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Dependence of the Life Span of the Honeybee (*Apis mellifica*) upon Flight Performance and Energy Consumption

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Summary. The life span of worker-honeybees is determined by the duration of the hive-period and of the foraging period (Figs. 1, 2). The duration of the foraging period is regulated in the following way: Total flight performance of the individual bee seems to be fixed. Daily flight performance strongly affects total flight duration. High daily flight performance decreases maximal flight duration and vice versa.

Foragers accumulate the highest glycogen reserves in the flight muscles compared to other stages (Figs. 3, 4). They use these reserves to overcome starvation or when growing old. Young foragers are able to restore glycogen reserves after sugar intake, whereas old foragers were found to have a reduced glycogen synthesizing ability (Fig. 5).

The results indicate that bees exhaust their energy-supplying mechanisms after a definite total flight performance.

Introduction

A worker-honeybees hive period, comprising a variety of physiological functions, is followed by the foraging period involving high expenditure of energy. The duration of these two periods is variable and adapted to the changing requirements of the community (von Frisch 1923; Lindauer 1952). This raises the question as to whether such variations have any influence on ageing and longevity of the worker bees. The different life span of summer- and winter-bees depends on the prolonged hive-period of the latter (Maurizio 1950); 98 percent of all bees are known to die during foraging (Lundie 1925). For this reason ageing and death might be initiated by intense flight in the later part of the life span. In addition, variations in the average life span are found in summer-bees (Free and Spencer-Booth 1959).

The foraging period might not only be characterized by its duration, but also by the total flight performance achieved. The relationship between flight duration and flight performance is of special interest.

Bees are asynchronous flyers and use only carbohydrate as fuel (Sacktor 1970). In addition to the carbohydrate content of the ventriculus there are glycogen reserves which increase during development from the newly hatched bee to the foraging bee (John 1958).

Flight duration in a number of insect species depends directly upon the amount of glycogen available (Miquel 1971; Rockstein 1950; Johnson and Rowley 1972). In ageing insects glycogen reserves may be diminished or the ability to metabolize glycogen in sufficient amounts becomes limited. Detailed data concerning the glycogen reserves of old bees are still lacking.

This paper deals with the life span and duration of foraging in summer bees living both under open-air conditions and continuous light conditions in a flight room. The glycogen content in the flight muscles was determined in bees of various ages, and glycogen synthesis in young bees, foragers and old bees was studied using tracer methods.

Materials and Methods

Life Span and Onset of Foraging of Summer Bees Under Open-Air Conditions. A five-comb hive of *Apis mellifica carnica* was supplied daily with 100 individually marked young bees from May to August. The marked bees had been hatched in an incubator at 34 °C. The hive was placed in an orchard. All bees were forced to walk to the hive entrance along a gangway covered by a glass roof. All marked bees departing from and reentering the hive were recorded daily between 10.00 and 12.00 a.m. from May to September.

The first flight of every marked bee was regarded as onset of foraging, the time interval between first and last flight representing duration of flight.

About 50% of all bees introduced into the hive originally could be recorded in this way.

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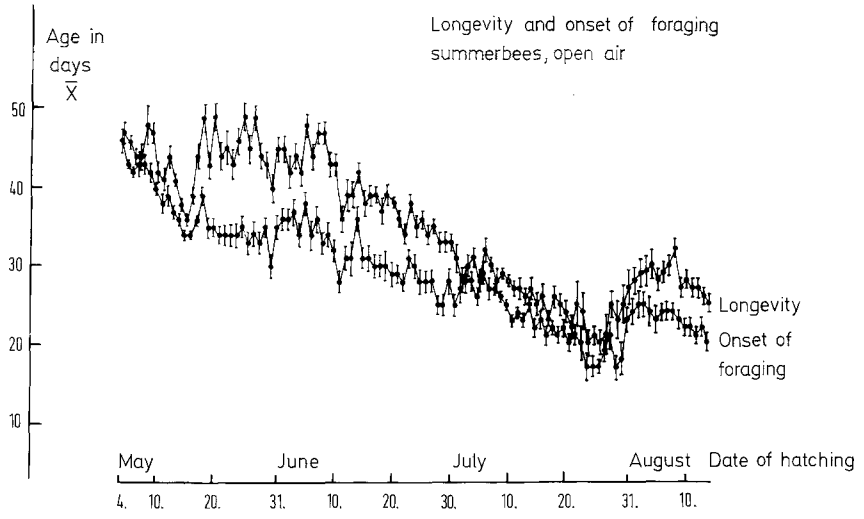

Fig. 1. Relation between date of hatching, onset of foraging and life span of bees in open-air conditions. Means ± S.E.M. Total number of bees observed: 4614

Life Span and Onset of Foraging of Bees Kept in a Flight Room. The flight room (2 × 3 × 2 m) was provided with controlled conditions of light (continuous illumination at 1,300 lx measured at 1 m height (no UV)), temperature (27 ± 3 °C), and relative humidity (55 ± 9%). Food, in the form of 1 M sucrose solution and dried, grounded pollen and water were available *ad libitum*. 50 individually marked young bees were introduced into a five-comb hive daily from May to August. Flying bees were recorded daily at the hive entrance and at the feeding place. Dead bees were collected every evening. 75% of the marked bees were relocated.

Flight Performance and Flight Duration of Bees Under Open-Air Conditions. During a period of 3 weeks all young bees hatched from an open-air hive were marked individually. Foragers were trained to visit a feeding place at 1,500 m distance from the hive in order to recruit the marked bees to the feeding place. The bees were offered a 2 M sucrose solution for 2 h per day. In a second study in the following year the bees were fed for 5 to 8 h per day. All approaches of marked and unmarked bees were recorded. The age of every marked forager and the flight performance and flight duration of all foragers could be determined in this manner.

The mean temperatures during the time of investigation in both years were 25 ± 4 and 21 ± 4 °C, respectively.

Quantitative Determination of Glycogen. In June, bees of known physiological condition from an open-air hive and bees of known age from the flight room were studied. Old bees, whose inability to fly had been proved previously, were collected from the floor of the flight room. Foragers were starved by caging them individually in wooden cases until they become unable to fly. All bees were killed by freezing in liquid nitrogen.

The glycogen reserves in the flight muscle were determined according to Roe and Dailey (1966).

Sugar Utilization and Glycogen Synthesis in Relation to Age and Flight Performance. Young bees were collected immediately after hatching and foragers were captured immediately after landing at the feeding place (to minimize dilution of labelled sucrose by sugar solution). Old bees and starved foragers, whose ventriculus is almost empty in most cases, were obtained as mentioned in the preceding paragraph.

All bees were held at the thorax without anaesthesia and were fed 20 µl 1 M sucrose solution (2.78×10^5 dpm per bee). Subsequently the bees were placed individually into wooden boxes in an incubator at 34 °C. After varying times they were killed by freezing in liquid nitrogen. The ventriculus, midgut, one half of the flight muscle and the remaining carcass were each dissolved in 1 ml Soluene 350 (Packard) for 24 h at room temperature. After mixing with 10 ml Dimilume 30 (Packard), the radioactivity was determined in a Packard Tri Carb scintillation counter. Each value is expressed as the percentage of the total activity applied per bee.

To determine the amount of labelled sugar incorporated into glycogen, the second half of the flight muscle was treated according to Roe and Dailey (1966). The isolated glycogen was dissolved in 0.1 ml water and 1 ml Soluene 350 and radioactivity measured as above.

Foragers were treated analogously, half of them being kept resting with tarsal contact for 20 min. The other half was made to fly against an airstream of 4 m/s for the same time interval. All bees were frozen immediately afterwards and studied as described above.

^{14}C-Sucrose (uniformly labelled) was obtained from Amersham Buchler, Braunschweig.

Statistics. Statistical comparisons were made using a test according to Tukey (in: Sachs 1973). Differences between age groups were considered to be significant when $P = 95\%$.

Results

Life Span and Onset of Foraging in Summer Bees Under Open-Air Conditions

The life span depends almost exclusively upon the duration of the hive period, whereas the duration of the foraging period remains constant to a large degree (Fig. 1). Two series of observations in two consecutive years demonstrated that the highest life spans are attained by bees hatched in May and June, and the shortest life spans by bees hatched in July. The onset of the foraging period runs nearly parallel to the curve obtained for total life span in each case.

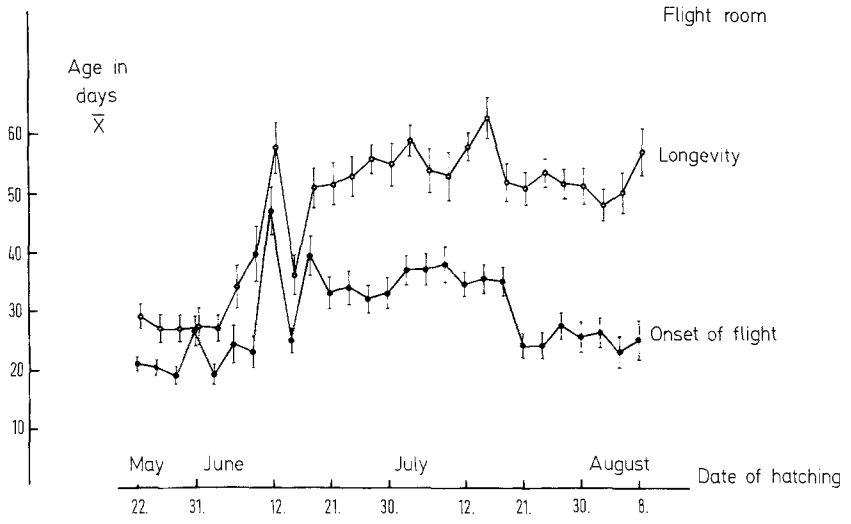

Fig. 2. Relation between date of hatching, onset of foraging and life span of bees kept in a flight room. Means ± S.E.M. Total number of bees observed: 991

Life Span and Onset of Foraging of Bees Kept in a Flight Room

During May and the first half of June, the life span of the worker bees is shortened, possibly caused by the changed environment (Fig. 2). Subsequently, there appeared a marked increase of the average life span. However, this is not due to a later onset of foraging. On the contrary, the foraging period is prolonged, its onset being similar to that of open-air bees.

Flight Performance and Flight Duration of Bees Under Open-Air Conditions

The bees which flew for 2 h per day achieved an average flight performance of 240 ± 166 km, maximum flight performance being 838 km. On average the 141 foragers observed flew 10 ± 5.6 days, maximum flight duration being 30 days. The data of all bees give an average distance of 21.5 ± 6.9 km flown per day per bee.

Even when a very high flight performance was achieved by an individual bee, a reduction of efficiency towards the last days of life was not observed.

Flight duration and flight performance were highly correlated ($r=0.88$; $n=141$). On the other hand, the chronological age of the bees at the onset of foraging did not influence flight duration ($r=0.05$).

In a second experiment the daily feeding interval was extended to 5–8 h per day. In this case, the 32 foragers observed flew an average of 487 ± 266 km. The average flight performance was twice that of the first series, maximum flight performance being again ca. 800 km. The bees attained a maximum flight duration of 13 days, average flight duration being 8 ± 3.5 days. On average 59 ± 13.7 km per day and bee were flown.

None of the bees showed a drop in performance in the last days of their life span.

Flight duration and flight performance were again highly correlated ($r=0.97$; $n=32$); likewise, flight duration and chronological age were independent in these bees ($r=0.07$).

Glycogen in the Flight Muscle at Different Physiological Conditions and Ages

The flight muscles of newly hatched bees, building bees, nursing bees and foragers reared in open-air conditions showed an increasing glycogen content (Fig. 3), attaining a maximum in foragers.

Analogous studies of bees of known chronological ages kept in the flight room also demonstrated an increasing glycogen content with age (Fig. 4).

Aged bees, which were obtained in the flight room only, showed a strongly reduced glycogen content in the flight muscles, similar to starved foragers regardless of age (Fig. 4).

Sugar Utilization and Glycogen Synthesis in Bees of Different Ages

In order to observe the distribution and utilization of ^{14}C-sucrose in the worker bees and to compare sugar utilization and glycogen synthesis in the different age groups, it would be desirable to study time intervals greater than 24 h. However, because of the high mortality of old bees – mortality after sugar intake being zero in all other groups – this comparison is restricted to a maximum interval of 24 h.

Immediately after intake of labelled sucrose 99 to 100% of the activity was present in the ventriculus as expected. After 24 h (Table 1) the ventriculus contained only a small fraction of the total activity, but

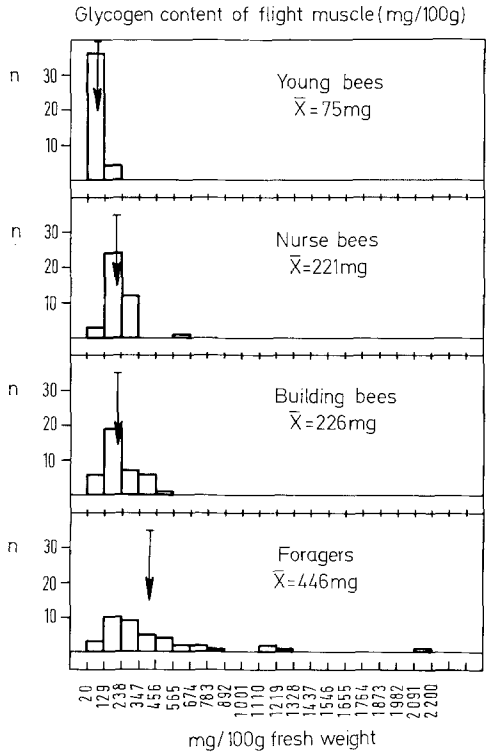

Fig. 3. Glycogen content in the flight muscles of bees in open-air conditions of various physiological stages. Average values are indicated by arrows. $n=40$ per group

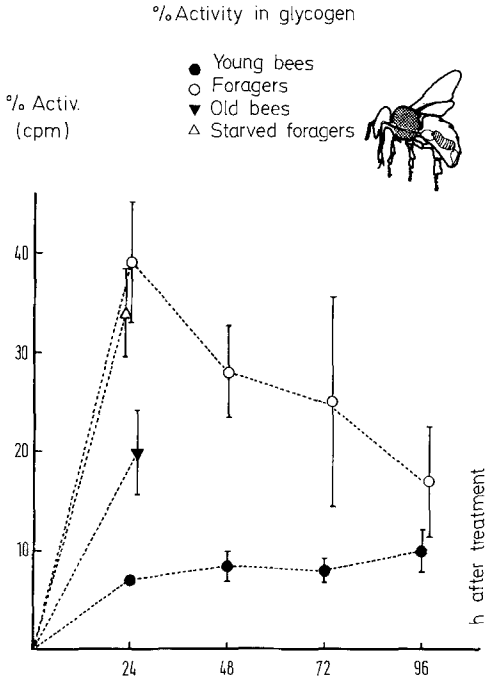

Fig. 5. Incorporation of ^{14}C-sucrose into glycogen. Each bee was fed 20 µl of labelled sucrose solution. The total radio activity appearing in flight muscle after 24 h was assumed to be 100%. Percentage of activity found in isolated glycogen is shown. Means ± S.E.M. ($n=10$ to 15)

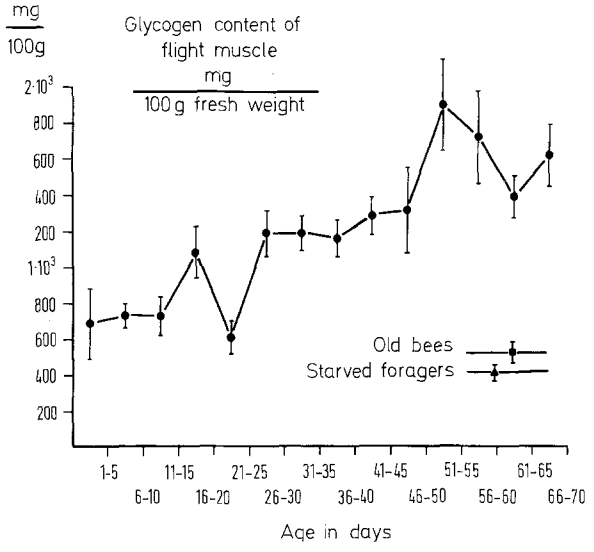

Fig. 4. Age dependence of glycogen content in the flight muscles of bees kept in a flight room. The values for old bees and starved foragers are indicated at the lower right (not related to age). Means ± S.E.M. ($n=10$ to 20)

variability was too great to allow any conclusions to be reached regarding differences between the groups.

In the midgut there appeared to be comparable amounts of radioactivity in foragers, starved foragers and old bees, whereas young bees retained a significantly greater quantity.

With respect to total activity incorporated into flight muscle, young bees again stored significantly higher amounts of labelled sugar compared to the three other groups.

If the percentage of radioactivity incorporated into glycogen is related to the total activity accumulated in the flight muscle, a most striking age dependence is apparent: After 24 h, foragers and starved foragers incorporated the same amount of radioactivity into glycogen, both also showing comparable quantities of labelled sugar in the flight muscle. In contrast, young bees incorporated very small amounts of ^{14}C-labelled sugar into glycogen. Old bees apparently had a reduced ability to utilize the sugar intake when compared to foragers; incorporation of label into glycogen was decreased or delayed despite their forager-like uptake of radioactivity into flight muscle (Fig. 5).

Influence of Flight Activity on Sugar Utilization and Glycogen Synthesis

Resting and flying foragers showed a significant difference in radioactivity remaining in the ventriculus after 20 min. In all parts of the body the amount of radioactivity was essentially the same in both

Table 1. Distribution of radioactivity 24 h after feeding ^{14}C-sucrose to bees of various ages. Data expressed as percent of total radioactivity; 'Glycogen synthesis' is defined as the percentage incorporated into glycogen, of the total radioactivity found in the flight muscle. Means ± S.D.

	% of applied radioactivity				Glycogen synthesis	*n*
	Ventriculus	Midgut	Flight muscle	Glycogen		
Young bees	5.6± 6.1	4.4±1.3	8.7±3.0	0.7±0.2	7.0± 1.7	10
Foragers	8.0±12.1	1.5±0.6	2.6±1.6	1.1±0.6	39.0±18.1	13
Starved foragers	6.3± 7.8	1.9±0.7	3.2±1.7	1.1±0.7	34.3±12.6	8
Old bees	1.0± 1.7	1.7±1.3	3.4±2.1	0.8±0.2	19.7±15.2	16

Table 2. Distribution of ^{14}C-labelled sucrose in foragers after 20 min flight or rest. Data expressed as percent of total amount of radioactivity sucrose fed to the bees; 'glycogen synthesis' is radioactivity in glycogen expressed as percent of total radioactivity found in the flight muscle. Mean values ± S.D.

	Ventriculus	Midgut	Flight muscle	Glycogen	Glycogen synthesis	*n*
Flight	59.8±15.2	6.0±2.7	3.6±1.3	0.4±0.3	9.3±6.1	15
Rest	77.7±11.8	3.8±1.9	2.4±1.0	0.2±0.1	9.1±5.7	15

groups of bees. Neither was there a difference in glycogen radioactivity, expressed as a percentage on the basis of total radioactivity transferred to the flight muscle (Table 2).

Discussion

Factors known to affect the length of the hive-period of bees include the brood size (Free and Spencer-Booth 1959) and the amount of pollen and nectar available (Jeffree and Allen 1957).

Bees starting to forage were found to have developed the greatest volume of corpora allata and the highest titers of juvenile hormone (Jaycox et al. 1974). It is also known that artificially high titers of juvenile hormone cause bees to forage earlier which results in a shorter life span (Rutz et al. 1977).

On the other hand, the maximum duration of the foraging period is determined by the daily flight performance. Lower daily foraging requirements mean that foraging can extend to as much as 30 days, whereas higher requirements reduce maximal flight time. Lower flight times of hard-working bees are undoubtedly a result of the limiting effect of high physical activity on the life span of insects (Sohal 1976; Ragland and Sohal 1975).

Uncontrolled flight activity beyond the training times, which would falsify the flight performances, is very small compared to the maximal flight activity within the training times (Martin et al. 1978). Therefore such flights make practically no contribution to the total flight performances. In open-air conditions the probability of accidents may decrease true total flight times. A comparison with flight room bees shows that bees are able to fly for long periods with low performance and low probability of accidents.

However, the chronological age at the onset of foraging has no influence on flight duration and flight performance. This observation is compatible with the findings on life span and onset of foraging: The hive phase exerts a greater influence on life span than the duration of foraging. The data of Maurizio (1950) on winter bees, whose prolonged life span is based on an extended hive phase, give further evidence for the hypothesis that the hive phase is variable within considerably broader limits than the duration of foraging. Just at the beginning of foraging an unknown 'physiological clock' seems to start, the operation of which can be accelerated or delayed by the flight activity of bees.

Bees starting to forage have accumulated the highest amounts of glycogen in their flight muscles. Bees aged by previous flight performance have a reduced or delayed glycogen synthesis combined with a high mortality. During foraging, glycogen reserves could help hungry bees to return home. Such bees are able to replenish their glycogen stores as starved foragers demonstrated. A nectar-laden bee can synthesize glycogen even during flight, an ability also found in mosquitoes (Nayar and van Handel 1971). This phenomenon could be due to a feedback inhibition of trehalose synthetase by elevated trehalose levels following a sugar meal. Excess UDP-glucose now allows glycogen synthesis (Sacktor 1970). However, this chance of recovery is not unlimited. After having

achieved a total flight performance of about 800 km the bees die quickly without a preceding drop of performance.

Working with open-air bees does not allow differentiation between the various causes of death such as starvation, senescence and accidents. Only in old, weak bees taken from a flight room can it be observed that there exists a difference between flight inability due to starvation, with the chance of recovery, and flight inability due to senescence, without a chance of recovery.

These findings suggest that the enzymic mechanisms of carbohydrate metabolism may be exhausted after a certain flight performance (Rowley and Graham 1968). Enzymes of the respiratory chain could also play an important role in age-dependent changes of flight and resting metabolism. Old *Phormia regina* were found to become defective in electron transport (Bulos et al. 1972). Such changes could be expressed by lowered ATP-levels and increased O_2-consumption (Tribe 1967a, 1972). Furthermore, genetically programmed enzyme activities (Rockstein 1972), appearance of inactive enzyme molecules (Burcombe 1972) and a lowered hormonal sensitivity of target cells (Adelman 1971) could be of importance. The mechanisms of age-dependent changes in honeybees are a subject for further investigation.

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