A RARE CASE OF OVERDOMINANCE IN DEFENSIVE BEHAVIOUR OF WORKERS IN HONEYBEE COLONIES

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S u m m a r y

Fifteen experiments were performed over five years. In each of them, the defensive behaviour of 3 nucleus colonies, which were artificially composed of gentle and defensive bees mixed in the following proportions: 80% : 20%, 50% : 50%, 20% : 80% was compared with the defensive behaviour of two homogenous nucleus colonies consisting of 100% defensive and 100% gentle bees. Fifteen repetitions of the ball test were performed in each of the fifteen experiments and also different combinations of various bees were used in each of them. Worker genotypic interactions were evaluated on the colony level. The interactions were additive in 13% and nonadditive in 87% (dominance 66%) for the time to the first sting (seconds) and they were additive in 33% and nonadditive in 67% (dominance 40%) for the sting number. Moreover, overdominance was observed in 2 out of 15 experiments, which had not been reported previously. Different aspects of such rare case of nonadditive worker interactions were discussed. It is interesting, that either defensive or gentle bees could be dominating, depending on the season and type of the bees.

Keywords: defensive behaviour, mixed-colony, genotypic variation, worker-interactions, *Apis mellifera*.

INTRODUCTION

Because of polyandry (Triasko 1951, Woyke 1955, Alber at al. 1955) honey bee colony consists of several groups of workers with various genotypes and, therefore, within each colony there is a certain variability known as the genotypic variation. This variation is further increased when brood combs are exchanged between hives, when colonies are united (Gromisz et al. 1978, Ostrowska 1985, Bruder Adam 1983) or by bee drifting (Taber 1988, Brodschneider et al. 2004). If the expected value of a given trait in a given bee colony is the weighted average of its values in the bee groups constituting the colony, then the worker interaction is regarded as additive. If, however, the value of this trait in this colony is significantly different from that weighted average, such interactions are nonadditive (Guzman--Novoa and Page 1994). The so-called behavioural dominance is a special case. It occurs when the value of the examined trait within a given colony does not differ from its value in one of the worker groups (usually the best one) constituting the colony. If the value of the trait monitored on the colony level is significantly different from the weighted average that has been mentioned above, but at the same time it is significantly different from the values of this trait in all the worker groups constituting the colony, then we suggest that such a domination should be defined as a semi--domination.

It is symptomatic that the terminology used in characterising genotypic variation in honeybee colonies is identical with that used in describing the genetic variation in single individuals (compare Falconer 1981, Guzman-Novoa and Page 1994). In both cases we speak about additive and non-additive effects, including the effects of dominance. However, the question arises whether inter-genotypic worker interactions could have the overdominance character, as it is the case in inter-gene interactions. Such a phenomena have not been described in the previous papers (Collins at al. 1988, Guzman-Novoa and Page 1994).

Therefore we examined genotypic inter-worker interactions concerning the colony defensive behaviour to see whether overdominance may happen.

MATERIAL AND METHODS

Fifteen experiments were conducted at the Agricultural University of Lublin, Poland, over five consecutive years 1999-2004. In each of the experiments defensive behaviour of five, artificially made, nucleus colonies was quantified and also the same experimental pattern/procedure was applied. At first, two strong source colonies were selected. One of them consisted of highly defensive hybrids whereas the other was very gentle. Next, the five experimental nucleus colonies were established. Two of them were homogenous, one consisting of 100% defensive and the other of 100% gentle bees. The remaining three nuclei were made up of gentle and defensive bees, physically mixed in the following proportions (gentle to defensive): 80% : 20%, 50% : 50% and 20% : 80%. In each experiment the nucleus colonies were being prepared in the following way: Bees from the defensive and the gentle source colony were shaken into two separate travelling boxes after the sunset. They were then mixed and sprinkled with water and mint extract. Next, the bees were sampled from each travelling box, using a measuring cylinder, and poured into the Langstroth hives (two litres of bees + 2 combs with supplies + 1 brood comb + a queen cell before emerging) in the proportions specified above. Nucleus colonies prepared in this way were put in a cellar for 48 hours and then transported to new locations, 11 kilometres away from the apiary, where they were placed in such a way as to avoid bee drifting. After the orientation flight, during the subsequent 15 days, the „ball test" was carried out once a day (15 repetitions) in the following way: bees were disturbed by knocking three times in the front wall of the hive. Then, time to the first sting (seconds) into an approximately 500 cm^2 leather glove moved not far away (about 20 cm) from the hive entrance was recorded. The number of stings within two minutes after the first sting had been made was also counted. During each experiment, brood was being removed just before its emergence, so that the emerging bees would not change the artificially made worker proportion.

As it has been described above, fifteen repetitions of the stinging test were performed in each of the fifteen experiments. Consequently, the obtained database consisted of: 15 experiments x 15 repetitions x 5 assayed nucleus colonies. The data were processed with STISTICA 6.0 software using ANOVA, multiple range Turkey tests and Chi^2 procedures.

Generally, four genetically different defensive hybrids (Native, Norwegian black bee, Caucasian, of a unknown origin) and four genetically different gentle purebreds (Italian, Caucasian, Cordovan, Italian-2) were used over the five years of the study. The difference between fifteen consecutive experiments lies in the fact that different combinations of bees were used in the consecutive experiments and that each experiment was performed under different environmental conditions.

Defensive hybrids of native *A. m. mellifera* (D_{MM}) and gentle purebreds A. m. caucasica (G_{CAU}) were used in experiments 6 and 10, which are analyzed in detail in this paper. Consequently, two homogenous nucleus colonies respectively consisting of 100% D_{MM} (**1D$_{MM}$**) and of 100% G_{CAU} (**1G$_{CAU}$**), as well as three mixed nucleus colonies respectively composed of 80% D_{MM} x 20% G_{CAU} (**8D$_{MM}$/2G$_{CAU}$**), 50% DMM x 50% G_{CAU} (**5D$_{MM}$/5G$_{CAU}$**), and, finally, 20% D_{MM} x 80% G_{CAU} (**2D$_{MM}$/8G$_{CAU}$**) were assayed in experiments 6 and 10.

RESULTS

Table 1 shows that the commonest worker interaction was dominance (66% for time to the first sting and 40% for number of stings). The interactions were additive in 13% and 33% for the time to the first sting and the number of stings, respectively. Only in two out of fifteen experiments, namely in experiment 6 and 10, such types of worker genotypic interactions were observed, which could be regarded as behavioural overdominance.

Table 2 shows that only in experiments 6 and 10, where overdomination was observed, bees from defensive source colony

Table 1

Types of worker genotypic interactions in mixed colonies assayed for defensive behaviour in 15 experiments conducted from 1999 to 2004

	EXPERIMENT														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
TFS	D*	D*	D*	D*	D*	OD*	D*	A	D*	OD*	D*	A	D*	D*	SD*
±se	2,5	7,5	9,9	8,4	9,1	3,4	2,3	5,3	4,3	13,3	0,5	2,3	5,5	9,8	6,2
NS	A	D*	A	A	D*	OD*	SD*	A	D*	OD*	A	D*	D*	D*	SD*
±se	9,6	4,3	4,1	6,9	11,0	8,8	13,4	9,9	2,8	6,8	7,8	13,1	8,8	7,3	10,5

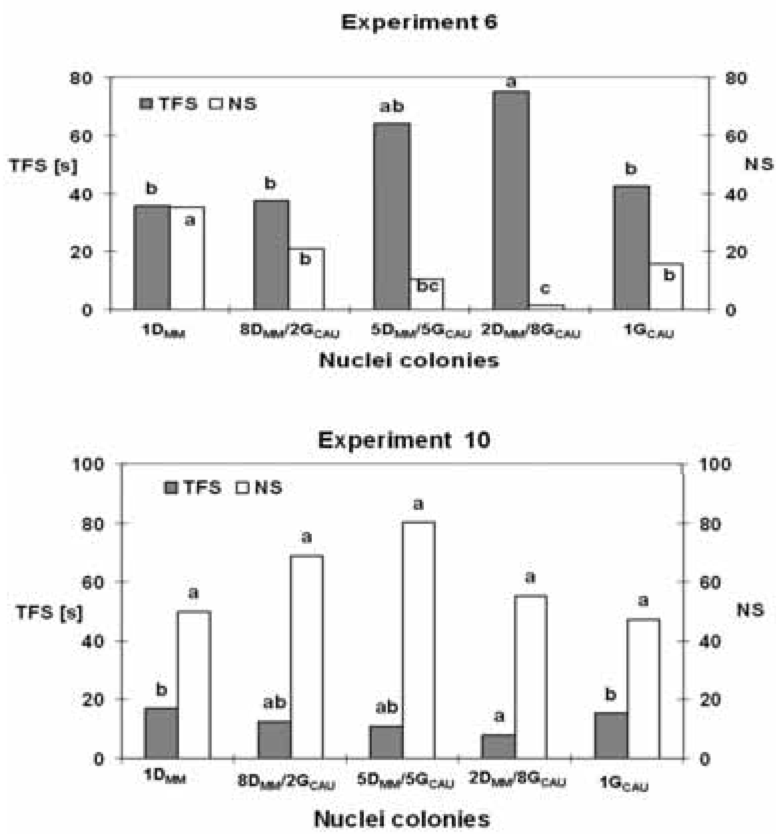
A – additive. SD – semi-dominance. D – dominance. OD – overdominance.
se – standard error from the one-way ANOVA for differences between experiments has been included.
(*) – the observed mixed colony value is significantly different from its expected value, which is the weighted average of the values of the homogenous colonies, - for $P<0.05$ (Chi^2). TFS – time to the first sting (seconds). NS – number of stings.
Fifteen repetitions of stinging test were performed in each of the fifteen experiments.

Table 2

Percentage of the values of the colony defense characteristics in the homogenous, defensive bee nucleus colony, in the ratio to the value of the homogenous, gentle bee nucleus colony

	EXPERIMENT														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
TFS	9*	6*	23*	8*	22*	86	20*	36*	62	112	24*	48*	18*	9*	8*
NS	1926*	2145*	712*	1712*	873*	224*	264*	265*	163*	106	1941*	346*	790*	995*	1271*

TFS – time to the first sting. NS – number of stings.
(*) – Value of the trait observed in homogenous gentle nucleus colony is significantly different from that observed in the homogenous defensive nucleus colony for $P<0.05$ (Tukey multiple range test).
Values of the colony defense characteristics were calculated from the fifteen repetitions of the stinging test performed in each of the fifteen experiments. Take the note of the fact that when the defense response is high, the number of stings is also high but the time to the first sting is short.



TFS – time to the first sting. NS number of stings. Columns represent means calulated from the 15 repetitions of the stinging test, performed in each of the two experiments. Different letters at columns indicate significant differences between the means ($P < 0.05$).

1D$_{MM}$ – homogenous, defensive *A. m. mellifera* bees.

1G - homogenous, gentle *A. m. caucasica* bees.

8D$_{MM}$/**2G**$_{CAU}$ – mixed, 80% D_{MM} and 20% G_{CAU}.

5D$_{MM}$/**5G**$_{CAU}$ – mixed, 50% D_{MM} and 50% B_{CAU};

2D$_{MM}$/**8G**$_{CAU}$ – mixed, 20% D_{MM} and 80% G_{CAU}.

Fig. 1. Results of the stinging tests in experiments 6 and 10

distinctly decreased their defensive response (in relation to the gentle bees) when they were placed in a experimental homogenous nucleus colony. As a result, 3 out of 4 means, did not differ significantly from bees in the homogenous, gentle nucleus colonies. It could so happen in our small nuclei, which were populated by only 2 litres of bees, that the decrease in the worker population caused the decrease of defense of the nucleus colonies.

However, in the remaining thirteen experiments, bees from defensive source colonies were still defensive in the small nuclei. Consequently, it is clearly visible that the mechanisms of defensive behaviour of the nucleus colonies tested in experiment 6 and 10 differ from those observed in the remaining experiments. Therefore, a more precise data, are presented in Fig. 1.

In experiment 6, bees from the mixed nucleus colony 5D$_{MM}$/5G$_{CAU}$, and to even a greater extent those from $2D_{MM}/8G_{CAU}$ were characterised by the longest time to the first sting (FST) and the lowest number of stings (NS). They were, then, even gentler than $1G_{CAU}$ composed of 100% gentle bees. In that case, we may speak about behavioural overdominance of the gentleness of bees. In experiment 10, on the other hand, we may observe a clear tendency indicating, that in three mixed nucleus colonies, the time to the first sting (TFS) was shorter than that in both homogenous nucleus colonies. In $2D_{MM}/8G_{CAU}$ that difference was statistically significant. The number of stings was clearly higher in three mixed nucleus colonies, and $5D_{MM}/5G_{CAU}$ bees were making the most stings. Since abnormally high variation occurred only in this case (sting number, experiment 10, see also "se" at table 1), the statistically significant differences were not found, but the tendency was obvious. It is, then, clear that in experiment 10, there were also worker interactions of the overdominance nature, however, in this case, overdominance of the defensiveness of bees occurred. It is worth of noticing that in both experiments, 6 and 10, we used D_{MM} and G_{CAU} bees to settle down the experimental colonies. The only difference between experiment 6 and 10 was, that experiment 6 was performed in late spring and experiment 10 in early autumn. It is commonly believed that the colony defense increase from spring to autumn. Therefore, probably just the colony mixed of defensive and gentle bees may be defensive in autumn but gentle in spring. Some other our observations (unpublished yet) may support this suggestion.

DISCUSSION

Authors of earlier studies do not report overdominance interactions. (Collins at al. 1988, Guzman-Novoa and Page 1994). Thus, such phenomenon occurred only incidentally. In the present study we were able to notice this phenomenon because we were analysing numerous combinations of bees being of eight different genetic types (4 deffensive and 4 gentle) under different environmental conditions over 15 consecutive experiments. Since defensive and non-defensive bees could combine differently (Stort 1974, Collins at al. 1988, Guzman-Novoa and Page 1994, Paxton et al. 1994), and the colony defense may be dependent on the actual weather conditions (Villa 1988), it is possible that among so many different cases examined by us, conditions might have occurred, which were necessary for the emergence of overdominance.

According to Woyke (1992) the decrease in defensiveness was influenced by the decrease in the colony strength. In our experiments bees were sampled from defensive and gentle source colonies and placed in 2-litre nuclei. The worker population was reduced in this way. However, in twelve out of fifteen performed experiments the difference between defensive and gentle colony was still relevant. Only in the remaining three experiments the gentle and the defensive colony did not differ from each other. Interestingly, in two of those three experiments overdomination was noticed.

The comparison of our results with those published earlier shows that overdominance of defensive behaviour in mixed populations of various worker bees is a rare phenomenon. In our studies its occurrence was noticed and statistically confirmed on the basis of 15 repetitions only in 2 (6^{th} and 10^{th}) out of 15 performed experiments. Since both experiments, 6 and 10 were carried out in different weather conditions and the results were proved statistically, the overdominance interaction cannot have been accidental. Its background, which is difficult to account for, may support the thesis of Gary (1999) that what we know about bee ethology is barely 1% of what we should still find out.

CONCLUSIONS

1. In the case of honey bee colony defensive behaviour, interactions between genetically different worker groups within particular colonies take most frequently the form of behavioural dominance/semi-dominance.
2. However, worker interactions of the overdominance nature may be encountered occasionally. The nature of such phenomenon requires further investigation.

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RZADKI PRZYPADEK NADDOMINACJI W ZACHOWANIU OBRONNYM ROBOTNIC W RODZINACH PSZCZELICH

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S t r e s z c z e n i e

W ciągu 5 lat przeprowadzono 15 doświadczeń. W każdym zastosowano te same procedury. Różnice polegały na wykorzystaniu różnych pszczół w różnych doświadczeniach i prowadzeniu kolejnych doświadczeń w różnych warunkach. W każdym doświadczeniu porównywano zachowania obronne oszacowane w dwu rodzinkach jednorodnych, utworzonych ze 100% pszczół łagodnych i ze 100% pszczół agresywnych, z zachowaniami obronnymi w trzech rodzinkach mieszanych zsypanych z pszczół łagodnych i agresywnych w następujących proporcjach: 20% : 80%; 50% : 50%; 80% : 20%. W każdym z kolejnych doświadczeń do utworzenia pięciu rodzinek używano tych samych pszczół. Zachowania obronne szacowano stosując klasyczny „ball test" wykonując 15 powtórzeń tego testu (po jednym powtórzeniu dziennie) w każdym z 15 doświadczeń. Określano czas od podrażnienia do wbicia pierwszego żądła (sekundy) i liczbę żądeł pozostawionych w ciągu 2 min w atakowanym celu. Najczęściej obserwowaną formą współdziałania robotnic była dominacja lub semi-dominacja behawioralna (tabela 1). Współdziałanie, które można określić mianem behawioralnej naddominacji zaobserwowano tylko w 2 z pośród 15 doświadczeń (rycina 1, tabela 1). Jedno z tych dwu doświadczeń przeprowadzono wiosną, a drugie jesienią, w obu używając tych samych pszczół. Znamienne, że w przypadku tylko tych dwu doświadczeń pszczoły o gwałtownych reakcjach obronnych zatraciły je po umieszczeniu w słabszych rodzinkach doświadczalnych (tabela 2). Na wiosnę (rycina 1) pszczoły z rodzinek mieszanych były łagodniejsze niż te z rodzinek składających się w 100% z pszczół łagodnych, natomiast jesienią, przejawiały gwałtowniejsze reakcje obronne niż pszczoły z rodzinek składających się w 100% z pszczół obronnych. Tak więc na wiosnę mieliśmy do czynienia z naddominacją ze strony pszczół łagodnych, a jesienią, ze strony agresywnych. Uzyskane wyniki wskazują, że w tych dwu omawianych doświadczeniach, w których zaobserwowano naddominację mieliśmy do czynienia z odmiennymi mechanizmami zachowań obronnych niż tymi występującymi w pozostałych doświadczeniach. Jednak duża liczba wykonanych w ciągu dwu tygodni powtórzeń sugeruje, że nie mogło być to przypadkowe. Dalsze badania są potrzebne dla wyjaśnienia tych zagadnień. Może nie być to łatwe z powodu rzadkiego występowania zjawiska naddominacji behawioralnej w przypadku zachowań obronnych pszczół.

Słowa kluczowe: behawior obronny, rodziny mieszane, genotypowa zmienność, interakcje, *Apis mellifera*.