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*The Effect of Worker Genotypic Interactions on Correlations Between Results of Different Hoarding Efficiency Tests in Honeybees**

Wpływ genotypowych interakcji między robotnicami na korelacje pomiędzy wynikami różnych testów w pozyskiwaniu pokarmu u pszczoły miodnej

Honey yield is a complex trait comprising both the bees' ability to forage nectar and also to process it into stores. It is more dependent on the collective effort/behaviour/cooperation of the bees belonging to a colony than on the efficiency of individual workers [2]. As a result of polyandry, a bee colony is made up of various worker groups, each having different, genetically determined, behaviour [3]. Natural diversity within a bee colony is further increased as a result of bee drifting [1, 11] and of such beekeeper's activities as exchanging brood combs or worker groups among bee colonies. Therefore, bee colonies are composed of genotypically diverse worker groups entering into interactions with one another. Interactions between worker groups may be of both additive and non-additive character [4], and getting acquainted with those interdependencies is important in the evaluation of the queen values since it helps estimate the value of the colony that is composed of more and less efficient foragers.

In order to simplify the genetic value evaluation of the queens and to eliminate the environmental influence, cage tests were carried out during which food hoarding rate was examined under laboratory conditions [6, 7]. However, some researchers obtained a positive correlation between the field and the laboratory test results, whereas others point out to the lack of such a correlation [5, 7, 9]. Further research concerning the conditions affecting the conformity of

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field and laboratory tests are, therefore, necessary. In the present study, the authors decided to find out whether the considerable genotypic diversity, involving interactions between different worker groups, will exert an influence on the effectiveness of food hoarding and accumulation being assessed by field and laboratory tests. Syrup foraging efficiency and the amount of syrup accumulated in the combs by bee colonies that were placed under the flying tents (field test) were compared with the candy intake rate measured in the labolatory cage tests. In both cases, homogenous worker groups were compared with those composed of a mixture of genotypically diverse workers.

MATERIAL AND METHODS

The bees that were used in the experiments came from four different genetic groups, each of them having the same queen. They were: 1) native bees with an *A. m. mellifera* the component (MM), 2) crossbreeds of a Norwegian queen *A. m. mellifera* and *A. m. caucasica* drones (Nor), 3) *A. m. caucasica* (Cau), 4) *A. m. ligustica* (IT).

FIELD TESTS

Four independent comparisons were carried during two subsequent seasons. In comparisons 1 and 2, MM and IT bees were used. Three types of nucleus colonies were set up for each comparison: the first was homogenous and contained 100% MM bees, the second was a mixture of 50% MM and 50% IT (MM/IT) and, finally, the third was homogenous, composed of 100% IT bees. Each nucleus colony was made up of 2 litres of bees that were placed on two dried and weighed Langstroth frames. The age structure of workers in each group was identical, since bees that were to be settled there were sampled late in the evening upon the completion of flights. Queens kept in the cages made of the queen excluder, which prevented laying the eggs, were then introduced to the so formed colonies. Subsequently, the colonies were transported to a new location and placed individually under mesh flying tents (100 x 200 x 150 cm) together with calibrated feeding stations. The tests were performed under the tents in order to eliminate the competition from other insects. The quantity of sugar syrup (1:1) was recorded daily to estimate the foraging rate, and every second day, all the combs were individually weighed in order to estimate the quantity of the accumulated stores. The measurements were continued for 13 days. Identical procedures were applied in comparisons 3 and 4, except that in comparison 3, MM and Nor bees were used, and Cau and IT bees in comparison 4.

Foraged nectar is stored or used for physiological colony needs and the colony's honey yield depends on the quantity of the foraged nectar and on the effectiveness of the processing it into stores. That is why the foraged syrup processing efficiency coefficient (EP%) was calculated with the formula:

$$EP\% = \frac{\text{Supplies accumulated in combs}}{\text{Syrup foraged from the feeding station}} \times 100$$

LABORATORY TESTS

One-day old bees emerged in the incubator (29°C; H=65%) were anesthetised with CO_2 and then placed in wooden cages (12.5 x 12.5 x 4.8 cm with vents and openings for a feeder with candy), 50 workers per cage. An experimental pattern was the same as in the field tests. In each comparison three groups of 20 cages each were set up. In comparison 1, the cages of the first group contained 100% MM, those of the second, a mixture of 50% MM and 50% IT (MM/IT) and the third 100% IT bees. By analogy, the second comparison comprised MM and IT bees, and the third comparison MM and Nor. Thus, always three groups of cages were set up, two of them containing only one kind of bees and the third one containing a mix.

Monitoring the candy hoarding rate was commenced after a two-day adaptation process during which dead bees (mechanical damage, anaesthesia) were replaced with living ones. In order to do so, feeders were weighed every second day, and candy was systematically replenished. Water was given through the vents every day. The monitoring was carried out for 43 days.

RESULTS AND DISCUSSION

The results of the field test are shown in Fig. 1. In comparison 1 (C1), the MM foraged the most syrup and the IT and MM/IT achieved considerably worse results. In comparison 2 (C2), MM and IT foraged similar quantities of syrup, whereas MM/IT foraged less of it. Therefore, the difference between the results of C1 and C2 consisted in different behaviour of the IT bees, which in C1 foraged large and in C2 small quantities of syrup. MM/IT were consistently worse than MM and always foraged the least syrup. During C1 all bees accumulated the maximum quantity of syrup between the 1st and the 4th days and then the weather deteriorated. As a result of adverse weather conditions, the quantities of the foraged syrup decreased and the stores began to diminish, since the bees started eating the syrup (MM and IT). The MM colony made the most of the good weather period, but its reaction to the weather deterioration was also the strongest. What is interesting, after the initial growth, the quantity of the stores accumulated by MM/IT remained constant despite the worsened weather conditions. During C2, the weather was good and in such conditions IT, whose food stores were growing steadily like those of MM, achieved better results. In the case of MM/IT, after the initial growth the quantity of accumulated supplies remained constant again despite favourable weather. In the third and fourth comparisons (C3 and C4), the most syrup was foraged and stored by the Nor and Cau colonies respectively. In both those comparisons IT achieved the worst results. With regard to accumulating the stores, mixed colonies (Nor/IT and Cau/IT) were no different than the better of the homogenous colonies. As far as the foraging rate is concerned, the Nor/IT colony was slightly worse and the Cau/IT considerably better than the better of the homogenous colonies. It is worth noting that during both C3 and C4 weather conditions were changeable.

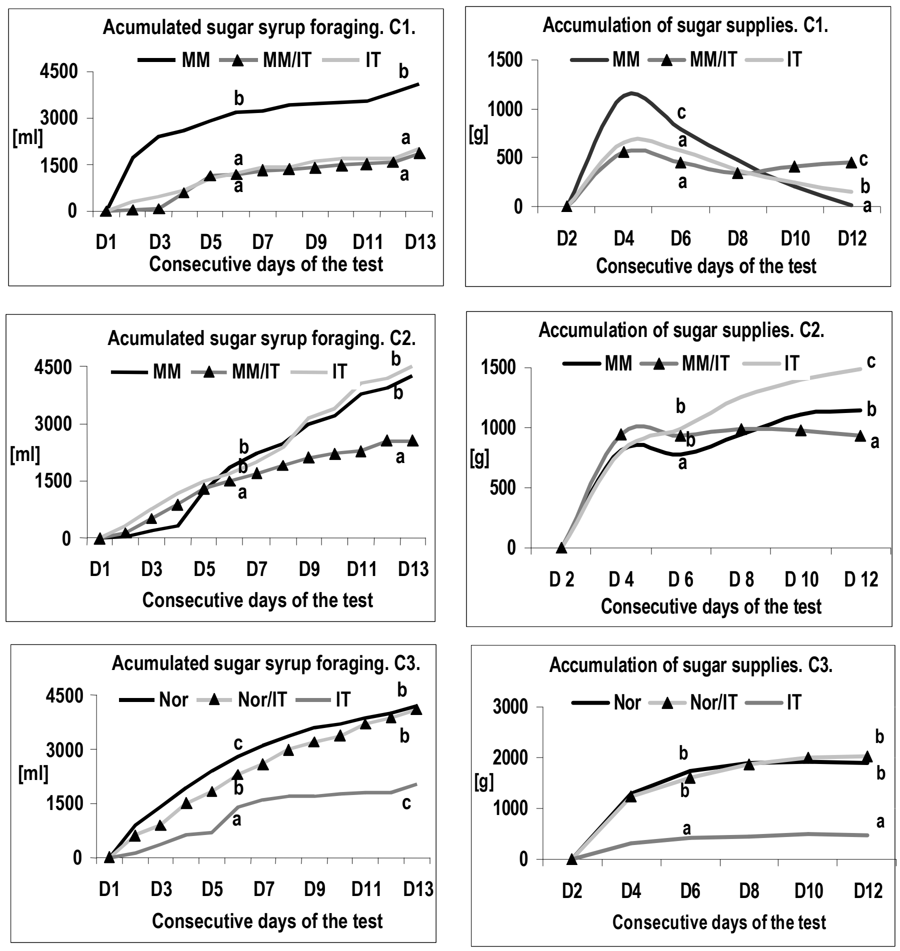

Figure 1. Foraging and accumulation of sugar syrup from the beginning of the field test to each of its consecutive days; MM – nuclei colonies consisting of 100% native bees with the component of *A. m. mellifera*; IT – nuclei colonies consisting of 100% *A. m. ligustica*; Nor – nuclei colonies consisting of 100% bees from the Norwegian queen *A. m. mellifera* inseminated by *A. m. caucasica* drones; Cau – homogenous nuclei colonies consisting of 100% *A. m. caucasica* bees. Mixed colonies were described as follows: e.g. MM/IT stand for the mixed colonies consisting of 50% native bees with the component of *A. m. mellifera,* and 50% of *A. m. ligustica* bees
[a,b,c]Differences between the tested colonies are statistically different ($p \leq 0{,}05$) for results obtained after 6[th] and 12[th] days of the test (ANOVA and Tukey test). C1, C2, C3, C4 – four independent, consecutive comparisons

Because honey yield depends on the effectiveness of the processing of the foraged nectar/syrup into stores the values of EP% were given separately for the two consecutive periods (from the 1[st] to the 6[th] and from the 7[th] to the 12[th] days of the test) in Table 1. The EP% values of individual colonies differed considerably but all of them were higher in the first than in the second period of the test. Most probably, in the first period, the bees did not yet manage to evaporate all the water from the syrup. It is interesting that in the first periods of C2, C3 and C4, the EP% of the mixed colonies (MM/IT, Nor/IT, Cau/IT) was the highest and only in C1 (MM/IT) its value was average. In the second period, the values of EP% in C2 and C3 were the highest and in C1 and C2 they were average. It is clearly visible that the EP% was generally higher in the mixed than in the homogenous colonies.

Table 1. The efficiency coefficient of sugar syrup processing into accumulated supplies (EP%)

	Tested colonies		EP %	
			1^{st} – 6^{th} day	7^{th} -12^{th} day
C1	Homogenous	MM	35^{a}	21^{a}
C1	Mixed	MM/IT	43^{ab}	29^{ab}
C1	Homogenous	IT	46^{b}	34^{b}
C2	Homogenous	MM	42^{a}	29
C2	Mixed	MM/IT	63^{b}	37
C2	Homogenous	IT	58^{b}	36
C3	Homogenous	Nor	62^{b}	48^{b}
C3	Mixed	Nor/IT	70^{b}	52^{b}
C3	Homogenous	IT	29^{a}	29^{a}
C4	Homogenous	Cau	46^{a}	39
C4	Mixed	Cau/IT	52^{b}	33
C4	Homogenous	IT	40^{a}	29
Mixed colonies total			57^{b}	38
Homogenous colonies total			45^{a}	33
All colonies total			49	35

^{a,b}Differences between tested colonies are statistically different for $p \leq 0,05$. The Bliss: $y = \arcsin[x/100^{0.5}]$ transformation was applied. C1, C2, C3, C4 – four independent, consecutive comparisons

The results of the laboratory tests are shown in Fig. 2. During C1, MM foraged the most and IT the least candy. The situation was opposite during C2. During C3, the IT bees were worse than the Nor. Thus, the results obtained in the field and in the laboratory were convergent (with slight exceptions) when homogenous colonies (field) were compared with homogenous worker groups (cage test). On the other hand, unlike mixed colonies assayed in the field tests, the mixed worker groups (MM/IT, Nor/IT) tested in the laboratory achieved average results in comparison with the homogenous worker groups. In that instance, the results obtained in the field and in the laboratory were divergent.

When the expected value of a given trait in a mixed colony is the average value of that trait in the groups of bees constituting that colony, such an interactions between those groups are additive [4]. When the value of the trait in a mixed colony differs from such an average, the interactions between the worker groups are non-additive. If such worker groups are genetically different, the interactions are described as genotypic worker interactions. In the field tests carried out in our study, the values of the mixed colonies was not average in relation to the values of the homogenous colonies in any of the four comparisons. Therefore, the interactions between various worker groups constituting the mixed colonies were non-additive. Furthermore, this phenomenon was much more clearly visible with regard to the process of syrup accumulation than in foraging. It may have been caused by the higher EP% value in the mixed colonies. It is impossible to judge, however, whether the better or the worse forager bees were behaviourally dominant [8, 4]. In the 1st and 2nd comparison, the value of the mixed colony was similar to that of the worse homogenous colony, whereas in the 3rd and 4th it was similar to the value achieved by the better homogenous colony. In other researches [4, 10], mixing diverse worker groups produced various results regarding both defensive behaviour, and the behaviour concerning foraging and accumulation. Hence, in the case of foraging and accumulation of sugar syrup, non-additive interactions between worker bees had a specific character. It is, then, difficult to make conclusions about the potential effects of mixing good and poor foragers within a single colony as the result of such a mixing may depend on the genetic types of bees being mixed and also on the environmental conditions [12]. In the laboratory tests, unlike the field tests, the value of the mixed colonies (candy hoarding) was average in relation to the value of the homogenous groups, and only in comparison 3 it was closer to that of the better homogenous colony. Therefore, in the cages, workers representing various groups tended to co-operate additively and no interactions between them took place.

A comparison of the results obtained in the field experiments with those obtained in the laboratory show that non-additive interactions between various worker groups occurred in the field rather than in the laboratory. It is clearly visible that if the comparison were limited only to homogenous colonies in both test types, the group/colony ranking established on the basis of the field and the laboratory test would be similar. The bees that foraged more candy in the laboratory were also the ones to forage and accumulate more syrup in the field. What is interesting, such a consistency was not observed in the case of mixed colonies and cages containing a mix of bees. Since non-additive interactions between worker groups occurred mainly in the filed, it may be inferred that those interactions may be one of the reasons for the inconsistency between the test results obtained in the filed and in the laboratory. This observation resulting from our experiments is an important contribution to the discussion on the possibility of using laboratory assessment in the process of breeding value estimation in bee queens [5, 6, 7, 9].

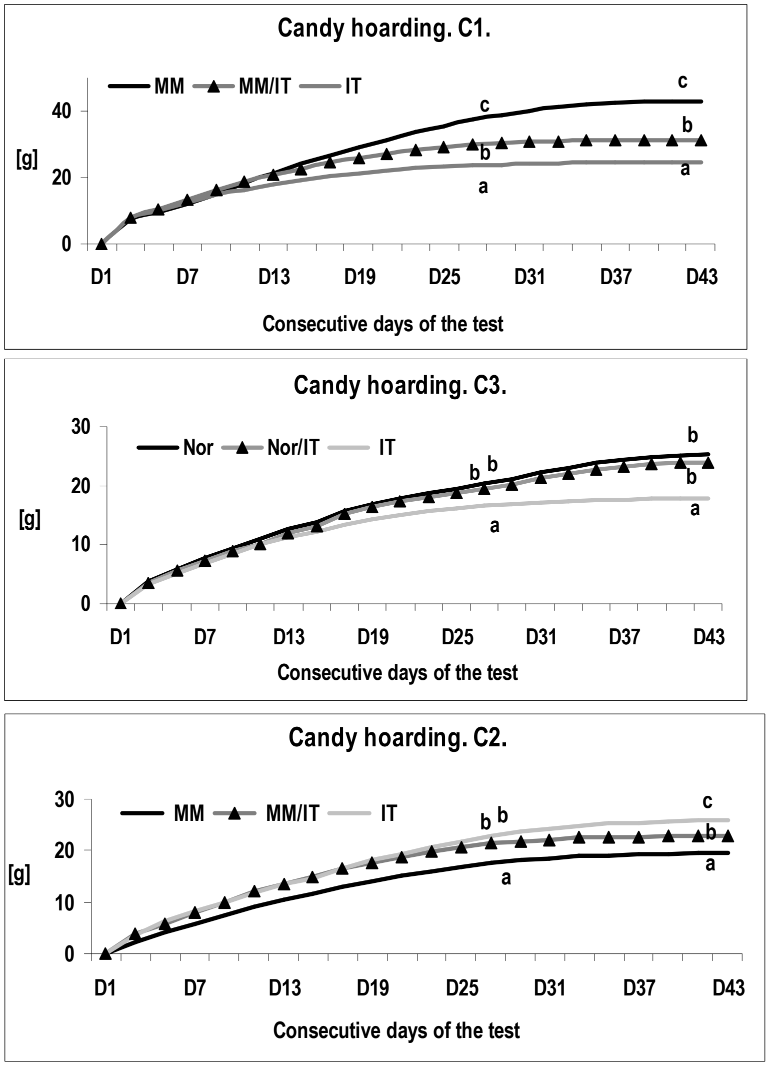

Figure 2. Honey-sugar candy hoarding from the beginning of the cage test to each of its consecutive days

MM – cages that included 100% of native bees with the component of *A. m.* mellifera; IT – cages that included 100% of *A. m. ligustica* bees; Nor – cages that included 100% of bees originating from the Norwegian queen *A. m. mellifera* inseminated by *A. m. caucasica* drones; MM/IT – cages that included a mix 50% of native bees with the component of *A. m. mellifera,* and 50% of *A. m. ligustica* bees; Nor/IT – cages that included a mix 50% of bees originated from the Norwegian queen *A. m. mellifera* inseminated by *A. m. caucasica* drones and 50% of *A. m. ligustica* bees. ^{a,b,c}Differences between the tested groups are statistically different ($p \leq 0{,}05$) for results obtained after 28th and 42^{ed} day of the test (ANOVA and Tukey test). C1, C2, C3 – three independent, consecutive comparisons

It is interesting that syrup processing efficiency (EP%) was higher in mixed than in homogenous colonies. Hence, in the case of EP%, interactions between worker groups (field tests) were substantial. Perhaps the efficiency of mixed colonies was higher as a result of co-operation between diverse worker groups. A detailed mechanism of that phenomenon is difficult to be proved. Research carried out by other authors and concerning other behavioural traits, however, showed that a colony that consists of diverse worker groups may take advantage of that diversity. Higher genotypic variation within a colony increases the chance of different tasks to be performed effectively [8]. It is worth noting in this context that under adverse weather conditions (C1) it was the mixed colony that was able to maintain the highest level of accumulated supplies even though that colony did not forage the highest quantities of syrup, thus the diverse environmental conditions in the field tests may stimulate co-operation among various worker groups. That phenomenon is not encountered in the laboratory. It may, therefore, be presumed that varying EP% values may be another reason for the discrepancy between the results of the field and the laboratory tests.

CONCLUSIONS

1. In field tests, an influence of one worker group on another was observed when they were mixed within a colony. Such an influence had the nature of non-additive interactions. Under laboratory conditions, diverse worker groups which were mixed in a single cage tended to co-operate in an additive way.

2. Results of mixing good and poor foragers is difficult to predict, since it depends on the genotype of the bees being mixed and on the environment.

3. The conformity between the results of the field and the laboratory tests may be considerably influenced by worker interactions, which usually can take place only in the field, and by breed factors.

REFERENCES

1. Brodschneider R., Arnold G., Hrassingg N., Crailsheim K.: Alien honeybees do their job well: Scouting and recruiting in drifted bees. Proc. of the First European Conference of Apidology, Udine 57, 2004.
2. Calderone N. W., Page R. E.: Effects of interactions among genetically diverse nestmates on task socialization by foraging honey bee *(Apis mellifera).* Behav. Ecol. Sociobiol., 30, 219–226, 1992.
3. Fewell J., Page R. E. Jr.: Genotypic variation in foraging responses to environmental stimuli by honey bees, *Apis mallifera.* Experienta, 49, 1106–1112, 1993.

4. G u z m a n-N o v o a R., P a g e R. E.: Genetic dominance and worker interactions affect honeybee colony defense, Behav. Ecol. 5, 91–97, 1994.
5. M i l n e C h. P.: An improved laboratory measurement of hoarding behavior in the honey bee. Amer. Bee *J*. 117, 8, 502–507, 1977.
6. M i l n e C h. P.: An estimate of the heritability of worker longevity or length of life in the honeybee. J. Apic. Res. 24, 3, 140–143, 1985.
7. M i l n e C h. P.: The need for using laboratory tests in breeding honeybees for improved honey production. J. Apic. Res., 24, 4, 237–242, 1985.
8. P a g e R. E., R o b i n s o n G. E.: The genetics of division of the labour in honey bee colonies. Adv. Insect Physiol. 23, 117–169, 1991.
9. P a l e o l o g J., F l i s W.: Wpływ genotypu i środowiska na zdolność pobierania wziątku u pszczoły miodnej w testach polowych i laboratoryjnych. Annales UMCS, sectio EE, vol. XVII, 17, 289–294, 1999.
10. P a x t o n R. J., S a k a m o t o C. H., R u g i g a F. C.: Modification of honey bee (Apis mellifera L.) stinging behaviour by within-colony environment and age. J. Apic. Res. 33, 2, 75–82, 1994.
11. T a b e r S.: Drifting, Gleanings in bee culture. Am. Bee J., 6, 398–399, 1988.
12. T h o m C., S e e l y T. D., T a u t z J.: A scientific note on the dynamics of labour devoted to nectar foraging in a honey bee colony: numbers of foragers versus individual activity. Apidologie, 31, 737–738, 2000.

STRESZCZENIE

W testach polowych porównano pozyskiwanie i magazynowanie syropu cukrowego w rodzinkach będących mieszaniną pszczół z dwu różnych grup genetycznych (po 50%) z rodzinkami jednorodnymi, zawierającymi robotnice tylko z jednej z tych dwu grup (100%). W testach laboratoryjnych porównano pobieranie syropu cukrowego w klatkach z mieszaniną pszczół z dwu różnych grup (50%) z klatkami zawierającymi pszczoły tylko z jednej grupy (100%). Postanowiono sprawdzić, jak interakcje genotypowe pomiędzy grupami robotnic wpłyną na wyniki uzyskane w polu i w laboratorium. W teście polowym (ryc. 1) ilość pobranego/zmagazynowanego syropu w rodzinkach mieszanych nigdy nie była pośrednia w stosunku do tej odnotowywanej w rodzinkach jednorodnych, co wskazuje na nieaddytywne interakcje między grupami robotnic. Efekty wymieszania lepszych i gorszych zbieraczek były różne i zależały od ich pochodzenia (genotypu). Efektywność przetwarzania przyniesionego syropu w zapasy (tab. 1) była wyższa w rodzinkach mieszanych, co wskazuje na specyficzne interakcje między robotnicami. Podczas testów laboratoryjnych (ryc. 2) pszczoły z klatek, w których wymieszano robotnice z dwu różnych grup genetycznych, pobierały pośrednie ilości ciasta w stosunku do klatek, w których były pszczoły tylko jednej z grup, czyli współdziałały w sposób addytywny. Zatem nieaddytywne interakcje pomiędzy robotnicami następowały jedynie w warunkach polowych, co mogło rzutować na zgodność wyników uzyskanych w polu i w laboratorium.