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EVALUATION OF THE GROOMING BEHAVIOR OF *Apis mellifera* *remipes* AND *Apis mellifera cerana* AGAINST *Varroa jacobsoni* BY

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The parasitic mite *Varroa jacobsoni* on the European honeybee (*Apis mellifera*) and the Africanized honeybee (*Apis mellifera cerana*) which is also supposed as the original host of the parasite.

Low *Varroa* infestation in *Apis cerana* indicates that the association between the host and the parasite during evolutionary process created certain resistance mechanisms including the actual removal of mite's infested workers brood and killing of phoretic *Varroa* by effective grooming behavior (Prag et al., 1987; Rosenkranz and Towarson, 1993). Bechler et al., (1992) compared grooming behavior in *Apis cerana* and *Apis mellifera* and found 75% successful mite removal in *Apis cerana* and 12.0% in *Apis mellifera*.

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EVALUATION OF THE GROOMING BEHAVIOR OF *Apis mellifera yemenitica* AND *Apis mellifera carnica* AGAINST *Varroa jacobsoni*
BY

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ABSTRACT

The grooming behavior of the two honeybees races *Apis mellifera yemenitica* (Indigenous) and *Apis mellifera carnica* (Carniolan) was evaluated against *Varroa jacobsoni* in the apiary of Agriculture Faculty, King Saudi University Riyadh, Saudi Arabia. The variations in grooming behavior during active season (April to September 2001) were estimated by counting of damaged mites dropped on "Inserts" placed under brood nest. The findings of the research revealed that the average percentage of all dropping mites that were injured was 35.54% and 33.14% for *Apis mellifera yemenitica* and *Apis mellifera carnica*, respectively. The study also indicated that the percentage of damage was more to legs of mite than other body parts as 13.25% and 16.29%, dorsal damage was 10.24% and 12.29% where as the appendages damage percentage was 12.05% and 4.75% in *Apis mellifera yemenitica* and *Apis mellifera carnica* bee colonies, respectively.

INTRODUCTION

The parasitic mite *Varroa jacobsoni* infests both the European honeybee (*Apis mellifera*) and the Asian honeybee (*Apis cerana*) which is also supposed as the original host of the parasite (Koeniger *et al.*, 1981).

Low *Varroa* infestation in *Apis cerana* indicates that the association between the host and the parasite during evolutionary processes created certain resistance mechanisms including the actual removal of mite's infested workers brood and killing of phoretic *Varroa* by effective grooming behavior (Peng *et al.*, 1987; Resenkrantz and Tewarson, 1993). Buchler *et al.*, (1992) compared grooming behavior in *Apis cerana* and *Apis mellifera* and found 75% successful mites removal in the case of *Apis cerana* and 48% in *Apis mellifera* by grooming behavior.

Apis cerana is more effective in both removing mites and causing damage to mites than *Apis mellifera* (Fries *et al.*, 1996). Zaitoun *et al.* (2001) determined the degree of damaged mites in *Apis mellifera syriaca* (the native bees in the area of study) they found that the average percentage of injured mites was

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**EVALUATION OF THE HYGIENIC BEHAVIOR IN TWO HONEYBEES
RACES- *Apis mellifera yemenitica*, *Apis mellifera carnica* AND THEIR
HYBRID AGAINST *Varroa jacobsoni*.**

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ABSTRACT

Hygienic behavior of two honeybee races Indigenous bees (*Apis mellifera yemenitica*), Carniolan bees (*Apis mellifera carnica*) and their hybrid, containing 16-colonies from each race were studied during late summer, 2001 in Saudi Arabia. The results revealed that the hygienic effectiveness was 100%, 86.5% and 81.25% after 48-hours for Indigenous, Carniolan and hybrid bees, respectively.

INTRODUCTION

Beekeeping is being practiced in Saudi Arabia since long ago. Presently, the beekeepers rear both the indigenous bees (*Apis mellifera yemenitica*) and Carniolan bees (*Apis mellifera carnica*). One of the major problems, facing beekeeping industry in Saudi Arabia is the infestation of honeybee colonies with parasitic mite *Varroa jacobsoni*. The initial observations have indicated that indigenous bees race is less susceptible to Varroa mites as compared to the imported one and this quality may be attributed to its more efficient defensive behavior.

Spivak (1996) found a relationship between hygienic behavior and the removal of mites from brood cells in the field. Test colonies with queens selected for hygienic behavior had fewer mites than the control colonies (Spivak and Reuter, 1998). Hygienic behavior is the main mechanism by which workers detect the diseased brood, un-cap the cells and remove the infested larvae or pupae (Spivak and Gilliam, 1998). This behavior forms one of the bases of balanced host parasite relationship in *Apis cerana* (Flores *et al.*, 2001).

It is known that the expression of hygienic behavior is influenced by some environmental conditions. For example, incoming nectar increases the rate of removal of dead and diseased brood (Thompson, 1964; Momot and Rothenbuhler, 1971). Also, hygienic behavior is heritable ($h^2 = 0.65$) as described by Harbo and Harris (1999). They recorded that it is a good candidate for selective breeding of bees, for resistance to *Varroa jacobsoni*.

22.8%. The grooming activity of *Apis mellifera Syriaca* provides evidence of active mechanism of resistance toward the parasitic *Varroa* mites.

The present study was conducted to investigate the grooming behavior of indigenous honeybee (*Apis mellifera yemenitica*) and Carniolan honeybee (*Apis mellifera carnica*).

MATERIALS AND METHODS

The experiment was conducted at the apiary of Faculty of Agriculture, King Saud University, Saudi Arabia during a constant warm and dry weather (April to Sept., 2001). Ten colonies from two races (5-colonies from each race): *Apis mellifera yemenitica* (Indigenous) and *Apis mellifera carnica* (Carniolan) were used. The colonies were similar in strength (6-combs covered with bees containing an average of 4-brood combs).

The mites that had fallen onto bottom-board traps from naturally infested colonies were collected with 7-days intervals to quantify the active grooming reactions by the bees towards the mite under natural conditions. Inserts made of 2-mm mesh screen wire were placed under the brood nests to cover the whole bottom board of the hives. The edges of these inserts were smeared with vaseline to prevent the dropping but still living mites from escaping. The inserts were renewed every 7 days, without smoking, in order to avoid disturbance of the colony. All collected mites were carefully examined under stereomicroscope at 40X magnification for injuries and abnormalities.

RESULTS AND DISCUSSION

Table 1 and Fig. 1 revealed the average number of *Varroa* mites collected from the *Apis mellifera yemenitica* colonies during the active season. Twenty-two observations were recorded with a week interval from 16-April to 11-Sep., 2001. The first record during April month showed the maximum number of dropped mites on bottom board (22 mites/colony) then the number of dropped mites decreased sharply during the month of May whereas no mites were collected during June, July, August and September. Only 2.67 mites/colony were recorded on Aug., 7. The percentages of damaged mites ranged between 8.25 to 50%. The average numbers of collected mites recorded during active season in Indigenous colonies differed significantly.

Table 2 and Fig. 1 presented the average of dropped mites and grooming activity of Carniolan bees. The maximum number of dropped mites were collected on the second observation (April, 23), then the numbers of dropped mites fluctuated and decreased sharply during May. Another two peaks were recorded during May, 14 and Aug., 14. Dropped mites were collected throughout the tested period except on June, 18 and 24 and July 23 and 30. The average number of collected mites recorded during active season in Carniolan colonies differed significantly.

Table 3 shows the total number of collected mites from two races of honeybee colonies and the percentage of damaged mites. A total of 166 mites were collected from indigenous bee colonies during the tested period. 64.46% of the total mites were found healthy while 35.54% were subjected to different types of damage due to the grooming activity. Similarly, a total of 350 mites were collected from Carniolan honeybee colonies as naturally dropped mites throughout the tested periods. 66.86% of the total mites were found healthy where as 33.14% were subjected to different types of damage. Types of damage were identified on legs, dorsal and appendage as 16.2, 12.29 and 4.57% respectively.

Results indicated that the indigenous honeybee colonies showed more effective defensive mechanism (35.53%) as compared to 33.14% Carniolan honeybee colonies. The grand mean of the collected mites was 2.52 mites/colony/week for indigenous bees while it was 5.30 mites for Carniolan bees. The percentage of damaged mites in indigenous bees was higher as compared to the damaged mites in Carniolan bees.

Table (1): Seasonal variations in the Grooming Behavior of Native honeybees (*Apis mellifera yemenitica*) colonies, Riyadh, Saudi Arabia 2001.

Sampling Date	Avg. No. of Mites collected/ Day.	Average percentage of mites				
		Healthy %	Damage %	Type of damage		
				Legs	Dorsal	App
16 April	22	63.63	36.37	13.64	10.61	12.12
23	20.33	62.39	37.70	11.47	8.20	18.03
30	1.33	50.00	50.00	25.00	0.00	25.00
7 May	1.00	67.00	33.00	0.00	33.00	0.00
14	4.00	91.75	8.25	0.00	8.25	0.00
21	2.67	62.50	37.50	25.00	12.50	0.00
28	0.67	50.00	50.00	50.00	0.00	0.00
4 June	0.67	50.00	50.00	0.00	50.00	0.00
11	0	0	0	0	0	0
18	0	0	0	0	0	0
25	0	0	0	0	0	0
2 July	0	0	0	0	0	0
9	0	0	0	0	0	0
16	0	0	0	0	0	0
23	0	0	0	0	0	0
30	0	0	0	0	0	0
7 Aug.	0	0	0	0	0	0
14	2.67	62.50	37.50	25.00	12.50	0
21	0	0	0	0	0	0
28	0	0	0	0	0	0
4 Sep.	0	0	0	0	0	0
11	0	0	0	0	0	0

Table (2): Seasonal variations in the Grooming Behavior of Carniolan (*Af mellifera carnica*) honey bee colonies, Riyadh, Saudi Arabia 2001.

Sampling Date	Avg. No. of Mites collected/ Day	Average percentage of mites.				
		Healthy %	Damage %	Type of damage %		
				Legs	Dorsal	App.
16 April	14.00	64.29	35.71	19.05	7.14	9.52
23	30.00	56.67	43.33	18.89	14.44	10.00
30	5.00	73.33	26.67	20.00	6.67	0.00
7 May	5.67	88.24	11.76	5.88	5.88	0.00
14	9.33	85.71	14.29	3.57	10.77	0.00
21	2.00	66.67	33.33	33.33	0.00	0.00
28	1.33	50.00	50.00	25.00	25.00	0.00
4 June	2.33	57.14	42.86	14.29	28.57	0.00
11	1.00	67.00	33.00	33.00	0.00	0.00
18	0.00	0	0	0	0	0
25	0.00	0	0	0	0	0
2 July	1.00	33.00	67.00	67.00	0	0
9	1.00	33.00	67.00	0	67.00	0
16	1.67	80.00	20.00	20.00	0	0
23	0.00	0	0	0	0	0
30	0.00	0	0	0	0	0
7 Aug.	13.00	48.72	51.28	25.64	20.51	5.13
14	12.33	75.68	24.32	13.51	8.11	2.70
21	9.67	82.76	17.24	3.45	13.79	0.00
28	3.00	66.67	33.33	11.1	22.2	0.00
4 Sep.	3.00	88.89	11.11	11.11	0	0
11	1.33	75.00	25.00	25.00	0	0

Table (3): Total number of collected mites from two races of honeybee colonies and percentage of damage.

Race of Honeybee	Total No. of mites collected (mean)	Avg. of mites (%)				
		Healthy mites	Damaged mites	Type of damaged %		
				Legs	Dorsal	App.
Native bees	166 (2.52) A	64.46%	35.54%	13.25b	10.24d	12.05c
Carniolan Bees	350 (5.30) A	66.86%	33.14%	16.29a	12.29bc	4.57e

Mean followed by the same letter does not differ significantly at the 5% level probability.

The findings of the study were in agreement with other researchers as Buchler *et al.* (1992) found that 48% of the mites were removed by grooming activity where as, Fries (1996) recorded 12.5% damaged mites in Carniolan honeybee colonies against 30% in *Apis cerana* colonies Khattab (2000), Zaitoun *et al.* (2001)observed 22.5% damagedJmites in *Apis mellifera* *Syriaca* colonies.

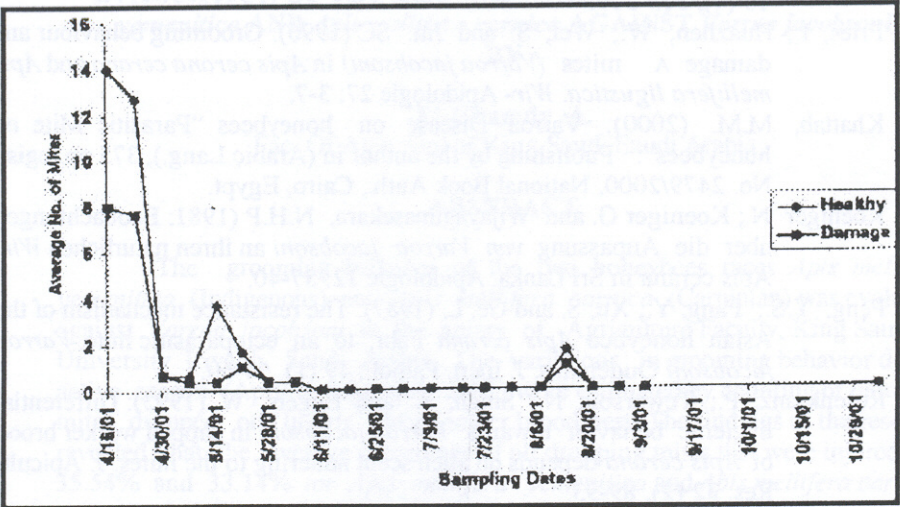


Figure (1): Seasonal variation in grooming behavior of Indigenous bees during 2001 in Saudi Arabia.

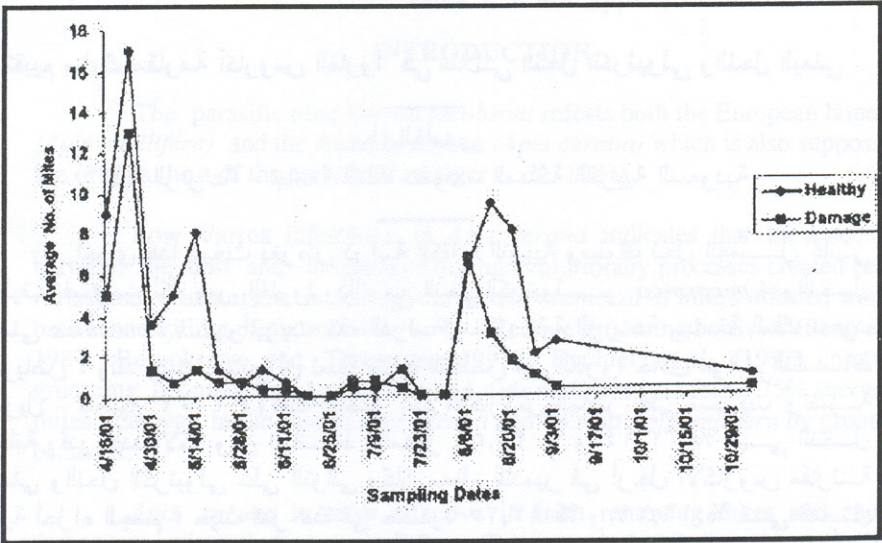


Figure (2): Seasonal variation in grooming behavior of Hybrid bees during 2001 in Saudi Arabia.

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تقييم سلوك مقاومة أكاروس الفاروا في سلالتى النحل الكرنيولى والنحل اليمنى

أ. الغامدى

كلية الزراعة - جامعة الملك سعود - المملكة العربية السعودية

أجرى هذا البحث بغرض دراسة الكفاءة النسبية وسلوك نحل العسل على تنظيف نفسه من أكاروس الفاروا وذلك مع النحل الكرنيولى *A.m.carnica* والنحل اليمنى *A.m.yemenitica* وأجريت هذه الدراسة بمنحل كلية الزراعة بجامعة الملك سعود بالرياض ، ولقد بينت النتائج أن نسبة نشاط التخلص من الفاروا خلال مواسم النشاط (أبريل - سبتمبر ٢٠٠١) وذلك بحصر عدد الأكاروس المدمر على شيت أرضية الخلية وقدر عدد الأكاروس المتساقط والمدمر ٣٥,٥٤ % و ٣٣,١٤ % فى النحل اليمنى والنحل الكرنيولى على التوالى وكان معظم التدمير فى أرجل الأكاروس مقارنة ببقية أجزاء الجسم ؛ حيث قدر هذا فى حدود ١٣,٢٥ % و ١٦,٢٩ % فى النحل اليمنى والنحل الكرنيولى ، وكانت نسبة التدمير فى الدرقلة الظهرية ١٠,٤٢ % ، ١٢,٢٩ % بينما كانت هذه النسبة فى زوائد الفاروا الأخرى ١٢,٠٥ % ، ٤,٧٥ % فى كلا طوائف السلالتين على التوالى .

The main objective of this study was to compare the hygienic effectiveness of indigenous, Carniolan bees and their hybrid bees.

MATERIALS AND METHODS

The experiment was conducted in Riyadh, Saudi Arabia during warm and dry weather in Sept., 2001 to ascertain the hygienic behavior in two honeybee races; the indigenous bees (*Apis mellifera Yemenitica*), the Carniolan bees (*Apis mellifera carnica*) and their hybrid.

Hygienic behavior was determined by the method described by Spivak and Downey (1998). This method depends on determination of the time required for honeybee workers to remove freeze-killed brood.

Sixteen honeybee colonies from each race were used. Comb sections of sealed brood (6 x 5.5 cm) containing 100 pupae/side were cut out from one healthy colony and frozen at -20°C for 24- hours before it was placed in the nest of tested colony. The number of removed pupae, uncapped but un-removed pupae and capped pupae were counted in each tested colony after 24 and 48 hours for comparison between the different races in hygienic behaviour. Only those colonies which removed > 95% of freeze-killed brood after 48 hours were considered hygienic.

Data was statistically analyzed by F-test and significance between the means was compared by Duncan Multiple range test (DMRT) (1955).

RESULTS AND DISCUSSION

The evaluation of the hygienic behavior, by using freeze-killed brood assay was carried out with three honeybee races. Data presented in Table 1 exhibited that the hygienic behavior of honeybee workers varied according to the honeybee race and the time needed for cleaning cells. Results also indicated that the hygienic behavior of indigenous bee (*Apis mellifera yemenitica*) was more efficient as compared to others. 14- colonies out of 16- colonies scored 100% of hygienic effectiveness after 24 hours and only 2-colonies gave 94% hygienic behavior. The percentages of cleaned cells after 48-hours were 100% in all indigenous colonies under study.

The hygienic effectiveness of Carniolan bees ranged between 21-93% with an average of 58% after 24- hours from the beginning of the treatment. The percentage of cleaned cells increased to 86.5% after 48-hours.

The hygienic behavior of hybrid bees after 24- hours showed a wide hygienic effectiveness (27-90%) with an average mean of 55%. After 48- hours, the percentage of cleaned cells ranged between 37% and 100% with an average mean scored 81.25%. Spivak and Downey (1998) obtained the wide range of removal rates between the honeybee colonies.

Table (1): Effectiveness of hygienic behavior in three honeybee races by using Freeze-killed brood.

Type of cells	Race of honeybees	Time after treatment	Rang	Total cells	Mean*	No. of colonies tested	No. of hygienic colonies >95%
Cleaned Cells	Indigenous bees	24 h.	100 - 94	1588	99.25a	16	14
		48 h.	100- 100	1600	100.00a		16
	Carniolan bees	24 h.	100 - 21	928	58.00bc	16	2
		48 h.	100 - 53	1384	86.50		8
	Hybrid bees	24 h.	90-27	880	55.00 be	16	0
		48 h.	100-35	1300	81.25 ab		8
Opened cells but not Cleaned	Indigenous bees	24 h.	5-0 0-0	10 0	0.625g		
		48 h.			0.00		
	Carniolan bees	24 h.	62-0	244	15.25def		
		48 h.	44-0	176	11.00defg		
	Hybrid bees	24 h.	73-0	652	40.75c		
		48 h.	65-0	298	18.63de		
Scaled brood Cells	Indigenous bees	24 h.	1-0	2 0	0.125g 0		
		48 h.	0-0				
	Carniolan bees	24 h.	79-0	428	26.75d		
		48 h.	11-0	40	2.50efg		
	Hybrid bees	24 h.	15-0	68	4.25efg		
		48 h.	1-0	2	0.125g		

* Means followed by the same letter do not differ significantly at 5 % level of probability.

From statistical analysis, it could be concluded that the mean number of cleaned cells by indigenous bees (*Apis mellifera yemenitica*) after 24- hours were differed significantly with means number of cleaned cells by Carniolan and hybrid bees.

After 48-hours, the mean number of cleaned cells by indigenous bees increased to 100% but insignificantly differed with mean number of cleaned cells by Carniolan and hybrid bees.

According to the description of hygienic effectiveness depicted by Taber and Gilliam (1987) and Spivak and Gilliam (1998), the tested colonies were classified as hygienic and un-hygienic. Some colonies presented high hygienic behavior than other both within and between the races (Table 1). All indigenous colonies were hygienic (100%) whereas in Carniolan honeybee colonies, 8- were found un-hygienic. The same result was obtained with the hybrid honeybee colonies.

The examined races were ranked according to their hygienic effectiveness as indigenous bees (100%), Carniolan bees (86.5%) and the hybrid bees (81.25%). These findings confirmed the results of many researchers who found that hygienic behavior was different between species and races of honeybee as the Africanized and Asian honeybees which were more hygienic than European bees (De Jong *et al.*, 1984; Vandame *et al.*, 1997; Khattab, 2000 and Flores *et al.*, 2001).

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تقدير كفاءة سلالتين من نحل العسل (النحل اليمني والنحل الكرنيولي)
وهجينهما في مقاومة أكاروس الفاروا

أ. الغامدى

كلية الزراعة - جامعة الملك سعود بالرياض - المملكة العربية السعودية

درست كفاءة وصحة سلالة النحل اليمني والنحل الكرنيولي وهجينهما في
مقدرتها على مقاومة أكاروس الفاروا وأوضحت النتائج مقدرة الطوائف على تنظيف
حضنة النحل المجدد من أكاروس الفاروا خلال ٤٨ ساعة كانت ١٠٠ % ، ٨٦,٥ % ،
٨١,٢٥ % في السلالتين وهجينهما على التوالي بمنحل كلية الزراعة بجامعة الملك
سعود بالرياض.

Hygienic behavior of two indigenous races indigenous bees (*Apis mellifera yemenitica*, *Apis mellifera carnica*) and their hybrids containing in colonies from the last year during his summer, 2001 in Saudi Arabia. The results showed that the hygienic effectiveness was 100%, 86.5% and 81.25% after 48 hours for indigenous, *Carnica* and 1-1/2 races, respectively.

INTRODUCTION

Beekeeping industry originates in Saudi Arabia since long ago. Presently, the beekeepers rear both the *Apis mellifera yemenitica* and *Carnica* bees (*Apis mellifera carnica*). One of the major problems facing beekeeping industry in Saudi Arabia is the infestation of honeybee colonies with parasitic mite (*Varroa destructor*). The hygienic observations have indicated that indigenous bees race is less susceptible to *Varroa* mites as compared to the imported ones and this might be due to its more efficient hygienic behavior.

Spivak (1994) Spivak is a relationship between hygienic behavior and the removal of mites from brood cells of the cells. Test colonies with queens selected for hygienic behavior had 3-fold lower rates than the control colonies (Spivak and Rogers 1993). Hygienic behavior is a self-maintenance by which workers reject the diseased brood, among the cells and remove the infested larvae or pupae (Spivak and Gilliam, 1993). This behavior is one of the bases of balanced brood genetic relationship in the honeybees (Al-Jarrah et al., 2001).

It is known that the development of hygienic behavior is influenced by some environmental conditions. For example, increasing rearing increases the rate of removal of dead and diseased brood (Thompson, 1964; Menzel and Reischmüller, 1971). Also, hygienic behavior is heritable ($h^2 = 0.63$) as described by Harbo and Harbo (1995). They reported that it is a good candidate for selective breeding of bees, for resistance to *Varroa destructor*.