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REVIEW OF TESTS USED IN HONEY BEE SELECTION (*Apis mellifera* L.)

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Abstract: Modern beekeeping is very interested in improving honey bee traits, especially bees' resistance to diseases and their production capacity. Honey and comb productivity can be increased by making detailed plans and selection programs in which good genetic material is the key factor for creating highly productive bee colonies in favorable environmental conditions. In order to choose the preferred characteristics for the purpose of selection, queen bee traits should be carefully analyzed, because she transfers them to her descendants. Selection programs should include genotypes with good production characteristics, but also those with a higher disease resistance and lower swarming tendency and lower aggression. Measuring and evaluating these traits require high accuracy, and the collected data must be processed by appropriate statistical methods.

This paper aims to demonstrate the basic principles of honey bee traits inheritance as well as to present an overview of the tests used in their selection such as: test for hygienic behavior, test for the determination of brood quality, test for the determination of bees' level of aggression, test for the determination of swarming tendency and test for the determination of honey production.

The implementation of all these tests is a necessary prerequisite for successful honey bee selection, as the results provide information about individual differences among bee colonies in the apiary, which is then used for choosing the best colonies which will be taken for further selection programs.

Key words: selection, tests, honey bee, productivity

Introduction

Honey bees (*Apis mellifera* L.) live in colonies with a very large number of members, where the behavior and survival of each individual bee depends on the other cohabiting members. From the genetic and evolutionary aspect it can be said that adaptive value of each individual bee, when removed from the colony, is zero, indicating that they would not

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survive alone outside the hive. A honey bee colony has a very complex organization, which implies that bees are very connected to one other, and therefore selection can be conducted only at the level of the colony (Moritz and Fuchs, 1998). Modern beekeeping is very interested in improving bee production traits, which is possible thanks to developed selection plans and programs. The goal of artificial selection is to change the frequency of certain genes in the population through favoring of genotypes with higher reproductive capability, i.e. those that give more offspring in the next generation. The success of selection depends on knowledge of mechanisms of inheritance and expression of genes responsible for phenotypic traits. The development of most honey bee traits not only depends on the genes but also on environmental conditions, which may modify this process. Therefore, it is important to know the heritability coefficient of the traits that are to be selected. If the value of the heritability coefficient is near 1 (100%) then the given phenotypic trait is more influenced by genes, the frequency of which in the population can be changed faster by selection, but if this value is close to 0 (0%), the success of selection drastically decreases (Harbo, 1999). The results of analyses of the heritability coefficient of some phenotypic traits in honey bee populations in Serbia showed that the average values of this coefficient are the following: for honey production (35.90%), swarming (28.62%), aggressiveness (35.90%) and robbing behavior (39.53%), which shows that environment significantly affects the expression of these traits (Jevtić et al., 2012). Good colony development requires optimal and stable environmental conditions, because the value of the heritability coefficient increases this way.

Control of the biological and production characteristics of a colony includes an assessment of the queen's reproductive capacity through brood quantity and quality, evaluation of overwintering ability, swarming propensity, aggression, hygienic behavior, as well as honey and wax production (Petrov, 2015). Selection is done by maternal line, ie. only colonies that have a highly productive queen bee, high honey production, good overwintering, low aggressiveness and good hygienic behavior are taken into account (Petrov, 2015). If the breeder has good genetic material, he may, in favorable environmental conditions, create a highly productive and a highly disease-resistant bee colony. Honey bee traits that are included in selection programs are resistance to diseases, swarming tendency, aggression, production traits, etc. The measurement of these traits should be precise and clear, and the collected data analyzed using adequate statistical methods. By summarizing the obtained data, the decision about the best colony that will be used in further selection is reached. Tests have been developed for assessing colony quality and the most important of these in practice are: the test for hygienic behavior, the test for grooming behavior, the aggressiveness test, the test for honey production, the swarming tendency test and the brood quality test.

Tests for evaluating colony traits

Testing hygienic behaviour. Hygienic behavior of honey bees is a trait potentially related to resistance to the *Varroa destructor* mite (Boecking et al., 2000). Mite infections can be reduced in colonies, at least for some time, by using acaricides. However, the long-term solution to the problem lies in the selection of lines resistant to this mite (Degrandi-Hoffman et al., 2002). Hygienic behavior of honey bees entails detection and removal of infected brood from the hive (Rémy Vandame et al., 2002). In other words, honey bees with hygienic behavior can detect diseased larvae in comb cells, open wax caps and remove infected larvae and pupae from cells. Hygienic behavior is expressed mostly by bees which are 15-17 days old and about 18% of all bees in the colony are involved in the performance of this task (Arathi et al., 2000). Stanimirović et al. (2008) carried out an experiment with a view to calculating the coefficient of inheritance of honey bee hygienic behavior in Serbia. The experiment entailed the monitoring of similarity in the expression of this trait in the offspring (daughters). The results showed that colonies selected for hygienic behavior have the same brood area and produce the same amount of honey as non-selected colonies. Boecking et al. (2000) found that h^2 for removal of mite-infected larvae from cells was 0.18 and that h^2 for removal of dead larvae from cells (larvae killed by application of the "pin-killed" method) was 0.36. This implies that hygienic behavior is somewhat regulated by genes, but also under the significant influence of the environment. There are several methods for testing hygienic behavior, all of them based on calculating the percentage of deliberately killed larvae in cells that are removed. One way to kill larvae is the "freeze kill" method, which entails freezing the brood with low temperatures (Spivak and Reuter, 1998) and the other is "pin kill" method, which entails piercing larvae with a needle (Spivak and Gilliam, 1991). The test for expression of hygienic behavior is measured by counting the number of cleaned cells over 24 hours. A colony that has cleaned over 95% of cells is considered super-hygienic, while a non-hygienic colony is one that has cleaned less than 90% of cells (Spivak and Downey, 1998). Hygienic behavior is a significant factor of resistance to the varoa mite, but also to American fulbrood and chalkbrood (Spivak and Reuter, 2001). The results obtained by Spivak (1997), showed that colonies selected for hygienic behavior had lower incidence of chalkbrood in comparison to non-selected commercial colonies. Furthermore, in their experiment hygienic colonies produced more honey than non-selected commercial colonies, which means that it is possible to perform selection for hygienic behavior without negatively impacting honey productivity.

Testing of grooming behavior. Varroa destructor is a honey bee ecto-parasite on which causes serious losses of bee colonies around the world and the long-term solution to this problem is breeding of lines resistant to varroa (Degrandi-Hoffman et al., 2002). A mite attack, without periodical treatment, would cause most colonies in a moderate climate zone to die within 2-3 years (Rozenkranz et al., 2010). Varroa mite feeds on the hemolymph of adult bees and larvae, and reproduce in the brood (Boecking and Spivek, 1999). Stanimirović et al., (2010), who conducted experiments with a view to calculating the heritability coefficient of honey bee grooming behavior in Serbia, found that it is a low heritability trait, i.e. a trait largely influenced by the environment. Considering that selection is only effective when the heritability coefficient is over 0.25, selection of bees based only on this trait is not recommended if the ultimate goal is resistance to *V. destructor* (Stanimirović et al., 2010). Research conducted by Ruttner et al. (1992) proved that *Apis mellifera carnica* has behavioral, anatomic and physiological possibilities for removing and killing mites in bee colonies. In this process, the mites' legs can be cut off or their cuticle can be damaged, using the mandibulae, which causes them to fall from the bee (the host). Thus, bees show certain resistance to varroa and that resistance can be defined, according to Rémy Vandame et al., 2002, as the adaptive response of the host aimed at actively preventing further development of the parasite. Bees remove parasites from their own body or other colony members (Rémy Vandame et al., 2002). Considering that direct tracking of grooming behavior is very difficult in practice, an indirect method can be used instead which entails calculating the percentage of damaged mites in the total number of fallen mites. Grooming behavior can be measured by counting alive and damaged mites on the hive's floors (Boecking and Spivek, 1999). However, only adult mites should be counted, because young individuals might have been damaged during their removal from the infected brood, which is related to hygienic behavior (Stanimirović et al., 2010). According to Bienefeld et al. (1999), mites lighter than the brown ochre color are classified as young individuals, while darker mites are adults. Only colonies with a highly expressed grooming behavior and good reproductive and production traits should be used for honey bee selection.

Testing of colonies for honey productivity. Honey production is a very complex bee trait, regulated by genes but also influenced by the environment. Thus, according to Bienfield and Pirchner (1990), the heritability coefficient for honey production has the value $h^2=0.26$. In practice, productivity is assessed by measuring the colony's weight in intervals. Measuring is quick; the method does not take long to learn and it does not interfere with the colony, so it can be conducted at any time of the year. Frequent and precise measuring of the colony can yield very useful information about the dynamics of nectar uptake (Meikle et al., 2008). Colonies can be measured during winter or summer, depending on whether we want to monitor the decrease in food storage during winter or the rate of nectar uptake in the summer. In Serbia, assessment of colony strength in terms of honey production is conducted during the season, using the Szabo method, which entails measuring the bee colony twice in three days, and the difference in weight is the value of the test (Pravilnik, 1996).

Testing of brood quality. In commercial beekeeping, natural mating sites are primarily used. The honeybee (*Apis mellifera*) queen mates during nuptial fights, in the so-called drone congregation area where many males from surrounding colonies gather. (Baudry et al., 1998). This way of reproduction is of vital importance for maintenance of genetic variability, because mating of the queen bee with drones from the same colony leads to 50% mortality for fertilized eggs (Page, 1980). As in other animal species, inbreeding in honey bees causes degradation of many traits, the occurrence of inbreeding depression and a decrease in colony quality. The consequences of inbreeding can be easily noticed through the occurrence of "shot brood" (Adams et al., 1977), with highly reduced viability. Brood quality, which depends both on the mating process and the quality of the queen bee, is assessed based on the brood surface. According to a generally adopted international standard[3], brood quality is assessed on a scale of 1-4, as follows: 1 if the brood is present on less than 25% of the comb, 2 if the brood is present on 25-50% of the comb, 3 if the brood is present on 50-75% of comb and 4 if the brood is present on over 75% of comb.

Testing of aggressiveness (temperament). Defensive behavior of the honey bee (*Apis mellifera* L.) is a complex trait which involves the behavior of individual bees and the coordinated response of the entire colony (Andere et al., 2002). In the event of stronger

[3] At the Apimondia Symposium "Controlled mating and selection of the honey bee", in Linz in 1972, technical recommendations for colony performance evaluation methods were developed (Ruttner, 1972); they are used today as an international standard for colony performance evaluation.

stimulus, bees quickly become aggressive and they send an alarm to other bees to respond. Other bees are recruited by the secretion of alarm pheromones and by flying, which serves as a visual stimulus (Guzman-Novoa et al., 2004). In practice, less aggressive colonies are more desirable, therefore, selection is directed toward developing gentle colonies. Assessing colonies for this trait, in the field, is not difficult and does not require special expertise, expensive equipment or personal experience (Moritz et al., 1987). The generally adopted international standard for assessing bee aggression entails observing bee colonies and giving scores on a scale of 4-1, as follows: 4 for very calm colonies (during hive inspection, bees do not leave combs and move calmly), 3 for calm colonies (during hive inspection, bees are a somewhat restless but do not attack), 2 for restless colonies (bees are very restless, they fly around and try to attack (they move from the brood to honey), 1 for very restless colonies (bees leave combs or cluster inside or outside the hive). The behavioral assessment must be repeated 3-6 times during season, regardless of the specific conditions such as weather, nectar uptake, etc. The average value of all assessments is calculated at the end of the season and taken as the value of the test.

Testing of swarming tendency. According to Büchler et al., 2010, the most important traits in the majority of selection programs are economic traits (honey production and colony strength) as well as traits desirable in modern beekeeping (gentleness and low swarming tendency). At the level of the colony, the European honey bee (*Apis mellifera* L.) multiplies by swarming, that is natural division of the colony. During colony division, an old fertilized queen is in the first swarm that comes out, while each subsequent swarm, if any, contains unfertilized queens (Fries et al, 2003). Natural swarming in beekeeping is not a desirable occurrence, as a large part of the bees leaves the hive, and the remaining colony produces much less honey. The generally adopted international standard for assessing swarming tendency entails giving marks on a scale of 1-4 during every inspection of the colony, as follows: 4 for colonies without swarming tendency (colonies that did not swarm), 3 for colonies in which queen cells have appeared, but where swarming tendency can be stopped by moving brood frames and adding new comb frames, 2 for swarming colonies where swarming can be stopped only by adding more frames or an entire hive box, 1 for swarmed colonies where all the conducted anti-swarming measures had no effect.

Conclusion

The most important step in honey bee selection is the right choice of desirable traits that will be favored and the accurate evaluation of bee colony performance. Tests are used to determine which colonies are the best and thus which can be considered for further selection. The best colonies will give queen bees of the highest quality, and several generations of careful selection will result in the creation of lines with a combination of desirable traits. Honey bee selection tests are an indispensable tool in the evaluation of colony performance, where good quality is characterized by highly expressed hygienic and grooming behavior, good productivity, a quality brood, calmness and low swarming tendency.

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