

**HERITABILITY OF GROOMING BEHAVIOUR IN GREY HONEY BEES (*Apis mellifera carnica*)**

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*Grooming behaviour is considered an important defensive mechanism of honey bees against Varroa mites. The aim of this study was to reveal whether grooming behaviour is a useful criterion in breeding of Varroa-tolerant bees. To obtain a reliable evaluation the environmental influences were excluded. The degree of grooming potential was estimated by the percentage of damaged mites in the total number of fallen mites. The heritability of grooming behaviour throughout the three consecutive generations of queens was assessed by mother-daughter regression method. Among unselected queens, expressed grooming behaviour was recorded only in colonies with F1 queens (36.27%), but not in colonies with P queens and F2 queens (33.69%, 31.66%, respectively). Significant differences in grooming behaviour were found between colonies of P and F1 queens ( $p < 0.001$ ), and between colonies of P and F2 queens ( $p < 0.05$ ).*

*However, all of the three generations of selected queens showed expressed grooming behaviour (37.99%, 39.42% and 38.58% in Ps, F1s and F2s, respectively) without significant ( $p > 0.05$ ) difference among them. Nevertheless, the relatively low heritability of grooming behaviour in the three generations of queens examined ( $h^2_{yx} = 0.49 \pm 0.02$ ;  $h^2_{zx} = 0.18 \pm 0.01$ ;  $h^2_{zy} = 0.16 \pm 0.01$ ) indicate that breeding colonies for grooming behaviour only cannot be advised to beekeepers whose aim is to breed bees highly tolerant to Varroa mites.*

*Key words: Apis mellifera carnica, heritability, grooming behaviour, Varroa*

INTRODUCTION

The ectoparasitic mite *Varroa destructor* Anderson and Trueman is doubtless one of the most serious worldwide pest of the honey bee *A. mellifera* L. Since it is deleterious to honey bees, colonies die from varroosis within a few years if the mite population growth is not regulated by the beekeeper (Fries *et al.*, 1996) and chemical control (with acaricides and/or organic acids) is restricting (Milani, 1999; Wallner, 1999; Gregorc *et al.*, 2004; Stanimirovic *et al.*, 2005a, 2007; Pejin *et al.*, 2006; Gregorc and Smodiš-Škerl, 2007), it is of particular interest to breed bees for higher resistance to this mite (Boecking and Spivak, 1999,

Stanimirović *et al.*, 2008). Selection and breeding bees highly resistant to *V. destructor* are considered long-term solutions to the problem (Boecking and Spivak, 1999; Ibrahim and Spivak, 2006). Among several mechanisms that contribute to the resistance to the mite, the most important are the hygienic and grooming behaviour. Furthermore, selection for hygienic behaviour alone is not a sufficient mechanism of resistance to *V. destructor* (Stanimirović *et al.*, 2008). Consequently, bees may require multiple mechanisms to confer resistance; i.e., to survive the infestation without treatment (Mondragon *et al.*, 2005). Thus, selecting bees for another trait, such as grooming behavior, which would limit the number of mites, may also be worthwhile (Ruttner and Hänel, 1992; Thakur *et al.*, 1997; Arechavaleta-Velasco and Guzmán-Novoa, 2001; Mondragon *et al.*, 2005; Stanimirović *et al.*, 2007, 2008). Selective breeding for mite resistance can proceed with the characteristics that are heritable ( $h^2 > 0.25$ ). However, genetic investigations of grooming behaviour are scarce. According to Frumhoff and Baker (1988) there is some degree of genetic determination of grooming specialization, and workers groom full-sisters more readily than half-sisters (Frumhoff and Schneider, 1987). Besides, Moretto *et al.* (1993) reported that grooming behaviour is a heritable characteristic ( $h^2 > 0.71$ ). Besides, the expression of grooming behaviour is strongly influenced by environmental factors (Stevanović, 2007; Currie and Tahmasbi, 2008) indicating the questionability of heritability. To solve the dilemma whether grooming behaviour is a useful criterion in breeding of *Varroa*-tolerant bees it is necessary to evaluate its heritability.

Grooming behaviour implies the ability of adult bees to detect and remove phoretic mites from themselves (auto-grooming) or from nestmates (allo-grooming) (Peng *et al.*, 1987). In the process, the legs of the mite may be cut off or the cuticle may be damaged by the bees' mandibles causing the fall of damaged mites from their hosts (Ruttner and Hänel, 1992). The main type of damage is amputation or mutilation of one or more mites' legs (Ruttner and Hänel, 1992; Lodesani *et al.*, 1996; Rosenkranz *et al.* 1997; Correa-Marques *et al.*, 2002; Stanimirovic *et al.*, 2003, 2005b), but there are also injuries of the mites' idiosoma or gnathosoma (Stevanović, 2007). There is video evidence of aggressive behaviour of bees against *Varroa* mites (Thakur *et al.*, 1997). However, direct recording of defensive behaviour in hives is extremely time-consuming and unfeasible in practical bee breeding (Fries *et al.*, 1996; Bienefeld *et al.*, 1999). For this reason, an indirect method for assessing grooming behaviour is proposed: the calculation of the proportion of damaged mites among naturally fallen mites (Hoffman, 1996; Moosbeckhofer, 1997). However, not all damages of naturally fallen mites are the resulting from active defensive grooming behaviour of bees (Szabo and Walker, 1995; Bienefeld *et al.*, 1999) and environmental factors affect the ability of honey bees to remove the parasitic mite *V. destructor* (Currie and Tahmasbi, 2008). Thus, in assessing grooming potential as a defence mechanism the influence of environmental factors should be excluded. Besides, only adult mites should be considered since immature mites might be damaged during the removal of infested brood (a consequence of hygienic behaviour). However, hitherto never have all precautions been taken in the investigation of grooming behaviour. Therefore, the aim of the present study was the assessment of

grooming potential and its heritability in conditions devoid of any environmental influence.

#### MATERIAL AND METHODS

The research was conducted in an apiary in the vicinity of the city of Gornji Milanovac (Šumadija, the central part of Serbia) from 2004 to 2006. The degree of grooming potential of honey bees was estimated by the percentage of damaged mites among the total that fell from bees onto the protected sampling sheets placed under the hive. All mites that fell were collected and examined microscopically (Bio-Optica, Italy, type 1000) under the magnification of 40x (Rosenkranz *et al.*, 1997). Adult mites were counted and the percentage of damaged mites among them was calculated.

According to Hoffman (1993) colonies with 36% or more damaged mites in the total number of fallen mites were considered colonies with expressed grooming behaviour whilst those with the percentage less than 36% were considered as colonies without grooming behaviour.

To obtain a reliable evaluation of the degree of the honey bee grooming potential, the following recommendations of Bienefeld *et al.* (1999), Mondragón *et al.* (2005) and Currie and Tahmasbi (2008) were taken into consideration:

1) Only colonies absolutely free of wax moth larvae were assessed in order to avoid the possibility of damages made by those larvae.

2) Secondary damages of fallen and/or dead mites were prevented with anti-varroa screened bottom board in each hive (Calderone and Lin, 2003). The board which was placed under the hive consisted of a screen mesh and bottom drawer beneath. Sampling metal sheet placed on the bottom drawer was stained in white and smeared with vegetable grease. Such an equipment prevents the re-attachment of fallen mites to the bees as well as possible predators (ants and other scavengers) from making secondary damages on fallen mites.

3) The mites were collected in 24h intervals and analysed immediately afterwards in order to exclude the environmental influence (especially temperature and humidity) that is proven to affect both the grooming abilities of bees (Currie and Tahmasbi, 2008) and the extent of the damage in warm and damp conditions (Bienefeld *et al.*, 1999).

4) The mites were collected from May to September to exclude the influence of the season. In that period numbers of fallen mites are quite similar and significantly higher than in the rest of the year (Mondragón *et al.*, 2005).

5) At least 30 mites were used for the calculation of grooming potential since that was the necessary minimum for reliable estimation of average damage rates (Bienefeld *et al.*, 1999).

6) In accordance with the recommendation of Bienefeld *et al.* (1999) to prevent additional damages all mites were removed with a fine brush.

7) Exclusively adult mites were used in the evaluation of grooming potential since injuries on immature mites are not considered a consequence of typical defensive behaviour of bees against *Varroa* (Bienefeld *et al.*, 1999). As

recommended by Bienefeld *et al.* (1999) mites brighter than "ochre brown" were classified as immature while darker ones were considered adult.

Following those recommendations, we excluded completely the environmental influences that could contribute to the overestimation of the active defence of bees against the *Varroa* mites.

Only colonies with expressed grooming behaviour as well as good reproductive and productive features were chosen as breeder colonies for rearing queens in this experiment. The queens were marked with enamel paint and numbered on the thoraces. The experiment began with eight unselected commercial lines of the grey carniolan bee. Ten daughter-queens were propagated from each of those eight mother-queens. These 80 queens were the unselected parental queens (P) whose colonies were tested for grooming behaviour. Eight queens from colonies that showed the best grooming potential were selected as breeder mother-queens (Ps queens), and 80 daughter-queens (F1 queens) were propagated from them and tested for grooming behaviour. The best eight daughter-queens were selected (F1s queens) and used for rearing 80 granddaughter-queens (F2 queens). Their colonies were also tested for grooming behaviour and the best eight granddaughter-queens were selected (F2s queens). All of the queens were mated naturally with high-quality drones reared according to the procedure of Laidlaw and Page (1997).

The heritability of grooming behaviour monitored through three generations of queens was estimated from one-parent-offspring regression method (mother-daughter regression method). The following equations were used for the calculation of heritability:

$$b_{yx} = \frac{\Sigma xy - \frac{(\Sigma x) \cdot (\Sigma y)}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}$$

$$b_{zx} = \frac{\Sigma xz - \frac{(\Sigma x) \cdot (\Sigma z)}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}$$

$$b_{zy} = \frac{\Sigma xy - \frac{(\Sigma y) \cdot (\Sigma z)}{n}}{\Sigma y^2 - \frac{(\Sigma y)^2}{n}}$$

- x – the mean value of the grooming behaviour of Ps queens
- y – the mean value of the grooming behaviour of F1s queens
- z – the mean value of the grooming behaviour of F2s queens
- n – the number of queens
- $b_{yx}$  – the heritability of grooming behaviour from F1s to Ps
- $b_{zx}$  – the heritability of grooming behaviour from F2s to Ps
- $b_{zy}$  – the heritability of grooming behaviour from F2s to F1s

The statistical analyses, the one-way analysis of variance (ANOVA) and Tukey pairwise multiple comparisons, were performed by GraphPad prism 4.0.

## RESULTS

The results of the grooming behaviour in honey bee colonies with unselected queens are summarised in Table 1 and Figure 1. Based on the average percentage of damaged mites in the total number of fallen mites colonies with P queens and F2 queens (33.69% and 31.66%, respectively) did not express grooming behaviour, whilst colonies with F1 queens did (36.27%).

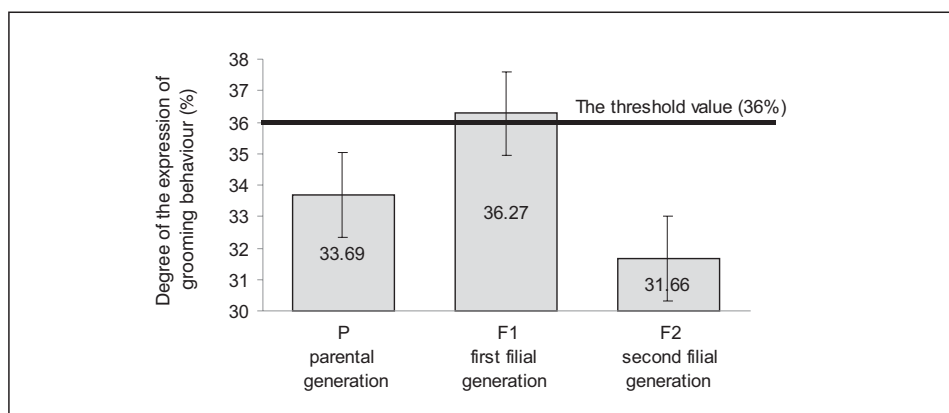


Figure 1. The mean values of grooming behaviour in colonies with unselected queens

The analysis of variance (group test) is done because there were three groups of colonies (P, F1 and F2). F-test proved the existence of significant ( $p < 0.001$ ) differences among all the colonies assessed ( $F = 9.373$ ). The differences between groups were tested by Tukey-test. The test revealed a significant ( $p < 0.001$ ) difference in the expression of grooming behaviour between P and F1 queens and a significant ( $p < 0.05$ ) difference between P and F2 queens. In contrast, the difference between F1 and F2 queens regarding grooming behaviour was not significant ( $p > 0.05$ ).

The results of grooming behaviour in honey bee colonies with selected queens are summarized in Table 2 and Figure 2. In all of the three generations of colonies with selected queens grooming behaviour was pronounced, i.e. the proportion of dented mites in the overall number of fallen mites was  $\geq 36\%$ . The exact corresponding percentages were 37.99%, 39.42%, and 38.58% in colonies with Ps, F1s and F2s queens, respectively.

The analysis of variance (group test) proved no significant difference ( $p > 0.05$ ) in grooming behaviour among the selected queens. Similarly, Tukey-test revealed no significant differences in the extent of grooming behaviour among the three generations of selected queens ( $p > 0.05$ ).

Table 1. Descriptive statistics of the grooming behaviour expression in colonies with unselected queens

Queen generation	No. of colonies	Grooming behaviour						SE	SD	CV (%)
		min		max		mean				
		No. of damaged mites/ total number of fallen mites	(%)	No. of damaged mites/ overall number of fallen mites	(%)	No. of damaged mites/ total number of fallen mites	(%)			
P	80	80/315	25.40	78/202	38.61	86.58/257	33.69	1.56	13.98	16.23
F1	80	69/247	27.94	63/159	39.62	74.41/205	36.27	1.30	11.62	15.66
F2	80	49/318	15.41	63/160	39.38	78.08/270	31.66	1.77	24.77	31.72

SE, Standard Error; SD, Standard Deviation; CV, Coefficient of Variation

Table 2. Descriptive statistics of the grooming behaviour expression in colonies with selected queens

Queen generation	No. of colonies	Grooming behaviour						SE	SD	CV (%)
		min		max		mean				
		No. of damaged mites/ total number of fallen mites	(%)	No. of damaged mites/ overall number of fallen mites	(%)	No. of damaged mites/ total number of fallen mites	(%)			
Ps	8	113/301	37.54	78/202	38.61	100.67/266	37.99	5.88	16.63	16.47
F1s	8	60/153	39.22	63/159	39.62	75.69/193	39.42	5.53	15.65	20.65
F2s	8	113/299	37.79	63/160	39.38	96.06/249	38.58	10.13	28.65	29.84

SE, Standard Error; SD, Standard Deviation; CV, Coefficient of Variation

The heritability of grooming behaviour throughout the colonies of three consecutive generations of queens was assessed by mother-daughter method of regression. In the current study the heritability of grooming behaviour from Ps to F1s generation ( $h^2_{yx}$ ) was  $0.49 \pm 0.02$ , the one from Ps to F2s ( $h^2_{zx}$ ) was  $0.18 \pm 0.01$ , whilst the one from F1s to F2s ( $h^2_{zy}$ ) was  $0.16 \pm 0.01$ .

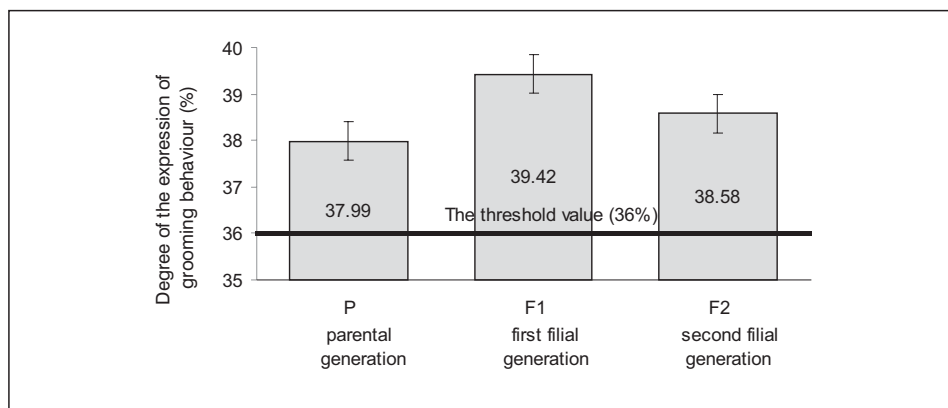


Figure 2. The mean values of grooming behaviour in colonies with selected queens

In addition, colonies bred for grooming behaviour had similar populations and brood areas and produced as much honey as the unselected colonies and suffer no apparent costs in reproductive and productive performances.

## DISCUSSION

The defensive behaviour of honey bees against *Varroa* mites consisting of auto-grooming and allo-grooming leads to the injury and death of mites (Ruttner and Hänel, 1992; Thakur *et al.*, 1997). The proportion of damaged mites in natural mite fall was considered a useful criterion in breeding of *Varroa*-tolerant bees (Hoffman, 1996; Moosbeckhofer, 1997). In addition, many authors considered breeding for grooming behaviour worthwhile besides other traits that contribute to reduction of the number of mites on adult bees (Ruttner and Hänel, 1992; Thakur *et al.*, 1997; Arechavaleta-Velasco and Guzmán-Novoa, 2001; Mondragon *et al.*, 2005). In this work the degree of grooming behaviour and its heritability was evaluated in colonies of grey honey bees in conditions devoid of any environmental influence.

Among unselected queens, expressed grooming behaviour was recorded only in colonies with F1 queens (36.27%), but not in colonies with P queens and F2 queens (33.69%, 31.66%, respectively). Significant differences in grooming behaviour were found between colonies of P and F1 queens ( $p < 0.001$ ), and between colonies of P and F2 queens ( $p < 0.05$ ) while the difference between colonies of F1 and F2 queens was insignificant ( $p > 0.05$ ).

However, all of the three generations of selected queens expressed grooming behaviour, as the proportion of damaged mites in the total number of fallen mites was >36% in each, Ps, F1s and F2s (37.99%, 39.42% and 38.58%, respectively) without significant ( $p > 0.05$ ) difference among them.

The results of heritability estimation showed the relatively low heritability of grooming behaviour in the three generations of queens examined ( $h^2_{yx} = 0.49 \pm 0.02$ ;  $h^2_{zx} = 0.18 \pm 0.01$ ;  $h^2_{zy} = 0.16 \pm 0.01$ ), which implies that grooming is a changeable, polygenic trait slightly influenced by genes. Since selection can affect the expression of genetically determined traits only if their coefficients of heritability are  $h^2 > 0.25$ , beekeepers cannot be advised to breed colonies for grooming behaviour due to low coefficients of heritability obtained in this research.

Our results may be explained by specific division of labor in honey bee colonies, based on polymorphism, temporal polyethism or differentiation among individuals of the same size and age class. As in most species of advanced social insects, honey bee workers show "temporal polyethism", performing different sets of tasks at different ages. Young individuals typically work close to the center of the nest, middle-age ones work in the periphery, while the older work outside the nest, mainly foraging (Seeley and Kolmes, 1989). However, not all workers in temporal polyethic societies, like colonies of honey bees, exhibit identical patterns of behavioural development. Inter-individual variation among honey bee workers of the same age is thus another form of division of labor (Trumbo *et al.*, 1997, Loengarov and Tereshko, 2008). Differences in rates of behavioral development are apparent; some show precocious behavioral development, while others develop more slowly (reviewed by Robinson, 1992; Rivera-Marchand *et al.*, 2008). There is also inter-individual variation in the degree of task specialization at a particular age or stage of behavioral development. Such inter-individual variation is most exerted in middle-aged workers (2-3 weeks of age) whose common tasks are food storage and wax working, but only a small percentage of them are engaged in guarding the nest entrance, removing dead bees from the nest and bees specialized for nestmate grooming (allo-grooming). It is interesting that allo-grooming is mostly performed by bees specialised in the task, unlike autogrooming which is performed by all bees (Winston and Punnett, 1982; Frumhoff and Baker, 1988). Grooming specialists who perform most of allo-grooming were initially reported by Winston and Punnett (1982). Afterwards, Moore *et al.* (1995) described highly specialized social grooming honey bees. In both investigations, those grooming specialists presented a small percentage of a colony's population (Winston and Punnett 1982; Frumhoff and Baker 1988). In the study of Kolmes (1989) grooming specialists performed common tasks (inspecting and feeding larvae, food storage) significantly less frequently than other bees and groomed themselves significantly less than non-specialists. They spent most of their time in allo-grooming, thus probably contributing to the greatest extent to the grooming potential of the colony. Having considered the aforementioned as well as the recent findings about close coupling of nutritional status and behaviour (Amnet *et al.*, 2008), it may be supposed that the grooming behavior could be genetically determined and heritable, but probably only among



those highly specialized allo-grooming honey bees which are full-sisters being a small percentage of every honey bee colony (because of the multiple mating of the queen). For those reasons, our results indicating low heritability of grooming potential are explicable, especially having in mind that the grooming behavior was assessed on colony level and grooming specialists are not numerous.

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## HERITABILNOST NEGOVATELJSKOG PONAŠANJA SIVE MEDONOSNE PČELE (*Apis mellifera carnica*)

STANIMIROVIĆ Z, STEVANOVIĆ JEVROSIMA, ALEKSIĆ NEVENKA i STOJIĆ V

### SADRŽAJ

Negovateljsko ponašanje se smatra značajnim mehanizmom odbrane pčela od *Varroa* krpelja. Cilj ovog rada je bilo ispitivanje negovateljskog ponašanja, procena njegove heritabilnosti i mogućnosti povećanja ekspresije te osobine putem selekcije. Radi dobijanja pouzdanih rezultata korišćenja je metodologija kojom se uticaj spoljašnjih faktora isključuje. Ispoljenost negovateljskog ponašanja procenjavana je na osnovu procenta oštećenih u ukupnom broju otpalih krpelja. Heritabilnost negovateljskog ponašanja praćena na kroz generacije matice i procenjavana metodom regresije majka-ćerka. Među neselekcionisanim maticama, negovateljsko ponašanje bilo je izraženo samo kod matice F1 generacije (36,27%), ali ne i kod P (33,69%) i F2 generacije (31,66%). Statistički značajne razlike u negovateljskom ponašanju zabeležene su između društava P i F1 matice ( $p < 0,001$ ) i između društava P i F2 matice ( $p < 0,05$ ).

Međutim, selekcionisane matice sve tri generacije (Ps, F1s, F2s) su imale izraženo negovateljsko ponašanje (37,99%, 39,42% i 38,58%) bez statistički značajnih ( $p > 0,05$ ) razlika među njima. Ipak, nizak koeficijent heritabilnosti praćene osobine ( $h^2_{yx} = 0,49 \pm 0,02$ ;  $h^2_{zx} = 0,18 \pm 0,01$ ;  $h^2_{zy} = 0,16 \pm 0,01$ ) ukazuje da se pčelarima ne može preporučiti selekcija pčela samo na negovateljsko ponašanje ako je njihov cilj uzgoj pčelinjih zajednica povećane otpornosti na *Varroa* krpelje.