The Standardization of the Honeybee Colonies Evaluation Methodology, with Application in Honeybee Breeding Programs, in Romanian Conditions

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Abstract

It is well known that breeding is based on phenotypic and behavioural performance assessed at the level of each honeybee colony. By selection, the genes responsible for the desired characters have to be favoured, by evaluation and classification of all colonies involved in a breeding program. Generally, in the beekeeping practice, the most applied method of selection is the mass selection regarding the main objective- honey production. Some more elaborated programs use selection simultaneous selection on several characters. Until now, a standard method for honey bees evaluation and selection on several characters could not be generalized, every breeder establishing the selection method depending on proposed goals which could be different especially when we speak about different races and environmental conditions. Taking into account the selection objectives in Romania it was conceived a standardized methodology for the selection on several characters in Romanian condition

Keywords: breeding programs, traits, honeybee, selection, standardization

1. Introduction

In honeybees *(A. mellifera L*) the breeding activity is based on the performance evaluation at the level of each honeybee colony [1, 2, 3]. The evaluation consist, generally, in the appreciation of economic and behavioural traits in order to be clasified and make selection of the best parrents. In order to correctly establish the genetic value of the individuals, in selection, it is very important that the environmental and management factors to be standardized [4, 5]. As it is known, the complications of a genetic honey bee colony evaluation protocol are produced by some general and particular biological characteristics [5, 6, 7, 8]. Some of them are:

- Honey bees genetic populations are components of different ecosystems and each is adapted to their ecosystem; man cannot control more than 10 % of bee's ecological conditions (climate, flora, diseases). As a result, honey bees evolution is more than other species of economic animals under the natural selection control, under the ecosystem control.
- The observed value of a colony is the result of two components – genetic, transmissible to offspring, and specific environmental (apiary location, unequal conditions, past etc.) not transmissible; the bigger production can be the result of a better special environment. In honeybees, the candidates to selection, the "individuals", are the honey bee colonies composed by subfamilies with different degrees of relatedness; as a result the bee genetic populations are not so large and the inbreeding

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has an increased incidence due also to the specific sex determinism;

- The genetic value of a colony is the result of the queen genetic value and of the drones which she mates. The honey bees' open natural mating system, heterospermia, haplo-diploid system, sex determination make more difficult the identification of best parents for the new generation.
- Establish as much as possible a simple objective of selection (not too many traits), accept characteristics with a high genetic control (high h^2), use correlated records or traits instead of costly or difficult measurements of honeybee recording traits [9, 10, 11], establish the most suited selection methods and decrease the interval between generations to one year.
- Avoid inbreeding by increase the population size to the minimum number of selected parents of the next generation and, in some situations avoid the strong, long reproductive isolation.
- The measurement of different characters can be costly. To avoid some of these complications, to increase the estimated value accuracy and decrease its costs, some procedure are generally adapted. Among them we mention the following**:**

- Avoid as much as possible the variability of environmental conditions for the colonies candidate to selection (use queens from the some ages, the same strength of the colonies, the same treatments, etc.).
- Eliminate major variability induced by environment by transformation of the absolute recorded value of traits (x = kg, numbers etc.) of each potential parent of the next generation in relative ones (z = score) as percentage (%) or standard deviation (s) from the average (M) of their contemporaries tested colonies [5]:

$z = [(x-M)/M] 100$, (when testing colonies from the some apiary, with the some environmental conditions), or

$z = (x-M)/s$ (when testing colonies from different apiaries);

Consequently, the average of apiary becomes 0, and the rest of values are ranged from –3 to +3, being thus comparable for the identification of the best parents.

Data recording and analysis are of a great importance in breeding process, as organizational matters [12].

Considering all these aspects, many honey bee relatively genetic closed population breeding programs have been elaborated, most of them using selection index mating control by instrumental insemination [13], or modern BLUP animal model approach [14].

Generally, all these programmes are scientifically correct, but often too complicated for a professional but not a scientist or technocrat beekeeper. For this reason we made this most elementary study on the evaluation of honeybee colonies, developing a standardized methodology and an electronic database for recording and processing data with application in honeybee breeding programs.

2. Materials and methods

The selection objective (the traits that must be conserved or improved) must be clear, constant, and stable for a long time, as well as simple, including just the economic and biologic essential traits, the characters with a high heritability or using not costly, genetic positive correlated traits [15, 16].

Taking into account the specificity of different breeding programme goals (economic, behavioural and race traits) and the new challenges regarding the adaptability to diseases and natural conditions there are different characters that can be included in an evaluation and selection protocol:

1. **Race specific morphology traits**: colour, proboscis length, tarsal index, cubital index, discoidal shift; generally, there are more measurements, including genetic markers which can be performed depending on the applied methodology of discrimination between races.
2. **General behaviour:** nest organization, honey capping mode, gentleness, calmness on the combs, swarming tendency etc.;
2. **Colony development**: the viability of brood, wintering ability, spring build up, colony strength in terms of quantity of honey bees and brood surface, honey bees longevity;
3. **Productivity**: honey and pollen production as the main productive traits, pollination activity;
4. **Resistance to diseases**: hygienic behaviour for diseased brood, suppressed mite reproduction (SMR), grooming behaviour, mites

infestation degree (the honey bees mites-*Varroa destructo*r and *Acarapis woodi*)[17, 18, 19, 20];

3. Results and discussion

I. The selection objectives - Traits to be recorded, included into the selection programme and the database. Following the presented situation and nationally interest to improve productive traits concomitantly with the specific race and adaptability ones it was considered useful to establish the following traits presented in the evaluation protocol and data base as breeding objectives:

(1) **The economic (productivity) traits:** the honey and pollen production (*table no 1.* z4 and z5). The honey represent the main economic objective in our national breeding program, but the existence of specialised beekeepers for pollen production in some areas make necessary to include in the evaluation data base and selection objective also the pollen production as a secondary important trait, or as first, the importance trait in a second type of breeding program.

(2) **The colony development in spring. (*table 1* - z2, z3), the viability of brood and the wintering ability (z1).**

(3) **The resistance to diseases traits (*table no 1* -z6, z7) –** in terms of hygienic behaviour for killed brood and level of *Varroa spp* infestation), as importance, the second group of traits accepted as the selection objective. Together with the productive traits we consider that it must receive some 85 % of selection pressure. The colony development indicators in spring are especially connected with the acacia honey flow, which represents an important goal for honey production. The blooming period for acacia in Romania (around 10th May) does not overlap with the maximum of the colonies development, so that the precocity of this trait has to be favoured in the selection process. A better development at this moment will lead to a better productivity, so the interest in selection is to obtain colonies with as much as many foragers workers, being directly depended on colony strength and development.

(4) **The general behaviour traits** (nest organization, honey capping, gentleness, calmness on the combs, swarming tendency), have received in the last time more and more the attention of breeders, thus a multitude of behavioural characters are of great interest for breeding. Some are not correlated with honey production and the inclusion of all can diminish the genetic gain for the main objective-honey production. For this reason we must accept that it is useful to include in the objective of selection just the behavioural traits positively correlated to honey and only if there are strictly necessary the others traits, too.

(5) **The morphological traits:** colour, proboscis length, tarsal index, cubital index, discoidal shift and some genetic markers (mtDNA and microsatellites); generally the morphological traits have no economic importance but they are very important for the preservation of local honeybees, which is considered to be the best adapted to local natural conditions. In Romania the national breeding program [21] specify the selection in pure race in order to avoid the introduction and multiplication of foreign biologic material. The genetic traits were included into the program to be evaluated by specialists once will be set up an expertise laboratory for molecular biology for honey bees.

II. Recording; Database software

The envisaged traits were classified as groups of characters and evaluated by a unitary system (selective data notes and scores), following the laboratory and field measurements, in order to classify and eliminate the colonies with undesirable traits. All data can be managed by a database which can work on 100 honey bee colonies, based on the structure in table no 1. The data base was created in Microsoft Office Access computer software, helping the breeder to manage a great number of data in an efficient way, being also accessible in html format to be accessed by different users by internet network. The data processing can be completed by Microsoft Office Excel software. The Office Access computer application is composed by a data recording system and a data displaying system, being conceived for a better management and the primary processing of data (data recording, queens identification, sorting, filtering, average and standard deviation calculations). The selection index will be calculated in Office Excel software in order to create a data hierarchy and to

compare for a better decision regarding the selection of the parents.

III. The selection methods

By selection, the genes responsible for the desired characters have to be favoured, by evaluation, classification of all colonies and selecting the parents of the next generation.

Generally, in beekeeping practice, the most applied method of selection is the mass selection (own performance), especially when the selection is performed for one complex, additive character as honey production with a relative good heritability and genetic correlated with many other characters – queen fertility, viability, honey bees longevity, hoarding behaviour, hygienic behaviour.

When selection for several traits is practised, three methods are available [22]:

(a) Selection by independent culling levels (each trait can be selected for separately and simultaneously),

(b) Tandem selection (one trait at a time could be selected in succession) and,

(c) Selection index (selection on all traits carried out simultaneously).

Following our inventory of traits and the succession of their evaluation in time we think that the best solution for our breeding objectives is a type of tandem selection for groups of characters: **two groups of traits could be selected at a time in succession:**

(1) Firstly, a selection by independent culling levels has to be done.

Taking into account the specific of measurements and one of the specific goals to maintain the selected populations in pure race it was established **an initial phase of colonies selection based on morphometric characteristics,** eliminating the colonies that are not conformed to race standards, **and some of the undesirable behavioural characteristics** (e.g. aggressiveness, swarming tendencies).

At the beginning of the program, the colonies with less than **85% genetic viability** of brood will be eliminated. Subsequently, if we notice a decreasing of viability toward this level, we will introduce tested queens to increase the number of sex alleles.

(2) Secondly, a selection index has to be calculated including the main character - the honey production and others characters included in selection objectives, preferable genetically correlated with the honey production [5, 23, 24].

Selection index (I_{sel}). According to Rinderer (1986) [5], the most rapid way to improve a stock, when several characters are to be selected, is the selection index, the method being known also as simultaneous selection on several characters [15].

It will be calculated by totalling the z scores obtained for the productive, development and resistance to diseases indicators, and the colonies will be classified.

In the simultaneous selection, the selection index is established by totalling the z scores for all characters ($z_{1...}$ z_n), each multiplied by a value, depending of his heritability, genetic correlation and economic value.

According to the mentioned authors, in more than two characters, the formula is more complicated using the multiple regression and covariance.

As the result of this complexity the selection index could be obtained by excluding the heritabilities and correlations, especially when these parameters are difficult to be estimated as there are a multitude of influence factors specific to honey bees' particularities.

In this situation, the selection index can be calculated based on the z scores and economic values of the main characteristics.

In order to emphasize the colonies with the best results in honey production (the main economic value) the "z" score for honey will receive a greater value (e.g. V=4) as compared to the others.

In this study we included the following z scores in the selection index:

Of course, the correct economic value that can be attributed to the main characteristics or to the other in the index selection construction need more elaborated studies.

In order to select the best parents the selection index will be also compared with other behavioural measurements (notes), the final decision being under the breeder analysis.

As basic rule, taking into account the relative modest heritability of honey production (0,22-0,25), in order to have genetic progress the most productive colonies have to be selected and the other characters have to receive constant attention to fix them in the breeding population [24, 25].

Table 1. The groups of traits and their evaluation system for methodology standardization

no. crt	Groups of traits	The traits	Measurements	Classification as data, note, and scores
0	1	2	3	4
1	**1. The morpho—genetic traits for eliminatory evaluations**	Colour	+ (desired)/-	Selection +
		Cubital index	number	Selection over 2,3
		Proboscis length	number	Selection over 6,3
		Tarsal index	number	Selection over 1,6
		discoidal shift	0/+/-	Selection +
		Genetic profile-mtDNA	C1/C2/C3 etc	specific
		nuclear DNA -microsatellites	Specific polymorphism	specific
2	**2.General behaviour traits**	Nest organization	Compactness and concentricity	5-1
		Honey capping	Dry- wet capping	5-1
		Gentleness	Gentleness degree	5-1
		Calmness on comb	Calmness degree	5-1
		Swarming tendency	Swarming degree	5-1
I.	**Behavioural Note**			**5-25**
3	**3. Colony development traits**	Genetic viability	%	Selection over 85%
		Frame intervals of honey bees - March	number (grams) x_1	
		Frame intervals of honey bees- April	x_2	
		Frame intervals of honey bees- May	x_3	
		Frame intervals of honey bees -October	x_4	
		a. ability to winter	$a= x_1/x_4$	$z_1=[(a-a_a)/a_a]$ 100 a_{a-} =*average of a values*
		b. spring build-up (1)	$b= x_2/x_1$	$z_2=[(b- a_b)/a_b]$ 100 a_b=*average of b values*
		c. colony development for first honey flow –acacia (2)	$c=x_3/x_1$	$z_3=[(c-a_c)/a_c]$ 100 a_c =*average of c values*
4	**4. Economic traits**	d. honey production (honey flows: I, II or III)	Weight gain test -kg **(d)**	$z_4=[(d-a_d)/a_d]$ 100 a_d =*average of d values*
		e. pollen production	quantity **(e)**	$z_5=[(e-a_e)/a_e]$ 100 a_e =*average of e values*
5	**5. Resistance to diseases traits**	f. hygienic behaviour	% **(f)**	$z_6=[(f-a_f)/a_f]$ 100 a_f = *average of f values*
		g. varroa infestation degree	% **(g)**	$z_7=[(g-a_g)/a_g]$ 100 a_g=*average of g values*
II.	**The selection index for honey production**		$I_{sel}= z_{1a}+z_{2b}+z_{3c}+Vz_{4d}+ z_{5e} + z_{6f}+z_{7g}$ where, V= economic value for the main character	

4. Conclusions

1. The standardized methodology is based on the inventory of most important traits according to the selection objective of Romanian honey bee breeding program.

2. The standardized methodology have the following advantages:

- it could be used as an unitary system in applying the selection programs, aiming the genetic

improvement, protection and preservation of local, Romanian honeybees;
- it increase the efficacy of classification and selection process of honeybees colonies with good performances, using an electronic data base;
- the major variability induced by special environment especially can be eliminated by standardising the conditions for evaluation and transformation of the absolute recorded values of traits into relative ones (% or standard deviation from the contemporaries) using the specific formulas included in the electronic program.
3. The standardized methodology include several characters as possible objectives of selection, grouped as follow: race specific morphology traits, general behaviour, colony development, productivity and resistance to diseases.
4. The following selection methods could be used in the program:
(a) Firstly, a selection has to be done by independent culling levels on morphometric characteristics, eliminating the colonies that are not conformed to race standards, and those who have some undesirable behavioural characteristics (e.g. aggressiveness, swarming tendencies).
(b) Secondly, there is a selection index that has to be calculated including the productive character (honey production, pollen production) and others characters included in selection objectives (colony development, resistance to diseases), usually genetically correlated with the honey production.

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