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Original Article

HYGIENIC BEHAVIOUR OF HONEYBEE COLONIES WITH DIFFERENT LEVELS OF POLYANDRY AND GENOTYPIC COMPOSITION

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Abstract

Honey bee queens were inseminated with diluted, homogenised semen collected from a few dozen drones. This procedure was carried out to increase the diversity of the queens' offspring, which is in comparison to the offspring of queens inseminated with semen from only a few drones coming from one colony. Queens and drones were mated within carniolan bee (*Apis mellifera carnica*) subspecies, but 3 selected lines were used. Queens were reared from one line and drones from the same line, and two additional lines differing in hygienic behaviour wherein in one of them that trait was strongly evident. The aim of this study was to examine whether the level of enhanced genetic variability in colonies and simultaneously the participation of hygienic bees, would increase the performance of hygienic behaviour. Overall hygienic behaviour of colonies with a lower and greater genetic variability did not differ significantly and amounted to 52.1 and 47.0%, respectively. Colonies within the lower variability group, in which drones from line selected in hygienic behaviour performance were used for inseminating queens, had a significantly greater percent of cleaned pupae than other colonies (63.2%). Hygienic behaviour in other colonies was more dependent on the gene quotas of hygienic bees in the colonies rather than on the level of polyandry.

Keywords: genetic diversity, genotypic variation, hygienic behaviour, instrumental insemination.

INTRODUCTION

Susceptibility of bees to diseases is genetically determined. Different genotypes of bees within races or lines characterise with varying degrees of susceptibility to pathogens, such as *Ascosphera apis* (Gilliam et al., 1988), American foulbrood (Rothenbuchler and Thompson, 1956), and the following parasites: *Varroa destructor* (Guzman et al., 1996), *Acarapis woodi* (Gary and Page, 1987), *Nosema apis* (Woyciechowski et al., 1994). One of many elements identifying bee resistance to diseases is hygienic behaviour. Therefore, it is important to check the usefulness of the various methods for assessing this trait (Olszewski and Paleolog 2007; Olszewski et al., 2013). Hygenic behaviour is based on detection of diseased or already dead brood, uncapping and removing such brood from the cell. This trait is highly heritable and can be selected in a number of bee populations (Rotenbuchler, 1964; Büchler, 1996; Spivak and Reuter, 1998). This mechanism of resistance is the most important in brood diseases and varrosis, in which exceeding a certain level of pathogens is crucial for the occurrence of clinical symptoms, such as ascospherosis (Gilliam et al., 1983). The effectiveness of this resistance mechanism is determined by those bees that develop hygienic behaviour in a colony. Already 25% of the highly hygienic bees in relation to the unhygienic ones, influenced the increased percentage of removed dead larvae from 26 to 46% (Arathi and Spivak, 2001).

In addition to the genetic variation in the population influencing phenotypic variation, there is a natural diversity of genotypes of bees in colonies. This natural diversity is a consequence of evolutionarily developed multiple mating. Polyandry is important

for natural selection, and without it, the colonies would not adapt easily to changing environmental conditions (Page and Robinson, 1991). One of theories about the genetic diversity benefits concerning the offspring in a colony is that the diversity increases disease resistance (Sherman et al., 1988; Shykoff and Schmid-Hempel, 1991; Schmid-Hempel, 1998; Baer and Schmid-Hempel, 1999; Tarpy, 2003; Tarpy and Seeley, 2006; Seeley and Tarpy, 2007). Increased genetic diversity lowers the variation in disease prevalence and mortality among colony members (Hamilton, 1987; Sherman et al., 1988; Schmid-Hempel, 1994; 1998; Tarpy, 2003). But a queen that mates multiple times produces genetically diverse workers that carry different genes from their respective fathers. By doing this, a queen minimises the risk that all of her worker-offspring will be sired by males that carry highly susceptible genes. Genetically diverse workers increase the probability that the colony as a whole, will survive. Thus, polyandry yields benefits by reducing the variance in disease-prevalence among colonies, not necessarily the average proportion of infected individuals (Sherman et al., 1988).

There are many researchers devoted to dealing with the impact of both: the degree of polyandry and selection for hygienic behaviour. However there is still little scientific work using the bees selected for hygienic behaviour to increase polyandry. The aim of this study was to examine whether increasing the level of genetic variation and simultaneously increasing the participation of hygienic bees in colonies, will increase hygienic performance.

MATERIAL AND METHODS

Experimental design

The study was performed in the 2009 - 2010 time period, at the Institute of Horticulture, Apiculture Division, in Puławy, Poland. In 2009, sister queens originating from a Carnica commercial line Marynka (Ma) were reared. Then, they were divided into two groups and instrumentally inseminated to obtain two levels of genetic diversity in the offspring within colonies. The queens from one group (SCS - single colony semen) were inseminated with semen collected from drones from one of 30 paternal colonies belonging to the same strain as the queen (Ma), and additionally from two unrelated strains Nieska (Ni) and GR-1 (Gr) which was selected on hygienic behaviour. The source of drones was systematically varied between the queens. This gave the three sub-groups: SCS-Ma, SCS-Ni, and SCS-Gr. The queens from the second group were inseminated with semen collected from drones coming from each of the 30 colonies (the MCS - mixed colony semen). Semen from 30 drones was collected by syringe, then, released into a glass vial, diluted with Hyes solution (composition described by Ruttner (1976)), and then mixed according to Skowronek et al. (1995). Queens from both groups were inseminated twice with a dose of 4 µL of semen each. Note that in the MCS group 4 µL of diluent was added to the injection volume. The queens were inseminated at the age of seven and eight days. Throughout the period from emergence to the start of oviposition, the queens were kept in a Kirchhain-type mating nuclei. At the start of oviposition, the queens of the two groups were introduced into the newly created colonies. A total of 102 colonies were set in Dadant hives with wax foundation frames. The colonies of the two experimental groups were randomly placed in two apiaries located in the areas of Wola Bukowska (W) 51°40'09"N 22°21'22"E and Sielce (S) 51°26'23"N 22°04'46"E.

In June 2010, the hygienic behaviour of bees, which is interpreted as the rate of cleaning out the cells with dead brood, was tested using method similar to Gilliam et al. (1983). A piece of comb with approximately 200 cells with freeze-killed pupae at -20°C, was placed into the colonies. Hygienic behaviour was expressed as a percentage of the completely cleaned brood cells 24 hours after the piece of comb had been inserted. The hygienic behaviour ratio, was measured on the basis of photographs. The images were analysed and the ratios calculated with computer software (MultiScanBase v. 18.03).

Statistical analysis

Multi-factor ANOVA model was used to test the difference between the groups of colonies and locations in hygienic behaviour performance. In order to achieve the assumptions of the analysis of variance, some data were subjected to the ArcSin(x) formula. Bartlett's test was used for equality of two and more variances. The means were compared using Tukey's post-hoc test. All computations were performed using the Statistica package v. 9.1.

RESULTS

Differences between the groups of colonies (SCS, N = 39) and (MCS, N = 46) in percentage of those cells cleared of dead pupae, were not significant $F_{(1,81)} = 1.366$, $p = 0.24$ (Fig. 1). Colonies cleared after 24 hours 52.1% and 47.0% cells, respectively.

The colonies varied strongly in hygienic behaviour. The rate of the cleaned cells ranged from 14.5 to 100%, but the variance in the two groups was similar (Bartlett's test p = 0.11). The environment had a significant effect on the hygienic behaviour $F_{(1,81)}$ = 9.30, p = 0.063. In the W apiary, bees removed 56.1% of dead brood while in the S apiary, only 43.0%. However, there was no interaction of environmental "apiary" and genetic "group" factors on hygienic behaviour $F_{(1,81)}$ = 0.04, p = 0.82.

The nectar flow in 2010 was very low in both apiaries. The nectar net income in the control colony controlled on hive scales from the 1st of May to the 31st of July, was 12.5 kg in the W apiary and 11.4 kg in the S apiary. During the hygienic behaviour test as well as three days prior the test, the nectar net gain was significantly different in the apiaries. In Wola Bukowska the nectar net gain was only 0.3 kg and in Sielce 2.2 kg (Fig. 3).

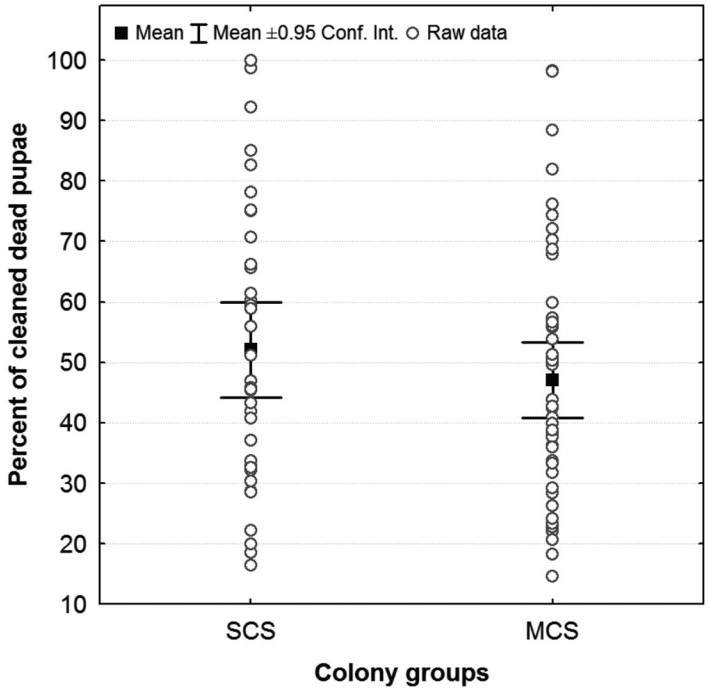

Fig. 1. Hygienic behaviour as a percent of cleaned dead pupae within 24 hours in colonies of each experimental group. SCS - queens instrumentally inseminated using semen from drones of single colony. MCS - queens instrumentally inseminated using mixed semen from drones of each of 30 colonies.

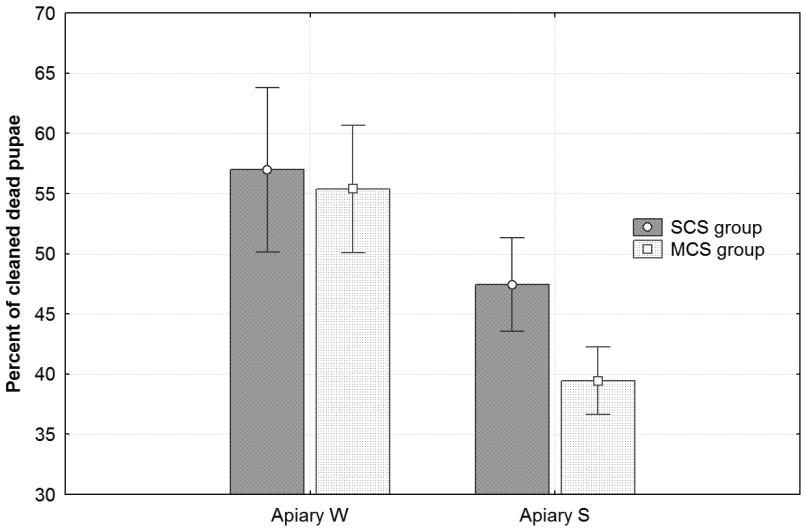

Fig. 2. Hygienic behaviour as a percent of cleaned dead pupae within 24 hours in colonies of each experimental group in two apiaries. Vertical bars indicate 0.95 confidence intervals. SCS - queens instrumentally inseminated using semen from drones of single colony. MCS - queens instrumentally inseminated using mixed semen from drones of each of 30 colonies.

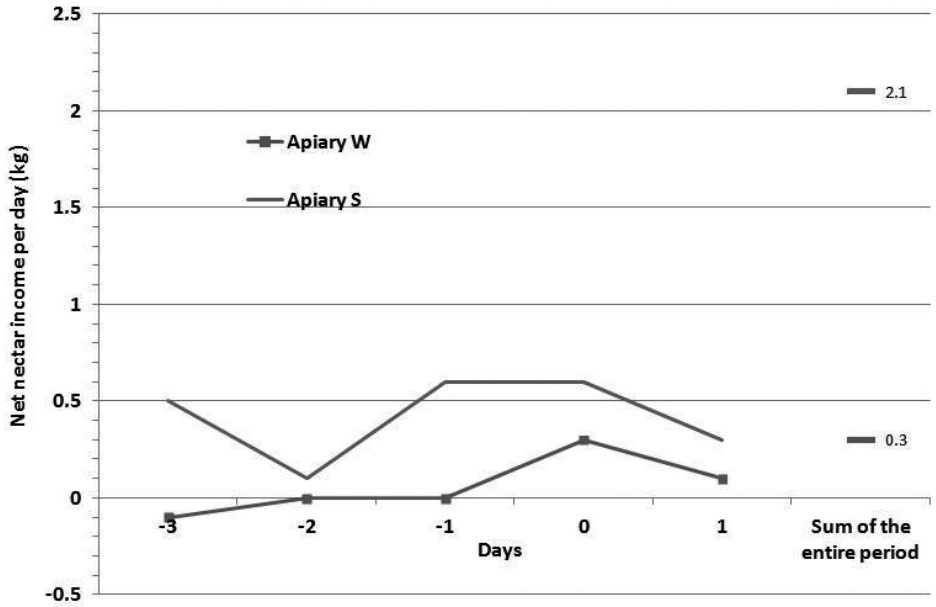

Fig. 3. The nectar net income in the control hives controlled on hive scales for three days before the test started (-3 days) to the end of the test (day 1). The day marked as day 0 is the day in which the tests were initiated.

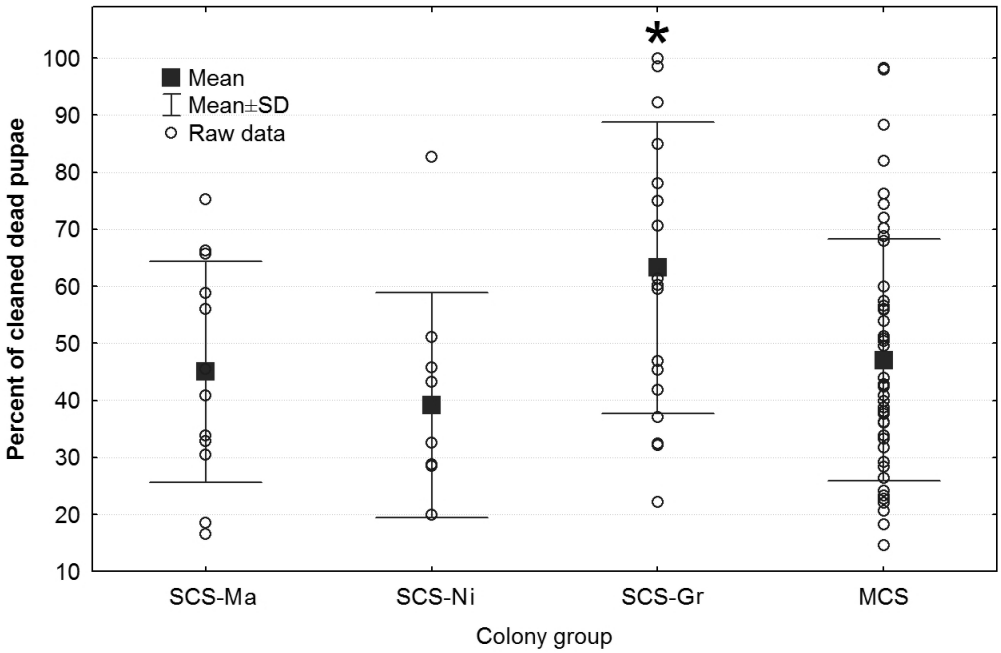

Fig. 4. Hygienic behaviour as a percent of dead pupae cleaned within 24 hours in colonies of each experimental group depending on the paternal line. SCS -queens instrumentally inseminated using semen from drones of single colony. The letters Ma, Ni, and Gr indicate certain strains of drones used for insemination. MCS - queens instrumentally inseminated using mixed semen from drones of each of 30 colonies. According to the Tukey's test, * marks the means which differed significantly from the others.

The effect of the origin of drones significantly affected the hygienic behaviour of the colonies $F_{(3,77)} = 4.59$, $p = 0.005$. Figure 4 shows the hygienic behaviour of colonies where the SCS group was divided according to the origin of drones used for insemination. It was found that colonies in which Gr line drones were used for insemination, had significantly greater hygienic behaviour (63.2%) than colonies where Ma (45%) and Ni (39.1%) line drones were used, and where colonies of the MCS group (47%) were used. The variance in the groups when a group of the SCS divided in terms of the

paternity component, was different, however not significant (Bartlett's chi-squared test for equal variances, p = 0.058). Paternal Gr component probably affected higher cleaning rate in this group. In addition, an interaction was found between the genetic factor "group" and the environmental factor "apiary" $F_{(3,77)}$ = 3.13, p = 0.03, Fig. 5).

A significant difference between colonies of the SCS group as far as the cleaning rate was concerned, was stated in favour of the SCS-Gr subgroup (Fig. 4). On the other hand, this trend was as expected, since this bee line (stain Gr) was selected based on hygienic behaviour. Different percentages of cleaned cells with dead brood after 24 hours, are presented

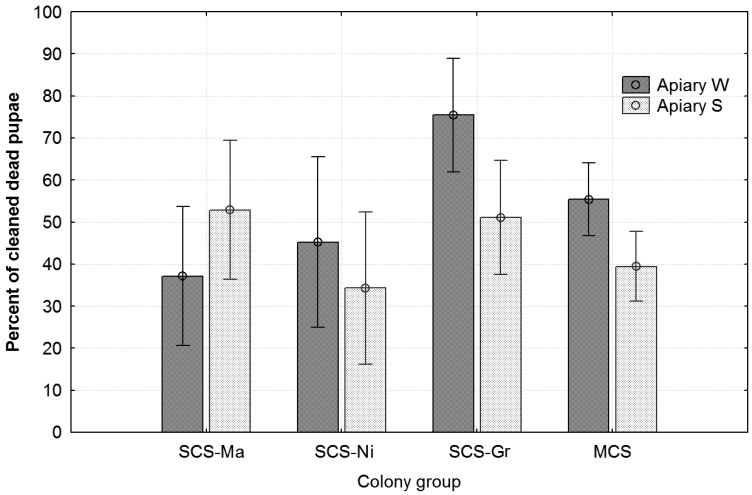

Fig. 5. Hygienic behaviour of colonies as a percent of dead pupae cleaned within 24 hours in each experimental group in two apiaries, depending on the paternal line. SCS - queens instrumentally inseminated using semen from drones of single colony. The letters Ma, Ni, and Gr indicate certain strains of drones used for insemination. MCS - queens instrumentally inseminated using mixed semen from drones of each of 30 colonies.

DISCUSSION

The hygienic behaviour of bees from groups with a lower and higher genetic diversity of workers did not differ significantly. Similar results were obtained in earlier studies (Page et al., 1995; Tarpy, 2003). None of the studies concentrated on bees selected towards hygienic behaviour. Tarpy (2003) analysed the variability in two groups of colonies. He found that increased genetic diversity within colonies limits the variability. However, these data are not fully comparable due to the experiment design. In this project and in the project of Tarpy (2003), the source of variation of genotypes was similar, 30 and 24 drone colonies. Genetic variation in the group of colonies with a lower genetic variability of offspring differed significantly between experiments. Tarpy (2003) inseminated sister queens with semen from only one drone. The offspring of these queens had a greater coefficient of relatedness (G = 0.75). Whereas, in the present experiment, queens were inseminated with semen from many drones (8 - 10) from a single colony that the progeny was related to (G = 0.5).

in different studies. The results are not comparable due to significant differences in the genotype of the tested bees (Panasiuk et al., 2009), methods of killing brood (Olszewski et al., 2013), or environmental conditions concerning the conditions inside the nest, for example the width of cells (Olszewski et al., 2014). We predicted, according to Arathi and Spivak (2001), that the natural composition of bees in a real colony with various genotypes, selected and unselected for hygienic behaviour (group MCS), affects the performance of the hygienic behaviour of the whole colony. However in this research this phenomenon was not observed.

It could be seen (Fig. 5), that in addition to considerable differences in hygienic behaviour between groups at particular apiaries, the interaction between genetic and environmental factors was detected. It should be noted though, that the differences occurred only in the subgroup SCS-Ma, where the queens and drones used for insemination were related. This probably influenced why the bees were unstable and why they were the most dependent on the environment.

The differences in the hygienic behaviour of bees in both apiaries were striking. Although the difference

was only 13% more cells cleaned in apiary W, the difference was statistically significant compared to apiary S. According to Panasiuk et al. (2009) the hygienic behaviour of bees depends on the natural flow, especially if the nectar flow occurs immediately prior to testing. In this experiment, the opposite relationship was observed because in apiary W, during the test period and three days prior to the test, there was a lack of nectar flow, or it was minimal in comparison to the nectar flow in apiary S. A possible explanation for this situation is the effect of other weather factors, such as the intensity of the sun, or the hours of sunshine during the day. The flight activity of the bees is significantly related to the above factors which encourage the bees to clean the cells.

CONCLUSION

The performance of the hygienic behaviour of honeybee colonies as colonies with a natural set of individuals having various genotypes, is more dependent on the selection than degree of polyandry. Hygienic behaviour in colonies is the result of characteristics of individual components, and can vary significantly in different environmental conditions.

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