The changes in foraging behaviour at the beginning of a bees foraging career most probably are the effects of learning. It is known, however, that insects chemically mark visited flowers and use this information during foraging (Giurfa 1993, Goulson et al. 1998). Because I did not remove the chemical signals, it is possible that the changes at the beginning of foraging were caused by the accumulation of

TABLE II

Correlation between variables describing foraging of workers and number of flight, for flights Nos. 1-50. R, Spearman's coefficient of rank correlation; P, significance level

Worker No.	Mean time to collect one portion of food		Number of visits per flight		Time to fly from flower to hive and back	
	R	P	R	P	R	P
G28	-0.521	0.000	0.373	0.008	-0.106	0.471
B92	-0.200	0.164	0.571	0.000	-0.206	0.161
B14	-0.227	0.113	-0.242	0.091	0.313	0.069
R47	-0.648	0.000	0.600	0.000	-0.145	0.319
R13	-0.513	0.000	0.253	0.076	0.016	0.913

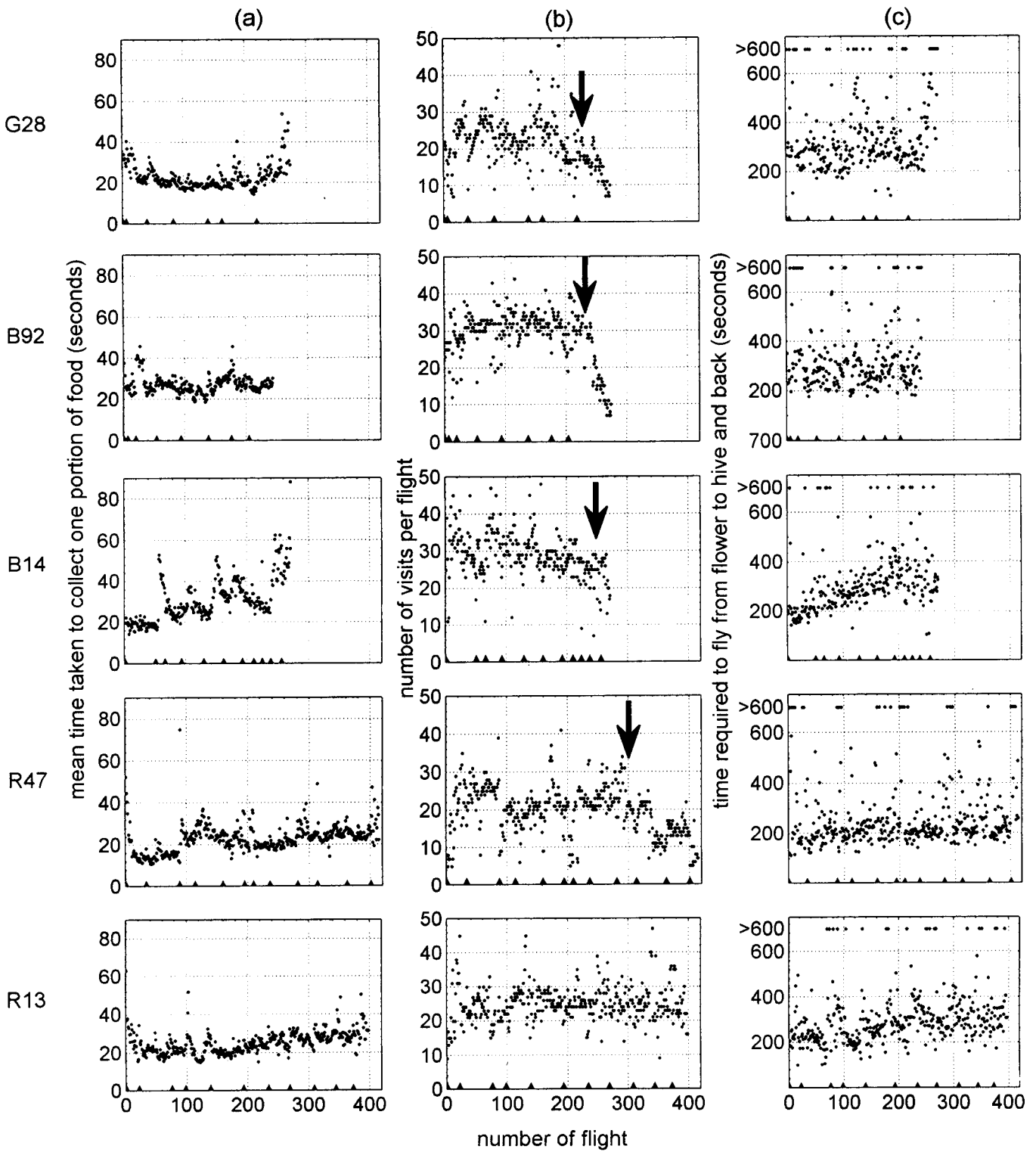


Fig. 2. Mean time to collect one portion of food (column a), number of visits per flight (column b) and time required to fly from the artificial flower to the hive and back (column c) during consecutive flights from the beginning of foraging to death. Each row corresponds to one worker, the number of which is indicated at left. Arrows indicate abrupt changes in the number of visits to the artificial flower per flight. Triangles at the horizontal axes indicate beginning of consecutive days of foraging.

TABLE III

Correlation between variables describing foraging of workers and number of flight, for flights Nos. >50. R, Spearman's coefficient of rank correlation; *P*, significance level

Worker No.	Mean time to collect one portion of food		Number of visits per flight		Time to fly from flower to hive and back	
	R	<i>P</i>	R	<i>P</i>	R	<i>P</i>
G28	0.395	0.000	-0.535	0.000	0.296	0.000
B92	0.126	0.081	-0.065	0.370	0.087	0.273
B14	0.472	0.000	-0.479	0.000	0.318	0.000
R47	0.337	0.000	-0.415	0.000	0.175	0.001
R13	0.665	0.000	0.005	0.928	0.323	0.000

those signals or at least were partly related to that phenomenon.

The age-dependent changes of behaviour of workers near the end of their life can be caused by physiological effects such as degradation of enzymatic mechanisms of carbohydrate metabolism (Neukirch 1982), changes in the nervous system (Crnjar et al. 1990, Withers et al. 1993), or degradation of non-regenerating morphological structures e.g. wings (Cartar 1992). The abrupt changes in behaviour of foragers during their last flights (Fig. 2) suggest that death was caused by senescence and not by unfavourable weather, predators, or losing the way to the nest. It cannot be ruled out, however, that the longevity of foragers was affected by pathogens.

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