THE EVALUATION OF HONEYBEE COLONIES, USING A DATABASE WITH APPLICATION IN HONEYBEE BREEDING PROGRAMS.

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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.
This evaluation often is a very labour and time consuming process, depending on number of measured characters, methods of evaluation, time, repetitions, registration and processing of the obtained data for colonies differentiation.
To make this process easier a breeding data base was created (Access software) for data registration, identification and classification, following the assessment of each colony.
This software was conceived to work on 100 bee colonies and can process data for the main characters grouped as follow: honey production from the main flows, population, brood viability, brood quality and quantity, gentleness, way of capping, swarming tendency, disease resistance, pollen production, morphometric measurements and genetic analyses.
A series of collected and processed data, from a breeding apiary belonging to Beekeeping Research and Development Institute from Bucharest, using this software will be also presented in this paper.

Keywords*: honeybee breeding, selection, evaluation, data base software.*

I. GENERAL ASPECTS IN HONEY BEE BREEDING

The honeybee colonies evaluation and classification is a usual, spontaneous preoccupation of every beekeeper; it is done somehow, for savage but also for apiaries colonies, by the natural selection. The problem becomes more complicated when the comparative evaluation must be done with a deliberate breeding purpose, with the purpose of genetic improvement of a bee genetic population, of a honey bee breed, with the purpose to obtain a genetic progress for each new generation.
As it is known, the complications of a genetic honey bee colony evaluation protocol are produced by some general and particular biological characteristics. Some of them are:

- Honey bees genetic populations <u>are components of different ecosystems and each is adapted to their ecosystem</u>; 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.

- 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 is more possible 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.
- 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:
  $z = [(x-M)/M]\ 100$, (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.
- 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, 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.

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 (NWC programme, Cobey 2006), or modern BLUP animal model approach (Bienefeld et al 2007).
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 database software with application in honeybee breeding programs.

II. SELECTION OBJECTIVE

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. It is known that when the selection is done for uncorrelated traits, the genetic gain for each of them is just $1/\sqrt{n}$ if the selection was made just for one important trait (Hazel and Lush 1943).

However, in attention of honey bee breeders are much more traits. Before analyzing the recommendable breeding objective, we made an inventory of characters that are in the attention of breeders**.**

II.1. The general traits of the honey bee colonies in the breeder's attention.

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 morphologic traits**: colour, proboscis length, tarsian index, cubital index, discoidal shift; here there are many other 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;

3. 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;

4. Productivity: honey and pollen production as the main productive traits, pollination activity;

5. Resistance to diseases: hygienic behaviour for diseased brood, suppressed mite reproduction (SMR), grooming behaviour, mites infestation degree (in acarapiosis and varoosis);

II.2. The heritability and genetic correlation for some honey bee traits.

Table no 1 and 2 show a general situation from the literature regarding the main studied characteristic in order to present their h^2 and genetic correlation with honey production.

Table no.1. The heritability of some traits in honey bees.

Trait	**Heritability ($h^{2)}$**	**According to**	**Source**
Honey production	0,23	Pirchner et all (1962)	Rinderer (1986)
Honey yield	0,35	Vesely and Siler (1963)	Rinderer (1986)
Brood	0,35	Pirchner et all (1962)	Rinderer (1986)
Brood 6 weeks before flow	0,30-0,41	Vesely and Siler (1963)	Rinderer (1986)
Worker longevity	0,196	Milne (1985)	Rinderer (1986)
Worker longevity	0,32	Rinderer (1983)	Rinderer (1986)
Hygienic behaviour for Varroa	0,18	Boeking, Bienefeld (2000)	Boeking, Bienefeld (2000)
Hygienic behaviour for dead brood	0,36	Boeking, Bienefeld (2000)	Boeking, Bienefeld (2000)
Suppressed mite reproduction	0,46	Harbo (1999)	Harbo (1999)
Initial activity of bees to alarm pheromone	0,04-0,12	Collins (1984)	Collins (1984)
Speed of bee's reaction	0,3/ 0,83/1,28	Collins (1984)	Collins (1984)
Defensive behaviour in field	0,1-0,93	Collins (1984)	Collins (1984)
Colour	0,21-0,39	Szabo (1992)	Szabo (1992)
Proboscis length (*A.m.meda*)	0,64	Mohamad (2006)	Mohamad (2006)
Cubital index (*A.m.meda*)	0,5	Mohamad (2006)	Mohamad (2006)
Number of hamulli	0,68	Oldroyd (1983)	Rinderer (1986)
Corbicular area	1,01	Milne 1985	Rinderer (1986)

Table no. 2. The correlation of some traits with honey production, generally the main trait in the breeding programmes.

Trait	**Genetic correlations with honey**	**According to**	**Source**
Honey production in spring	1,02	Soller and Bar Cohen (1967)	Rinderer (1986)
Winter brood	1,12	Soller and Bar Cohen (1967)	Rinderer (1986)
Whole year brood	0,77	El Banby (1967)	Rinderer (1986)
Wintering ability	**0,17/0,15/-07**	Bienefeld K (1991)	Bienefeld K (1991)

Spring development	0,47/0,17/0,04	Bienefeld K (1992)	Bienefeld K (1992)
Colony size	0,32/0,21/0,16	Bienefeld K (1991)	Bienefeld K (1991)
Swarming tendency	**-0,04/0,13/0,11**	Bienefeld K (1991)	Bienefeld K (1991)
Aggressiveness during inspection	**-0,06/0,03/0,11**	Bienefeld K (1991)	Bienefeld K (1991)
Calmness during inspection	0,31	Bienefeld K (1991)	Bienefeld K (1991)
Cubital index	0,00	Bienefeld K (1991)	Bienefeld K (1991)
Corbicular area	0,587	Darcet Costa (2002)	Darcet Costa (2002)

It is necessary to take into account that when the correlation of two traits is positive or nearly 0, the selection for both characteristics will improve them simultaneously, but when the correlation is negative the selection has little chances to improve the specific characteristics simultaneously. (Rinderer, 1986).

II.3. Breeding objectives - Traits to be recorded, included in 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 (z4 and z5). The honey represent the main economic objective in the national 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) Colony development in spring. (z2, z3 and, the viability of brood, wintering ability **z1) and resistance to diseases traits (z6, z7-** hygienic behaviour for killed brood and level of varroa 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 10 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.
(3) 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.
(4) The morphological traits: colour, proboscis length, tarsian 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 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.4. 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 3.

Table no. 3

Crt No	Groups of traits	Characters (traits)	Measurement	Classification 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**
		Tarsian 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*

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. 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 (Szabo, 1988).

When selection for several traits is practiced, three methods are available (Lush 1947):

(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. **Afterwards 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 - honey production and others characters included in selection objectives, preferable genetically correlated with the honey production.

Selection index (I_{sel}). According to Rinderer (1986), 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 (Draganescu, 1979).

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 ($z_{1 \ldots} z_n$), each multiplied by a value, depending of his heritability, genetic correlation and economic value.

Generally, in more than two characters, the formula is more complicated using the multiple regression and covariance (Rinderer 1986). As the result of this complexity the selection index could be obtained by excluding the heritabilities and correlations, (Rinderer, 1986 and van Engelsdorp, 2000) 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 (Rinderer, 1986).

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 in general could be different.

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:

II.	**SELECTION INDEX for honey production**	$I_{sel} = z_{1a}+z_{2b}+z_{3c}+Vz_{4d}+z_{5e}+z_{6f}+z_{7g}$ V= economic value for the main character

Of course, the **correct economic values 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.

CONCLUSIONS

1. The honeybee colonies evaluation and classification is a usual, spontaneous, preoccupation of every beekeeper and generally empirically practiced. The problem becomes more complicated when the beekeeper intends to obtain a genetic improvement of some important characters. In this case the beekeeper should to:
   - Pay attention to the biology of bee production and reproduction, which makes, along with the larger control from the natural selection, the genetic improvement more complicated than those of others farm animals.
   - Elimination of major variability induced by special environment especially by standardising the conditions for evaluation and transformation of the absolute recorded values of traits into relative ones (% or standard deviation from the contemporaries).
2. As possible objectives of selection, 5 main characters groups were accepted in our study: race specific morphologic traits, general behaviour, colony development, productivity and resistance to diseases. Their heritability, genetic correlations and economic importance were underlined.
3. A breeding data base was created (Access software) for data registration, identification and classification, following the assessment of each colony. This software was conceived to work on 100 bee colonies.
4. It is suggested that colonies can be selected by two groups of traits at a time in succession (tandem):
   (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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