Original Article

PERFORMANCE OF BEE COLONIES HEADED BY QUEENS INSTRUMENTALLY INSEMINATED WITH SEMEN OF DRONES WHO COME FROM A SINGLE COLONY OR MANY COLONIES

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Abstract

The aim of the study was to determine the effect of honey bee worker diversity within the colony on: development, honey productivity, and wintering. Two different levels of diversity within the colony were tested. The appropriate levels of diversity within the colony were obtained by selecting drones for inseminating the queens. Lower genetic diversity was obtained in the colonies headed by a queen inseminated with semen collected from drones originating from a single colony. Higher genetic diversity was obtained in the colonies with queens inseminated with semen from drones of 30 different colonies. Colonies with a higher genetic variation of workers in the colonies had greater levels of functional characteristics. However, apart from the number of dead bees in winter, the genetic diversity level of the workers on the colony development and honey production, did not have a significant influence. There was an averaging effect observed concerning that male component in the colonies with a higher genetic variation of workers - on honey yield, when compared to the non-additive effect of the best drones.

Keywords: **genetic diversity, honey yield, instrumental insemination, polyandry, wintering.**

INTRODUCTION

Multiple copulation is a common phenomenon among the species of social Hymenoptera (Strassmann, 2001). Among such species, bees of the *Apis* genus are the most well-known for copulation frequency and for having the highest degree of polyandry (Moritz et al., 1995; Oldroyd et al., 1995; Oldroyd et al., 1996; Oldroyd et al., 1997; Oldroyd et al., 1998; Rinderer et al., 1998). Simmons and Siva-Jothy (1998) recognised that the benefits which bee queens gain from copulating with multiple drones must be more advantageous than the risk of performing multiple mating flights. The risk of multiple mating includes increased exposure to predators, risk of injury to the body, and some disease transmission. One of the proposed benefits to the queen could be that more sperm is collected, which should be sufficient

for many years (Crozier and Page, 1985). Multiple mating reduces the chance that all the queens' offspring will come from poor males, especially when there are genetically less valuable males in the drone population (Page et al., 1995; Jennions and Petrie, 2000). One of the theories about the genetic diversity benefits concerning the offspring in a colony is that the diversity facilitates specialisation and division of labor (Fewell and Page, 1993; Page et al., 1995; Mattila et al., 2008), and 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). General genetic diversity among progeny may be advantageous in highly variable environments. This includes circumstances where progeny compete very intensely (Williams, 1975; Ridley, 1993). Genetic diversity ensures the bees

adapt better to the environment. Multiple mating may reduce the risk of inbreeding (Stockley et al., 1993).

Females of *Apis mellifera* mate while taking part in several mating flights. Females may even mate with 17 drones (Woyke, 1960). The average number of matings is 12 (Estoup et al., 1994; Tarpy and Nielsen, 2002). It is important to note that in natural conditions, it is unlikely that a queen will mate with drones from the same colony. In contrast, in the case of instrumental insemination, the queens are usually inseminated with semen collected from drones from a single colony.

The unexplained phenomenon of honey-bee colony losses have been observed for the last several years all over the world. In Poland, bee loss mainly takes place in the autumn and winter. The parasite, *Varroa destructor*, and the accompanying viral infections are the main reason for the colony losses (Topolska et al., 2008; Pohorecka et al., 2011). The decrease in viability of bee colonies may be due to the lower genetic variation of individuals within the colony. Less variation leads to a reduced adaptability of bees. To date, many experiments have been performed to find out the influence of genetic variation within bee colonies the on performance. However, the results obtained in these experiments were various. There have been varying results because the experiments were performed either in small bee colonies (Fuchs and Schade, 1994; Page et al., 1995) or the queens were instrumentally inseminated with a small amount of semen (Fuchs and Schade, 1994; Page et al., 1995; Tarpy, 2003; Tarpy and Seeley, 2006; Mattila and Seeley, 2007). Sometimes, the coefficient of relatedness in experimental queens, was changed in an unnatural way (Oldroyd et al., 1992). There was no correlation between the copulation frequency of queens and colony strength, and disease resistance (Neumann and Moritz, 2000). In the case of colonies which significantly exceeded the natural variation (queen inseminated with mixed semen from 200 drones) an even lower colony development was observed (Fuchs et al., 1996). Averaging the positive and negative characteristics of bee colonies, instead of only the positive and desired characteristics, may indicate that multiple mating and polyandry in bees tends to stabilise the colonies and reduce the negative effects of random natural selection (Page et al., 1995; Fuchs et al., 1996).

The aim of the research was to determine the effects of bee worker diversity within the colony, on the development, productivity, and overwintering ability. Tests were done on two groups of bee colonies in which the individuals had different levels of genetic diversity. The appropriate levels of diversity within the colony were obtained by selection of drones for insemination of the queens. The lower genetic diversity of workers was obtained in colonies in which the queens were inseminated with semen from drones of a single colony. The higher genetic diversity of workers was obtained in colonies in which the queens were inseminated with drone semen collected from 30 different colonies. The semen had been collected and then mixed.

MATERIAL AND METHODS

Rearing and insemination of the bee queens

The study was performed in the 2009 - 2012 time period, at the Institute of Horticulture, the Apiculture Division in Pulawy, Poland. The study continued over two full beekeeping seasons. Three commercial strains of carnica bees (M, N, and G) were used in the experiment. Sister queens (coefficient of relatedness 0.5), daughters of a queen mated with drones obtained from a single mother queen from a line M, were reared in June 2009. Virgin queens, soon after hatching from queen cells, were individually introduced into a 3-skewer trapezoid styrofoam mating nuclei with a capacity of about 1.5 dm^3. The queens were colonised with about 1000 bees and kept in prior to laying eggs. Drones were raised at the same time, in 30 colonies, 10 in each of the bee strains used. The experimental queens were divided into two groups. Then, the queens were instrumentally inseminated to achieve two levels of genetic diversity in the offspring. Queens from both groups were inseminated twice with a dose of 4 µL of semen each. The first time the queens were inseminated was at the age of seven days. Insemination was repeated 24 hours later. For both the first and second insemination, the queens were subjected to 3 minutes of carbon dioxide anesthesia. Each time, drone semen from the same colonies was injected. The queens from one group (SCS - single colony semen) were inseminated with semen collected from drones from one of 30 paternal colonies. For each insemination, semen from 4 - 5 drones was used depending on how many of them were needed to collect a 4 µL dose of semen. The above information means that the source of drones was systematically varied between the queens. The queens from the second group were inseminated with semen collected from one drone from each of the 30 colonies, and the semen was then mixed (the MCS - colony semen mixed group). Semen from

30 drones was collected into a syringe, and then let out into a glass vial, mixed, and diluted with Hyes solution prior to inseminating the MCS queen group (Skowronek et al., 1995). One portion of mixed ejaculate and solution was enough to inseminate 3 - 4 queens. This manipulation was repeated till all the queens were inseminated.

Establishment of experimental apiaries

At the end of July 2009, 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 placed in two apiaries (Tab. 1) located in areas with different flow conditions: in the village of Wola Bukowska (W) 51°40'09"N 22°21'22" E and the suburban area of Sielce (S) 51°26'23"N 22°04'46"E. A varroa control treatment was applied in all of the colonies each autumn to eliminate most of the parasites and equalise the level of invasion.

Apiary observations and laboratory tests

The nectar flow was monitored in both experimental apiaries from May to July of 2010 and from May to July of 2011. On the basis of the control-hive scale measurements, the periods and abundance of nectar flow were described and calculated to characterise the monthly net nectar income in kg. The spring-summer development of colonies, according to brood area (dm^2), was evaluated 3 times per season, starting from mid-May and repeated every 3 weeks. The honey yield was the main indicator of the colony productivity. The colony productivity consisted of the sum of the honey centrifuged in kg together at each honey harvest, and the honey left in the nests after the last harvest. Overwintering of colonies was assessed on the basis of the number of bees fallen during the winter (determined just before spring flight activity), and the percentage rate of change of the nest size on the basis of the frame number in spring and autumn. Measurements were made of the brood surface and honey left in the hive with the help of photographs taken. The images were analysed and calculated using a computer program (MultiScanBase. v.18.03).

Statistical analysis

To analyse the impact of genetic variation on the number of brood in colonies, the repeated measures ANOVA was used. Assumptions of the model were tested by the Mauchly's sphericity test. Complete cases were taken into account in the analysis. For the colonies, an average honey yield and the number of bees lost in the winter were calculated by aggregating two years. Differences between groups were analysed with the two-way ANOVA. Homogeneous groups were created using the Fisher's NIR test. Some indicators of the colonies' overwintering did not have a normal data distribution. For the data analysis, the nonparametric Mann-Whitney tests were used. All calculations were performed using the Statistica package (StatSoft, Inc.).

RESULTS

Nectar flow in experimental apiaries

The nectar flow was very low in 2010 in both apiaries. The nectar net income in the control hives was 12.5 kg in the W apiary controlled on hive scales from the 1st of May to the 31st of July and amounted in the subsequent months to: -0.9 kg, 10.2 kg, and 3.2 kg. In the S apiary, net nectar income was 11.4 kg, and in 3 months 5.1 kg, 5.6 kg, and 0.7 kg, respectively.

Table 1.

Experiment design and the number of colonies in particular groups

Apiary	Groups of colonies			
	SCS			MCS
	SCS-M*	SCS-N*	SCS-G*	
Wola Bukowska	9	8	10	27
Sielce	8	8	8	24
Total		51		51

The SCS colonies with queens instrumentally inseminated using semen from drones of single colony. The MCS queens instrumentally inseminated using mixed semen from drones of 30 colonies.

SCS - queen inseminated with single colony semen; MCS - queen inseminated with multiple colony semen

*SCS group divided in terms of the paternity component; letters M, N, and G indicate certain strains of drones used for insemination.

In 2011, the nectar flow was much higher than in the previous year, while the distribution of the flow in the season was similar with the low nectar flow in July. The nectar net income in the control hives from 1st May to 31st July was 44.8 kg in the W apiary: 10.9 kg, 33.8 kg, and 0.1 kg in 3 months, respectively. In the S apiary, net nectar income was 40.9 kg, 23.5 kg, 20.4 kg, -3 kg, respectively.

Brood area

There was no significant difference in brood area between colonies with different genetic variation within the colony, however, the brood area was slightly bigger in the MCS group than in the SCS group: 73.7 and 69.6 dm^2, respectively. No difference between apiaries and no interactions between the groups and the environmental factors were detected (Tab. 2, Fig. 1). Obvious variation between measurements of the brood area taken in different seasons was confirmed. In contrast, no interaction was observed between groups and the measurements (Tab. 2, Fig. 2). Figure 3 shows the average brood area in individual colonies, where the SCS group was divided according to the origin of drones used for insemination. It was found, that colonies in which M line drones were used for insemination produced slightly less brood than colonies from other groups. However no significant differences and interaction with location were detected (Tab. 2, Fig. 3).

Table 2.

Brood area and synthetic results of the repeated measures ANOVA

Effect	The SCS group without splitting the paternity component		The SCS group divided in terms of paternity component	
	df	p	df	p
Group	1	0.24	3	0.59
Apiary	1	0.21	1	0.12
Group x Apiary	1	0.39	3	0.70
R1	5	<0.01	5	<0.01
R1 x Group	5	0.8	15	0.28
R1 x Apiary	5	<0.01	5	<0.01
R1 x Group x Apiary	5	0.15	15	0.13
Error	160		140	

R1 - measurement, p - probability of F statistic, df - degree of freedom; for other abbreviations see Table 1.
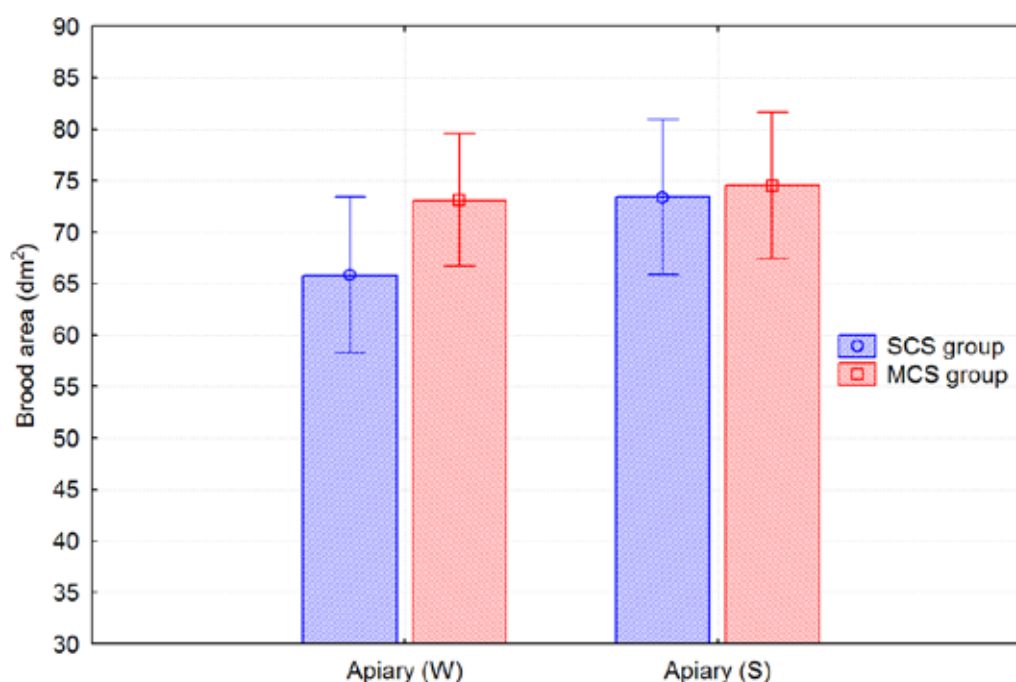

Fig. 1. The average brood area in colonies of both groups in each apiary. The means were calculated on the basis of repeated measures ANOVA. Vertical bars indicate 0.95 confidence interval. For abbreviations see Table 1.

Honey production

Considering the total for two years, the colonies with lower genetic variation (the SCS group) produced, on average, about 2 kg less honey than colonies with higher genetic variation (the MCS group); 33.7 and 35.6 kg, respectively. The differences in honey production between the groups were not significant (Tab. 3). When taking into account both years of the study, the average honey yield of colonies in the S apiary, was almost two times lower than in the W apiary; 23.5 and 43.7 kg, respectively. (Tab. 3). The two-way ANOVA showed only an environmental "apiary" effect on honey yield, and interaction with the genetic factor "group" was close to significant (Tab. 3, Fig. 4). There was found to be a very large range of honey yield in the colonies, regardless of the group. In the first year of the study, the range was from 2.3 kg to 21.8 kg. In the second year, the range was even higher, from 1.3 kg to 54.7 kg. However, the variance in the two groups was similar (Bartlett's test for equal variances, p = 0.6). The Pearson correlation coefficient between the honey production in individual colonies in both years (calculated separately for each group) was: in the SCS group r = 0.4, p = 0.6, and in the MCS group r = 0.7, p≤0.05. These results indicate that the colonies from the MCS group were less diverse than colonies from the other group and the production results of the MCS group repeated year after year. Table 3 and Figure 5 show honey production in

Table 3.

Honey production and number of dead bees found in winter debris. Synthetic results of the multifactor ANOVA. Analysis was performed on means, by aggregating two years

Effect	Traits							
	Honey production				Number of dead bees found in winter debris			
	df	p	df*	p*	df	p	df*	P*
Group	1	0.7	3	<0.01	1	<0.01	3	0.03
Apiary	1	<0.01	1	0.01	1	<0.01	1	0.07
Group x Apiary	1	0.06	3	0.2	1	0.8	3	0.07
Error	42		42		55		51	

p - probability of F statistic, df - degree of freedom; for other abbreviations see Table 1.
*Statistics calculated for the group when a group of the SCS divided in terms of the paternity component.
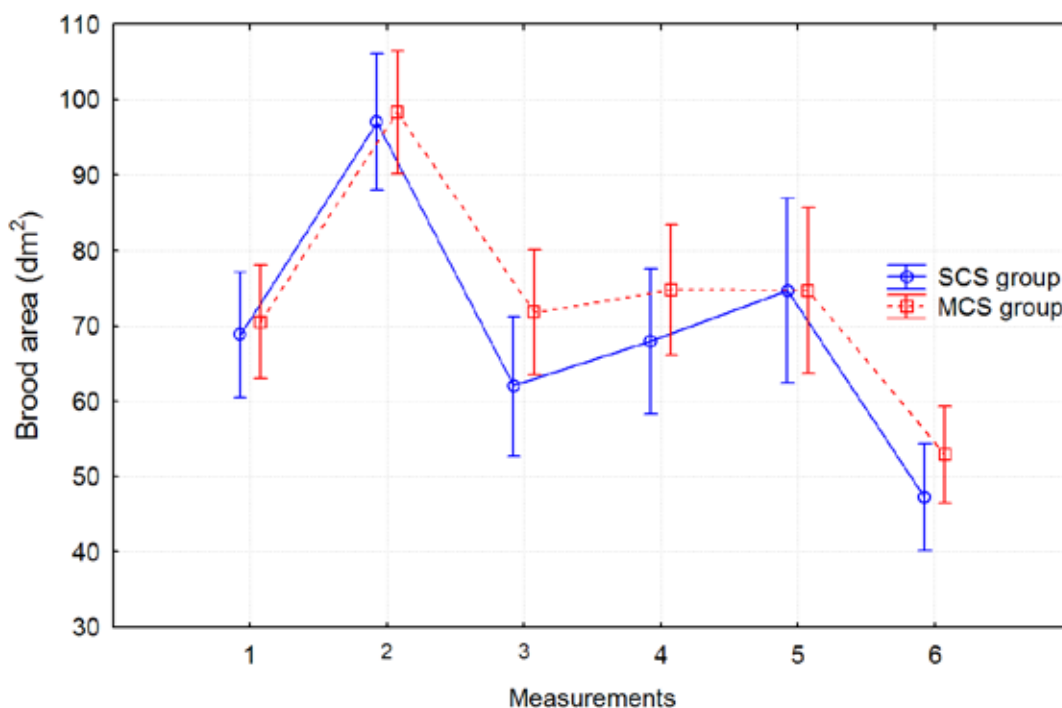

Fig. 2. The average brood area in colonies of both groups in each measurement. The means were calculated on the basis of repeated measures ANOVA. Vertical bars indicate 0.95 confidence interval. For abbreviations see Table 1.
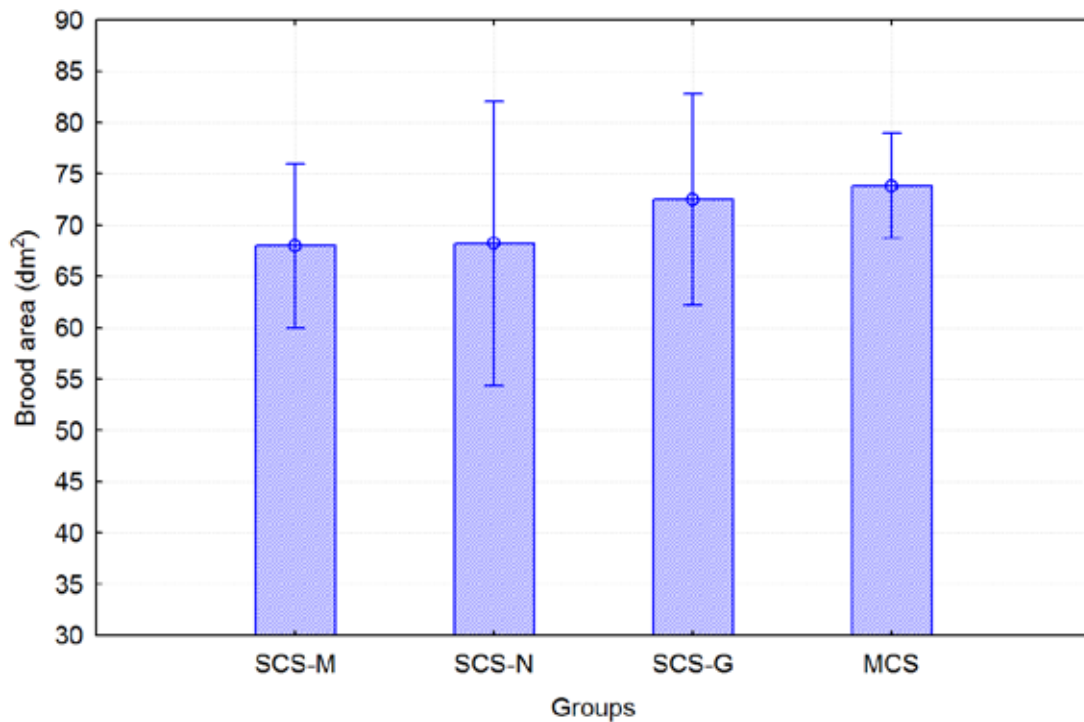
Fig. 3. The average brood area in colonies of both groups. The SCS group divided in terms of the paternity component. The means were calculated on the basis of repeated measures ANOVA. Vertical bars indicate 0.95 confidence intervals. For abbreviations see Table 1.
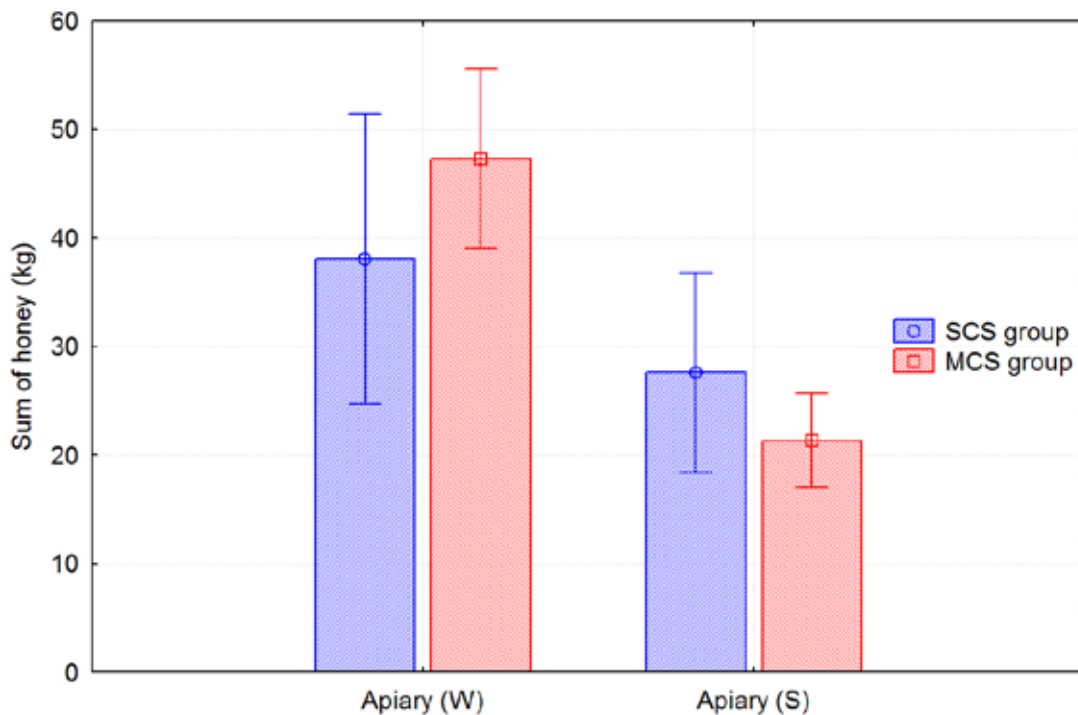

Fig. 4. Honey production in colonies of both groups in each apiary. The means were calculated for the two-year total. Vertical bars indicate 0.95 confidence intervals. For abbreviations see Table 1.

individual colonies where the SCS group was divided according to the origin of drones used for insemination. It was found, that colonies where G line drones were used for insemination produced significantly less honey than colonies where M and N line drones were used, and significantly less honey than colonies of the MCS group.
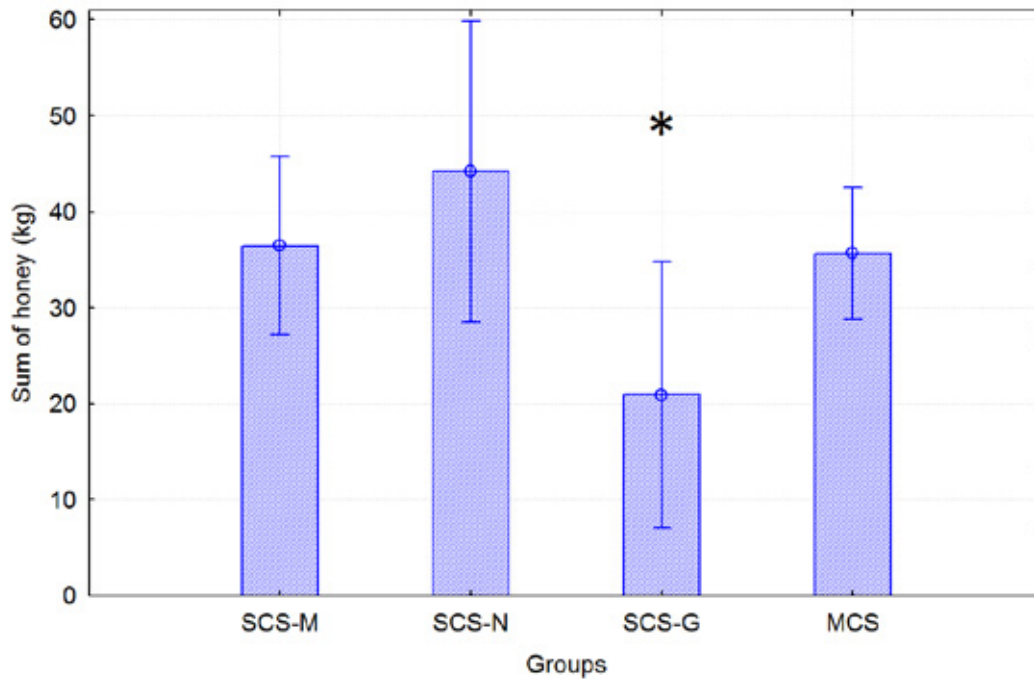
Fig. 5. Honey production in colonies of both groups. The SCS group divided in terms of the paternity component. The means were calculated for the two-year total. Vertical bars indicate 0.95 confidence intervals. According to the LSD test, (*) marks the means which differed significantly from the others. For abbreviations see Table 1.
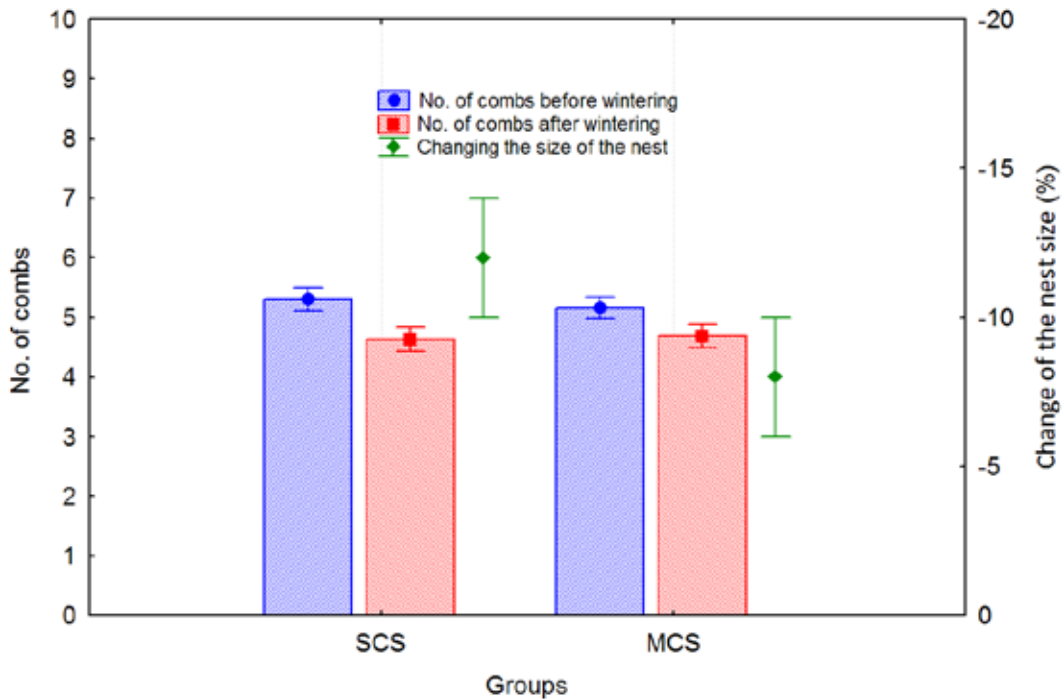

Fig. 6. Qualitative descriptors of wintering colonies from both experimental groups in both apiaries during the wintering. The data for the two years was averaged for one winter period. For abbreviations see Table 1.

Overwintering

Data concerning the size of the colony nests before and after overwintering as well as the changes in the size of the nest, did not have a normal distribution. Differences between means were tested by the non-parametric Mann-Whitney U test at $\alpha = 0.05$. Bees from the SCS and MCS groups wintered on a similar number of combs; 5.3 and 5.1, respectively. In spring, bee nests of the SCS colonies were reduced, on average, by an 11.2% of combs while the nests of the MCS colonies by 8.6%. No significant differences in the strength of these colonies
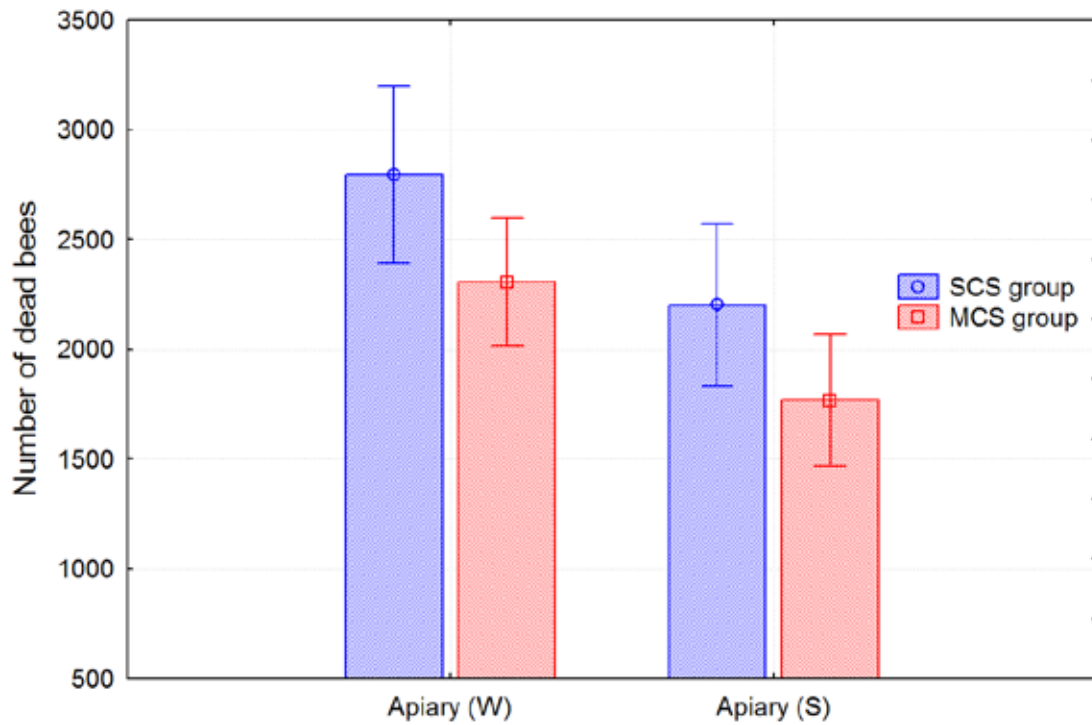
Fig. 7. Number of dead bees found in winter debris in bee colonies from both experimental groups in both apiaries. The means were calculated for the two-year total. Vertical bars indicate 0.95 confidence intervals. For abbreviations see Table 1.
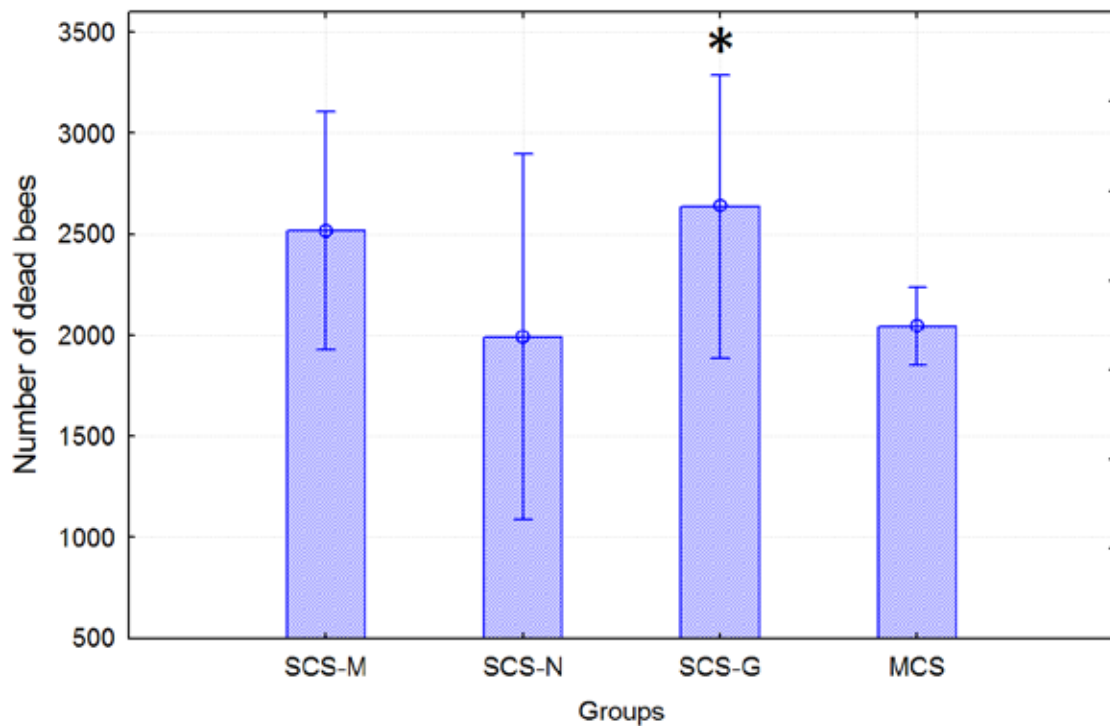

Fig 8. Number of dead bees found in winter debris. The SCS group is divided in terms of the paternity component. The means were calculated for the two-year total. Vertical bars indicate 0.95 confidence intervals. According to the LSD test, (*) marks the means which differed significantly from the others. For abbreviations see Table 1.

were stated at both measurements, before and after wintering (Fig. 6). The colonies in the village of Wola Bukowska were significantly stronger in autumn than the colonies in the suburban area of Sielce. Bees occupied an average of 5.3 combs in the W apiary and 5.0 combs in the S apiary. However, the average loss of bees was higher in bee colonies in the W apiary compared to loss of bees in the S apiary (Fig. 7). Consequently, in spring, the bees covered a similar number of combs.

Bees lost during the overwintering period were found on the bottom board just before the first flight in spring. The number of bees lost during the over-wintering period for the two years, differed significantly between the groups. Colonies from the SCS group lost significantly more bees than the colonies from the MCS group; 2470 and 2043 individuals, respectively. Regardless of the group, there were significantly more bees lost in the W apiary compared to the S apiary; 2473 and 1940 individuals, respectively. There was no "group" and "apiary" interaction. (Tab. 3, Fig. 7). Figure 8 shows the number of bees of individual colonies lost during the overwintering period; the SCS group was divided according to the origin of drones used for insemination. Colonies in which the drones of the G line were used, lost significantly more bees than: the colonies where the M and N line drones were used, and the colonies of the MCS group.

DISCUSSION

The colonies with lower worker genetic diversity (the SCS group) raised less brood compared to colonies with higher diversity (the MCS group), but the difference was not significant. Similar results were obtained in many other experiments (Fuchs and Schade, 1994; Page et al., 1995; Fuchs et al., 1996;

Neuman and Moritz, 2000). A significant increase in the amount of brood raised in the colonies with greater genetic diversity was obtained by Oldroyd et al. (1992). In nature, the level of polyandry depends on the number of matings, and this is related to the volume of semen that the queen takes. Richards et al. (2007) and Niño et al. (2013) showed that semen volume affects the performance of queens and colonies. In the present study, semen volume was standardised. Standardisation might be the reason why the main effect (the demonstration of the advantages of the polyandrous queens) was not illustrated.

The colonies with the lower genetic diversity of workers were slightly weaker in honey production compared to colonies with a higher genetic diversity of workers. The breeding value of drones used for insemination was different (Fig. 5). There were significant differences in honey yield in colonies in which the queens mated with a different line of drones from single colonies (the SCS group). There was an averaging effect observed, of the individual male component in the colonies with queens inseminated with mixed semen (the MCS group) with a non-additive effect of the best drone values. Similar conclusions have been found by Fuchs and Schade (1994), Page et al. (1995), and Fuchs et al. (1996). Such a trait expression in the offspring reduces the adverse failed selection effect of the parents. Repeatability of the production of specific colonies in the subsequent years was observed only in the colonies of the MCS group (r = 0.7). The issue of the honey production in colonies with a high genetic variety of individuals, has not been entirely explained. The interaction (close to significance) of genetic traits with environmental factors may prove this (Fig. 3). Colonies from the MCS group showed a greater honey production in the apiary and the

Table 4.

Collective list of the average level of examined traits for the factor group and apiary.

Factor analysed	Brood area (dm^2)	Honey production (kg)	Change in the size of the nest in spring (%)	Number of dead bees in winter debris
	Mean	Mean±SD	Mean±SD	Mean±SD
SCS	69.6 a	33.7±16,2 a	-11.2±13.7 a	2470±831 b
MCS	73.7 a	35.6±17,9 a	-8.6±15.1 a	2043±582 a
Apiary (W)	70.0 a	43.7±17,0 a	-15.8±12.6 a	2473±693 b
Apiary (S)	74.0 a	23.5±9.5 a	-5.9±15.2 a	1943±633 a

The SCS colonies with queens instrumentally inseminated using semen from drones of a single colony.
The MCS queens instrumentally inseminated using mixed semen from drones of 30 colonies.
Different letters in columns separated by a blank row indicate significant differences at α = 0.05.
For abbreviations see Table 1.

honey had a later and slightly more abundant flow. Genetic diversity of workers within colonies had a significant effect on the number of dead bees found in the debris after wintering. Therefore, it appears that it is justified to apply appropriate selection to receive a higher genetic diversity within colonies. The higher diversity improves the rate of overwintering. However, based on the number of nest combs present in spring, in both groups, there was no significant difference between groups.

CONCLUSIONS

Colonies with a higher genetic variation of workers had greater levels of functional characteristics (Tab. 4). However, apart from the number of bees dying in winter, there was no significant influence of the level of genetic diversity of workers - on the colony development and honey production. There was an observed averaging effect of the male component, in the colonies with a higher genetic variation of workers, on honey yield, with the non-additive effect of the best drones.

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