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Effect of population size on the expression of hygienic behavior in the Iranian honey bee (*Apis. mellifera meda*)

Javad Najafgholian [1*], Gholamhosein Thahmasbi [2], Abbas Pakdel[1], Gholamali Nehzati[1]

[1] *University of Tehran, University Collage of Agriculture and Natural Resources, Faculty of Agricultural Sciences and Engineering, Department of Animal Sciences, Karaj, Iran*

[2] *Research Institute of Animal Sciences, Department of Honeybee Genetics and Breeding, Karaj, Iran*

Correspondance: Javad Najafgholian, University of Tehran, University Collage of Agriculture and Natural Resources, Faculty of Agricultural Sciences and Engineering, Department of Animal Sciences, Karaj, Iran, Email: najafgholian.javad@yahoo.com, Tel:+98(441)3441868, +989143478297

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Abstract

As one of the main race characteristics of Iranian honeybees, brood rearing is attenuated at the end of summer and as a result adult population is declined noticeably in the autumn. On the other hand, in the middle of summer with rising in temperature *Varroa* mite reproduction decreases and at the end of summer with declining in the temperature this rate increases. Therefore, declining in the population of Iranian honeybee is concurrent with surging of *Varroa* mite population. Without regarding the honeybee adult population size, one of the important genetic mechanisms of honeybee colonies against *Varroa* mite is hygienic behavior (uncapping and removing). During the uncapping, young and middle-aged honeybees detect capped cells which infected with *Varroa* and in the other level, deletion, honeybees remove these polluted cells. In this situation, if hygienic

Introduction

Social insects have a plenty of strategies to disturb parasites. Their defenses can include multiple mating to increase the genetic heterogeneity of offspring, individual immunity, antibiotic secretions, nest hygiene and other behavioral and social mechanisms of infection control [1-13]. Disease resistance may also be socially strengthened. For instance, in the dampwood termite *Zootermopsis angusticollis* and the leaf-cutting ant *Acromyrmex echinatior*, the survivorship of individuals living in groups is easier than that of isolated [4,12,14]. Mechanisms that may account for this improved resistance include allogrooming, the transmission of immune factors between nestmates and/or the amplification of individual antimicrobial secretions [5,7,8,12,14]. Despite, an alternative mechanism, density-dependent prophylaxis (DDP) described in some gregarious insects could explain the enhanced survival of grouped *Z. angusticollis* [15].

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DDP involves greater investment in immune function at high population densities; individuals likely use cues associated with density to determine the optimal allocation of resources to immunity [15]. DDP, also, has been demonstrated in locusts [16], noctuid moth caterpillars [17,18] and mealworm beetles [19].

Moreover, in the honey bees constitutional defense mechanisms such as the chitinous cuticle which serves as a barrier between internal and external environment and the intestinal microflora of the bee gut can protect each individual bee against infectious diseases [20, 21]. Cellular defense mechanisms (haemocytes) and humoral reactions (enzyme and antimicrobic factors) can contribute to resistance toward infections [22]. The proventricular valve enables the bees to filter ingested spores, which serves as a mechanism of physiological resistance to diseases [20]. These individual responses, coupled with the short life-span of the bees and their rapid replacement with healthy individuals, can limit the spread of infections between bees within colony [23].

Besides individual resistance mechanisms, honeybees, also, have special resistance behavior related to population which is called hygienic behavior. Behavioral defense of the honey bee *Apis mellifera* against ectoparasitic mite *Varroa destructor* involves two important mechanisms: uncapping and removing. In this procedure, firstly hygienic honey bee workers have the ability to detect diseased brood, uncap the wax covering over the brood cells and secondly remove infected larvae or pupae. Hygienic behavior was described as the main mechanism by which *A. mellifera* resist the brood diseases like American foulbrood and chalkbrood. Afterwards, it has been demonstrated that hygienic bees detect and remove pupae infested with the parasitic *Varroa* mites [24]. Arathi *et al.* (2000) found that hygienic behavior is predominantly performed by the 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 [25]. The removal of infested pupae interrupts the reproduction of the fertile mites inside sealed brood cells. The colonies of *A. mellifera* die from varroasis (disease caused by *V. destructor*) within a few years if the mite population growth is not regulated by the beekeeper. Chemical control has its problems and limitations - reduced efficacy and development of resistance to chemical control by *Varroa* mites [26, 27]. Therefore, the only possible solution to the problems of honey bee varroasis is the identification and use of resistant stocks of honey bees, and their selection for enhanced resistance toward that disease, which could be acquired with stimulating hygienic and grooming behaviors, without loses of reproductive-productive features of honey bee colonies [28].

behavior is affected by population, slim colonies will be died or they will not capable of wintering ability; And in this position, if colony have wintering power in the beginning of spring will be weak. In this study, we evaluated the effect of colony population size on the expression of hygienic behavior (uncapping and removing) of Iranian honeybees which were maintained in the queen breeding Institute in western Azerbaijan province, Urmia city. The result of this study showed that effect of population size on the expression of uncapping and removing of infected larvae was very significant ($p<0.0001$). Furthermore, solely populous colonies, treatment 8 and 9, were regarded as hygienic colonies (removing rate >%95) and there was not seen any significant mean differences between these two treatments at the second day of study. Despite, all other treatments, 1 to 7, were non hygienic (removing rate <%95). Therefore, it is supposed that with direct selection for population size we expect to take correlated response for hygienic behavior and ultimately colonies genetic[1] resistance against pests and disease, *Varroa* mite, American foulbrood and chalk brood, in following years.

Figure 1. Evaluation of hygienic behavior in the Iranian honey bees.

Since there has not been conducted a great deal of researches in the field of honeybee population size on the resistance of colonies against *Varroa* mite, and we saw noticeable conflict between 2 biological life cycles, honeybee population and reproduction of ectoparasitic mite, we inspired to survey quantity of hygienic behavior in Iranian honeybee in relevant to different population sizes.

Materials and methods

Original breeding stock

This study was conducted on the existed breeding stock in the western Azerbaijan province, Urmia city. In breeding stock, every generation those quality queens which showed gentle behavior, less swarming behavior and high honey production selected and propagated for production of daughter queens. Since there had not been performed any breeding practice in the breeding stock against disease and pests, we determined to survey hygienic behavior as a criterion of disease resistance in this stock.

Hygienic behavior evaluation

Colonies were tested for hygienic behavior by freezing a circular section of sealed worker brood containing 160 pupa cells within the comb by using liquid nitrogen [29]. Within 2 days, the number of dead pupae that were in the process of being removed (were uncapped and/or partially removed), and the number completely removed from the cells were recorded each 24 hours separately for trial colonies *(Figure 1)*. In the current study only those colonies that were uncapped and removed more than 95% of the freeze-killed brood within 48 hours was considered as hygienic. 48 hours after pouring liquid nitrogen for hygienic behavior assessing, we noticed that there is the great phenotypic variation for hygienic behavior expression in the trial colonies. Some colonies were capable to remove freezed and killed brood completely (hygienic) but certain not (non hygienic).

Adult bee population

Adult worker bee population was visually determined by estimation of combs number covered by bees in each colony. The frames that covered thoroughly in 2 sides by adult bees considered as 1 frame whilst the less populous frames accounted fraction of 1 frame [30] *(Figure 2)*.

Table 1. Descriptive statistics of uncapping and removing rate at the first and second day of study.

Variables	Treatment (Adult population per comb)	Mean (%)	Standard Deviation	Maximum (%)	Minimum (%)
Uncapping rate at the first day of study	1(2)	38	7.43	55	30
	2(3)	46.7	8.81	60	33
	3(4)	62.7	5.39	69	50
	4(5)	70.7	1.41	73	68
	5(6)	76.8	1.98	79	73
	6(7)	82.4	2.31	85	79
	7(8)	88.2	1.47	90	86
	8(9)	92.2	1.22	94	90
	9(10)	98.1	2.37	100	94
Removing rate at the first day of study	1(2)	31	8.79	50	20
	2(3)	39.7	7.49	50	27
	3(4)	58.7	5.85	65	45
	4(5)	68.7	2.11	72	65
	5(6)	75.3	1.82	78	73
	6(7)	80.9	2.07	84	78
	7(8)	87.3	1.56	89	85
	8(9)	91.6	1.26	94	85
	9(10)	97.4	2.22	100	94
Uncapping rate at the second day of study	1(2)	38.3	7.48	55	30
	2(3)	47.2	8.52	60	34
	3(4)	64.2	4.73	69	53
	4(5)	73.1	1.59	76	71
	5(6)	79.3	1.82	81	76
	6(7)	85.15	2.28	88	82
	7(8)	91.9	1.52	94	89
	8(9)	96.95	1.16	99	95
	9(10)	99.8	1.42	100	99
Removing rate at the second day of study	1(2)	33	8.69	59	26
	2(3)	41	7.11	51	29
	3(4)	59.8	5.49	65	46
	4(5)	70.9	1.85	74	68
	5(6)	77.9	1.85	80	76
	6(7)	83.4	1.83	86	81
	7(8)	90.9	1.59	93	88
	8(9)	96.4	1.42	98	94
	9(10)	99.5	0.7	100	98

Experimental design

For having similar experimental units, one breeder queen which mated naturally was used for generating 90 daughter queens. Interestingly all daughter queens after mating had similar population size ranging from 7 to 8 combs. Thereafter, we made different colonies based on population size with comb transferring and colonies were arranged from 2 to 10 combs. Finally, we studied effects of 9 populations (2 to 10 combs) as a treatment based on completely randomized block design. 10 colonies were assigned for each treatment simultaneously in 10 different and nondescript geographical zones and these regions were considered as an experimental block. The study as conducted on following model.

$$Y_{ij} = \mu + T_i + R_j + e_{ij}$$

In this model Y_{ij} is the proportion of uncapped cells or removed pupa in 2 following days in different zones, μ is the population mean, T_i is treatment effect (population size), R_j is block effect and e_{ij} is random error term.

The PROC GLM in SAS program was used for data analysis. A Duncan test, also, was used for post-hoc comparison of the means.

Figure 2. *Measuring of adult bee population*

Results and Discussion

Descriptive statistics of uncapping and removing rate for colonies with different population size has been shown in *Table 1*. On average, solely treatment 8 and 9 with 96.4 and 99.5 percent removing killed pupa were considered hygienic ones respectively (removing rate >%95). Nonetheless, other treatments, 1 to 7, were non hygienic (removing rate <%95).

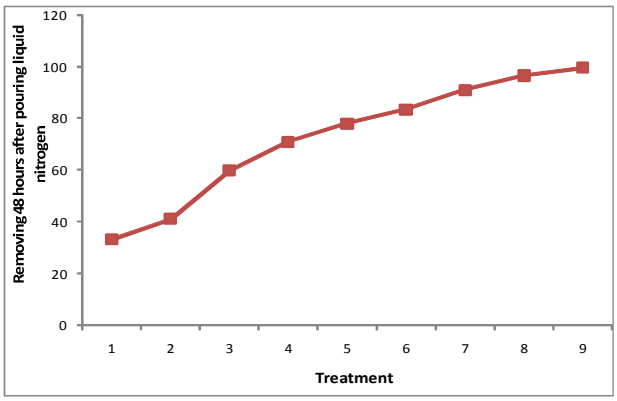

Figure 3. Effect of population size on the expression of uncapping at the first day of study.

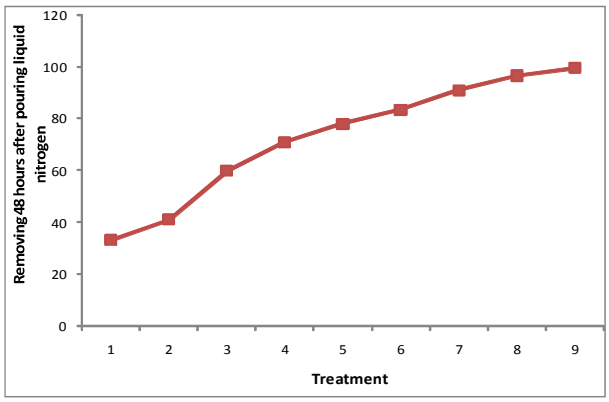

Figure 4. Effect of population size on the expression of removing at the first day of study.

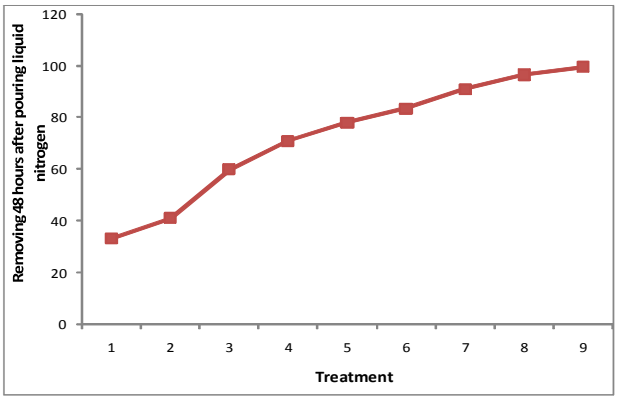

Figure 5. Effect of population size on the expression of uncapping at the second day of study.

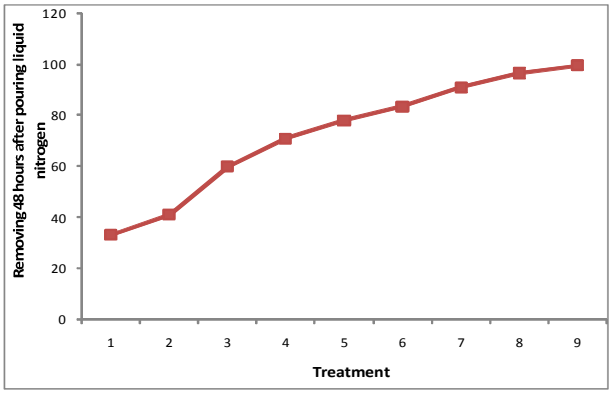

Figure 6. Effect of population size on the expression of removing at the second day of study.

In addition to, conducted study clarified that population size has very significant effect on the expression of hygienic behavior ($p<0.0001$). Mean comparison, also, for both uncapping and removing, demonstrated significant differences among treatments at the first day of study ($p<0.0001$). Despite, there was not seen any significant differences between treatment 8 and 9 in the second day of study. At the first day of study populous colonies exhibited the more hygienic traits (*Figure. 3, 4*). In addition to, similar trend followed at the second day of study *(Figure. 5, 6)*. On the other hand, blocks had significant effect in this experiment ($p<0.0001$) and that specify this statistic design.

Hygienic behavior, an external task performed by middle-aged worker bees, is an important behavioral mechanism of resistance to disease and pests such as *Varroa destructor*, an ectoparasitic mite [23, 29]. The results of hygienic behavior analyses obtained in this study are in accordance with those of Spivak and Gilliam [30] and point to an indisputable relationship between expression of hygienic behavior and the strength of honey bee colonies, which means

that the potent colonies have more expressed hygienic behavior and consequently greater resistance to many diseases.

Furthermore, our result is in compatible with the finding of Stanmirovic et al. Stanmirovic et al. [32] in the study of hygienic and grooming behaviors of Syenichko-Peshterski honey bee ecotype analyzed 440 honey bee colonies from 11 localities. At each locality 40 honey bee colonies were investigated: 10 potent colonies with one year old queen, 10 potent colonies with two-year old queen, 10 medium potent and 10 weak honey bee colonies. Hygienic behavior was expressed in a range from 95.12% to 99.50% in potent honey bee colonies with one-year old and two-year old queens. Statistically highly significant ($p<0.01$) differences were registered among the analyzed honey bee colonies at the investigated region, in favor of the potent honey bee colonies, compared to the medium potent and weak colonies. Also, statistically highly significant ($p<0.01$) differences were recorded between potent colonies with one-year old queens and colonies with two-year old queens, in favor of the colonies with one year old queens. Conducted research by Stanmirovic et al. [32] clarified that populous colonies are very potent to cope with pests and disease.

Such super hygienic potent honey bee colonies from all investigated localities can be used as breeding colonies for rearing quality queens. If those queens are naturally mated to unselected drones, their offspring will actively defend themselves against the mites. However, according to Spivak and Reuter [33], such behavioral response is possible only at low mite levels (<15% of worker brood and <15% of adult bees) in the first year of service period (for up to 1yr without treatment). Conversely, if mite infestation is higher (>15% of worker brood and >15% of adult bees) hygienic colonies eventually will collapse unless treated, because at high mite levels, the bees may habituate to the odor cues that elicit the hygienic behavior and are not able to detect individually infested cells. In this case, the bees may cease to detect and remove the infested brood [34]. Therefore, it is proven that evaluated populous colonies for hygienic behavior can be used for improving breeds selection and for organic beekeeping in Iran.

It is currently unreasonable to assume that honey bees bred for hygienic behavior will survive indefinitely without some sort of periodic treatment. However, it is encouraging that lines bred for hygienic behavior may require less frequent treatments that unselected lines. Any reduction in pesticide use within colonies translates into lower operating costs for the commercial beekeeper and decreased risk of contaminating honey and hive products [33]. Indeed, generating Iranian honeybee resistant line in Mid East is

our ultimate goal and we are hopeful to introduce such resistant line in following years.

Conclusion

Study showed, population size was more effective in increasing the hygienic behavior ratio and decreasing the probability of *Vorroasis*. We suggest that populous breeders which have been tested for economic traits must be used as quality queens in queen breeding institutes.

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