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Studies of Different Methods of Evaluation of Cleaning Behaviour in Honeybees – Apis Mellifera

Badania różnych metod szacowania zachowań związanych z czyszczeniem gniazda u pszczoły miodnej *Apis mellifera*

Starting with works of Rothenbuhler (8), who made the first attempt to describe the genetic background of hygienic behavior, breeders all over the world are interested in the evaluation of this trait for a selection of bees which are resistant to numerous diseases (9,10,6). In each of the proposed test methods, they examined how fast a colony of bees cleaned out a sample of a dead sealed brood introduced into the nest. Before such an introduction, the still living brood is removed from a hive and then killed either by deep-freezing (9,2) or by puncturing it (needle-test/pin-killed brood assay) with needles (6), or by applying microinjections of various chemicals (11). In order to make the estimate of the genetic-population parameters of the hygienic behaviour easier, laboratory tests were proposed lately as well (3). In those tests, worker bees were sampled from the colonies, caged, and given pieces of combs with a killed brood to be cleaned out. Nevertheless, a selection for the hygienic behaviour is performed only in a few breeding programmes because most of the queen breeders considered those original test methods as too difficult, complicated and time consuming. Moreover, in connection with the discussion that has just begun regarding the ethic aspects of experiments in invertebrates (7), we should try to find some "alternative methods" for the tests described above.

In most papers the term "cleaning behaviour" is limited only to cleaning out the cells by worker bees. However, bees are also able to remove foreign bodies introduced into the nest. Therefore, it is especially interesting whether cleaning

the cells and cleaning the nest are linked traits. Several years ago, some beekeepers introduced pieces of cardboard into the nests with drugs against Varroa when they were fighting these mites. They believed that the bees that removed cardboard faster would also clean the cells from the damaged brood more efficiently (5). If it were the truth, a simple test of hygienic behaviour could be worked out. Thus, in the present studies it was decided to compare the efficiency of removing both paper and cardboard from the nest with the efficiency of cleaning out the killed-brood from the cells. Two different breeds of bees were used.

MATERIAL AND METHODS

Ten colonies of the Buckfast Bees and ten colonies of the Caucasian x Carniolan crossbreed were used. The colonies were approximately of the same strength and age structure and they were headed by a one-year old instrumentally inseminated queen, which had been introduced in the previous season.

Three tests of cleaning behaviour were performed: 1) A classical pin-killed brood assay that is commonly used to evaluate the cleaning behaviour 2) A test of cardboard removal 3) A test of paper removal. Tests 2 and 3 were specially designed to be performed during the present studies. In order to obtain a reliable database, four repetitions of each of these three tests were carried out.

1. Hygienic behaviour was evaluated as follows: first the section of a comb with capped brood from each colony was removed, then the brood (100 cells) was killed by pricking each cell, then the combs were returned to their colonies and finally, all the cells cleaned out partially and completely (pin-killed brood assay) were counted. The combs with the killed-brood sections were inspected 6, 12 and 24 hours after their introduction.

2. To perform the test of cardboard removal (TCR), a piece of soft cardboard, 9 x 9 cm (81 cm^2), previously weighed, was inserted into each of the examined colonies and hung in the nest between two combs with the open brood. It was decided to use cardboard beer rests to make sure that the same, standardized pieces of cardboard were inserted every time. Then, the pieces were removed from the colonies and weighed again, after the span of 6, 12, and 24 hours. The amount of the removed (cleaned out) cardboard was recorded.

3. The test of paper removal (TPR) was carried out in the same way as the TCR test except that the sheets of graph paper, 5 x 10 cm (50 cm^2), were inserted into the nests instead of cardboard pieces. The screen printed on the sheets enabled us to calculate losses of the paper (expressed in the surface units) after 2, 4 and 6 hours.

RESULTS AND DISSCUSSION

Some high, significant correlation (Tab. 1) was found between the amounts of the cardboard removed after 6, 12 and 24 hours respectively in TCR. That finding suggests that cardboard removal is connected with the systematic and continuous worker activity and that in the next studies only one weighing of cardboard pieces (after 10 to 12 hours) could be performed. On the other hand,

Table 1. Correlation coefficients between the examined behavioral traits estimated for all examined colonies after 6, 12 and 24 hours respectively

partly 6 h	not 6 h	comp. 12 h	partly 12 h	not 12 h	comp. 24 h	partly 24 h	not 24 h	card. 6 h	card. 12 h	card. 24 h	
-.36^{xx}	-.11	.79^{xxx}	-.79^{xxx}	-.15	.46^{xxx}	-.45^{xxx}	-.17	.09	.12	.04	comp. 6 h
	-.89^{xxx}	-.21	.28	-.42^{xxx}	.05	-.05	-.11	.02	-.02	-.04	partly 6 h
		-.16	.09	.52^{xxx}	-.28	.28	.20	-.06	-.03	.02	not 6 h
			-.99^{xxx}	-.28	.57^{xxx}	-.57	-.20	.04	.08	.01	comp. 12 h
				.13	-.55^{xxx}	.55^{xxx}	.19	-.04	-.06	.01	partly 12 h
					-.29	.29	.12	-.06	-.13	-.15	not 12 h
						-.99^{xxx}	.00	.02	-.06	-.10	comp. 24 h
							-.02	-.02	.06	.10	partly 24 h
								-.05	-.05	-.04	not 24 h
									.87^{xxx}	.71^{xxx}	card. 6 h
										.90^{xxx}	card. 12 h

The number of cells cleaned out completely (comp.), partly (partly) and not cleaned (not) and the weight of the cardboard removed from the nest (card.); (xx) - significant for $P \leq 0.01$; (xxx) - significant for $P \leq 0.001$

Table 2. Correlation coefficients between the examined behavioral traits estimated separately for Buckfast and Caucasian x Carniolan bees after 6, 12 and 24 hours respectively

	comp. 6 h	partly 6 h	not 6h	comp. 12 h	partly 12 h	not 12 h	comp. 24 h	partly 24 h	not 24 h	card. 6 h	card. 12 h	card. 24 h	
comp. 6 h		-.38	.02	$.80^{xxx}$	$-.81^{xxx}$	.12	$.48^{xx}$	$-.48^{xx}$	-.14	.03	-.10	-.14	comp. 6 h
partly 6 h	-.34		$-.93^{xxx}$	-.25	.28	-.28	-.02	.03	$-.46^{xx}$	.09	.02	-.06	partly 6 h
not 6 h	-.24	$-.83^{xxx}$		-.04	.01	.25	-.16	.16	$.55^{xxx}$	-.11	.01	.12	not 6 h
comp. 12 h	$.80^{xxx}$	-.16	-.31		$-.99^{xxx}$	-.01	$.60^{xxx}$	$-.60^{xxx}$	-.17	-.08	-.21	-.27	comp. 12 h
partly 12 h	$-.79^{xxx}$	.28	.18	$-.99^{xxx}$		-.11	$-.59^{xxx}$	$.59^{xxx}$	.17	.07	.21	.27	partly 12 h
not 12 h	-.32	$-.60^{xxx}$	$.81^{xxx}$	$-.46^{xx}$	.31		-.08	.08	.04	.07	.02	-.03	not 12 h
comp. 24 h	.39	.20	$-.43^{xx}$	$.54^{xxx}$	$-.49^{xx}$	$-.47^{xx}$		-1.0^{xxx}	-.07	.01	-.18	-.28	comp. 24 h
partly 24 h	-.39	-.20	$.43^{xx}$	$-.53^{xxx}$	$.48^{xx}$	$.47^{xx}$	$-.99^{xxx}$		.06	-.01	.18	.28	partly 24 h
not 24 h	-.24	.08	.05	-.24	.23	15	.02	-.04		-.04	.05	.13	not 24 h
card. 6 h	.19	-.16	.05	.29	-.26	-.27	.03	-.02	-.10		$.93^{xxx}$	$.81^{xxx}$	card. 6 h
card. 12 h	.35	-.09	-.11	$.46^{xx}$	$-.43^{xx}$	-.30	.07	-.07	-.16	$.72^{xxx}$		$.95^{xxx}$	card. 12 h
card. 24 h	.09	.03	-.09	.28	-.25	-.29	.03	-.03	-.20	$.58^{xxx}$	$.83^{xxx}$		card. 24 h

The number of cells cleaned out completely (comp.), partly (partly) and not cleaned (not) and also the amount of the cardboard removed from the nest (card.); (xx) - significant for $P \leq 0.01$; (xxx) - significant for $P \leq 0.001$; shadow part – Buckfast; bright part - Caucasian x Carniolan

as the correlation coefficients were lower in Buckfast bees than in Caucasian x Carniolan crossbreeds (Tab. 2), such a factor as breed must have influenced that correlation. Exactly the same situation was observed in TPR (Tab. 3), except that the influences of the breed were not noticed. As for the amount of the removed paper, it could also be evaluated just once but after the span of 4 to 6 hours. The breed also influenced variability (Tab. 4) but only the traits examined in TCR. Higher variability was observed in Caucasian x Carniolan bees. Additionally, it was observed that bees intensively moisturized both cardboard and paper before they cut and removed it from the nest.

Table 3. The correlation coefficients between the amounts of paper removed from the nest (paper) after 2, 4, and 6 hours respectively and the number of cells cleaned out completely (comp.), partly (partly) and not cleaned (not), after 6, 12 and 24 hours respectively

	paper 2 h			paper 4 h			paper 6 h		
	I	II	III	I	II	III	I	II	III
comp. 6 h	-.19	-.07	-.16	-.29	.00	-.15	-.18	.08	.00
partly 6 h	.09	.03	.07	-.06	-.16	-.12	-.11	-.15	-.14
not 6 h	.02	-.01	.01	.22	.17	-.19	.21	.12	.15
comp. 12 h	-.08	-.21	-.16	-.28	-.07	-.17	-.10	.01	-.02
partly 12 h	.05	.23	.16	.26	.06	.16	.06	-.01	.00
not 12 h	.21	-.11	.05	.18	.06	.12	.23	-.02	.10
comp. 24 h	-.38	-.14	-.25	-.38	-.37	-.37^{xx}	-.32	-.35	-.31
partly 24 h	.37	.14	.25	.37	.37	.36^{xx}	.31	.35	.31
not 24 h	.24	.13	.17	.29	.18	.23	.20	.19	.17
card. 6 h	-.24	.04	-.06	-.01	.09	.05	.02	.17	.13
card. 12 h	-.04	.05	.01	.00	.13	.08	.02	.21	.19
card. 24 h	-.11	.01	.01	-.10	.16	.06	-.16	.24	.14
paper 2 h				.61^{xxx}	.62^{xxx}	.61^{xxx}	.67^{xxx}	.62^{xxx}	.61^{xxx}
							.80^{xxx}	.92^{xxx}	.87^{xxx}

(xx) - significant for $P \leq 0.01$; (xxx) - significant for $P \leq 0.001$; I – for Buckfast bees; II – for Caucasian x Carniolan crossbreed bees; III – for the both groups

No correlation was found when the results of TCR (Tab. 1) and TPR (Tab. 3) were compared with the results of the pin-killed brood assay. When the correlation coefficients were calculated separately within each of the two examined breeds, the values obtained for each breed were similar to each other so, in this case as well, the breed did not affect the correlations significantly. Therefore, the TCR and TPR tests do not seem to be useful as the "alternative tests" during the selection for hygienic behaviour (cleaning out of the killed-brood cells). Thereby, observations of some Polish and German beekeepers on the links be-

tween the efficiency of hygienic behaviour and efficiency of cardboard removal (5) find no confirmation, at least (see further) not in the present experiment (in the studied population). Moreover, and this is very surprising, no correlation was found between the efficiency of the cleaning observed in TCR and that observed in TPR. Could the cleaning out of a nest from paper and from cardboard be determined in a different way? On the other hand, Buckfast bees always cleaned out cardboard, paper, and killed-brood cells a little bit faster than Caucasian x Carniolan (Fig. 1) but only for "the cardboard after 24 hours" that difference was significant (Tab. 4). Maybe, if we had compared the bees selected previously for hygienic behaviour (1, 6) with the "not hygienic" bees, we would have finally been able to answer whether cleaning out the nests from paper/cardboard was correlated with cleaning out the killed-brood cells. This is the topic for further studies because in the population like we had, the genetic variance of the examined traits could be too low to result in a significant covariance (correlation).

Table 4. Results of statistical comparisons (ANOVA) of differences between the basic parameters of the examined behavioral traits in Buckfast and Caucasian x Carniolan bees. The probabilities of the "F" function were shown for the means, while the results of the homogeneity test (P) were presented for variability

	Pin-killed brood cells								
	6 hours			12 hours			24 hours		
	comp.	partly	not	comp.	partly	not	comp.	partly	not
Mean	.02	.58	.06	.33	.30	.82	.14	.14	.46
Variability	.51	.04	.07	.97	.68	.03	.33	.33	.00
	Cardboard				Paper				
	6 hours	12 hours		24 hours	2 hours		4 hours	6 hours	
Mean	.86	.34		.02	.51		.80	.31	
Variability	.00	.01		.06	.12		.20	.00	

Cells cleaned out completely (comp.), partly (partly) and not cleaned (not)

Significant negative correlation was found between the number of the killed-brood cells cleaned out partly and completely (Tab. 1). These results suggest that the bees which were efficient cleaners uncapped the kill-brood cells inefficiently and *vice versa*. It is believed that hygienic behaviour consisted of two different traits: uncapping behaviour and cleaning behaviour and that those both types of behaviour have different genetic background Moritz (4), Rothenbuhler (8). In our studies, partial cleaning was the result of only uncapping behaviour, whereas complete cleaning was the result of both cleaning and uncapping behaviour. On the other hand, as opposed to our results, positive correlation between these two

traits was reported (6). Unsystematic influence of the breed of bees on the correlations between the characteristics evaluated during the pin-killed brood assay was noticed (Tab. 2) even if such influence was visible for individual comparisons. In Buckfast bees, a little bit more significant correlation coefficients were noticed.

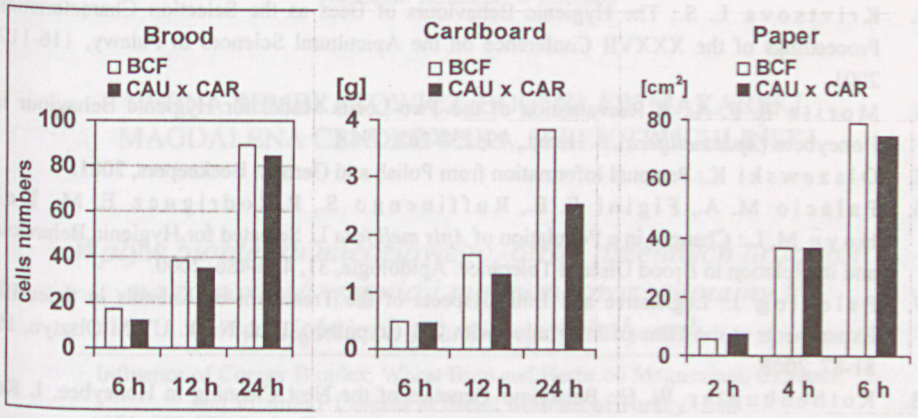

Fig. 1. The number of the killed-brood cells that were completely cleaned out (killed-brood), the weight of the cardboard that was removed from the nest (cardboard), and the surface of the removed paper, in the three consecutive measurements in Buckfast and Caucasian x Carniolan bees separately

The only high correlation coefficients were noticed for the numbers of cells that were completely cleaned out after 6, 12 and 24 hours, respectively (Tab. 1) although it was less visible in Buckfast (Tab. 2). The correlation coefficients for the same comparisons but for the cells cleaned out partially were insignificant and low except the correlation between the cells cleaned out after the period of 12 and 24 hours. A similar situation was observed when numbers of the uncapped cells were compared.

CONCLUSIONS

1. Hygienic behavior did not seem to be linked to the cleaning behavior connected with the paper and cardboard removal.

2. Surprisingly cleaning out the nest from paper and from cardboard were two different activites. Thus, all the examined traits were not linked.

3. Bees that were efficient cleaners uncapped the kill-brood cells inefficiently and *vice versa*.

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STRESZCZENIE

Porównano efektywność czyszczenia (częściowego i całkowitego) komórek z martwym czerwiem (test igłowy) z efektywnością usuwania z gniazda zarówno tektury (TCR), jak i papieru (TPR). Użyto pszczół Buckfast i kaukaska x kraińska, po dziesięć rodzin każdej rasy. Przeprowadzono cztery powtórzenia każdego testu. Plastry z obszarami nieżywego czerwiu oraz kawałki tektury były oceniane w 6, 12 i 24 godz. (trzy kolejne pomiary) po ich włożeniu do gniazda. Papier był oceniany w po 2, 4 i 6 godzinach. Nie znaleziono korelacji pomiędzy efektywnością czyszczenia komórek (częściowego i całkowitego) z zabitego czerwiu a TPR i TCR. Nieoczekiwanie nie wykazano istnienia korelacji pomiędzy TPR i TCR. Okazało się, że istnieje wysoka, istotna, ujemna korelacja pomiędzy liczbą komórek wyczyszczonych częściowo a wyczyszczonych całkowicie. Wysoką, istotną dodatnią korelację pomiędzy trzema kolejnymi pomiarami wykazano dla liczby całkowicie wyczyszczonych komórek z zabitym czerwiem, TPR i TCR. Istnienia takiej korelacji nie stwierdzono w przypadku komórek wyczyszczonych częściowo. Wpływ rasy był nieznaczny, ale widoczny w przypadku niektórych korelacji. Pszczoły Buckfast były nieznacznie bardziej efektywnymi czyścicielami, szczególnie w przypadku tektury.