

## MODERN METHODOLOGY FOR QUANTIFYING THE BIODIVERSITY IN OVINE SPECIES BASED ON GENETIC MARKERS USING THE CONCEPTS OF INFORMATIONAL STATISTICS

Gheorghe HRINCA

Research and Development Station for Sheep and Goat Breeding, Popauti-Botosani, Romania  
Corresponding author: ghrinca@yahoo.com

Accepted August 28, 2015

The main target of this study is elaboration of a new experimental methodology utilising the informational statistics concepts for a more accurate evaluation of the genetic patrimony in ovine species; the investigational support of this methodological construction is represented by some biostructures with discontinuous variability (biochemical-genetic, immunogenetic and molecular-genetic systems). The analysis of these genetic systems, from point of view of informational statistics, in an integrationist, poly- and interfactorial manner and with the highest accuracy, allows a more precise characterization of hereditary dowry of different animal groupings than the studies based on morphological, osteological and hybridological features. The proposed methodology focuses on two major objectives: 1) on the one hand, the measurement of *genetic similarity and differentiation degree* among the sheep breeds as well as among different ecotypes within the same breeds by means of the *informational correlation* ( $C_{x,y}$ ) by calculating the *informational correlation coefficient* ( $R_{x,y}$ ): this parameter is more efficient than the *genetic distance* ( $D$ ), indicator used in the classical statistics; 2) on the other hand, the *quantification of diversity* or of *polymorphism degree* ( $d$ ) of different genetic structures within each sheep breed by means of the *informational energy* ( $e$ ). This indicator completes an information void, not only of the classical statistics but even of an older concept of informational statistics – the *informational entropy* ( $H$ ). From applicative point of view, the methodology proposes to perfect, on modern bases, a sustainable management of genetic patrimony and of biodiversity conservation in sheep species, in full conformity with the European policy directives in the field of farm animal breeding.

*Keywords:* biodiversity, genetic markers, informational statistics, sheep.

### INTRODUCTION

The vast diversity of biological world, the surprising richness of living forms, which are so different in the respect of their external appearance and genetic constitution, among which the most various interrelations are established and which achieve the most unexpected relations with their environment, as well as the continuous transformation of organic matter are explained by the action of primary forces of evolution – mutation, migration, natural selection and genetic drift – and by the intervention of speciation mechanisms, especially by allopatry. The phenomenon dynamics and complexity of living field occurring at micro- and macrobiological level impose a thorough analysis for whose accuracy

there is required the use of operational tools of maximal precision, with great generalization and synthesize force to protect the conclusions stemming from these analyses of errors and to provide them the fullest reasonableness. These tools are provided by statistics.

The statistics is the science which studies the quantitative and qualitative aspects of mass phenomena which are subjected to action of statistical laws that manifest in variable concrete conditions in time and space. In the current stage, in which the multidisciplinary character of scientific investigation has considerably increased, the statistics remains an indispensable modality to study the random phenomena, irrespective of field that they belong. New scientific disciplines (especially those of border or intersection) have occurred in the last decades on the scientific-technical revolution

background in full expansion. In this context, it seemed that the statistics will loose much of up-to-dateless or moreover will become out-date. But, surprisingly, the statistics succeeds not only to readjust to contemporaneous conditions step by step, but also to renovate its methodological device. The cybernetics, system theory, mathematical modelling and programming, in their different variants, reiterated its importance, obviously in a modern vision.

The statistics develops today as a quasi independent science: it has the feature – common to all sciences – to be, in the same time, science and method utilizable in other sciences too. Strongly controversialist, outbid by some people, minimized by others, the statistics becomes indispensable to knowledge *the mass phenomena* generated by multiple and complex causes.

Given the exponential development of the science and the enhancement of its inter- and multidisciplinary content, determining the statistics place in the present context and defining the specificity of object and their methods acquire a special importance.

Phenomena of the living world have greatly benefited and still use the precepts and tools of the statistics. As a general rule, the statistical processing within the biological sciences and of their applicative branches (agronomy, animal husbandry, human and veterinary medicine etc.) used, as primary data, aleatory variables representing sizes, concrete measurable characters. These represent the action field of the *classical (mathematical) statistics*. The mathematical statistics is a branch of applied mathematics that deals with grouping, analysis and interpretation of the data related to a certain mass phenomenon in order to obtain some previsions concerning its subsequent deployment. The element with which the mathematical statistics works is the statistical population. But the live matter presents spectacular changes and evolutions so that much more flexible methods are required to know the complexity of these phenomena. Thus, a complete and complex evaluation of the essence and dynamics of biological world would be realizable by utilization of some concepts derived from the *informational statistics* which does not use measurable sizes, but qualities, frequencies, variables with a high abstracting degree<sup>14</sup>. The creator of the information theory in the mathematics field is Shannon<sup>40</sup> who used in 1948 the *entropy* as average estimator of information, being elaborated in the statistical

mechanic field. With aid of this parameter he succeeded the measurement of information transmitted and elaborated the mathematical method of perturbation decrease which influences the information transmission on the basis of the theory and calculus of probabilities. Well-known properties today gave the possibility to Shannon to construct the *information theory* concerning all its essential elements by means of entropy.

Ten years later Kolmagorov (in 1958)<sup>20</sup> stood out the central place occupied by the information concept in cybernetics. By identifying the communication relations among the system sections (either biological, either mechanic or social) the functioning and stability of the system against the outside (perturbing) influences are ensured.

The Shannon entropy has drawn attention to statisticians which taken over it in the study of the most various structures, becoming one of the most used indicators in these researches. The statistician Kullbach<sup>22</sup> used in 1959 the entropy as a general canon around which he succeeded to construct a statistical edifice.

In 1966, Onicescu<sup>32</sup> replaced the informational entropy with another estimator – *informational energy* – conferring a greater concision to information. By introduction of this new estimator (parameter) he created a new branch of statistics – *informational statistics* – in whose centre two concepts are situated: the *informational correlation* that defines the relation intensity among different systems and the *diversity* that quantifies the variability degree (polymorphism) of the respective systems.

If Onicescu founded the informational statistics, Stefanescu (1979)<sup>42</sup> studied and widened the correlation and information concepts, giving them a certain extension for systematic construction of informational statistics to be able to approach the problems in some activity fields. Thus, he applied the concepts of this theory to areas of social life, as education, pedagogy, art, linguistics, economy, culture, population structure depending on certain criteria etc.

At international level, in biology and its applicative disciplines there are partial attempts of informational statistics utilization (but only in the respect of the genetic distances, which represent a primary form of informational correlation) in ovine species, starting with the 8<sup>th</sup> decade of the XX<sup>th</sup> century, effected especially in some countries of Europe: Spain<sup>33, 36, 37, 45</sup>, France<sup>29, 30</sup>, Poland<sup>21</sup>, Italy<sup>25, 49</sup>,

Romania<sup>47</sup> etc. In the other continents, studies on genetic distances between sheep breeds are fewer being reported in China<sup>13</sup>, Japan<sup>43</sup>, Australia<sup>24</sup>, South Africa<sup>38</sup>, Brazil<sup>34</sup>, Canada<sup>35</sup> and the USA<sup>48</sup>. Until now, the studies on the genetic diversity quantified by informational energy have not been reported.

Identification of biological material represents an indispensable phase in the estimation of existent genetic potential, as well as in the selection and improvement activities of animals. The identification and characterization methodology of animals was based and is still based on phenotypical quantitative characters, but these criteria present a high subjectivity degree. Our demarche aims to implement a more accurate method of animal identification, an earlier prediction of their evolution trends and a better characterization of sheep breeds in econogenic context.

## MATERIALS AND METHODS

### BIOLOGICAL MATERIAL

The necessary biological material to achieve the methodology can consist of more randomized populations belonging to more sheep breeds

The biological product examined for the identification of genetic markers is the blood collected by jugular vein puncture of animals, on heparin (to determine potassium and haemoglobin phenotypes), on sodium citrate (to determine the blood group factors), on EDTANa<sub>2</sub> (to determine the molecular proteins) and without anticoagulant (to determine the transferrin, albumin amylase phenotypes and other serum proteins).

### RESEARCH METHODS

#### Research methods of biochemical-genetic markers

The analysis of biochemical-genetic systems in sheep breeds takes into account the main proteins and minerals with polymorphic features, revealed electrophoretically and photometrically:

*The analysis of polymorph protein systems* (haemoglobin, transferrin, albumin, amylase etc.) is carried out by horizontal electrophoresis method, having as substratum the starch gel and using as electrolyte buffer solutions of Tris(hydroxymethyl) aminomethane, EDTA.Na<sub>2</sub> and boric acid, citric acid, lithium hydroxide or sodium hydroxide,

depending on the protein analysed. The identification of protein phenotypes is made depending on the migration speed of these protein fractions<sup>16, 17</sup>.

#### *The analysis of polymorph mineral systems.*

Relatively recent research revealed that in some breeds or species the variability of concentrations of some mineral elements (potassium, sodium, copper, iron) from the circulating blood has a discontinuous distribution, which is genetically determined. Thus, those elements behave like true genetic and biochemical systems, as proteins that present genetic polymorphism<sup>23, 46</sup>. Mineral polymorphism was revealed at best in sheep and had in view in particular the blood potassium system. The potassium polymorphism can be detected by flame photometric method. The K<sup>+</sup> cationic concentrations in whole blood of animals are expressed in mEq/l. Potassium phenotypes deceleration will be made depending on the discontinuity range of the potassium ion distribution<sup>16</sup>.

### RESEARCH METHODS OF IMMUNO-GENETIC MARKERS

The detection of blood group factors is performed by haemolytic test method, which is an immunoserological reaction of antigen-antibody type. Thus, the reactant elements are represented by: 1) sheep red cells, acting as antigens, will be used to prepare the erythrocyte suspensions according to the standard method; 2) the monospecific reagent sera obtained by isoimmunisation and heteroimmunisation methods will be used as antibodies; 3) in order to develop the antigen-antibody reaction under normal conditions, the complement of rabbit absorbed on sheep red cells will be added to the first two reaction components. At the same time, two serological controls (physiological serum control and complement control) will be made to avoid the pseudo-reactions. The identification of blood group factor phenotypes is done according to the lysis haematic reaction degree of immunoserological reactions<sup>18</sup>.

### RESEARCH METHODS OF MOLECULAR-GENETIC MARKERS

A DNA test consists in DNA extraction, PCR amplification, capillary electrophoresis and detection by fluorescence of the amplified fragments. The DNA extraction is applied to blood

samples whose genetic material will be then used like matrix for PCR amplification.

For each DNA marker the originate alleles from parents are detected and then genotyped by comparison with sets of standardized international markers. The PCR reaction is achieved with primers intended to amplify uncorrelated marker sets (microsatellites), with a high polymorphism and with low mutational levels. The PCR reactions are multiplex and suppose amplification achievement in one or two stages. One of primers of each microsatellite is fluorescently marked with one of the three available fluorochromes (FAM, JOE and NED) to ease the multiplex analysis of markers in one, two or more reactions. The reaction products are assembled with a molecular mass standard marked in its turn with the ROX fluorochrom that assures the accuracy and precision of determinations<sup>19</sup>.

In sheep, the number of markers presenting interest for biodiversity study is considerable: BM1314; BM6506; BM6526; BM757; BM8125; BM827; CSSM31; CSSM47; HUI616; ILSTS002; OarAE129; OarCP20; OarCP34; OarCP38; OarFCB128; OarFCB20; OarFCB48; OarHH35; OarHH41; OarHH47; OarHH64; OarJMP29; OarJMP8; OarVH72; OMHC1; RM4; TGLA137 etc.<sup>31</sup>

#### BIOSTATISTICAL METHODS FOR THE INTERPRETATION OF EXPERIMENTAL RESULTS BY INFORMATIONAL STATISTICS PARAMETERS

##### *Estimation of the heterozygosity degree*

The primary data to calculate the informational-statistical parameters are represented by the allelic frequencies of biochemical-genetic, immunogenetic and molecular-genetic systems, frequencies calculated in accordance with the co-dominance or dominance phenomena depending on the hereditary way of each system.

The first step undertaken in carrying out of this study must be estimating *the heterozygosity degree* of sheep populations of different breeds, as a first indicator of diversity, which is calculated by the following expression:

$$Ht = \left[ \frac{2n}{2n-1} \right] \left[ 1 - \sum_{i=1}^k x_i^2 \right], \text{ where:}$$

Ht = the heterozygosity of population  $k$ ;  
 n = the number of individuals;  
 x = the allelic frequency at the locus  $i$ .

The comparison of the empirical distributions with the theoretical ones will be achieved by means of the *Hi square test* ( $\chi^2$ ); the value of  $\chi^2$  test and its signification intensity will reflect the status of genetic equilibrium Hardy-Weinberg of the analysed populations.

$$\chi^2 = \sum_{i=1}^m \frac{(f_i - \varphi_i)^2}{\varphi_i}, \text{ where:}$$

$f_i$  – represents the observed (practical, empirical) frequency;  
 $\varphi_i$  – represents the expected (theoretical, estimated) frequency;

##### *Measurement of the genetic interrelationship intensity*

Until recently, to estimate the relations among different biological entities there was used the *genetic distance* ( $D$ ), which is calculated by the Nei method<sup>26,27</sup>:

$$D = -\log_e \frac{Jx \cdot y}{(Jx \cdot Jy)^{1/2}} = 2\alpha t, \text{ where:}$$

–  $D$  = standard genetic distance;  
 –  $t$  = differentiation time;  
 –  $\alpha$  – substitution percentage of genes per locus and per year, whose value is  $10^{-7}$ .  
 –  $Jxy$  = probability of genetic identity between two populations  $x$  and  $y$ ;

$$Jxy = \frac{1}{n} \sum_{j=1}^n \sum_{i=1}^k p_{xij} p_{yij}, \text{ with}$$

$l$  to  $k$  alleles within of one number from  $l$  to  $n$  loci, where  $p$  is the gene frequency.

–  $Jx$  – probability of genetic identity within the population  $x$ :

$$Jx = \frac{1}{n} \sum_{j=1}^n \sum_{i=1}^k (p_{xij})^2$$

–  $Jy$  – probability of genetic identity within the population  $y$ :

$$Jy = \frac{1}{n} \sum_{j=1}^n \sum_{i=1}^k (p_{yij})^2.$$

But a more precise commensuration of the genetic similarity or differentiation degree among different taxons is feasible using a concept derived from the informational statistics: the *informational correlation* ( $C_{x,y}$ )<sup>32</sup>. The correlation  $C_{x,y}$  between two populations is defined by the expression:

$$C_{x,y} = \sum_{i=1}^n f_{xi} f_{yi}$$

where,  $f_{xi}$  and  $f_{yi}$  are the frequencies for the first, respectively, for the second population.

Through the standardization of this correlation there is obtained the **informational correlation coefficient** ( $R_{x,y}$ ) calculated by the following formula:

$$R_{x,y} = \frac{\sum_{i=1}^n f_{xi} f_{yi}}{\sqrt{(\sum_{i=1}^n f_{xi}^2)(\sum_{i=1}^n f_{yi}^2)}}$$

The value of the informational correlation coefficient varies between 0 and 1 ( $0 \leq R_{x,y} \leq 1$ ). It results that the informational correlation coefficient  $R_{x,y}$  can reach the value 1 only if the frequency  $f_{xi}$  and its correspondent  $f_{yi}$  have the value 1, and  $R_{x,y}$  takes the value 0 only if the two populations have no common characteristic, so they are foreign to one another.

**Quantification of the genetic polymorphism degree**

To quantify the polymorphism degree of these systems there were used two parameters of informational statistics: informational energy ( $e$ ) and genetic diversity ( $d$ ).

Well-known properties today gave to Shannon<sup>40</sup> the possibility to construct the information theory with all its essential elements by means of the entropy ( $H$ ).

$$H = -K \sum_{i=1}^m p_i \ln(p_i); \text{ where } K=1/m.$$

For a very long time the indicator that was used to estimate the diversity of a system was the **informational entropy**. Consequently, the diversity coefficient is given by:

$$D = 1 - H.$$

The advantage of this method is that the diversity coefficient takes values between 0 and 1 and it does not require a correction to compare two structures with a different number of statuses (classes). The disadvantage is that if the frequency value of a status is 0, the parameter  $H$  can not be calculated because the logarithm function is not definite in point 0. Onicescu<sup>32</sup> noticed that, for the

purely statistical purposes, the probability itself can be kept as information (which is multiplicative for independent statuses) and not its logarithm which he named informational energy, which also is multiplicative for independent systems. Therefore, it was agreed that the most adequate indicator to quantify the diversity of the polymorph structures of some populational groupings is the **informational energy** calculated by the formula:

$$e = \sum_{i=1}^m p_i^2$$

$p$  = the probability (which ranges from 0 to 1);  
 $m$  = number of classes (statuses).

This indicator takes the maximum value ( $1$ ), when in the system a status has maximum frequency, the other statuses having null frequencies; on the other hand, it takes minimum value ( $1/m$ ), when all statuses have equal frequencies. So, the diversity coefficient can be defined as follows:

$$D = 1 - e.$$

The advantage of this method is that it allows the diversity measurement. The disadvantage of this method is that the diversity coefficient takes values between 1 and  $1/m$  (which may be close to 0, but never 0). To remedy this drawback an indicator called **informational energy corrected** ( $e_c$ ) was introduced in the mathematical literature. This parameter takes values between 0 and 1 allowing the reduction of the system to a simple status (class) and thus it can be calculated by the following mathematical monotony:

$$e_c = \frac{(\sum_{i=1}^m p_i^2 - \frac{1}{m})}{(1 - \frac{1}{m})}$$

Thus the diversity coefficient becomes:  $d_c = 1 - e_c$

By extension, the informational energy parameter is closely linked to that of informational correlation. In fact, from the quantifiable aspect, the informational energy is the correlation of a population with itself:

$$e = \sum_{i=1}^n p_i^2 = C_{x,y}$$

For a comprehensive perception of the polymorphism of all genetic systems there will be

calculated the *complex informational energy* ( $c_i$ ) and the *complex genetic diversity* ( $d_i$ ).

## RESULTS

The methodology elaborated will reflect the features of the informational correlation and genetic diversity in a systematic, comprehensive and intuitive manner under two aspects:

- analytical representation – as a relationship between the informational-statistical parameter and the allele incidence of polymorph systems;
- graphical representation – as a multi-D representation in a defined space: statistical-informational parameter – allelic frequency.

### a) Demonstrative model for informational correlation

Determination of the informational correlations ( $C_{x,v}$ ) and genetic distances ( $D$ ) among various breeds, populations, varieties, zootechnical lines or other ecotypes reflects the degree of genetic similarity or genetic differentiation among these taxonomic entities during their creation, development and strengthening. The value of these

genetic parameters, expressed through a series of synthetic indexes, is in accordance with the distributional gene curves of the polymorphic systems analyzed, as well as with the number of such systems.

If the genetic distance indexes record very low values, then there are very low degrees of genetic differentiation among the taxonomic entities examined (Table 1). At the same time, high values of informational correlation coefficients ( $R_{x,y}$ ) also more eloquently suggests that among the structures that make up the hereditary heritage of these entities there are genetic similarities of a high level (Table 1, Fig. 1). If the genetic distances among taxons are large, then among these there is a considerable genetic differentiation (Table 1). Similarly, the low values of informational correlation coefficients show that the genetic similarity among taxons is low (Table 1, Fig. 1). But it should be noted that, in terms of value, the informational correlation is much more suggestive to demonstrate the genetic similarity or differentiation among different taxonomic entities than the genetic distance.

Table 1

Genetic distances ( $D$ ) and informational correlation coefficients ( $R_{x,y}$ ) among different sheep breeds

Alleles (%)		Sheep breeds					
Allele A	Allele B		Breed A	Breed B	Breed C	Breed D	Breed E
100.00	0.00	Breed A		0.00024106	0.0000384	0.3873303	2.4637778
93.50	6.50	Breed B	0.9975		0.0000868	0.3172422	1.8707536
97.30	2.70	Breed C	0.9996	0.9991		0.3581432	2.1828666
48.04	51.96	Breed D	0.6788	0.7281	0.6989		0.2365108
7.87	92.13	Breed E	0.0085	0.1540	0.1127	0.7894	
$R_{x,y}$							

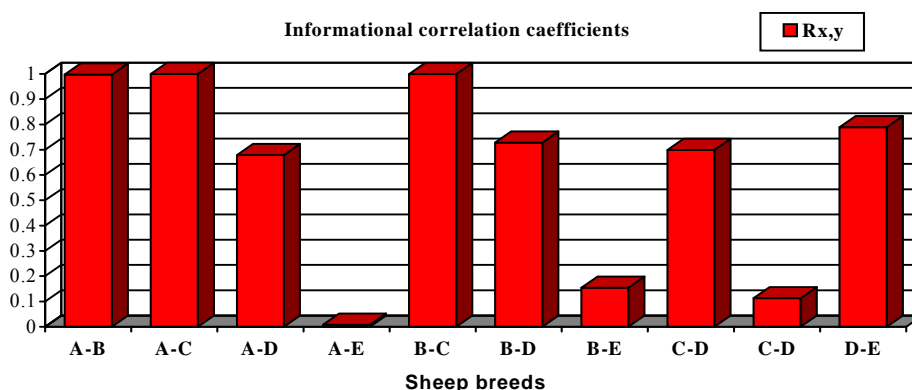


Figure 1. Informational correlation coefficients ( $R_{x,y}$ ) among different sheep breeds.

**b) Demonstrative model for genetic diversity**

The primary data to quantify the informational energy (*e*) and genetic diversity coefficient (*d*) in the sheep breeds are represented by the allelic frequencies of genetic systems studied.

The relationship among allele frequencies that make up the architecture of each system within each breed is decisive in defining the coefficient size of informational correlation of and genetic diversity. The genetic diversity indicates the variability degree of a system in a quantifiable manner, in this case representing a frequency distribution of all alleles of a genetic system. The energy information (*e*) and genetic diversity (*d*) are complementary operational concepts, their values being inversely proportional. Both indicators can take values from 0 to 1, depending on the allele incidences within the system. The more one of allele records higher frequencies over other alleles, the more increased the informational energy can be, but the genetic diversity decreases with the same measure unit and vice versa. When in the system only one allele is fixed the informational energy is absolute (1) and the genetic diversity is null (0); on the contrary, when in the system all alleles have equal representation the informational energy is zero and the genetic diversity is maximum (1). The data below show a greater accuracy for the genetic polymorphism quantification when using the informational energy in comparison with informational entropy (Table 2, Fig. 2).

Table 2

Values of genetic diversity (*d*) calculated by informational energy (*e*) and informational entropy (*H*) in different sheep breeds

Breed	Alleles (%)		Informational-statistical parameters			
	Allele A	Allele B	<i>e</i>	<i>d<sub>e</sub></i>	<i>H</i>	<i>d<sub>H</sub></i>
Breed A	79	21	0.34	0.66	0.26	0.74
Breed B	25	75	0.25	0.75	0.28	0.72
Breed C	0	100	1.00	0.00	0.98	0.02
Breed D	44	56	0.01	0.99	0.34	0.66
Breed E	12	88	0.58	0.42	0.18	0.82

For a more rigorous understanding and a more eloquent presentation of the biodiversity, both the genetic distances or the informational correlation and the genetic diversity can have a projection in bidimensional plane as:

- hierarchical structure by cluster analysis;
- topological structure by multidimensional scaling;
- extracted factor space by discriminatory analysis.

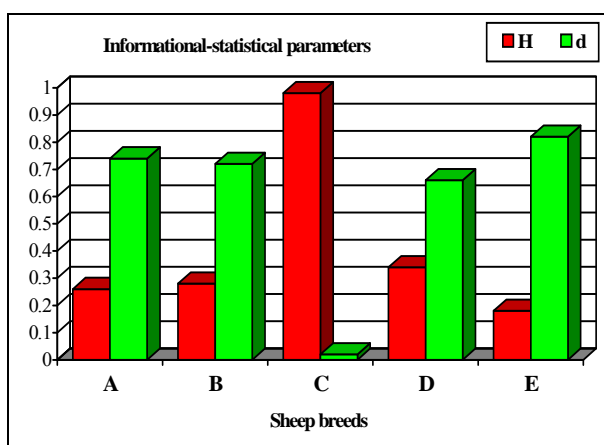
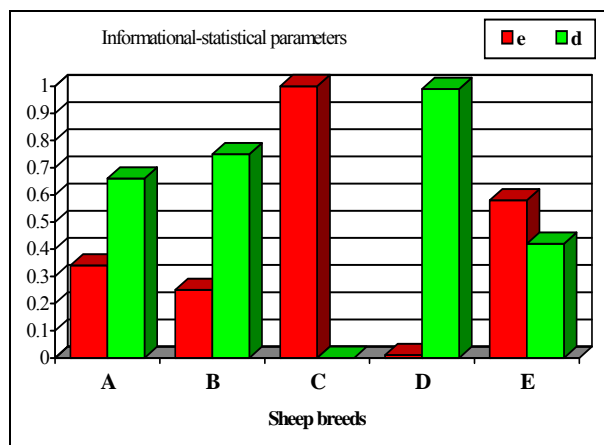


Figure 2. Values of genetic diversity (*d*) calculated by informational energy (*e*) and informational entropy (*H*) in different sheep breeds.

The *Cluster Analysis* (CA) is an algorithm intended for structuring the experimental data. The algorithm supposes “relaxation” of our criteria in such way than the classes do not contain a single element. In other words, we always descend the standard by which two elements are declared as belonging to the same class. It follows that we joint permanently more elements, increasing the non-similarity character. Finally, all objects are together joined in the same structure. When the data make up a clear structure and all object are included in a whole, then such structure reflects a *hierarchical tree with distinct branches* (Fig. 3). The method result is a diagram reproducing the biological system structure (system elements + relations among them). By the Cluster Analysis it can come

out the way in which the elements of biological system are differentiated among them or grouped with each other, the similarity degree etc.

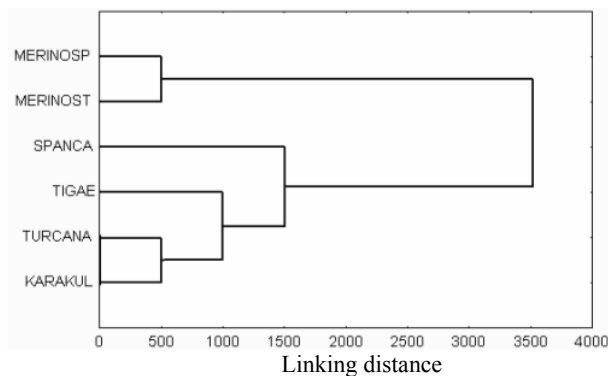


Figure 3. Hierarchical structure by cluster analysis.

The *Multidimensional Scaling* (MDS) is used to visually evaluate the distances among the component elements of a biological system. It reproduces (in a certain moment, in a space with a certain dimension number) the distances from the initial multidimensional space. The MDS is nothing but than an exact “rearrangement” procedure of objects in an efficient manner, so that the final configuration to be a better approximation of the observed distances. By the Multidimensional Scaling the objects are moved in a space with a requested number of dimensions so that the distances among objects to be reproduced in the new configuration. Technically, the method requires a minimization algorithm to evaluate different configurations with the aim to optimize the “arrangement”. This algorithm produces the *topological structure* of the system (Fig. 4). The distances among the biological system objects are identically maintained, irrespective of change in the number of dimensions. The user has the possibility to view the topological structure of the biological system.

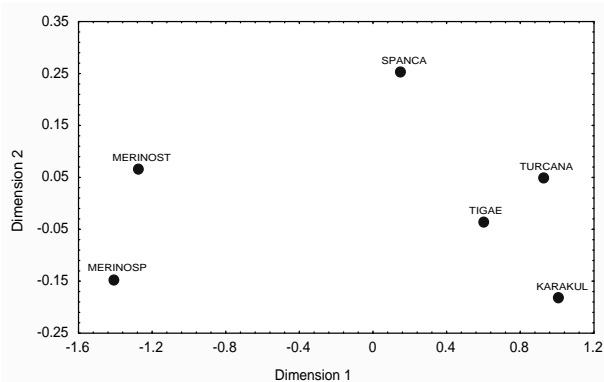


Figure 4. Topological structure by multidimensional scaling.

The *Discriminatory Analysis* (DA) is different from the other taxonomic algorithms because it does not deliver the hierarchic or topological structures of the analysed system but typifies the objects in classes (generally, in taxons). As a result, the algorithm produces discriminatory functions, the probable membership of an object to a certain object class (taxon) and a graphic representation of object in the *space of factors* (Fig. 5).

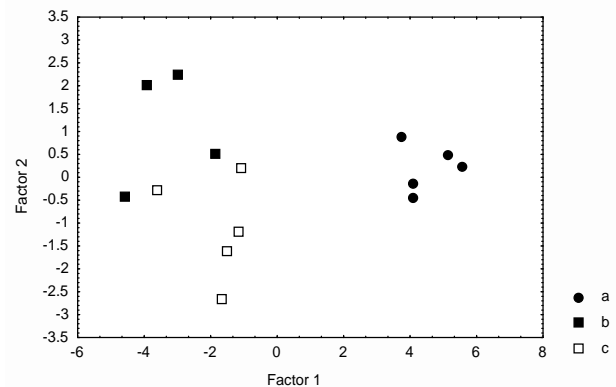


Figure 5. Extracted factor space by discriminatory analysis.

The factors are new variables obtained by the extraction of common variance. The factor extraction is performed by rotating the axes to maximize the variance. The diagram resulted represents the individual grouping (generally of the objects) in taxons. The algorithm utility is obvious: it can be used to determine the class (taxon) to which an object (individual) belongs, the discriminatory degree among taxons caused by different biological properties, characters etc., the taxon specificity in the space of analysed properties.

The presented parameters allow to user to analyse the biological systems which interest him either comparatively among them or in comparison with a set of known structural entities (species, breeds, populations, genotypes, biological products etc.) that can serve as bench-mark. The user can evaluate the distances among the biological entities mentioned in the space of analysed parameters, the differences and similarities among different classes (taxons), their typology. In other words, the structure of biological system analysed is rendered. In reverse order, starting from the classes composed of biological analysed entities, the “specificity” of each class can be determined in the analysed parameter space. On this basis, it can be determined subsequently for other biological entities the class to which they belong.



The algorithms have a very high abstracting degree and they can be used for any biological entity (species, breed, population, variety, ecotype, genotype, sex etc.). They are based on systemic methods that focus on the structure on analysed system, that on its essence. There are many other possible uses of the methods than those described here. The applications of these algorithms in production practice can be very diverse from the application in the general experimental technique to the typifying of species, breeds, populations, classification of drugs or different biological products, specification of diagnostic methods of pathological entities etc.<sup>15</sup>

## DISCUSSIONS

### CONNECTING THE METHODOLOGY TO THE IMPERATIVES OF GENETIC RESOURCE CONSERVATION

The modern breeding of animals is characterized by two defining features: on the one hand, the decrease of breed number, and on the other hand, a strong extension of reproduction biotechnologies (artificial insemination, embryo transfer, cloning etc.). The both trends, beside the positive aspects, lead to narrowing of the gene pool. Eliminating the rustic indigene breeds, which are less pretentious and yet possess valuable hereditary features concerning the adaptation to environmental conditions and to a more natural foraging, the genetic diversity of species that include the respective breeds is decreased. For example, in Romania only 3 cow breeds, 3–4 sheep breed, 4–5 pig breeds, 6–7 hen breeds are zoned, many of these being created in other countries and successively refreshed with reproduction material from imports. It came out that the repeatedly import of live material can generate some maladaptation or degeneration phenomena if the optimal breeding and exploitation conditions are not assured. This fact obliges to the preservation of autochthonous material in disappearance danger and its rational use, by infusion of genetic material utilising scientific actions that aim the genetic adaptation increase of improved imported breeds, which impoverished concerning the gene pool for resistance and adaptation in the process of their setting up and of improvement of production traits. Without doubt, the most rational and sustainable way to conserve the animal genetic resources is to

ensure that indigenous breeds remain functional parts of production systems, *i.e.* the *conservation through use*. This is possible only if economically important attributes of indigenous breeds are identified, studied and incorporated in breed improvement programmes. Identification of unique attributes should also increase interest for these genetic resources. On the other hand, in a certain period, by the agricultural transition from the small private farms to the big commercial (industrial) systems, the genetic diversity of farm animals decreased. The selection purposes and the production environments are very similar in the whole developed world. Thus, the modern reproduction biotechnologies (collection, refrigeration and freezing of sperm and embryos, artificial insemination, embryo transfer, fast and efficient distribution of germoplasma) or the selection systems (the selection criterion being the maximal production level in the global economy or designing and uncoiling the animal breeding programs depending on the profitability interests of national and international companies) determined a number decrease of reproducer sources, which each of them in the reproduction process comes with a limited and particular hereditary dowry leading to a restriction of character variability range in animal populations. The result? Nowadays, the intensive livestock is applied to only a few breeds of some species. From a lot of animal breeds, since are used in farming purposes, many of them have considerably decreased in number and others have completely disappeared<sup>5</sup>.

Exploitation, collection and scientific preservation of genetic resources are an urgent action of the utmost importance. To achieve this objective all researchers and people from all over the world must participate as rapidly as possible. In this action it must be implied, firstly, the big livestock enterprises (holdings), the national associations whose their object activity is the genetic resource conservation of animals, the governments (by legislative acts), the international organisms (FAO), but also the private sheep breeders. Thus, there was created an *international attitudinal major scientific current of management of genetic resources and biodiversity in animals*<sup>6, 9</sup>. So a new discipline appeared, *Econogeny*, which remarkably combines the molecular biodiversity analyses with socio-economic and geostatistical elements having in view the genetic resource conservation of sheep with the purpose of rural development in the European agroecosystems by

well-defined strategies. The study analyses the genetic diversity of sheep at molecular level, examines many local unstudied species and identifies the gene pools to map the conservation priorities. More maps of conservation and development priorities were achieved and the value of this biodiversity is estimated to justify the economic intervention and taking measures in this regard<sup>1,2</sup>.

For this purpose, the methodology that we propose meets these desiderata by providing a very efficacious tool on the assessment of genetic resources and biodiversity in animal populations using genetic markers by informational-statistical quantification and, alike, to prevent some genetic disturbances caused by the environmental conditions and technological and selection factors.

#### INNOVATIVE CONTRIBUTION OF THE METHODOLOGY

The population (biological population, local population, natural population, mendelian population, genetic population) is the base unit on which the primary forces of evolution (mutation, migration, natural selection and genetic drift) work. The population belongs to a group of population (a population system). A *population system* represents a reproduction unit, with a well outlined gene constellation, occupies a vast ecological zone and has a long existence period, profiling that biological entity named breed.

*The breed* can be defined as a subdivision of species grouping individuals that have in common a certain number of hereditary characters which distinguish them from the individuals of other groups. Therefore, the differences that characterize the breeds are essentially genetic. Because the gene number of each individual is well determined, the breed comparison must be based on the data that reflect the overall difference at the level of loci ensemble that constitute the genome.

Until recently, the characterial variability of different taxonomic entities, as well as their comparison were achieved exclusively on the basis of some external morphology, osteological and hybridological characters (the body aspect, the colour of skin, hair or wool, the presence or absence of horns and their configuration, the shape of face or ears, the aspect of head etc.); but, these phenotypic characters provide vague and belated conclusions concerning the hereditary patrimony of species, breeds, populations etc. because their

inheritance modality is generally complex and still not elucidated and because these characters can be influenced by environment factors. Instead, the biochemical-genetic (electrophoretic variants of some polymorph proteins and those flamephotometric of some mineral elements), immunogenetic (blood group factor antigens) and molecular-genetic (microsatellites) characters, whose expressions are not influenced by external (less in normal conditions) or internal (age, sex) factors, have a simple enough mode of inheritance and in according to the Mendelian genetics laws. Moreover, these structures are genetic systems that behave independently of each other.

The analysis of these genetic systems allows a characterization in a more precise manner of the hereditary dowry of different animal groups than the studies based on morphological characters. Otherwise, it is well-known that different animal populations have different frequencies of various alleles of these biochemical, immunological and molecular characters. This variation of allelic frequencies of different loci that control the blood polymorphism constitutes an efficacious means to calculate some synthetic indices which to reflect the genetic patrimony essence of taxonomic entities, as well as the global similarity and differentiation among populations or breeds. Until now, these phenomena were studied by means of the *classic (mathematic) statistics* which is based on the grouped comparison of allelic frequencies of the biochemical-genetic, immunogenetic and molecular-genetic systems, the analysis of allele number, their incidence within the systems, the variance analysis, the signification testing etc. These methods have only an estimative character. Therefore, the present study will try a new approach of these population genetic phenomena by means of the informational statistics methods and will include two sections: informational correlation and genetic diversity (genetic polymorphism).

The measurement of the relations among different sheep breeds is usually made by the determination of *genetic distances*. Also, to estimate the necessary time for strengthening these taxonomic entities the *genetic differentiation times* are measured. But, an accurate assessment of similarity and differentiation degree is conferred by other informational-statistical parameter – *the informational correlation*. The essential property of the *informational correlation coefficient* is that when it is equal to 1, the two allele repartitions (belonging to the two breeds) are identical.

A quantification of a higher accuracy of polymorphism degree of some genetic structures is achievable using the *informational energy* by means of which the *genetic diversity* is measured. The informational energy has the physical signification of the information quantity included by a certain structure. The informational energy indicator comes to complete an informative lacuna not only of the classical statistics, but even of an older concept of informational statistics – *informational entropy* – which was situated for a very long time to the foundation of information theory. In a certain extent, the informational energy is more sensible to the changes within the system than the entropy.

These new concepts represent a way to obtain as complex and complete as possible information within the one or more systems and give the possibility to extend the mathematic statistics in the qualitative systems in which the primary data are not represented by concrete measurable sizes but by qualities represented by frequencies and probabilities. With their aid the mathematic properties of energy and correlation are investigated both for discontinuous systems and for the continuous ones. Also, the theoretical problems of estimations and information are analysed from a new viewpoint and the correlation informational is applied to the study of *Markovian chains and processes*. In this framework, the informational energy appears as the correlation of one system with itself.

The informational statistics parameters give the real dimension of the *systemic polymorphism degree*. The allele number, but especially their frequencies within the genetic systems are determinant to define the size of informational energy and genetic diversity. The values of these operational concepts are complementary. Increasing the informational energy causes the diversity restriction; conversely, decreasing the informational energy has a consequence the diversity amplification.

The genetic diversity can be considered as an *indicator of population age (ancientness)*, because the structure polymorphism has the natural trend to increase in time (e.g. the mutations as a result of natural and artificial phenomenon action), but the affirmation must have a certain wariness.

The values of the two informational parameters point out not only the polymorphism degree but also the *hierarchy succession of the genetic systems*, this feature being a function of direct linearity with their polymorph character.

On the other hand, the dimension of the two informational statistics parameters shows the *systemic organization degree*, size that is directly proportional with the polymorphism degree (so with genetic diversity) of the incriminated systems and in reversely proportional with the informational energy. The polymorphism limitation has as result the organization degree increase of system; on the contrary, the polymorphism emphasis leads to the systemic entropy increase, so to the disorganization increase in system.

Besides the polymorphism degree of one genetic structure at any given time, the informational statistics can also *indicate the polymorphism evolution in different historical stages of a taxonomic entity*. There are two opinions concerning the evolution of the diversity of the polymorph structures. According to the first opinion, the structure polymorphism has the natural tendency to increase by fixation of some new alleles and by an equilibration of their spreading. By the second opinion, some structures have the tendency to restrict their polymorphism by artificial selection which can determine the elimination of some alleles and a considerable increase in frequencies of the others.

Up to now, this scientific investigation kind had in view only the species and breed. But, the change of the structural elements of the genetic systems under the natural and artificial selection pressure, as well as the apparition of different change types within the certain populations determine differentiations and discontinuities among these taxonomic entities; in this way, each population represents an independent evolutionary line that possesses their specific proper potentialities for further modifications. Following the biological and evolutionist significance of the biochemical and molecular system polymorphism, a hypothesis was emitted according to which *this polymorphism would confer the selection advantage to animal body in comparison with other biostructures that do not present discontinuous character variability*. In the speciation process, the protein and mineral polymorphism produces a species discontinuity as a result of the genetic structure change of populations. The change of the genetic structure of population at the locus of a certain protein or mineral system under the selection and mutation action determines either the total or partial elimination of some alleles, or their distributional resizing, or introduction of new alleles in genofond that were inexistent in population till then. In all situations, there is a

percentage repartition change of different alleles, because a new distribution takes place of whole genofond per the existent individuals in population. This makes also the phenotype for a certain character of individuals which compose the population to appear in a plurality of clearly differentiated configurations among them.

The evaluation of informational correlation, informational energy and genetic diversity on the basis of allelic structures of genetic systems in an integrationist, poly- and interfactorial manner and with the highest accuracy is appropriate to define the intensity and specificity of relations among the sheep breeds, as well as the genetic variability of the ovine species. The genetic variability is the result of the mutations, of the genetic material flux among populations and of the genetic recombination factors, all acting in connection with selection pressures that finalise the character of variations. In genetics, the character variability is the action field of selection and represents an essential condition in order to achieve genetic progress in the sheep population.

The estimation of genetic relations among the sheep breeds by means of the informational correlation and of the genetic distances *could elucidate the problem of their phylogenetic origin, the chronology of breed creating, their age, the genetic strengthening degree*. At the same time, the contribution of informational correlation is necessary to determine the similarity and differentiation degree among different sheep breeds, either autochthonous or imported, as well as the signification of interracial relations, these being the basic information in the crossing process to create new breeds, ecotypes and morpho-production types.

The polymorphism detection of some genetic structures in a quantifiable way by means of informational statistics concepts is necessary for the *assessment of the genetic resources* in order to allow by selection an increase of the productive performances, of the resistance to diseases and of the heterosis. At the same time, this inventory of genetic resources is useful for the substantiation of some programmes for the genetic potential conservation of the breeds presenting economic interest or for the improvement of this potential when it tends to decrease as a result of the homogenization of the animal populations. On the other hand, such genetic patrimony can be a base for the setting up of some new zootechnical lines, varieties, subpopulations or even ecotypes. Therefore, the indicators of informational statistics

point out not only the *intensity of the selection pressure*, but especially the *specificity of the selection process* in the animal improvement.

At the same time, analysing the temporary evolution of different genetic structures, we can evaluate the *answer to selection*, the mode in which the selection influences diversity, the possibility to accelerate the selection, the answer limitation at selection etc. But the informational quantification of genetic similarity and of genetic polymorphism, interpreted in evolutionary dynamics, will be able to clarify just how influential are the physiological peculiarities of animal body in relation with its ambient environment on the genetic patrimony, on the one hand, and the technological selection processes exerted to improve the breeds, on the other hand.

Given that the informational energy dimension is an organizing degree measure of biological systems, the evaluation of the informational correlation and genetic diversity is important for *knowing the genetic equilibrium of populations* within different breeds and for *forecasting of the population dynamics over generations*. Thus, the informational statistics is a very useful tool to maintain a high systemic organization degree of sheep populations and, at the same time, an alarm signal when the genetic unbalances caused by environmental conditions or selective and technological factors are foreseen; therefore, the prophylactic measures need to be promoted to diminish the systemic entropy and to install the genetic and physiological homeostasis by specific self-regulation mechanisms.

The achievement of such study would be useful in a multitude of situations such as:

- aid for thinking, communication, to founding and taking the decisions;
- prognosis tool;
- management, instruction and analysis means.

#### THE NEED FOR BIODIVERSITY CONSERVATION

Biodiversity is a broad term that refers to the variety of life and its processes. It includes all life forms, from single cell to complex organisms and processes, pathways and cycles that link living organisms into populations, ecosystems and landscapes.

The agricultural biodiversity includes all components of biological diversity of relevance for food and agriculture and all components of

biological diversity that constitutes the **agro-ecosystem**: the variety of animals, plants and micro-organisms at the level of genetic apparatus, species and ecosystem which are necessary to sustain the key functions of the agro-ecosystem, its structure and processes<sup>41</sup>.

Biodiversity of farm animals is a particular type of biodiversity created by man through artificial means of reproductive isolation, from which the breeds and strains (lines) of domestic animals resulted as populations.

Basically, it is well known that the industrial activities, especially the synthesis ones, supermechanized and chemicalized agriculture, inclusion in the life circuit of new technologies and numerous non-biodegradable substances have the most damaging secondary effects. Gradually, but with an accelerated intensity, this whole assembly of human activities (economic, social, technological, military) devastate the equilibrium of the ecosystems, necessary to survival of plant and animals, and pollute the human life environment. So, the ambient environment, bioresources and the men are severely threatened. All these uncontrolled activities impoverish the genetic resources, progressively eroding the gene dowry of the Terra, whereon is being a real "biological massacre". Degradation and destruction of the ecologic balance, of a life environment, caused by irrational human actions over the years scattered the number and structure of species. The exploration and research of flora and fauna of the Terra enabled the inventory of about 1.5 millions of species. However, it has been estimated that on Earth the number of animal and plant species could exceed three millions. But, throughout entire evolution of living matter numerous species disappeared, in fact they passed from reality in history, and the scientists consider that the number of plant and animal species that can disappear represents a really apocalyptical figure<sup>39</sup>.

The domestication is one of the major successes of humanity. The domestic animals provided to human species essential food, clothes and force traction permitting the civilization progress. The earliest breeders selected from "wildness" the species which had important features for human activities.

Domestic animal breeds are an important resource for economic development. The historical global increase in food production was based on the genetic improvement of domesticated animals and plants, greater farming inputs and the cultivation of more land. Oldenbroek (1999)<sup>31</sup> argues that in

future the genetic improvement of farm animals will have a fundamental role in increasing animal production in intensive farming systems. He further points out that the marginal rural areas, less favourable for agriculture, may also be used for food production in low-input farming systems. The ruminants as cattle and sheep are able to digest fibrous material produced by grasslands which are less able to digest by monogastric species. Moreover, future market demands for specialised forms of food production such as organic and regional farming may increase. European Union directives currently protect the local food cultures.

The rapid evolution of civilization, in the last two hundred years, has led to a loss of genetic diversity of domestic animals. This fact puts into danger the possibilities of improvement and future development of domestic animals and implicitly can affect, in the long-term, the durability of actual civilization development. Therefore, in the last decades, the biodiversity conservation problem was put in discussion like a component of environment conservation.

Beside the scientific interest, this gene pool represents an inestimable agricultural, chemical and pharmaceutical potential. The agricultural production intensification and the improvement activity extension, besides their useful and positive effects, eliminate from reproduction not only species, but especially subspecies, breeds, varieties. Disappearance of subspecific genotypes restricts the species variability, impoverishing it. Elimination from reproduction of some "rustic" populations has harmful effects, as 4/5 of food production of the world proceed from less two dozens of plant and animal species. The original genes of "wild" type, true "cement bricks" can serve to the biological status reformation of plants and animals used in present, which generally have a high efficaciousness, but genetically are vulnerable to diseases and pests<sup>6, 41, 44</sup>.

To make in the future the successful improvement of domestic animal populations under intensive and extensive circumstances and for specialised food production, genetic variation within domesticated species must be maintained and even developed<sup>10, 11, 31</sup>. In this context the socio-economic and cultural values of different animal breeds should also be considered. The social benefits of the conservation of endangered breeds can exceed the social costs involved<sup>3</sup>. The genetic improvement activity of plants and animals can have negative effects too. In themselves, these activities,

through selection of genotypes of more and more perfected, contributed, together with technology improvement, to the gradual progress of agricultural production. But the genetic improvement did not always carried out on scientific bases. Moreover, neither in present a rightful care is not accorded to the ecological equilibrium necessity. These aspects lead to the subspecies elimination of local populations, varieties and breeds with lower performances, but which are better adapted to environment conditions and simultaneously constitute an extremely precious source of genetic variability.

Approach of some top researches in the animal breeding field and implementation of testes based on the genetic markers (proteins and DNA) is absolutely necessary to harmonise the national and international policies concerning the identification and traceability of animals and of their productions, to identify the rare, harmful or in danger of disappearing species and breeds, to enrich and to preserve the biological resource from the compact and marginal rural zones to develop a multifunctional agricultural<sup>7</sup>.

Because the international organizations of the animal breeders impose the identity determination and origin verifying of animals by AND tests, it is possible the reassessment of positions occupied by sheep breeds, allowing the *settlement of some real criteria concerning their economic and econogenic importance, their evolutionary trends, as well as their impact on community*<sup>2</sup>.

The ONU Convention for biological diversity (1992) recognizes the importance of genetic resources of domestic animals, being institutionalized the strong connection of the biodiversity with food, agriculture and environment<sup>5</sup>.

## REASONS FOR GENETIC CONSERVATION

There are more major criteria that could be used in prioritization decisions of breeds for concrete conservation actions<sup>4, 8, 10, 11, 12, 28, 31, 35</sup>.

- the need to keep the potential of useful genes, specific genes and gene combinations;
- maintaining or increasing of heterosis (hybrid vigour) and use of its advantage in livestock practice;
- mitigating of genetic diversity capping caused by the selection pressure;
- traits of current economic value; a breed with a trait (or several traits) of current economic value would obviously be of interest for conservation

purposes. Generally, the breeds that are profitable from economic point of view are not jeopardized. Conversely, the breeds that are not profitable may be in danger. However, market conditions may change rapidly as traits with high economic value today may have a low value in the future, and vice versa;

- promoting an insurance policy against climate changes, spread of diseases (zoonosis), changing availability of feedstuffs, social changes (such as issues of animal welfare and environmental sustainability), selection errors (a widely use of a valuable sire induces the inbreeding phenomenon or the extended use of a sire with a heredity defect can spread a genetic disease throughout a population before the genetic defect to be identified);

- degree of endangerment; the degree of endangerment of a breed is determined primarily by its current population size. The breeds that have large population sizes and seem to have a fairly secure future would not be prioritized for conservation purposes. Monitoring of population sizes is important because it can indicate significant trends and give a first warning that some conservation actions may be needed. It is important to act before the breed becomes endangered.

- cultural and historical value; our history is closely linked to agricultural practices on use of particular breeds, specific to certain areas. Breeds are (especially the rare ones), to a large degree, human-made products because artificial selection by humans for specific colours, production traits or conformation has been the primary force in the development of breeds, especially in economically developed countries. As with all other human-made products (such as paintings or buildings) the breeds can be considered part of the cultural or historical heritage of a certain area, These breeds are now used as “living history” parks or “living museums”, both important in education and tourism;

- special landscape value; some breeds have been traditionally used in special landscapes that would otherwise not be used for food production. Over time, the breeds may become adapted to the specific characteristics of the environment; for example, some breeds that have a long tradition of grazing on mountain pastures in summer or on pastures in nature reserve parks may have a value for their contribution to the characteristic ecology of the region. They may also contribute economically to the region through tourism.

- traits of current scientific value: breeds, by definition, are populations of animals that (to some

degree) are genetically different from each other. This genetic variation can be of great value for research on traits of agricultural interest as well as on traits of human interest where the animals are used as genetic models. Researchers can use these breeds to understand the genetic mechanisms behind such traits (*i.e.* quantitative and qualitative production traits, disease resistance, adaptation to the environment, etc.). Unselected lines are used to measure the genetic progress in selection. Identification of specific genes, which control some traits such as production quality or health, is made easier by comparing very different groups. Economic evaluation of animal breeding programmes includes now sociological aspects, as part of a focus on sustainable rural development. Research on the role of minor breeds in such production systems is needed.

– genetic uniqueness: the small number of species domesticated by humans is essential for feeding the world population, which is increasing rapidly. Therefore, the maintenance of variation within these species by ensuring the continued existence of distinct breeds which to be genetically separate is important enough.

The conservation of biological diversity is a decisive factor in agricultural activities: at the core of the various biological processes utilised by agriculture, biodiversity allows farmers to produce foodstuff and non-food products as well as services. Biodiversity utilisation in agriculture allows the creation of new varieties and breeds for the achievement of economic, health, technical and ecological objectives. Conversely, the evolution of agricultural activity in certain cases enriches biodiversity. Thanks to selection and research of domesticated plant and animal species, it also develops their intraspecific variability (*e.g.* selection of resistant animals to diseases).

The first beneficiaries of the management planes of animal genetic resources become the farmers and national consumers, resources, which rationally used, will lead to an increase in food safety, environment protection, rural economy strengthening and generally to satisfaction of the community requirements. From these findings it arises a better understanding by farmers of sustainable husbandry systems and the role of indigenous and imported breeds to maintain these production systems.

Resorting to genetic material of high quality will lead to the productivity gain, keeping the available resource, minimizing the effects for environment

and to providing different types and qualities of products requested by the community. Also, the world economy is becoming more uniform as regards the use of genetic material by all countries, as well as the possibilities for improvement of this material.

Resolving these issues by implementation of this methodology would optimize some applications concerning the correlation of genetic markers with production, reproduction and health traits having in view fixing those genes with high selection value in population and eliminating those genes which diminish the animal productivity and biological vigour using the blood polymorphism. On the other hand, theoretically speaking, the diversity of these selective values can be used to a better understanding of necessity to maintain and to emphasize the genetic polymorphism in sheep populations. In this way, the existent stocks may be indicated to increase the genetic progress in ovine species.

## CONCLUSIONS

The paper substantiates a new, modern and flexible methodology to quantify the genetic diversity in sheep using the genetic markers in informational-statistical processing.

The requirements on which the new methodology is based are:

– identifying the genetic structures of individuals (biochemical-genetic, immunogenetic and molecular-genetic markers);

– developing the computation algorithms with concepts from informational statistics: the informational correlation ( $C_{x,y}$ ) and informational energy ( $e$ ).

– creating a database to manage and enable the processing of information derived from experimental results.

The redefinition and redimensioning of the relations among sheep breeds by means of the informational correlation would elucidate some problems such as: their phylogenetic origin, the chronology of breed setting up, their age, the degree of genetic strengthening etc.

The quantification of some genetic structures, using the informational energy, will allow evaluation of the genetic variability, systemic organization degree and evolution of genetic systems under the environmental, selection and technological factors so that, by selection, to have an increase of production and reproduction

performances, resistance to diseases, heterosis etc.

The inventory of genetic resources represents fundamental information in the interbreeding process to create new breeds, ecotypes and morpho-production types; at the same time, these inventory is useful for foundation of some programmes to conserve the genetic potential of some biological entities of economic interest when this potential tends to decrease as a result of disappearance of some breeds or of homogenization of animal populations.

## REFERENCES

- Ajmone-Marsan, P.; ECONOGENE CONSORTIUM, *Sustainable conservation of animal genetic resources in marginal rural areas: integrating molecular genetics, socio-economic and geostatistical approaches*. *ECONOGENE*, www.econogene.eu, **2000**.
- Ajmone-Marsan, P., *Overview of Econogene, a European project that integrates genetics, socio-economics and geostatistics for the sustainable conservation of sheep and goat genetic resources*. *International Workshop on the role of biotechnology for the characterisation and conservation of crop, forestry, animal and fishery genetic resources*, FAO, Torino, **2005**, 89-96.
- Cicia, G.; D'Ercole E.; Marino, D., *Costs and benefits of preserving farm animal genetic resources from extinction: CVM and Bio-economic model for valuing a conservation program for the Italian Pentro horse*. *Ecological Economics*, **2003**, 45: 445-459.
- Cohen J.E., *Population growth and Earth's human carrying condition*. *Science, New Series*, **1995**, 269 (5222), 341-346.
- Commission of the European Communities, *Biodiversity Action Plan for Agriculture. Communication from the Commission to the Council and the European Parliament*, **2001**, III, Brussels.
- Commission on Genetic Resources for Food and Agriculture, *Draft guidelines on molecular genetics. Characterization of animal genetic resources. Thirteenth Regular Session. Rome*, **2011**.
- European Commission for Nature and Biodiversity, *EU biodiversity strategy to 2020*. *Nature & Biodiversity*, **2011**, [http://ec.europa.eu/environment/nature/index\\_en.htm](http://ec.europa.eu/environment/nature/index_en.htm).
- European Federation of Animal Science, *49th EAAP Annual Meeting: Warsaw, Poland: Book of Abstracts*, 1988, 4.
- Galal S.; Hamond K., *The Global Program for the Management of farm Animal Genetic Resources. The III<sup>rd</sup> Iberoamerican Congress of Criollo and Native Breeds, Santa Fe de Bogotá, D.C., Colombia*, **1996**.
- Gandini G.C.; Oldenbroek J.K., *Genebanks and conservation of farm animal genetic resources*. Oldenbroek J. K. (ed.), *DLO Institute for Animal Science and Health, The Netherlands*, **1999**.
- Gandini G.C.; Villa E., *Analysis of the cultural value of local livestock breeds: a methodology*. *J. of Anim. Breeding and Genetics*. **2003**, 120, 1-11.
- Gandini G.C.; Oldenbroek J.K., *Strategies for moving from conservation to utilization*. In: *OLDENBROECK, K. (Ed.) Utilisation and conservation of farm animal genetic resources*. Wageningen Academic Publ. **2007**. pp. 55-74.
- Geng R.; Chang H.; Wang L.; Tsunoda K.; Yang Z.; Sun W.; Ji D.; Li Y., *Genetic differentiation of native sheep populations in East and South Asia*. *Biochem. Genet.*, **2007**, 45 (3-4), 263-279.
- Groza M.; Pădeanu I., *Studiu privind utilizarea energiei informaționale pentru calculul gradului de diversitate a unei structuri genetice*. *Lucr. Șt. "Zootehnie și Biotehnologii"*, **1999**, XXXII, Edit. Agroprint, Timișoara, 242-249.
- Groza M.; Hrinică Gh., *Metode taxonomice de clasificare numerică a populațiilor de ovine. A 30<sup>a</sup> Ses. de Comunic. Șt., Univ. de Șt. Agr. și Med. Vet. București*, **2001**, 164-168.
- Hrinică Gh., *Sistemele genotico-biochimice la ovine*. Edit. Agata Botoșani, **2004**.
- Hrinică Gh., *Polimorfismul genetic al enzimelor la ovine*. Edit. Agata Botoșani, **2007**.
- Hrinică Gh., *Grupele sanguine la ovine*. Edit. Agata Botoșani, **2012**.
- Kevorkian Steliana Elvira Maria, 2010., *Markeri moleculari la ovine*. Teză de Doctorat, Universitatea București.
- Kolmogorov, A.N., *A new invariant for transitive dynamical systems*. *Dokl. Akad. Nauk SSSR*, **1958**, 119, 861-867.
- Kowalska M.; Zatoń-Dobrowolska M., *Genetic distance between different breeds of sheep*. *J. Agrobiology*, **2008**, 25, 23-26.
- Kullback, S., *Information theory and statistics*. John Wiley and Sons, New York, NY, **1959**.
- Lipecka, C.; Pieta M.; Gruszecki T., *The potassium level in the blood of sheep and their productivity*. *J. Anim. Feed. Sci.*, **1994**, 3: 89-96.
- Manwell C.; Baker. C.M.A., *Genetic distances between Australian Merino and Poll Dorset sheep*. *Genet. Res.*, **1977**, 29, 239-244.
- Moioli Bianca, Napolitano F., Orrù L, Catillo G., *Analysis of the genetic diversity between Gentile di Puglia, Sopravissana and Sarda sheep breeds using microsatellite markers*. *Ital. J. Anim.Sci.*, **2006**, 5, 73-78.
- Nei M., *Genetic distance between populations*. *Amer. Naturalist*, **1972**, 106, 291-296.
- Nei M., *Mathematical models of speciation and genetic distances*. In "Population Genetics and Ecology", New-York, Academic Press, **1976**, 723 - 766.
- Nei M.; Tajima F.; Tatenò Y., *Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data*. *J. Mol. Evol.*, **1983**, 19, 153-170.
- Nguyen T. C., *Polymorphisme sanguin du mouton et distance genetique entre les races*. *Ann. I.N.R.A. - C.N.R.A, Lab. Genet. biochim., Jouy-en-Josas*, **1982**, 6, 248-254.
- Nguyen T.C., Morera L, Llanes D., Leger P., 1992 - *Sheep Blood Polymorphism and Genetic Divergence between French Rambouillet and Spanish Merino. Role of Genetic Drift*. *Anim. Genet.*, 23, 325-332.
- Oldenbroek, J. K. (ed.), *Genebanks and the conservation of farm animal genetic resources*. DLO Institute for Animal Science and Health. Lelystad, the Netherlands, **1999**, 119 p.
- Onicesco O., *Theorie de l'information. Energie informationnelle*. *C.R. Acad. Sci., Serie A, Paris*, **1966**, 263, 841-842.
- Ordas J.G.; San Primitivo F., *Genetic Variation in Blood Proteins within and between Spanish Dairy Sheep*. *Anim. Genet.*, **1986**, 17, 255-266.
- Paiva, S.R.; Faria D.A.; Silvério V.C.; McManus C.; Egito A.A.; Dergam J.A.; Guimarães S.E.F.; Castro S.R.;



- Albuquerque M.S.M.; Mariante A.S., *Genetic variability among Brazilian sheep using microsatellites*. **2005**, www.fao.org.
35. Patterson D.L.; Silversides F.G., *Farm animal genetic resource conservation. Why and how?. Canadian Farm Animal Genetic Resources Foundation Brighton, ON, Canada*, **2003**.
36. Rodero A., Garzón R., Llanes D., Zarazaga I., Vallejo M., Monge E., 1982 – *Genetic distance between Spanish sheep breeds*. Archivos de Zootecnia, vol. 31: 97-108
37. Rodero E.; Camacho M.E.; Delgado J.V.; Rodero A., *Study on the Andalusian Minor Breeds: Evaluation and Priorities of Conservation*. Anim. Genet. Resources Information. FAO, **1992**, 10, 41-45.
38. Sargent J.; van der Bank F.H.; Kotze A., *Genetic variation in blood proteins within and between 19 sheep breeds from southern Africa*. S. Afr. Anim., Sci. **1999**, 29 (3), 245-257.
39. Scher B.; Galal S.; Hoffmann I. (Editors), *Animal Genetic Resources. Food and Agriculture Organization of the United Nations*, **2012**.
40. Shannon C.E., *A Mathematical Theory of Communication*. The Bell System Technical Journal, **1948**, 27, pp. 379-423, 623-656,
41. Simberloff, D., *The contribution of population and community biology to conservation science*. Ann. Rev. fcol. Syst. **1988**, 19, 473-511.
42. Ștefănescu V., *Aplicații ale energiei și corelației informaționale*, Edit. Acad. Române, București., 1979.
43. Takezaki N.; Nei M., *Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA*. Genetics, **1996**, 144, 389-399.
44. Tapio Miika; Tapio Ilma; Grislis Z.; Holm Lars-Erik; Jeppsson S.; Kantanen Juha; Miceikiene I.; Olsaker Ingrid; Viinalass Haldja; Eythorsdottir E., *Native breeds demonstrate high contributions to the molecular variation in northern European sheep*. Molecular Ecology, **2005**, 14 (3), 3951-3963.
45. Vallejo M.; Zarazaga I.; Sierra I.; *Relaciones genéticas entre diversos ecotipos de la raza Aragonesa y las razas Merina y Manchega*. An. Fac. de Vet. Zaragoza, **1977-1978**, 11-12, 235-243.
46. Vicovan G.; Hrință Gh., *Tipurile de hemoglobină și potasiu sanguin la unele rase de oi indigene și importate*. Lucr. șt. ale ICPCOC Palas-Constanța, **1985**, V, 111-118.
47. Vicovan G.; Rașcu Dorina, *Distanțele genetice între rasele de ovine Țurcană și Țigaie*. Lucr. șt. ale ICPCOC Palas-Constanța, **1994**, VII, 41-47.
48. Worley K.; Strobeck C.; Arthur S.; Carey J.; Schwantje H.; Veitch A.; Coltman D. W., *Population genetic structure of North American thinhorn sheep (Ovis dalli)*. Molecular Ecology, **2004**, 13, 2545-2556.
49. Zanoti Casati M.; Gandini G.C.; Leone P., *Genetic variation and distances of five Italian native breeds*. Anim. Genet. **1990**, 21, 87-92.

