SELECTION CRITERIA IN AN APIARY OF CARNIOLAN HONEY BEE (APIS MELLIFERA CARNICA) COLONIES FOR QUEEN REARING

SELEKCIJSKI KRITERIJI V ČEBELNJAKU Z DRUŽINAMI KRANJSKE ČEBELE (APIS MELLIFERA CARNICA) ZA VZREJO MATIC

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ABSTRACT

Thirty six honey bee (Apis mellifera carnica) colonies were tested for gentleness, swarming behaviour, colony strength, racial characteristics, Cubital index (Ci), honey production, extension of capped brood, hygienic behaviour and the presence of Nosema spp. spores. The average value of Ci of all measures was 2.7 (±0.40). The average honey production was 9.5 kg (±6.6) and the area of capped brood was 7061 cm^2 (±2813). Colonies expressed hygienic behaviour observed 24 hours after killing pupae twice in May and July at the level of 83.4% (±11.2). Each of twelve colonies uncapped and removed more than 90% of killed pupae, and of these, eight colonies cleaned more than 96% of killed pupae. The highest Nosema spp. spore load was found during September. We conclude that establishing the colony performance factors, with maximal level of 34 points, is a suitable tool for ranking and selection of colonies in each queen rearing apiary.

Keywords: Carniolan bee, selection, racial characteristics, hygienic behaviour, Nosema spp.

IZVLEČEK

Šestintrideset čebeljih družin (Apis mellifera carnica) smo vrednotili glede na mirnost, rojivost, živalnost, rasne karakteristike, kubitalni indeks (Ci), pridelavo medu, obseg pokrite zalege, sposobnost čiščenja odmrle zalege in prisotnost spor Nosema spp. Povprečna vrednost Ci je bila 2,69 (±0,40), povprečna pridelava medu 9,52 kg (±6,64) in obseg pokrite zalege je v povprečju obsegal 7061 cm^2 (±2813). Družine so v 24 urnem testu čiščenja odmrle zalege, izvedenem v maju in juniju odstranile 83,4 % (±11,2) zalege. Nad 90 % odmrle zalege je bilo odstranjenih v dvanajstih družinah, nad 96 % odmrle zalege pa je bilo odstranjene v osmih družinah. Največja količina ugotovljenih spor Nosema spp. je bila v septembru. Na osnovi izvedenih testov, ugotavljamo, da je ocenjevanje in selekcija čebeljih družin na osnovi uporabljenih karakteristik, z največjo oceno 34 točk, ustrezna metoda za rangiranje in odbiro družin v vzrejališču čebeljih matic.

Ključne besede: kranjska čebela, selekcija, rasne lastnosti, higiensko vedenje, Nosema spp.

POVZETEK

Šestintrideset čebeljih družin (Apis mellifera carnica) nameščenih v poskusnem čebelarstvu Kmetijskega inštituta Slovenije smo vrednotili glede na mirnost, rojivost, živalnost, rasne karakteristike, kubitalni indeks (Ci), pridelavo medu, obseg pokrite zalege, sposobnost čiščenja odmrle zalege in prisotnost spor Nosema spp. Mirnost čebel na satju smo ocenjevali z ocenami od 1 do 4, dvakrat v letu, rojenje smo ocenjevali v maju in juniju z ocenami od 1 do 4. Živalnost izraženo v številu zasedenih ulic smo ocenjevali v aprilu in juliju. Zasedenost ulic v posamezni družini smo primerjali glede na povprečno zasedenost v vseh testiranih družinah. Oceni 1 in 2 predstavljata zasedenost pod povprečjem, 3 in 4 pa zasedenost nad povprečjem v čebelnjaku. Obarvanost obročkov zadka in Ci smo določali dvakrat v letu, v aprilu in juliju. Kubitalni indeks smo izmerili na vzorcu 1749 delavk iz 36 družin. Ocene smo določili glede na skladnost izračunanega Ci s standardom. Vzorci, ki so ustrezali standardu (od 2,4 – 3,0), so dobili oceno 1, vzorci, ki standardu niso ustrezali, so dobili oceno 0 in so bili izločeni iz nadaljnje obravnave. Tudi družine, pri katerih so bile ugotovljene delavke z oranžnimi obročki zadka so bile izločene. Donos medu je bil ugotovljen s tehtanjem medenega satja pred točenjem in po točenju medu. Razlika v teži je predstavljala količino pridelanega medu v posamezni družini. Obseg pokrite zalege je bil izmerjen za vsako družino trikrat in sicer aprila, maja in julija. Sposobnost čiščenja odmrle zalege smo ugotavljali s pomočjo "pin-kill testa". Uporabili smo dve polji s pokrito zalego ki sta obsegali po 100 satnih celic. V enem polju s 100 satnimi celicami smo usmrtili bube, ter po 16 in 24 urah prešteli satne celice, ki so bile očiščene odmrle zalege. Sposobnost čiščenja smo izrazili v odstotku očiščenih satnih celic v časovnem obdobju. Testiranje smo izvedli v maju in v juliju. Družine so bile glede na čistilno sposobnost razvrščene v pet skupin na osnovi deleža očiščenih satnih celic: 5 = > 95 %; 4 = 90 – 95 %; 3 = 80 - 89 %; 2 = 70 – 79 %; in 1 = < 70 %. Vzorec delavk iz vsake družine je bil odvzet z vrha čebelje gruče v januarju, in z brade panja v maju in septembru. Vzorci so bili preiskani na spore Nosema spp. Družine so bile ocenjene glede na količino ugotovljenih spor: ocena 4 = 0 x 10^6 spor/čebelo; 3 = < 8 x 10^6 spor/čebelo; 2 = 8 do 30 x 10^6 spor/čebelo; in 1 = > 30 x 10^6 spor/čebelo. Vrednosti ocen za posamezne lastnosti smo sešteli v končni Selekcijski Indeks čebelje družine. Povprečna vrednost Ci v testnih družinah je bila 2,69 (±0,40), povprečen donos medu 9,52 kg (±6,64) in obseg pokrite zalege je v povprečju obsegal 7061 cm^2 (±2813). Družine so v 24 urnem testu odstranjevanja odmrle zalege, izvedenem v maju in juniju odstranile 83,4 % (±11,2) zalege. Nad 90 % odmrle zalege je bilo odstranjenih v dvanajstih družinah, nad 96 % odmrle zalege pa je bilo odstranjene v osmih družinah. Največje število spor Nosema spp. je bilo v septembru. Na osnovi izvedenih testov, ugotavljamo, da je ocenjevanje in selekcija čebeljih družin na osnovi uporabljenih karakteristik ustrezna metoda za rangiranje družin in odbiro v vzrejališču čebeljih matic.

INTRODUCTION

Beekeeping production depends on the selection of honey bee colonies, so selection aims and criteria are important tools in bee breeding. Spring colony development, gentleness and a tendency to remain calm on the comb, over wintering ability, honey production and resistance against diseases, are the most important measurable colony characteristics. Selection and breeding of the indigenous Carniolan bee, Apis mellifera carnica, is a beekeeping priority and practice in Slovenia [5].

The first step in selection, as defined by breeding programme, is race confirmation using morphological and behavioural observations. Morphological analyses can include cubital index (Ci) calculations, the colouration of abdominal segments and non stinging behaviour as important characteristics. Ci is known to be race specific, and for Apis mellifera carnica averaged at 2.589 (±0.418) [17]. Results shown on linear diagrams are more informative than statistical calculations only. A pure race sample of worker bees will produce a graph with a single peak, whilst a curve with two or more distinct peaks is indicative of interracial breeding [17]. Apis mellifera carnica is recognized by beekeepers as a race with a strong swarming tendency but, as an economic characteristic, it can be reduced through breeding. The surface area of capped brood is a reflection of queen productivity that is an important indicator for colony build up ability and the production of honey, pollen, propolis and royal jelly.

Disease resistance is known to correlate with the "hygienic behaviour" of worker bees. This is the ability of workers to recognize dead brood and then remove infected or damaged brood. At the first stage bees remove the capping of the brood cell, and then remove the dead larvae or pupae [15]. This characteristic has been found to be regulated by two pairs of recessive genes [16], whilst later experiments showed three levels of hygienic behaviour: recognizing infected or dead brood; uncapping of capped brood; and the removal of brood from comb cells. Colonies with enhanced hygienic behaviour have been shown to be significantly less diseased [18, 19]. Differences in the ability to remove dead larvae and pupae after infection by American foul brood also exist between colonies. There are colonies with an immediate cleansing

ability and others which clean dead brood only after a few days or even weeks [16]. Colonies show different level of hygienic behaviour, and successful selection is an option to reduce the incidence of diseases and a reduction in the use of chemicals in honey bee colonies [3].

Nosemosis is a chronic bee infection caused by Nosema spp. Worker bees are affected by the continuous presence of Nosema spores, which are ingested by the bee via contaminated water or food. They are then passed by the proventriculus into the midgut where they enter the epithelial cells and multiply. There are approximately 30 to 50 million Nosema spores in the gut of a bee with a fully developed infection [2]. Infected bees do not show any characteristic signs specific to Nosema apis infections, but a subclinical infestation can impact upon an individual bee and on the normal functioning of an entire colony [8]. This is due to pathological changes in the epithelial cells of the gut and interference with digestion, which leads to malnutrition and premature death of the individual bees [10]. Nosema apis infections negatively affect colony overwintering [2].

The aim of our work was to determine whether testing different quality parameters for Carniolan honey bee colonies in an apiary would allow us to establish the criteria for ranking the colonies. We therefore attempted to study the pre-selection criteria for each apiary before starting queen rearing for requeening them.

MATERIALS AND METHODS

Thirty six colonies of Apis mellifera carnica located at the Senično apiary in the central region of Slovenia were tested for the following characteristics: gentleness and tendency to remain calm on the comb, swarming behaviour, colony strength, racial characteristics (the colouration of abdominal segments and Cubital index (Ci)), honey production, the area of capped brood, hygienic behaviour and the presence of Nosema spp. spores.

Gentleness and a tendency to remain calm on the comb were scored according to a four point system where: 4 = very gentle / very calm; 3 = gentle / calm; 2 = aggressive, where bees run on the comb edges or fly from the comb; and 1 = very aggressive where bees run away from the comb. This characteristic was assessed twice during the season and an average score was calculated.

Swarming behaviour was also scored on a four point system where 4 = the colony showed no swarming tendency through the entire season and did not construct any queen cells; 3 = queen cells with eggs and open queen cells were found but after the removal of these cells, no more were constructed; 2 = swarming could be prevented only by extensive intervention such as using an interim nucleus; and 1 = the colony swarmed. Swarming behaviour was evaluated during the swarming period in May and June.

Colony strength was determined twice a year; in April for colonies coming out of the winter and in July to determine colony strength in late summer, in the form of the relative number of occupied combs compared to the average number of occupied combs in all colonies at the apiary. Colonies with above average colony strength during the summer were used for further analyses and rearing. Colony strength was scored according to a four point system, where 1 and 2 represent below average values and 3 and 4 represent an above average number of occupied combs calculated for all colonies tested.

The colour of the abdominal segments of Apis mellifera carnica and the Ci of worker bees from each colony were assessed twice a year in April and July. The colouration of abdominal segments for Carniolan bees is shown in Fig. 1 and defined on a four point scoring system where: 1 = the majority of workers have two abdominal segments coloured yellow; 2 = the majority of workers have the first abdominal segment coloured yellow; 3 = the majority of workers have the first abdominal segment partially coloured yellow; and 4 = all abdominal segments are dark or grey.

Cubital index was measured in 1749 worker bees from 36 colonies, 50 (±5) workers per colony. The first right wing was fixed and photographed using a binocular microscope (Zeiss) with x 50 magnification and the PC program AxioVision 4.6. Ci was measured using the program Beewings 1.20. Results were then plotted on a diagram. Colonies with Ci within the standard for carniolan bees (2.4 – 3.0) received the score 1, and colonies with a Ci lower than the standard received the score 0 (Table 1). An additional criterion used was that only colonies with less

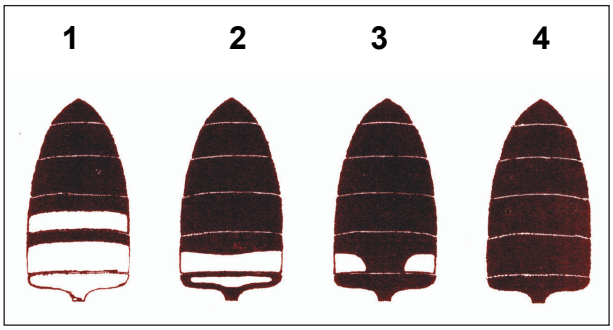

Fig. 1. Scoring of worker bees abdominal segment colouration.

Slika 1. Ocenjevanje obarvanosti obročkov zadka pri delavkah.

Table 1. Colony scores for individual observed characteristics during the experiment.
Pregl. 1. Ocene družin za posamezne lastnosti, ki so bile spremljane v času poskusa.

Hive (Panj)	Gentleness (Mirnost) (1-4)	Swarming (Rojivost) (1-4)	Colony strength (Jakost družine) (1-4)	Honey yield (Donos medu) (1-4)	Capped brood (Pokrita zalega) (1-4)	Hygienic behaviour (Higiensko vedenje) (1-5)	Coloration (Obarvanost) (1-4)	Ci (0-1)	Nosema (1-4)	CPF (max 34)
1	4	4	4	4	4	5	4	0	3	32
2	4	4	4	4	4	4.5	4	0	3	31.5
3	4	4	3.5	4	4	4	4	0	3	30.5
4	2	4	3.5	4	4	4.5	4	1	3	30
5	4	4	4	4	4	3.5	4	0	2.3	29.8
6	4	4	3.5	4	3	3	4	1	3	29.5
7	4	4	4	3	4	3	3.5	1	3	29.5
8	4	4	3.5	4	3	4.5	4	0	2	29
9	4	4	4	2	4	5	4	0	2	29
10	4	4	4	4	2	4	4	0	2.7	28.7
11	4	4	3.5	3	3	3	4	1	2.7	28.2
12	4	4	3.5	1	4	4	4	1	2.7	28.2
13	4	4	3.5	3	4	3	4	0	2.3	27.8
14	4	4	4	2	3	3	4	1	2.7	27.7
15	4	4	2	1	3	5	4	1	3	27
16	4	4	3	4	2	5	2	0	2.3	26.3
17	3.5	4	3	4	3	1.5	4	1	2	26
18	4	4	3	4	2	2	4	0	3	26
19	4	4	3	4	2	2	4	0	3	26
20	4	4	3	2	2	2.5	4	1	3.3	25.8
21	4	4	2	1	2	4	4	1	3.3	25.3
22	4	4	3	2	3	3	3.5	0	2.7	25.2
23	4	1	4	3	3	3	4	0	3	25
24	2.5	4	2.5	3	2	3	4	1	2.7	24.7
25	4	4	2.5	2	2	2.5	3.5	1	3	24.5
26	4	4	3	1	3	2	4	0	3	24
27	4	4	3.5	2	3	3.5	1.5	0	2.3	23.8
28	4	4	2.5	1	3	2	4	1	1.7	23.2
29	4	4	4	1	2	1	4	1	2	23
30	4	1	2.5	1	3	5	2	1	3	22.5
31	4	4	2	1	2	2	4	1	2	22
32	4	1	3.5	1	3	2	4	1	2	21.5
33	4	1	3	1	3	4	2	1	2.3	21.3
34	4	1	3	1	1	4	4	1	2.3	21.3
35	4	4	3	1	1	2	2	1	2.7	20.7
36	4	4	2	2	2	1	3	0	2.3	20.3

Ci = Cubital index (kubitalni indeks)
CPF = Colony performance factor (Selekcijski Indeks čebelje družine)

than 15% Ci in a sample bellow 2.40 were considered [14]. Colonies, where worker bees were found to have yellow coloured abdominal segment or with a non typical Ci, were not considered for further queen rearing.

Honey yield was determined for each colony by weighting all combs before and after honey extraction, the difference in kg being the amount of honey produced per colony. Colony honey production was scored according a four point system where 1 and 2 represented below average and 3 and 4 were above average honey production calculated from all colonies tested.

The area of capped brood in colonies was measured using square wire mesh on both comb sides, and the total calculated for each hive. Measurement was conducted on 36 colonies three times (in April, May and July).

Colonies were tested for hygienic behaviour using the "pin-kill test" [4]. Capped brood combs were selected from each colony to perform the hygienic behaviour test. Two fields with 100 capped cells (10 x 10) were used; one for testing and the second as a control field. Cell capping were perforated and pupae in the cells were killed using entomological needle. Sixteen and 24 hours after perforation, emptied cells in the tested and control brood areas were counted (Fig. 2). Hygienic behaviour was then calculated according to Goncalves and Gramacho [4]. Two evaluations were performed, the first in May (spring time) and the second in July (the summer period). Colonies were scored according to the proportion of cleaned brood comb cells 24 hours after killing the pupae on the following five point scale: 5 = > 95%; 4 = 90 – 95%; 3 = 80 - 89%; 2 = 70 – 79%; and

1 = < 70%.

A sample of 50 worker bees was collected from the top of the bee cluster of each colony in January and from the hive entrance in May and September and was examined for the presence of Nosema spores using a microscope. Samples were macerated in a mortar with 1 ml water per bee. A suspension of each bee sample was separately examined under 400 x magnification using a Bürker haemocytometer. The number of Nosema spores per bee was then calculated. Colonies were scored in accordance with the spore load on a four point scale where: 4 = 0 x 10^6 spores/bee; 3 = < 8 x 10^6 spores/bee; 2 = 8 to 30 x 10^6 spores/bee; and 1 = > 30 x 10^6 spores/bee. The scores for each colony were summarised and the 20% of colonies with the lowest spore load were selected for further queen rearing. Colony scores for the individual observed characteristics summarised as a final Colony Performance Factor (CPF) with a maximum possible score of 34. Colonies with the highest 20% of scores for CPF were selected for further breeding.

RESULTS

Colonies were all evaluated as gentle and calm except for three colonies, which in both evaluations received scores less than 4 (Table 1). Evaluation for swarming behaviour started in spring in the period of pollen and nectar flow, during May and June. Colony, which tend to swarm one or more times per season, and thus a swarming tendency with score 1 was observed in five colonies. Colony strength in terms of the average number of occupied combs was evaluated twice per year, and then scores were allocated according to the data distribution. Nine colonies received the highest score. Colouration of abdominal segments of workers from 36 colonies was examined in April and July, and four colonies were scored with less than 3 and were considered for further breeding. The average value of Ci in all measured samples was 2.69 ±0.40. The minimum value was 1.76 and maximum value was 4.56. Samples from 16 (44.4%) colonies had more than 15% of measured wings below the limit Ci value of 2.40 and were thus also scored as 0. Samples from 20 (55.6%) colonies corresponded to the criteria for Ci standard.

Honey production in 36 colonies ranged from a minimum of 1.07 kg to a maximum of 27.65 kg, the average being 9.52 ± 6.64 kg. Twelve colonies were scored the highest, 4, five scored 3, and 19 colonies were below average and received scores 2 or 1 (Table 1).

The area of capped brood in 15 colonies exceeded the average measured in all colonies. The area of capped brood in all of the 36 colonies ranged from a minimum 1810 cm^2 to a maximum of 11925 cm^2, the average being 7061 ± 2813 cm^2.

Testing of hygienic behaviour was performed twice during the beekeeping season, the first during spring colony development and the second at a time of poor forage availability. Relative hygienic behaviour after 24 hours was 84.7 ± 13.4% in May and 82.3 ± 16.3% in July, an average of 83.4 ± 11.2%. In both the May and July testing, twelve colonies expressed hygienic behaviour higher than 90%, with eight colonies removing more than 96% of killed capped brood cells. Colony 16 showed more than 95% removal in both May and July, whilst colonies 4, 8, 2, 30, 15, 21, 1 and 9 showed more than 90% (Table 1). Fig. 3 shows the number of colonies expressing various levels of hygienic behaviour in May and July.

Worker bees from 36 colonies were analysed for Nosema spores in January, May and September, the greatest infection being found in September. There was no colony without presence of Nosema spores and 15 colonies were found spore load les that 8 million.

Table 1 shows colony scores summarised as a final Colony Performance Factor (CPF). Seven colonies with specific race characteristics and the highest score levels were selected: 4, 6, 7, 11,12, 14 and 15.

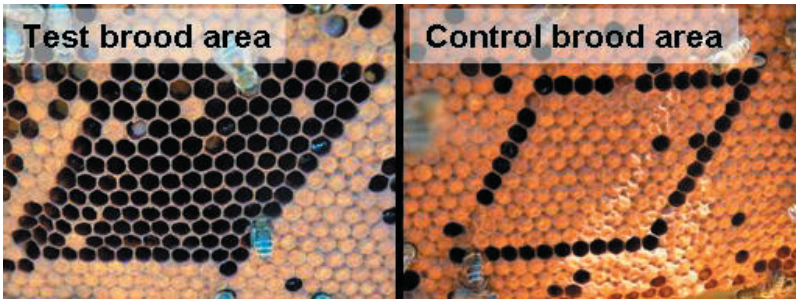

Fig. 2. Test and control areas 24 hours after perforation cell cups and killing pupae.
Slika 2. Testno in kontrolno polje 24 ur po perforaciji celičnih pokrovcev in usmrtitvi bub.

DISCUSSION

The majority of colonies were scored high for gentleness and a tendency to remain calm on the comb. Only colonies 4 and 24 were evaluated as aggressive. Swarming behaviour was expressed in colonies 33, 32, 23, 34 and 30. Swarming used to be desired characteristic of honey bee colonies some decades ago, but nowadays is not appreciated [5]. Colony strength estimated twice a year allows the possibility of ranking colonies for their economic potential [13]. Colony productivity and brood production depend on the quality of the queen and its age [21]. Measuring colony strength using the number of brood combs is a useful method for selecting productive colonies, and nine of the colonies received the highest scores.

Abdomen colouration and morphological characteristics are, as well as behavioural characteristics, specific for ecotype determination in Carniolan bees [17]. Colonies, where workers were found to have yellow abdomen coloration, were not considered for further evaluation. Cubital index, as a morphological criteria, was considered within the standard limits described by Ruttner [17]. In our study, 16 colonies (44%) had Ci lower than 2.40 in more than 15% of sampled bees. Therefore, they were excluded from further evaluation.

Average honey production was 8.8 kg per colony, and after grouping into four classes on this basis, 16 colonies were considered for evaluation and selection. It is supposed that large colonies with more occupied comb frames produce more honey whilst the smaller colonies produce the most brood. When a colony population increases, brood area per bee decreases. The optimal colony size is thus determined, with the rate of weight gain in colonies of this size being near to that of the largest populations and the rate of brood production being near to that of the smaller colonies [7].

Honey bee colonies can also be breed for hygienic behaviour. Selected colonies are considered to be able to resist the parasitic mite Varroa destructor, American foul brood and other diseases and parasites. Colonies, breed for hygienic behaviour, are able to detect, uncap, and remove damaged brood from the nest. Hygienic colonies actively defended themselves against mites and diseases expressed at low level [20]. The importance of

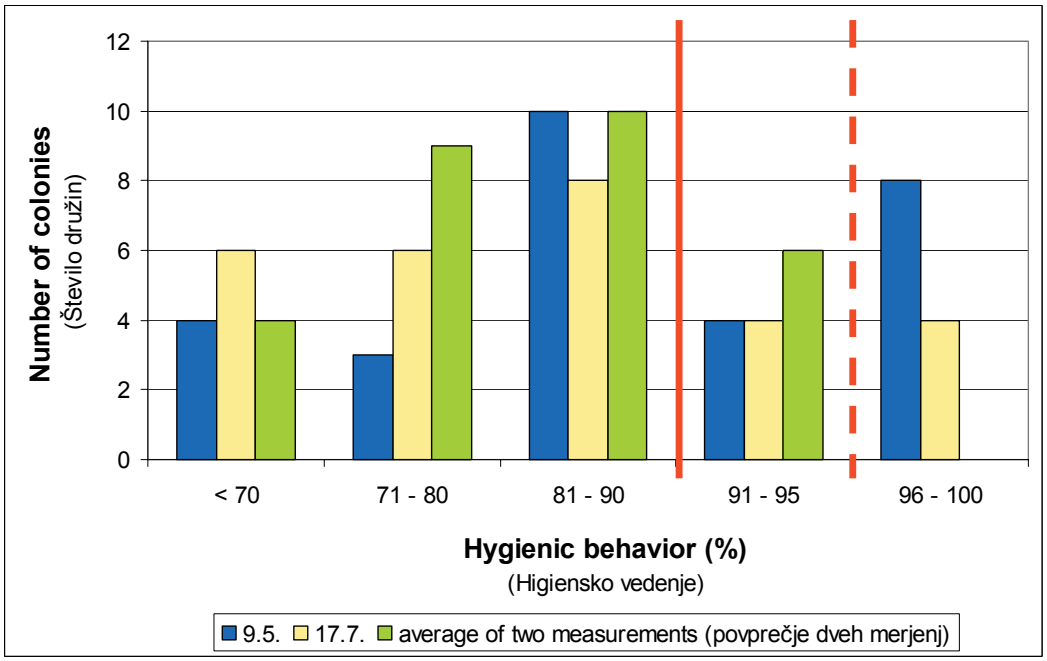

Fig. 3. Hygienic behaviour expressed as the number of colonies expressing various levels of removal of previously perforated brood cells containing pupae. Bars indicate No. of colonies at two measurements (9. 5. and 17. 7.) and No. of colonies with average hygienic behaviour established with two measurements. The solid red bar indicates colonies which expressed more than 90% hygienic behaviour.

Slika 3. V grafu je prikazano število družin z ugotovljeno čistilno sposobnostjo, ki odraža odstranitev odmrle zalege iz satnih celic, izraženo v odstotkih (%). Stolpci prikazujejo št. družin za posamezno meritev (9. 5. in 17. 7.) in št. družin z ugotovljeno povprečno čistilno sposobnostjo v dveh merjenjih. Desno od neprekinjene črte so družine, ki so kazale preko 90 % čistilno sposobnost.

selecting hygienic colonies is that they have similar adult populations and brood areas, produce as much honey, and have less brood disease than unselected colonies [20]. Among our colonies one exhibited >95% removal behaviour and three colonies exhibited >90% removal behaviour in both tests. It has been found that hygienic behaviour is predominantly performed by middle aged worker bees that have not yet begun foraging and that 18% of the bees in the colony are actually involved in the task at any given time [1]. In our experiments, workers removed on average 84% of perforated brood within 24 hours. It was found that non-hygienic honey bees removed only about 53% of dead brood [12]. Colonies that removed more than 80% of dead brood after 24 hours were selected for queen and drone production. Palacio et al. [11] reported that due to queen selection without mating control total hygienic behaviour was increased in a population from 66.25% to 84.56% in five years. Nosema infestation was quantitatively surveyed during the beekeeping season and the most heavily infected colonies were not used for further breeding. There was an influence of colony management on response to infection, but no difference was observed between races [9].

Our results on behavioural, morphological and economic characteristics were considered from the viewpoint of their ability to serve as selection criteria for the indigenous honey bee race. The average Ci was 2.79 which is characteristic for the local Apis mellifera carnica population [14]. Testing the hygienic behaviour in colonies is important in order to reduce incidence of brood diseases [11]. It was suggested that this trait can be used as a criterion in further selection in queen breeding apiaries. Colony No. 1 achieved the highest overall score. High scores were also achieved by colonies 8, 6, 11, 7, 14, 10, 17 and 12, which were suggested to be used for further observation and queen rearing.

It could be concluded that selected queens of race specific colonies in the experimental apiary demonstrate a high genetic potential for hygienic behaviour, and also demonstrate other desired qualities of gentleness, low swarming tendency and good honey production. Selected honey bee colonies in the apiary are thus the result of considering several characteristics. Improving breed selection is important for maintaining variability in different geographic regions of Slovenia through breeding activities and establishing isolated mating stations [6] as a potential for maintaining required variability in population and preservation of local adaptations.

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