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Department of Biological Basis of Animal Production, Agricultural University of Lublin

JERZY PALEOLOG, GRZEGORZ BORSUK, KRZYSZTOF OLSZEWSKI

Some Factors Influencing the Results of the Cage Tests of a Life Span and Food Intake in Apis Mellifera Workers

Niektóre czynniki wpływające na wyniki testów klatkowych na długość życia i pobieranie pokarmu u robotnic *Apis mellifera*

The worker's life span, as one of the components of colony dynamics, and therefore a component of many economic features (7, 10), was a subject of numerous studies. In order to obtain more reliable and standardized results, the laboratory tests were proposed to evaluate this trait and its genetic background (4,5). However, the results of these studies were different and appeared not to be so standardized as one could wish, probably because they were influenced by numerous genetic and environmental factors even if the tests were performed in the laboratory conditions. In the present study, which is the continuation of the former studies (6) in this field, the effects of some other factors on the worker's life span in comparison with the food intake were studied.

A bee colony is composed of worker bees that are of different genotypes. Most of the economic and functional traits result from the behaviour of the whole colony and researchers tried to learn how the relations between different groups of workers within one colony affect the expression of these traits (1, 8). Therefore, in experiment 1, the purpose of the study was whether the mixing of two different groups of workers in one cage could influence their life span, in other words, if there were any interactions between these two groups that could affect their life span. Additionally, it was attempted to find out how the life story of a brood could affect the life span of workers that emerged latter.

Usually, in the laboratory tests, the mortality of the caged workers was monitored (4, 5), but in a natural colony a queen and drones accompany the workers. Therefore, the queen's influence on the longevity of the caged workers was studied in our former research (6, 9). The other researchers examined the way in which drones could affect workers' life span. Therefore, it was decided, in the present research on the factors influencing the life span of the caged workers (Experiment 2), to separately examine first the singular effect of drones, then the singular effect of a queen and finally also the combined effect of drones + queens.

MATERIAL AND METHODS

In order to evaluate the life span of the worker bees under laboratory conditions, the workers were placed in wooden cages (12.5 x 4.8 x 12.5 cm) with some sections of combs, 50 bees per cage. The cages were kept in the air-conditioned room (temperature 30°C: humidity 65% : 24 hours of darkness). The front, movable wall of the cage was made of glass, so the holes necessary for ventilation, fixing a feeder and handling the bees were made in the sidewalls. Every second day of the test, the bees were supplied with water and candy made of honey and sugar. At the same time, dead bees were counted and the consumption of the candy was monitored.

EXPERIMENT 1

Both native crossbreed (*Apis mellifera mellifera*) and Italian bees (*Apis mellifera ligustica*) were used in order to establish three different groups in each of the two repetitions of Experiment 1. In the first group, only the native bees (NN) and in the second group only the Italian bees (IT) were caged, whereas in the third group, the mixture of 50% of the native and 50% of the Italian bees were settled into each of the cages (NN/IT). It was decided to use IT and NN bees because of their different geographical origin and because it was easy to distinguish the bees of these two breeds when they were caged together (because of their different colouring).

In Repetition 1, performed in early spring, young, no more than 2-day-old bees were picked up directly from the combs. The outward appearance of the worker body was a criterion of that selection. Then, under CO_2 anaesthesias (about 1 min of treatment), the selected workers were settled into the cages. After two days, some dead bees that were probably damaged or poisoned (manipulations / anaesthetic) were replaced by the alive ones. After that, the monitoring of mortality was initiated. In Repetition 2, performed in summer, the combs with just emerging brood were incubated and 1-day-old bees were collected. Then, under CO_2 anaesthesias, the collected bees were placed in the cages in the same manner as in Repetition 1.

Each of the three groups consisted of 15 cages in Repetition 1, and of 20 cages in Repetition 2.

EXPERIMENT 2

In this experiment, the Caucasian crossbreed bees were used in order to establish four experimental groups consisting of 20 cages per group. In the first group, the workers were caged without drones or a queen (W), in the second one, there was a queen but no drones (Q + W), in the third one, there were drones but no queen (D + W) and in the fourth group both drones and a queen were present (Q + D + W). Four to drones a cage were caged in D + W and Q + D + W groups. The bees were settled into the cages in autumn, the same way as in case of Repetition 2 of Experiment 1.

RESULTS AND DISCUSSION

EXPERIMENT 1

Some considerable influence of the repetition (Fig. 1a) on the diversity of the life span in NN, IT and NN/IT was observed because in Repetition 1 the diversity was large, while in Repetition 2 it was really small. Moreover, in Repetition 1, NN bees lived longer than IT bees, whereas in Repetition 2 the life span of the bees of these two breeds was much the same and even more, IT bees lived slightly longer than NN. The patterns of the curves indicating the life span of the workers in a cage show that mortality in Repetition 1 was rather of the linear function shape, while in Repetition 2 the patterns of the curves were similar to the logarithmic shape. In the former studies (6,9), it was also observed that the mortality/longevity curves could be of different shape depending on interactions *genotype x environment*. In order to perform statistical analysis (ANOVA, Duncan), the following characteristics of the examined trait were evaluated: L70%, L50%, L30% (the number of days from emerging to the day in which 70, 50, and 30% of workers survived, respectively). The results shown in Figure 2 statistically confirmed all the findings described above, especially the fact that the differences between NN and IT were big in Repetition 1 performed in early spring, whereas they were very small in Repetition 2 performed in summer. What is more interesting, all these differences resulted from the changes in the life span of IT bees because NN bees showed almost the same life span in Repetition 1 and Repetition 2, whereas IT bees revealed low longevity in Repetition 1 but high in Repetition 2. In early spring, NN bees reared not too many larvae while IT bees raised a lot. Therefore, IT larvae could not be well nursed and fed. In other studies, it was confirmed that the life history of a worker bee, particularly the history of its tasks, could influence its life span (2,7). However, our present results suggest that the life story of the brood a worker emerged from could also affect its life span.

A comparison of the results shown in Figure 1 and in Figure 2 did not reveal any distinct interactions between IT and NN bees (while caged together) because the life span in NN/IT was always intermediate in relation to NN and IT. On the other hand, some slight interactions between NN and IT could not be precluded because the bees from these two groups revealed a more similar life span when they were kept together in one cage than when they were held separately in different cages (Fig. 1b). This very interesting finding needs to be confirmed in the future studies. A situation was not so clear and some interactions between NN and IT could be taken into account when the food intake was analyzed (Fig. 4).

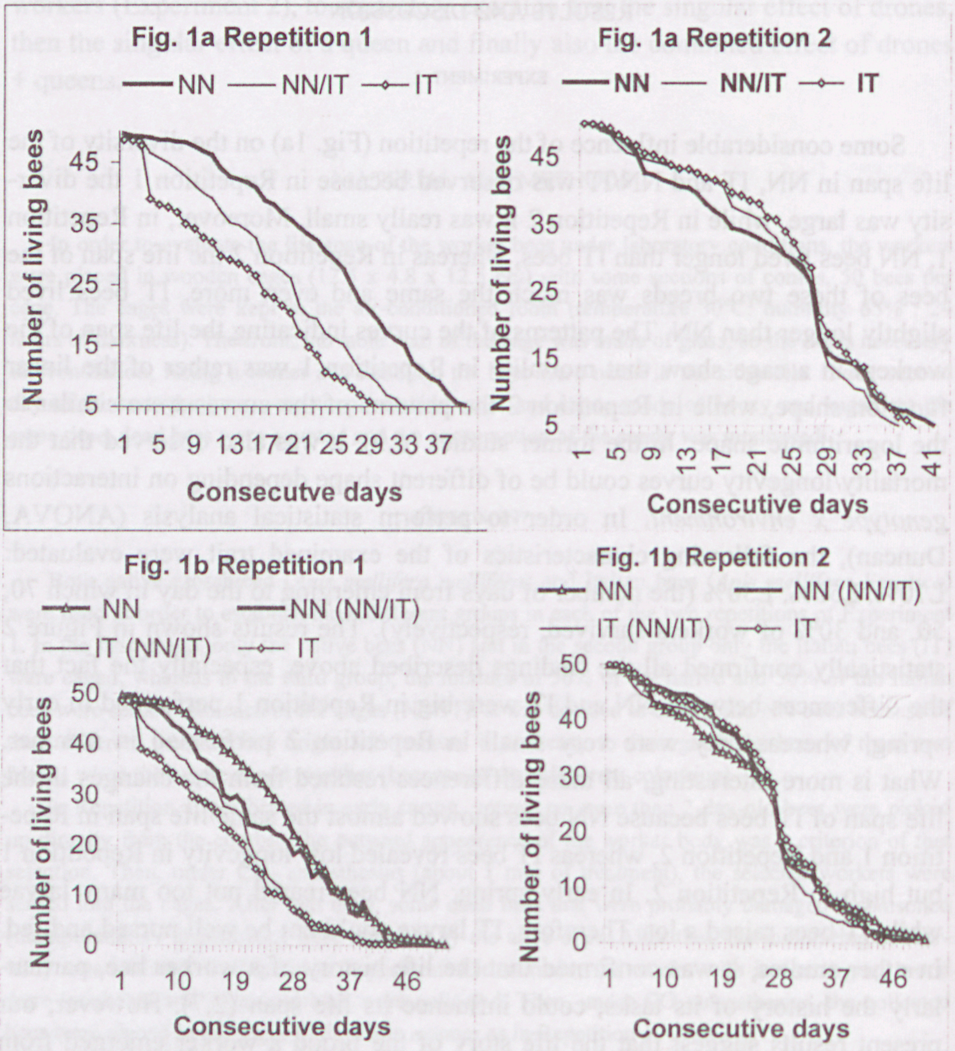

Fig. 1. Life span of the workers measured as a mean number of bees that stayed alive in the cages in the consecutive days of the laboratory test in two repetitions of Experiment 1; NN – only native crossbreed bees in a cage (*A. m. mellifera*); IT – only Italian (*A. m. ligustica*) bees in a cage; NN/IT – admixture (50%) of native and Italian bees in a cage; NN(NN/IT) – number of NN bees in a cage containing admixture of NN and IT bees; IT(NN/IT) – number of IT bees in a cage containing admixture of NN and IT bees; In order to make the comparison with NN and IT possible numbers of bees in NN(NN/IT) and IT(NN/IT) were multiplied by two

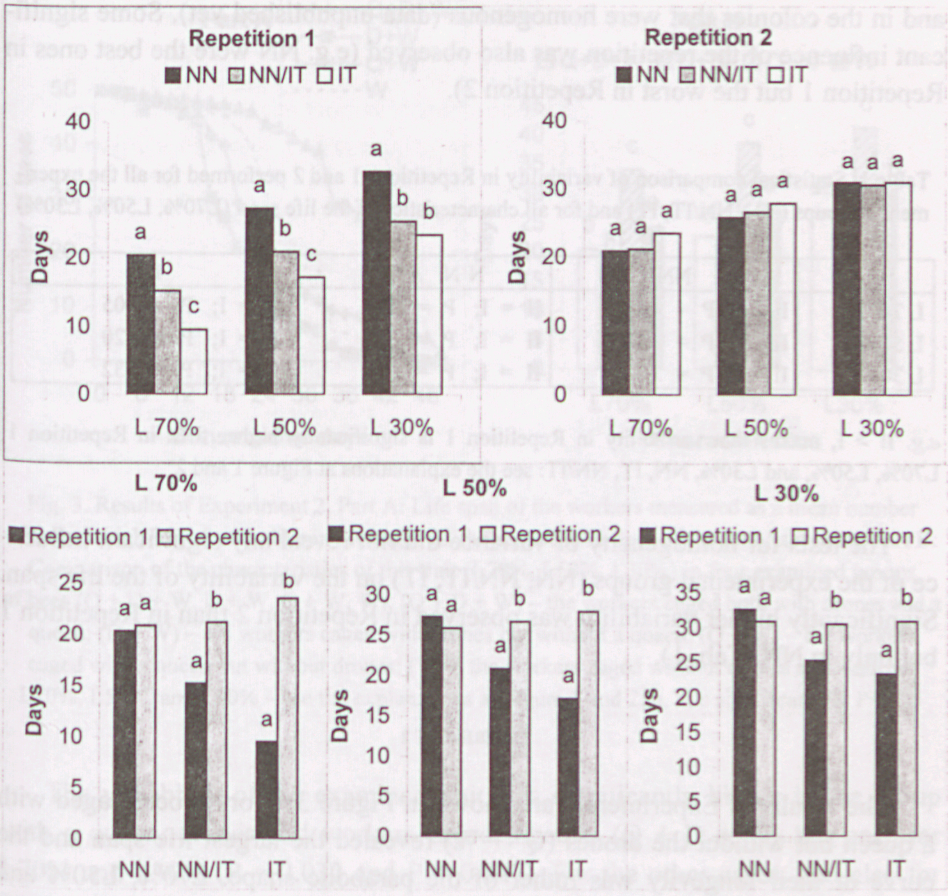

Fig. 2. Comparison of the characteristics of the life span of caged workers (L70%, L50%, L30%) in three examined groups of bees (NN, IT, NN/IT) as well as in two repetitions of experiment 1; NN, IT, NN/IT – see the explanations at Figure 1; L70%, L50%, L30% – number of days from emerging to the day in which respectively 70%, 50%, 30% of workers survived and stayed still alive within a cage; a, b, c significant for $P<0.05$

It was because in Repetition 2 the food intake in NN/IT was more similar to the one observed in IT, both when it was expressed as "intake per cage" and as an "intake per a single bee". What is even more important, in Repetition 1 the value of the examined trait expressed as "intake per cage" was intermediate in NN/IT but when it was expressed as "intake per a single bee" it appeared to be definitely the lowest one (in relation to NN and IT). It was impossible to explain this phenomenon, but we observed a similar situation in the other field experiments. In those experiments, the efficiency of the food intake from the outer feeders was monitored both in the colonies that consisted of admixture of different bees and in the colonies that were homogenous (data unpublished yet). Some significant influence of the repetition was also observed (e.g. NN were the best ones in Repetition 1 but the worst in Repetition 2).

Table 1. Statistical comparison of variability in Repetitions 1 and 2 performed for all the experimental groups (NN, NN/IT, IT) and for all characteristics of the life span (L70%, L50%, L30%)

	NN	NN / IT	IT
L 70%	II > I; P = .045	II = I; P = .407	II = I; P = .405
L 50%	II > I; P = .011	II = I; P = .055	II = I; P = .820
L 30%	II > I; P = .038	II = I; P = .618	II = I; P = .932

e.g. II > I, means that variability in Repetition 1 is significantly higher then in Repetition 1; L70%, L50%, and L30%, NN, IT, NN/IT: see the explanations at Figure 1 and 2

The tests for homogeneity of variance did not reveal any significant influence of the experimental groups (NN, NN/IT, IT) on the variability of the life span. Significantly higher variability was observed in Repetition 2 than in Repetition 1 but only in NN (Tab. 1).

EXPERIMENT 2

The results of Experiment 2 are shown in Figure 3. Worker bees caged with a queen but without the drones (Q + W) revealed the largest life span and the curve of their longevity was rather of the parabolic shape. L70%, L50% and L30% were also significantly the highest ones in Q + W. The curves of the workers' longevity in the other three groups were rather of the logarithmic or similar to the linear shape. Some similar influence of a queen on the shape of the worker longevity/mortality curve had been observed in our previous studies (9). Thus, it was confirmed that the presence of a queen in a cage decreased the early worker's mortality. On the other hand, the company of drones markedly decreased the life span of the workers caged with them and, which is interesting, that phenomenon was visible in both groups, those with and without a queen in a cage (Q + D + W and D + W). Although the workers' life span was slightly longer in the Q + D + W than in the D + W group, the values of L70%, L50% and L30% did not differ statistically between these two groups. Moreover, L70% and L50% were significantly higher in the group where only the workers were kept in cages (W) than in the Q + D + W and D + W groups. Thus, the influence of drones on the worker's life span was stronger than the influence of a queen.

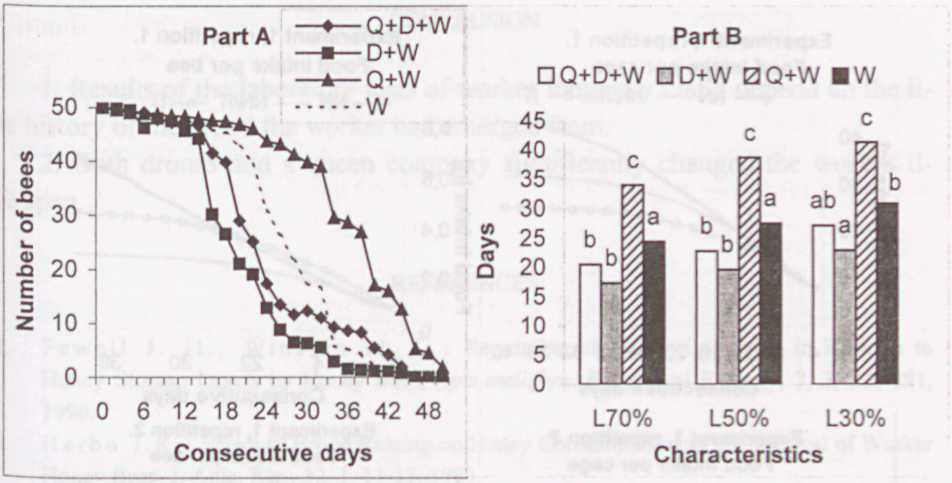

Fig. 3. Results of Experiment 2. Part A: Life span of the workers measured as a mean number of bees, which stayed still alive in the cages on the consecutive days of the laboratory test. Part B: Comparison of the characteristics of this trait (L70%, L50%, L30%) in four examined groups of bees (Q + D + W, D + W, Q + W, W); (Q + D + W) – the workers caged both with drones and a queen; (D + W) – the workers caged with drones but without a queen; (Q + W) – the workers caged with a queen but without drones; (W) – the workers caged without drones and a queen. L70%, L50%, and L30% – see the explanations at Figure 1 and 2; a, b, c significant for $P<0.05$

The variability of the examined trait was significantly higher in the group with a queen, drones and workers caged together (Q + D + W) but only for L70% and L30% ($P = 0.032$ and $P = 0.047$). For the other cases, and also for L50%, variability never differed statistically between the groups.

In the D + W group, workers rather accepted drones, whereas in the Q + D + W group workers mostly eliminated drones. Could the caged workers behave in the same way as the workers in the natural colony meaning eliminating drones when being queenright and not doing it when queenless in autumn? We did not remove the dead drones from the cages and, additionally, the living drones were introduced in order to maintain the pheromone concentration. In the groups with drones (Q + D + W and D + W), a cumulated "food intake per cage" was lower than in the other groups but "food intake per bee" was almost the same in all the groups (Fig. 4). Thus, the food intake (per cage) depended rather on the number of the caged worker bees but did not depend on drone or/and a queen presence in a cage. In other words, the workers which were accompanied by drones lived shorter not because of the necessity of feeding them but because of some other reasons.

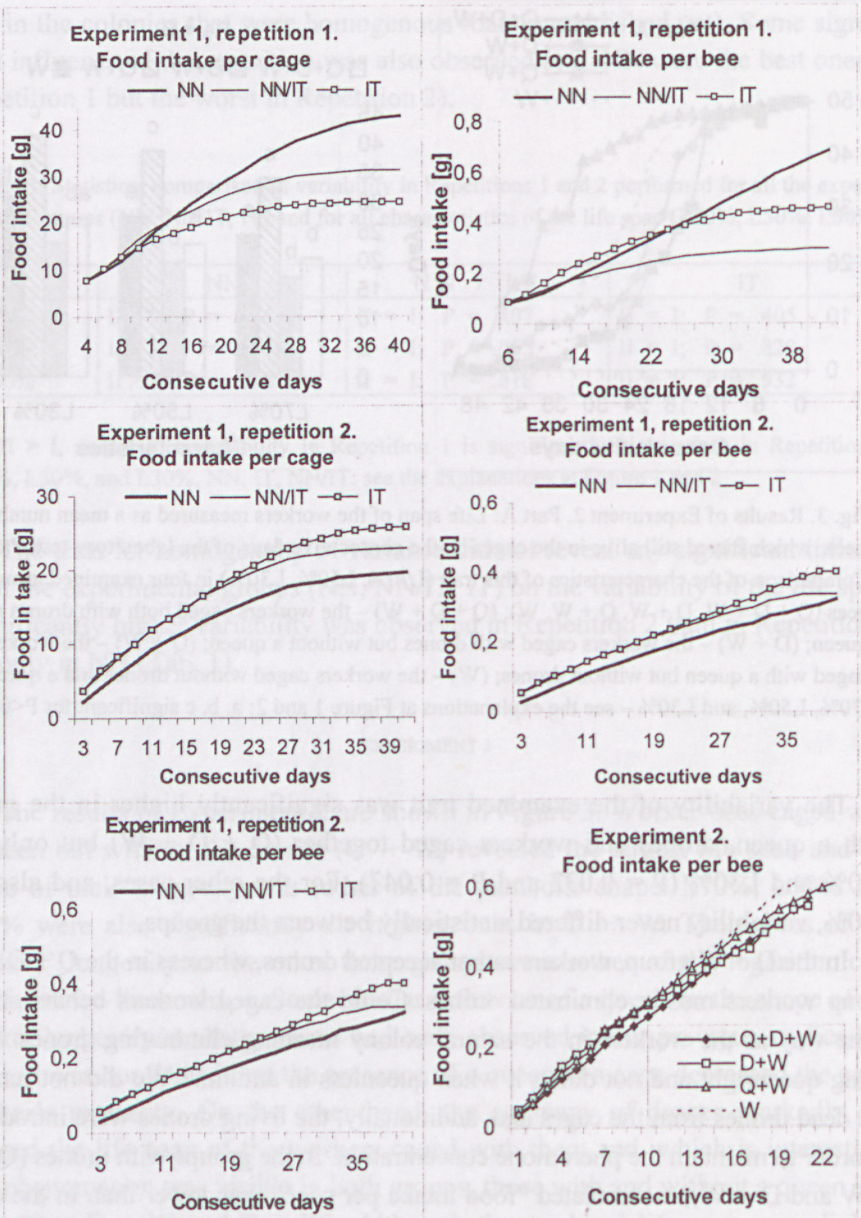

Fig. 4. A mean cumulated food intake in a cage and a single bee in Repetition 1 and 2 of Experiment 1and in Experiment 2; (1) mean food intake was usually calculated "per single cage" (with workers). However, after several days, due to workers' mortality, different numbers of bees were caged in different cages and more bees ate more food. To eliminate that problem, it was decided to calculate additionally food intake "per a single bee"; (2) cumulated food intake means summaring the food intake from the beginning to each of the following days of the test; (3) for explanation of all the symbols used, see figures 1, 2 and 3

CONCLUSION

1. Results of the laboratory tests of worker longevity could depend on the life history of the brood the worker had emerged from.

2. Both drones and a queen company significantly changed the workes life span.

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STRESZCZENIE

W testach laboratoryjnych zbadano, czy oddziaływanie na siebie dwu różniących się pochodzeniem i genotypem grup robotnic (miejscowe – NN i włoskie – IT) wpłynie na długość ich życia, gdy wymieszamy je w jednej klatce oraz jaki wpływ na wyniki takiego doświadczenia będzie miał termin (wiosna/lato) pobrania pszczół do badań (doświadczenie 1). Sprawdzono też, jak obecność w klatkach z robotnicami trutni, matki oraz obu tych postaci razem wpłynie na długość życia robotnic (doświadczenie 2). Nie zaobserwowano znaczących interakcji pomiędzy IT i NN, wpływających na długość życia robotnic (ryc. 1, 2). IT pobrane wiosną żyły krócej niż pobrane latem, czego nie obserwowano u NN. Może IT produkując wiosną więcej czerwiu nie mogły go dobrze odżywiać, co wpłynęło na długość życia wygryzionych później robotnic. Obecność matek wydłużała życie robotnic, a obecność trutni je skracała, zarówno w obecności matki,

jak i bez niej (ryc. 3). Zaobserwowano wpływ interakcji pomiędzy pszczołami NN i IT (zsypanymi w jednej klatce) jak też i pory roku, w której pobierano robotnice do badań, na ilość pokarmu pobranego w grupach NN, IT NN/IT (doświadczenie 1). Obecność matki i trutni (doświadczenie 2) nie wpływała znacząco na tę cechę (ryc. 4). Zmienność długości życia była większa w drugim powtórzeniu (doświadczenie 1), ale tylko u NN (tab. 1). W grupie matki + robotnice + trutnie zmienność była większa w stosunku do innych grup (doświadczenie 2).