

Universitatea de Științe Agronomice și Medicină Veterinară – București **Facultatea de Zootehnie** 

# LUCRĂRI ȘTIINȚIFICE SCIENTIFIC PAPERS

## SERIA D

## VOL. LI

## ZOOTEHNIE ANIMAL SCIENCE

## THE 37<sup>TH</sup> INTERNATIONAL SESSION OF SCIENTIFIC COMMUNICATIONS OF THE FACULTY OF ANIMAL SCIENCE

BUCUREȘTI 2008

#### KUP '3: 65/826:

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#### = ABSTRACTS =

## ON THE HISTORY OF ROMANIAN FARM ANIMAL SCIENCES AND EDUCATION

#### ISTORIA CONCEPȚIILOR ÎN ȘTIINȚA ȘI ÎNVĂȚĂMÂNTUL SUPERIOR ZOOTEHNIC ROMÂNESC

CONDREA DRĂGĂNESCU

**Key words**: science and education aims. Farm animal science paradigm:-dormant, autonomous, mature, intensive production and sustainable production. Mixed, autonomous, Bolognia processus education. Cultural models

**Cuvinte cheie**: obiectivele științei și învățământului. Paradigmele-concepțiile știnței zootehnice:empirică, autonomă, matură, producție intensivă, durabilă. Invățământ-mixt, autonom, procesul Bolognia. Modele culturale

> The aims of science are to understand, predict and control the development. Mayr 1999

#### SUMMARY

The science and education is one of the main forces of farm animal production progress. Science evolves change his content, his paradigm, vision. The study of history of scientific ideas can help to identify what is valuable and what is surpassed in a done moment. In the history of Romanian animal science and tecnology can be noticed some five paradigms: dormant, autonomous, mature, intensive production and sustainable production. In the dormant paradigm the Romanian shephard contributed to the outlive and unity of Romania people on a large Central and South-East European territory. Others paradigm passes with paradigm crisis. High education presents three paradigms: mixed, autonomous, Bolognia processus. For the education of new generation the history must present the scientists and technocrats with a real contribution to the idea and technological progress, but the problem is to notes them correctly. The result-of confusions can be a low profession cultural level.

The science is one of the main forces of humanitarian progress. It permit to understand, predict and control the socio-economic development. The Seventh Framework Programme of the European Community for research, technological development and innovations (2007-2013) underline that the new strategic goal of European Union for the next decade is to become the most competitive and dynamic knowledge-based economy in the world, capable of sustainable economic growth with more and better jobs and greater social cohesion. "The triangle of knowledge education, research and innovation — is essential for achieving this goal". This objective is valuable now also for Romania and his animal production..

In this ages of a predicted exponentially raised consumption of meat and milk in developing countries until 2020 (so-called "livestock revolution"-IFRI 2000), of global **competition**, in this ages of a strong necessity to preserve **the habitability of this planet**,

the Romanian Farm Animal production must introduce a developmental strategy adapted to this conditions. The production must be a competitive, **dynamic knowledge-based production**, capable of **sustainable** economic growth. Dynamic knowledge-based production means **science** –which is the basis of production **technology**. The scientific researches assure the science development, the innovation - the technological progress and **education the** transmission of science, technology and correct social behaviors.

Table 1

Science"	Component of	Developmental method	By
(growing body of	Science (truth,	Fundamental research	Scientist
knowledge "the organization	ideas)	Applied research	
and classification	Technology	Operational research	Technocrat
of knowledge on	(technique)	Technological innovations	Farmer
the basis of explanatory principles"	<b>High Education</b>	Transmission of science,	Scientist
Mayr 1999)	+ Extension	technology and correct social	
		behaviors	

## Science and education the sources for a sustainable economic growth of farm animal production

Science evolves change his content, his paradigm, vision. The study of history of science can help to identify what is valuable and what is surpassed in a done moment. The biography of scientific personality implicated in the scientific ideas apparition and promotion, is a cultural "models" for the young scientist. Generally there are many works connected to the events in zootechnical science history and to his scientific personalities (Cornevin 1880, Stefan 1981, Obrejan 1976, Pestean etc). The problem is if this works noticed correctly the paradigm evolution and the real cultural models.

Our paper intends to make a contribution to the clarification of some aspects of knowledge, conceptions evolution in Romanian animal science and to the notation of scientist, technocrats implied in this evolution probleme. That can help to understand, estimate and control a sustainable and competitive development of animal production in the country.

#### 1. IDEAS HISTORY IN ROMANIAN ANIMAL SCIENCE

"Who can be sure that many present truths will be tomorrow fable" Emil Racovita

The essence of science are the ideas and the visions, the theories, the paradigm are in a continuous change. New visions replace the old one; "Science build his future on his past foundation. It is born from his own death." the philosophers of science (Kuhn, Dimitriu ) noticed.. There are few spatio-temporally unrestricted visions. Sure the replacement of a vision imply scientific disputes,, altercation, a paradigm crisis. However not all altercations are a sign of scientific progres, but nevertless **the history of science is a history of ideas change**. Sure when we speak of scientific ideas we mean also tecnological one and we can't neglect some facts, same mans who promoted the ideas, the technologies, the inovations, some technocrats who imposed it and the education system who dispersed them. We think that in the evolution of Romanian animal science and technology history can be noticed some **five paradigm**, visions moments.

(a) ZOOTCHNIE BASICALLY DORMANT SCIENCE UNTIL TWENTIETH CENTURIES. Animal production empirical knowledge has been accumulated during some thousands of years with a minimum of written material. Cornevin (1880) presented a very interesting history of ideas in this period zootechny. He noticed four periods, from which three, up to some 1840 years, belongs to this empirical epoch, in which the farm animal knowledge were mixed with knowledge from others areas. We resume our approach just to some aspect connected to the Romania animal production history.

The "enigma and the historical miracle" who is the apparition and the persistence of Romanian people (Ferdinand Lot 1932, G.I.Bratianu 1988), seem to be connected, as some ethnographers suppose, with the pastoral life. The former Romans, inhabitants of former East Roman empire, rescued from the newcomers barbarian and from their Byzantine enemy retiring in marginal lands, especially in mountains. There the main existence source was the pastoralism. "Up to 1864 the agricultural production of the country was based on animal production" noticed N.A.Dumirescu (1924).

We must underline that local sheep breeds-Valachian and Tsigai- from a vast European area (from Ural to Bohemia and Peloponnese) are Romanian heritage, documents for their history (Draganescu). History is made not just on written documents, but on all thinks who belonged to man and was used by him (L.L.Fevre in Bratianu 1988). Even now the historians did-not noticed the historical importance of local sheep breeds and the animal scientist did not registered all the "science" of traditional shepherds. In the era of sustainable development, of breed and traditional production conservation, all this empirical "science" is important. We note however that, besaide the data presented by Cornevin, that there are some written material even in Romanian. Cantemir (1716) presented some data of Farm Animals in Moldavia, Molnar (1783), quoted by Lupsan, wrote the first Romanian book on Beekeeping.

(b) ZOOTECHNIE AS AUTONOMOUS SCIENCE. Expression Zootechny is, at is known, relatively recent (Gasparin 1844) and the history of Farm Animal Science as autonomous science and "provincial" (antonym to universal") is connected with the apparition of this expression (Cornevin 1880). There were practically to disciplines (is difficult to tell sciences): General Zootechnie and Special Zootechnie. Practically the Farm Animal Science started in 19<sup>th</sup> century and was introduced in Romania in 1870 with a book of Ion Ionescu de la Brad, practically in the some ages as in France,(Baudement 1869) and Germany. George Maior (1899) wrote under a German influence the first book (799p) on Farm Animal Science, he presented also his personal opinions on General Zootechny (Anatomy, Physiology, Exterior, some Genetics, Breeding, Nutrition) and Special Zootechny (cattle, sheep management, milk processing).

Generally the founder of Animal Science in Romania is considered. Nicolae Filip (G.Ionescu Braila 1924). He wrote in a very nice language a General Zootechny (1909,492 p.) and a study on Romanian breeds, organize for short time a first research institute and continued the Ionescu de la Brad organization of state breeding farms. He benefit in his life of a good social position, respect and he had some disciple. We must

note however that he had Neo Lamark ideas, did not accepted or noticed the new paradigm introduced in Animal Improvement by the Darwin Evolutionary theory, the apparition of Mendel Genetics, the progress in animal nutrition and the timid progress in farm animals management. The knowledge in this science was essentially based upon description, observation.

(c) START OF MATURE ZOOTECHNICAL SCIENCE (1920-1945). The unification of Romania Stat and the apparition of new paradigm in international Animal Science imposed in Romania a new vision, a new paradigm in Animal Science and Animal Production, the a evolution to a **mature animal science**.

"We are now at the beginning of a new era, we can tell that we start a new zootechnical school", "the zootechnicall science entered in complete new phase...who will persevere in empiricism will be lost" noticed **Gh.K. Constantinescu** (1923,1930), the man who responded to the necessities of this epoch. Especially in Animal Genetics and Animal improvement, introduced a new paradigm, the **Darwinian and Mendelian paradigm**, was the lieder of a real scientific school, organize a strong Zootchnical Research Institute, the systematic publication o scientific research papers, vulgarization publications and even monitoring of country farm animal improvement. During this period was introduced the experiment as mean of scientific research with some methodological rigor specific to a real science.An independent course of Animal Nutrition was introduced, experiments in artificial insemination and even appeared some tendency to look at animal management from an ecosystem view. We note, however that the fragmentation of rural propriety, imposed by socio-strategic-politic reason did not facilitate the production modernization (Garoflid 1924) and the new Neodarwinian, the Quantitative and Population Genetic apparition was not noticed, as in all Europe.

(d) SCIENTIFIC PARADIGMS OF LARGE SCALE, INTENSIVE PRODUCTION. The need of a rapid increase of food production (FAO"-War to hunger", Military blocks competition etc) imposed on the second part of 20-th century the development of intensive, large scale production, the change of the old scientific paradigm, the evolution of Animal Sciences to a **mature science**, to a more interdisciplinary connections with the fundamental and pure biological sciences. The department of Animal Nutrition from Agricultural Universities (IN-PG France etc) is also the scientific forum for human nutrition, Genetic Animal Improvement science is connected with the scientist of Genetic Research Institute from Edinburgh, even in Romania between the founders of local Biometric Society there are animal production scientists. Appeared a science of "biology of agricultural systems" (Spedding 1972)

The change to a new paradigm was normally, for subjective reasons, slow in many countries but it was more checked in Romania by some political mixture in science ("Mitsurin Genetics" etc) and by political actions for changing the agricultural structure by forced cooperativisation. It was more difficult to surpass the paradigm crisis. Lerner and Donald (1966,p 216) made strong critics of the proper design of research in Easter Europe made under the principle "try something without rhyme or reason and see what happens". Especially after 1968 the science received more attention and we attempted to introduce the new scientific paradigms. The name, sometimes even the content of some discipline was changed (General Zootechnie in Animal Improvement, Species

management in Exploitation as applied ecology) **but the paradigm crisis was not solved**. As a result the technocrats seldom rely on transferring some exogenous technology of large scale, intensive production rather to cooperate with they autochtonous scientists. Not all aspects of adapted technological solutions were the best, but at least in poultry and even in pigs intensive production Romania was classified between the first then countries of the world.

(e) ANIMAL SCIENCES FOR A COMPETITIVE SUSTAINABLE PRODUCTION. The grow trends in world population, industrialization, pollution, food production and resources depletion observed on the last century revised the problem of sustainability of this development to preserve the habitability of the Terra. In such conditions animal production must solve two contradictory imperatives:

•Maximize production and economic efficiency and competitively as an answer to the globalization, population growth, less land and to a real revolution in human nutrition toward farm animals food.

Minimization pollution, resources depletion, maximization animal welfare.

That mean it must increase production, intensify it in a sustainable way, there is in a SARD (FAO agency-Sustainable agriculture and rural development) definition develop an <u>agricultura and rural development ecological and economical viable, social correct, cultural and human responsive and based on a scientific approach.</u>

The science made important progresses (genomic, genetically modified organisms, nutrition physiology, ethology etc) but he must, paid more attention to an ecosystem approach of animal production, develop in sustainable manner the large scale intensive systems, maintain and develop especially in marginal area the old, traditional extensive systems (pastorals, even smallholders farming subsistence) for production and nature conservation. Start again a paradigm crisis, sustained in Romania, beside the old scientific inertias, by a new fragmentation of rural propriety.

#### 2. SCIENTISTS – THE CREATIVE FORCE OF ANIMAL SCIENCE

"Next to music and art, science is the greatest, most beautiful and most enlightening achievement of the human spirit"

Karl Popper

The science and the technology is the product of ideas of scientist, of scientific research and of technological innovators. Sure, Karl Popper was correct comparing the science to art and music, but that means that not all people have the same vocation for it as for art and music. For vainglory or base economical reasons there are people who like to pas as scientists even they have no vocation

(a) CHARACTERISTIC OF A SCIENTIST. The stereotype picture of as scientist is, as Lerner and Donald (1966) underlined, of a man driven by curiosity, whose equipment is intelligence, integrity, observation, thinking and creativity. Pastea (1972) present 18 quality from a scientist researcher, one of the first being the honesty

#### Some psychological attribute of a scientist

<b>Scientist</b> -Man driven by curiosity, to whom research is a hobby	Characteristics	Some quality
	Intelligence	Cognitive ability, discernment
	Integrity	Honesty, deontology (ethics)
	Observation	Rapid and correct
	Thinking	Nonconforming, critique
	Creativity	Original ideas,

Very few people who work in science have the scientist equipment developed to a high degree. Most could lay claim, like others human beings, to some degree of ignorance, bias, pour judgment, vanity and snobbery. Few scientists are non-conforming, original thinkers with a high integrity, professional deontology. The fact that a man works and use to work in a research didn't mean that he is a scientist (Pastea 1972). He can be utmost a research worker, an officer with any role or negative role in knowledge evolution. The correct evaluation and utilization of scientists is a difficult job, but it is however the basis of the scientific progress.

(b) "THE SCIENIC TRIBUNAL". The science history, but also the scientific forums have the mission to identify the really personality who have "vocation" contributed to the science evolution, the scientific cultural models. The mission is difficult because the new ideas can be not noticed or rejected by traditional thinkers, the nonconforming thinkers are incommode. We note again (2004) that the scientific, implicit the production progress can be checked by many non-ethically, even antisocially facts (<u>compilation, plagiarism, false authors, "rediscover</u>" of discovered facts) by which some "officers" try to pass as scientists. These facts are stimulated by the estimation of the man scientific value by the number of his signed papers, by the incapacity to appreciate the paper quality. It is necessary to make from zootechnical history "a science tribunal" as Schiller though on world history, which accept or reject a paper as scientific or a man as scientific personality. That is the only justification of scientific forums existence (Academy, Governmental agency etc). Every man who work in science must understand that if will succeed to elude the contemporary correct appreciation he can't elude the history appreciation. The education must underline the fact underlined also by Darwin (1871) that "of all the differences between Man and lower animals the moral sense or conscience is by far the most important.)

Ta	ble	3
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Papers and books type	Efect
Originale (new reserch facts ,opinions, scientific and	Scientific and production
techological ideas, critical synthesis)	progres
Compilation (syntesis without noticing the essential	
and new ideas, witout personal vision, realy subtile intelectual	Scientific and production
plagiat) or <b>unjustified research or paper</b>	erodation
Plagiat (intelectual thif); or fals author on a paper	

(c)CULTURAL MODELS The culture is an integrated system of social behaviors learns and transmitted from one generation to another by non-biological mechanism (Neculau 1987), by education, by cultural models. The history must present such models, but the problem is to notes them correctly. Many mans wont to become "historical" personality, with portrait in zootechical institutions; ppersonality with a role in the social, cultural, scientific human development not died altogether. However not allformer "power" mans deserve to be cultural models and many cultural models are not observed. There are also negatives models, which acted against the new vision in science, offered example of self-seekers and had life success. It deserve to be noted. Some people from the young generation can take them as "models". The result-of such confusions is **a low profession cultural level**.

A cultural model can be a scientist, a technocrat or even a profession. There are no perfect mans, but the cultural mans had a real contribution to the science, education, production or profession progress. In farm animals area there are scientific, technocrat and perhaps even profession cultural models. Generally N. Filip and G.K.Constantinescu are unanimously accepted. The problem of others is practically not solved and approached with subjectivity or under the pressure to have a "model", a "past". We have not the intention to solve the problem. With an inerrant subjectivity I writhed on G.K.Constantinescu, G.Maior, G. Moldoveanu, D.Contescu, E.Pastea, Em.Negrutiu. I presented a table with those to teach animal sciences up to some 1970. I intend to write on A. Tacu and to V. Beres (as professionals, his colleges build for him a statue). Sure many others can candidate to be cultural models. Especially I think from technocrats to G.Ionescu Braila, Stan Tarlea, G. Moldovan, who had a great role in technological development.

#### **3.ANIMAL SCIENCE EDUCATION; -THE BOLOGNIA PROCESSUS**

"It must be accepted for zootechnie a professional responsibility comparable to that of veterinary, agricultural, forestry etc sciences and to develop par consequence institutions, study programs, education courses correspondent"

FAO Recommendation Gottigen 1966

The education (high, professional, extension) is the main way of transmission of knowledge accumulated by science to the new generations of scientist, technocrats, and farmers. That is the explanation of the great attention received by it from all national and international institutions. We will pay attention in our paper just to high education, the staring point of all others.

It must be accepted that in education as in science there were and there are different vision, paradigm and thei are in a continuous change. In our opinions (1968...1999) all over the world use to be three leadingn high education system: French-academic, classic, German academic-pragmatic, Anglo-American adapted to the variation of student capacity and to the variations of production demand. Each of them evolved,

changed his content and diversity according to the scientific and production progress. In animal science high education history can be noticed three main periods.

(a) MIXED EDUCATION. Teaching farm animal science at the university level, in eterinary and agricultural schools, start in 19-th century, in the era of apparition of this autonomous sciences (1844-1870). There were two discipline General and Special Zootechnie, up to 1920 seldom teaches by the some professors at veterinary and agricultural schools. At the beginning of 20-th century, in Romania about in 1937, was introduced also as an independent discipline Nutrition (1926 Contescu and Puscariu translated the 1904 Kellner book) and Hygiene integrate the farm animal management. It was a competition, seldom dispute in zootechnical profession between the two specialization.

(b) AUTONOMOUS ZOOTECHNICAL HIGH EDUCATION. The production and farm animal science development imposed also the education development. It seem that the American developed the first autonomous farm animal science education in Farm animals, Dairy Cattle, with the specialization in science, production, processing and trade. The system was introduced in less pragmatical and scientific mode in Easter Europe and in others many countries, the graduate receiving diploma of B Sc (Ing.zoot) in Animal Science or specialization (Draganescu 1991). FAO organized a special workshop on Animal Science high education (Gottingen 1966) and the conclusion was the recommendation to accept for zootechnie a professional responsibility comparable to that of veterinary, agricultural, forestry etc sciences and to develop par consequence institutions, study programs, education courses correspondent".

In Romania the Zootechnical faculty was organized in 1948, on the former Zoo-Veterinary Institute. The old dispute for zootechie affected the new faculty, who, agains the will of his graduates, was transformed in section (1958) and eliminated (1962). In 1968 the faculty was again organized and started the adaptation to the scientific paradigm of intensive, large scale, intensive production system. Some eroneus scientific approach have been eliminated ("Mitsurin vision) the name, sometimes even the content of some discipline was changed but the "traditions" did not help the full elimination of paradigm crisis.

(c) TO A MATURE HIGH ANIMAL SCIENCE EDUCATION- THE BOLOGNIA PROCESSUS. The scientific revolution developed in the second part of 20th century, the large-scale intensive production, the development of European Union, imposed a new vision on education. Startig with the year 1974 a all system of European organization was devoted to education (Euridice, Eudised,Tese, Cedefop, European Schoonet, TEE, Eurovoc etc). Was clear that the high education, including the agricultural education start to change in the direction of the adaptation to the variability of student vocation, to the variability and demand of labour maket, to the new advances in scientific knowledge, to an interdisciplinary approach and sustainable development to assure the student mobility on the European area.

In 1990 we attempted to adapt the education plan to the exigence of a mature science, visible alredy: three cycle system-(bachelor, master, doctor)-, optional discipline directed in three direction (production, science animal breeding, science animal nutrition), transferable credits, necessary competences to face the challenges of the new conditions

and to the new millennium, utilization of docimological tests, analyze and assess consequence of various animal production systems (Dragoness 1991) This opinions were not originally. The EU Siena conference, (1992), The first Coference on Agricultural Education-(Wageningen1992, East European Conference on Agricultural Education (Warsaw 1992) stressed more or les the same problems.

The Romanian Minister of Education signet, with others 39 European Ministers, on 19<sup>th</sup> of June 1999 in Bolognia a **Joint Declaration on The European Education Area. The Bolognia Process aims to create a European Higher Education Area by 2010.** A series of reforms, principally in the direction presented by us before, intend to make European Higher Education more compatible and comparable, more competitive and more attractive to match the performance of the best performing systems in the world, notable the Us and Asia. The high education students of every country must be allowed to change the university or to work in any European country. All countries, including Romania, act to respond to the signet convention. The European Ministers of education analyze every two years the progress (Prague 2001, Berlin 2003, Bergen 2005, London 2007, Louvain-La-Neuve 2009). <u>The question is: the Romanian Zootechnical Faculty answered to the Bolognia imperatives ? Can be useful an analyze.</u>

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#### THE PREDICTION OF BREEDING VALUE IN A DAIRY SHEEP POPULATION USING THE TEST DAY ANIMAL MODELS

#### H. GROSU

Key words: random regression test day animal model; own performance; covariance components; accuracy; rank correlation

#### SUMMARY

The objective of this study was to predict the breeding values in a dairy sheep population in order to find the best individuals for the next generation. The breeding values were predicted using two methods: a) the *random regression test day animal model* and b) the average phenotypic performance. The data set consisted of 1375 TD records from 209 ewes in the first lactation. The whole population had 416 individuals, which the following structure: 32 sires, 175 dams and 209 offsprings (ewes with own performances). Totally, 209 ewes had records. The average number of TD per lactation was about 6,3. Data were edited and TD records were deleted if ewe' ID was unknown, if lactation number was not specified and if days in lactation for the TD record was < 60 or > 175 days. Also, a TD class had to have at least 4 observations. The two methods were compared using the accuracy of prediction of breeding values and the percentage of squared bias. For the data set accounted the best method was the random regression test day with the three order Legendre polynomials.

Best linear unbiased prediction (BLUP) applied to an animal model (AM) is the standard procedure for genetic evaluation. It has the advantage that all known information is optimally taken into account and selection or special mating has a small or no effect on the evaluation. This procedure is even more valuable in dairy sheep because, with natural mating, the number of progeny per ram is relatively small, which makes information from other relatives more important. Consequently, an increasing number of national evaluation systems is based on AM in dairy sheep: since 1990-1992, AM evaluation systems have been implemented in France for Lacaune, Basco-Bearnaise, Manech and Corsica breeds, in Italy for Sarda, Comisana and Langhe breeds, and Spain for Laxta and Carranzana breeds (Gabina et Barrillet, 1991; Barrilet et al, 1992; Sana et al., 1993; Pagnacco et al., 1991; Pinelli et al, 2000).

In recent years, attention has been drawn to use of test day (**TD**) records directly instead of using cumulative lactation yield calculated from them. There are several potential benefits from using this technology. First, conventional recording systems are costly and ways for a simplification of production recording schemes are desirable. Milk recording agencies may not need to collect many (bimonthly tests) yields per ewe per lactation, and this could result in lower costs to dairy producers. Second, generation intervals can be reduced as genetic evaluations can be performed sooner using all test day records available at a given time instead of waiting for complete lactation records. Third, the traditional approach of using complete lactation records has been criticized as inconsistent since record taken at defined locations and time are aggregated in a rather arbitrary way and are subsequently subjected to quite sophisticated statistical analyses targeted toward an optimum differentiation of all genetic and environmental effects.

The main objective of this study was to compare the efficiency of a two methods: a) the *random regression test day animal model* and b) the average phenotypic performance. Secondary, the genetic parameters were estimated.

#### **1. MATERIALS AND METHODS**

Data were restricted to first lactations, DIM on TD between 60 and 175 d, age at first calving between 22 and 30 mo, milk yield from 300 and 1200 ml. TD records were deleted if ewe' ID was unknown, if lactation number was not specified and if days in lactation for the TD record was < 60 or > 175 days. 8 test-date classes were represented. Also, a TD class had to have at least 4 observations. After editing, the final data set consisted of 1317 test day records from 209 ewes and the pedigree file consisted of 416 animals, including these 209 ewes and their ancestors, 32 rams and 175 dams. Average no of TD per ewe was about 6.3. The 209 ewes have had their first lambing on 2001.

Table 1

Numbers of test day (TD) records, fixed effects, animals, and mean daily milk yield in first lactation for a local dairy sheep line

	in first factation for a focal	J ~
Nr	Specification	
crt		
1	Total test day records, no	1375
2	TD fixed effects	8
3	Animal, no	416
4	Ewes with records, no	209
5	Mean TD record per ewe, no	6.3
6	Mean daily yield, gram	680.33
7	Standard deviation daily, gram yield	153.19
8	Variation coefficient, %	22.52

#### Models

The first type of model used for genetic evaluation is a random regression model:

$$y_{ijkl} = TD_i + \sum_{m=1}^{3} b_{mj} X_m + \sum_{m=1}^{3} \alpha_{mk} X_m + p_k + e_{ijkl}$$

where:

 $\mathbf{y}_{ijkl}$  = record l on ewe k made on DIM tjl of first lactation for a ewe belonging to class j of age at lambing;  $\mathbf{TD}_i$  is the fixed effect due to ewe tested in the same test-date i;  $\mathbf{p}_k$  is random permanent environmental effect associated with all TD yields of ewe k within lactation;  $\mathbf{e}_{ijkl}$  is random residual effect;  $\mathbf{b}_{mj}$  and  $\boldsymbol{\alpha}_{mk}$  are fixed and random regression coefficients, respectively.

Third-order Legendre polynomials  $(X_m)$  were used for both fixed and random regressions on the scale from 60 to 175 DIM.

In matrix notation, the model can be written as:

$$\mathbf{P} = \mathbf{X}_1 \mathbf{b}_1 + \mathbf{X}_2 \mathbf{b}_2 + \mathbf{Z}_1 \alpha + \mathbf{Z}_2 \mathbf{p} + \mathbf{e}$$

The Mixed Model Equations for this model are:

$$\begin{bmatrix} X_{1} \cdot X_{1} & X_{1} \cdot X_{2} & X_{1} \cdot Z_{1} & X_{1} \cdot Z_{2} \\ X_{2} \cdot X_{1} & X_{2} \cdot X_{2} & X_{2} \cdot Z_{1} & X_{2} \cdot Z_{2} \\ Z_{1} \cdot X_{1} & Z_{1} \cdot X_{2} & Z_{1} \cdot Z_{1} + G^{-1} \otimes A^{-1} & Z_{1} \cdot Z_{2} \\ Z_{2} \cdot X_{1} & Z_{2} \cdot X_{2} & Z_{2} \cdot Z_{1} & Z_{2} \cdot Z_{2} + I \cdot k \end{bmatrix} \cdot \begin{bmatrix} \widetilde{b}_{1} \\ \widetilde{b}_{2} \\ \widetilde{c} \\ \widehat{a} \\ \widehat{p} \end{bmatrix} = \begin{bmatrix} X_{1} \cdot P \\ X_{2} \cdot P \\ Z_{1} \cdot P \\ Z_{2} \cdot P \end{bmatrix}$$
$$k = \sigma_{e}^{2} / \sigma_{p}^{2}$$

#### The criterion to chose the methods

Two criterions were used in order to compare the efficiency of the two methods: the accuracy of the selection and the rank correlation.

The accuracy of evaluation was calculated from the inverse elements of the mixed model equations for the diagonal block corresponding to animal genetic effects (Jamrozik et al., 2000).

Rank correlation (Spearman) was used for the estimation of the correlation between the ranks occupied by the same animal on different models. (Co)variance components were estimated using REML method.

#### 2. RESULTS AND DISCUSSIONS

**Covariance components.** The parameters for the genetic covariance matrix of the random regression coefficients (G), permanent environmental variance and residual for milk yield are shown in table 2.

Table 2

	environmental (pe) and re	esidual variances for milk yield
	Parameters	Milk
α0	α	271.700
α0	$\alpha_1$	-11.127
α0	$\alpha_2$	0.089
$\alpha_1$	$\alpha_1$	4.335
$\alpha_1$	$\alpha_2$	-0.041
$\alpha_2$	$\alpha_2$	0.004
Perm.	env. variance	672.12
Resid	ual variance	2914.85

Estimates of genetic (co)variance for random regression coefficients, permanent environmental (pe) and residual variances for milk yield

**Predicted Breeding Values (PBV).** In the second stage of the analysis the breeding values and accuracy of EBV were predicted for all animals in the data set. Using BLUP methodology, these estimates are adjusted for all other effects included in the model. All effects included in the model are simultaneous estimated and predicted each others.

For the ewe evaluation with yields and pedigree information, all three sources of information could be available. Progeny performance is adjusted for merit of the mate by

subtracting half the mate's breeding value. For ram evaluation, only pedigree and progeny portions of the equation are used.

A sample of results is presented in table 3, representing the best 15 animals based on their breeding value and their accuracy, for both methods.

1	able	3

The best 15 sheep ordered after the two kinds of models						
		Random				
Nr		regression	Accuracy_of		Average own	
crt	ID	Breeding values	the selection	Rank_RR	performance	Rank_OP
1	93058	88.36	0.66	1	863.33	3
2	72512	82.41	0.70	2	865.00	2
3	72624	76.28	0.64	3	780.14	11
4	93056	76.25	0.64	4	766.67	14
5	82770	72.40	0.64	5	785.12	9
6	82872	67.76	0.77	6	795.00	7
7	61918	67.49	0.65	7	790.00	8
8	82834	65.52	0.74	8	775.00	12
9	51718	62.74	0.70	9	840.00	4
10	10154	61.16	0.69	10	803.33	5
11	82818	57.97	0.66	11	781.67	10
12	82998	57.77	0.71	12	754.25	15
13	93062	56.55	0.64	13	911.15	1
14	51500	56.24	0.62	14	796.00	6
15	82798	54.88	0.71	15	774.14	13

The best 15 sheep ordered after the two kinds of models

From the data shown in Table 3 it can be noticed that there is a big difference between the two methods, concerning the breeding values.

The accuracy of selection and rank correlation. For whole population the average of accuracy of selection was about 0.65 for random regression model and 0.59 for the average own performance. The gain obtained is about 10%. The rank correlation coefficient between the two methods is close to zero (0.15). This means that there is a small concordance between the two methods, concerning the breeding values predicted by test day random regression model and the own performance. The bigger discrepance between the two methods is for the animal with ID 93062, which has first position after the own performance but the  $13^{\text{th}}$  position using the test day model.

The animal model use all kinds of information: parents, collaterals, own performance and offsprings. Even the individual has a high own performance, if it has bad relatives, his breeding values will be close to zero, or negative. This is a possible explanation for the animal with ID 93062.

#### **3. CONCLUSIONS**

1. The TD animal models offer the opportunity to improve the genetic evaluation of dairy sheep.

2. TD models can account more precisely for the environmental factors that could affect the ewes differently during lactation.

3. TD model allows a ewe to be evaluated on the basis of any number of TD records during lactation.

4. The selection done using the two methods has an average accuracy of 65%.

5. The rank correlation between the two methods is low, close to zero (0.15).

6. Higher accuracy of the selection will help us to identify the best individuals in order to be parents for next generation.

7. Higher accuracy means more genetic progress at the population level; in this case the higher sheep milk production.

8. The TD animal models should be used for the genetic evaluation in sheep dairy milk.

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#### **IQ SCORE HERITABILITY**

GH. SANDU<sup>1</sup>, H. GROSU<sup>2</sup>, S. LUNGU<sup>2</sup>, I. RESCEANU<sup>1</sup> <sup>1</sup> Centre for Studies of Applied Theology – Craiova, Romania <sup>2</sup> University of Agronomic Sciences and Veterinary Medicine – Bucharest, Romania

The Internet plays an important role in the offensive of the Eugenics to conquer the public opinion in favor of their ideas, given its wide addressability. The possibility to remain anonymous makes a lot of websites produced by Eugenic circles to speak straightforward of improving the human species by selection. This is part of a long-term strategy to prepare the public opinion for a future acceptance of this monstrous solution.

The main criterion of the foreseen solution is the intellectual performance evaluated by intelligence quotients (IQ). In front of those willing to neglect the moral implications, the viability of this proposal depends only in the dimension of the expected effect. In order to demonstrate a considerable effect of the election on the average human intelligence, the Eugenics propose very high values for intelligence heritability (coefficient of hereditary transmission). Different sources cite for this genetic parameters values between 0.6 and 0.8. With such transmissibility the effect of selection would be promising and many intelligent people, but without moral scruples, might accept Eugenic measures against the less intelligent people, such as banning them from reproduction.

The everyday experience shows us that the odds the genies give birth to genies are not so high, and so are the odds that the less endowed people give birth la less endowed descendents. Such a complex trait as intelligence can not have such a simple genetic determinism so that its heritability is so high.

The purpose of this paper is to give a rigorously scientific evaluation of intelligence (IQ score) heritability.

#### 1. MATERIALS AND METHODS

A sample of 48 school children and 90 parents of these children responded to a Raven intelligence quotient. The Restricted Maximum Likelihood method (REML) was used to evaluate the variance components required to calculate heritability. The used biometric model is a mixed one, with a fixed factor (education level) and a randomized factor (genetic effect of the individual), according to the following equation:

$$y_{ijk} = \mu + \alpha_i + B_j + e_{ijk}$$

where:

 $y_{ijk}$  = observed (measured) value, k=138 observations

 $\mu$  = general average, common for all observations

 $\alpha_i$  = fixed effect, i=5 (G=gymnasium; L=high school; P=vocational school; S=higher education; F=under formation (for children))

 $B_j$  = randomized effect of the individual, j=318

 $e_{ijk}$  = error effect

In order to make use of the parent performances as well, they are used in calculations as descendants (because they have performance). Their consideration as descendants requires the specification without performance of their parents, namely two for each parent. It results in total: 180 grandparents + 90 parents + 48 children = 318. Working this way we worked on 318 observations.

Using a matrix notation the model may be written as

$$y = Xb + Za + e$$

where:

y = vector of the observations

X = incidence matrix; it associates the observations to the fixed effects

Z = incidence matrix; it associates the observations to the randomized effects

a = vector of the fixed effect (educational level of the individuals)

b = vector of the randomized effect

This mixed model has a correspondent in the following system of BLUP equations:

$$\begin{bmatrix} X' \cdot X & X' \cdot Z \\ Z' \cdot X & Z' \cdot Z + A^{-1} \cdot k \end{bmatrix} \cdot \begin{bmatrix} \widetilde{b} \\ \widehat{a} \end{bmatrix} = \begin{bmatrix} X' \cdot y \\ Z' \cdot y \end{bmatrix}$$

where:

 $\hat{a}$  = vector of the improvement values (318 values)

 $A^{-1}$  = inverse matrix of the kinship relations between the analyzed individuals  $K = (1 - h_a^2) / h_0^2$ 

 $h_0^2$  is the heritability in the basic population

The environment  $(\sigma_e^2)$  and additive genetics  $(\sigma_A^2)$  variances are obtained based on the solutions of the system of equations  $(\tilde{b} \text{ and } \hat{a})$ , according to the following relations:

$$\sigma_e^2 = \frac{y \cdot y - \tilde{b} \cdot X \cdot y - \hat{a} \cdot Z \cdot y}{n - r(X)}$$
$$\sigma_A^2 = \frac{\hat{a} \cdot A^{-1} \cdot \hat{a} + \sigma_e^2 \cdot tr(A^{-1} \cdot C_{22})}{q}$$

where:

tr = matrix trace (sum of the elements on a matrix diagonal)

- n = number of observations (138)
- q = total number of individuals (318)

r(X) = rank of X matrix (number of the linearly independent columns)

$$C = \begin{bmatrix} X' \cdot X & X' \cdot Z \\ Z' \cdot X & Z' \cdot Z + I \cdot k \end{bmatrix}^{-1} = \begin{bmatrix} C_{11} & C_{12} \\ C_{21} & C_{22} \end{bmatrix}$$
$$\mathbf{n}^2 = \sigma_1^2 / (\sigma_1^2 + \sigma_2^2)$$

Finally,  $h^2 = \sigma_A^2 / (\sigma_A^2 + \sigma_e^2)$ 

The calculations were done with a calculation algorithm developed by the authors under MATLAB-MATrix LABoratory software.

#### 2. RESULTS AND DISCUSSIONS

REML method is iterative. For iteration 1 we assign a start value for  $h_0^2$  either the value of the basic population (if known from higher education), or an arbitrary value (between -1;+1). At the end of iteration 1 we obtain a first heritability ( $h_1^2$ ), then we calculate a new value k ( $k_1$ ), which enters in the system of equations, an so on, until the final heritability is no longer different from the previous one.

In our calculations we assigned 5 starting values for the basic heritability. It is interesting to note that irrespective of the starting value, the final values stabilized around the value of 0.28-0.29 (Table 1). Obviously, the farther is the star value from the final value, the larger is the number of iterations.

Table 1

IQ score heritability						
Number of	Value of					
iterations	heritability at the end of					
	iterations					
12	0.2868					
60	0.2866					
81	0.2864					
124	0.2867					
131	0.2865					
	Number of iterations           12           60           81           124					

IQ score heritability

It results thus that the intelligence has middle towards low genetic determination. The results agree with American studies of 1980/81 cited by Cavalli-Sforza (Genes, Peoples and Languages, 2000), but neglected by the Eugenics because they assign to genetic causes only 1/3 of IQ variation (another 1/3 is assigned to the general environment of the cultural background of the individuals and the last 1/3 is assigned to the social environment associated to the different experience of the private life).

In order to be more convincing in front of non-specialists, the contemporary Eugenics no longer stress in the cleaning of the human gene fund of the unwanted genes (negative Eugenics), rather on the increase of desired genes frequency (positive Eugenics). The specialists realize, however, that the two sides go hand in hand: it depends on the perspective you take, to make one plane or another come in front.

To make better received a positive Eugenics regarding the intelligence of the human population, its followers cite excessively high values for IQ heritability (Burt 0.8-0.9; Nichols 0.6-0.7; Jensen, Eysenck and Vernon 0.8; Catell 0.5-0.8; Dickens and Flynn

0.75; Rijsijk 0.65-0.77; Posthuma et al. 0.69-0.85; Weight et al. 0.71-0.87 for psychosomatic aptitudes and 0.65 for memory).

Such large values might be explained either by a small number of loci involved in the genetic control of intelligence, or by a overwhelming dominance of the additive interactions between the genes from the involved polygenic complex.

Psychology showed however, that the global intellectual performance depends on a lot of simple traits, so the number of the involved loci can not be slam. The originality of genius's thinking doesn't seem either to be the priority result of the additive interactions between the genes, but rather the effect of extremely favorable non-additive interactions (over dominance, epistasis), but with very low odds of existence (for instance, Bernstein mentions that the famous Einstein had a mediocre IQ score in tests targeting the basic component of intelligence, the only which might be built particularly by additive interactions).

The results of this paper support the pertinent and competent expectations regarding intelligence heritability. With values between 0.2 and 0.3, the selection for own performance would have very modest results. All proposals which aim to increase the odds of marriage between people with high IQ consider only the self performance. Considering that the success of marriage direction is expected to be low unless abusive interventions occur in people's life, the intensity of the selection will also be low. Therefore, with a low heritability of the selection criterion and with a low intensity of selection, the effect of selection can only be almost insignificant.

For such low heritabilities, the increase of selection effect is usually done by considering the family performance. This would change, however, the marriage habits of the people taking them closer to the criteria used for directed mating in the populations of animals undergoing genetic improvement at the hand of the man.

#### **3. CONCLUSIONS**

1. Applying the best credited "free culture" test (Raven progressive matrixes) on a significant sample, as well as the most modern calculation technique (REML), IQ score heritability is rather low (0.28).

2. On the background of an intensity of the selection which can not be high unless abuses limiting human liberty to marriage are taken, with this heritability the effect of selection for intelligence in human populations will be very low.

3. Therefore, the proposal of the Eugenics to improve the average level of intelligence by selection for this criterion within human populations has no genetic substantiation, remaining a mere moral challenge to humanity.

#### REFERENCES

A large amount of references can be obtained on the Internet using the following keywords: heritability, IQ, as well as the names of the mentioned authors, or of other authors cited by the first accessed websites. The books of these authors lack, however, from our libraries.

#### PRINCIPII DE AMELIORARE A RASELOR DE SUINE ÎN CENTRUL DE SELECTIE SI HIBRIDARE DIN REPUBLICA MOLDOVA

## PRINCIPLES IN IMPOVING THE PIG'S BREEDS IN THE CENTER OF SELECTION AND HIBRIDIZATION FROM REPUBLIC OF MOLDOVA

#### I.ROTARU\*, V. HAREA\*\* \*STATE AGRARIAN UNIVERSITY, REPUBLIC OF MOLDOVA, \*\*"I.S MOLDSUINHIBRID"

**Cuvinte cheie**: hibrid, vier, carcasă, genotip, suine, rasă, hibridare, selecție **Key words**: fattening, hybrids, boors, carcass, genotype, pigs, breed, hybridization, selection

#### SUMMARY

To improve the production and quality indexes at pigs a special role has the improving work towards identifying effective parental combination, which largely depends on the progress and the level of genetic selection in pig populations. The most effective path is the technologies and biological material transfer to the actual producers in order to obtain the hybrids with a guaranteed productive genetic potential. To transfer the most advanced animals a study has been made at the qualitative production at the different breeds of pigs used to produce advanced biological material, in order to obtain competitive hybrids.

#### 1. MATERIALS AND METHODS

To conduct the research works of productive capacities in pigs the methods have been approved in research institutions of the Republic and other countries. Young pigs of different breeds have been appreciated by their performance, based on studies of the intensity of growth, consumption and specific precocity. In this effect was used to test the resort MLP Manager (company "Schauer", Austria).

#### 2. RESULTS AND DISCUSSIONS

The location and the multiplication of genetic resources of pigs in the units of selection and production is effective only after testing centers in the genetic material selection (tab. 1)

The pigs testing results reveal that the intensity of growth varies according to race. The difference on the average daily increase in paternal breeds Duroc and Hampshire, Yorkshire and Landrace Maternal the 71-91 g, which demonstrates paternal breeds are characterized by a greater precocity. The achievement of 100 kg corresponds to 211-229 days. The thickness of a fat layer is relatively low at all youth tested breeds and equalizes in Yorkshire and Landrace breeds with 17,8 to 19,1 mm, and the Duroc and Hampshire breeds 20,4-21,1 mm.

#### Table 1

Genotype	Broad head injury	Short trunk,1 head, at the end of testing, kg	Thick, cm	Fat layer, mm	Verage daily gaining, g
Yorkshire	349	82	115	14,7	459
Landrace	302	83	116,5	13,9	453
Hampshire	193	97	116	18,7	562
Duroc	90	98	118	17,4	624

Table 2

#### The characteristics of paternal lines of breeds according to their performance $(\overline{X} + S\overline{x})$

to their performance $(X \pm SX)$							
Line	Nr of	Head length	Weight,	Average	Thickness of	Reaching	
	heads	crop, cm	kg	daily gain,	fat layer,	age,100kg,	
				g	mm	day	
			Hamp	shire			
Нор	119	120,7 <u>+</u> 0,21	90,4	421,2 <u>+</u> 6,07	19,9 <u>+</u> 0,46	243,2	
Hun	65	121,9 <u>+</u> 0,77	97,1	435,4 <u>+</u> 10,46	18,6 <u>+</u> 0,58	229,7	
Hor	4	111,0 <u>+</u> 3,9	72,3	405,3 <u>+</u> 15,2	12,1 <u>+</u> 5,5	246,7	
			Dur	OC			
Dor	61	120,7 <u>+</u> 0,92	93,7	436,0 <u>+</u> 9,73	13,3 <u>+</u> 0,16	229,35	
Dog	19	127,7 <u>+</u> 2,66	106,6	477,8 <u>+</u> 15,1	14,1 <u>+</u> 0,69	209,1	
Dac	5	110,2 <u>+</u> 2,69	92,0	515,0 <u>+</u> 32,2	13,6 <u>+</u> 1,76	194,2	
Dud	3	127,0 <u>+</u> 4,04	134,6	580,0 <u>+</u> 78,6	17,6 <u>+</u> 0,72	165,6	

A bigger average daily gain was achieved from the Hun line which equals to 435 g at the Hampshire breed and the line Dud which was 580 g at the Duroc breed. The thickness of a fat layer at the youth lines HOP, HUN and HOR varied within the limits of 12,1-19,9 mm. Better results showed the young pigs, representative lines Dor, Dog, Dac and Dud at the race Duroc 13,3-17,6 mm. An excellent precocity showed the line Dud equal to 165 days.

The data presented in the table 3 reveals that the productive performances of the descendents vary by breed and genetic potential of boars breeders. A lower specific consumption -3,22 kg feed/kg gain was recorded in Yorkshire breed, being smaller in comparison with Duroc, Hampshire and Landrace by 0,75-1,32 kg. The biggest average daily increase occurred at Hampshire race, which was 657 g, but the differences between races are 24-29 g, which are not significant. The trunk length was higher at Yorkshire and Landrace breeds, Duroc has a medium length and the differences are 7-9 cm in comparison with Hampshire race. Very good results on the thickness of the fat layer were

obtained from the progeny of breed Yorkshire boars - 17,4-17,8 mm, while the young pigs from Duroc and Hampshire breeds formed a layer of fat equal to 20,7 mm.

Table 3

Race	Nr.of	Consumption	Average	Age achieve	The length of	Thickness
	animals	kg / feed / kg	daily gain	weight	crop, cm	of a fat
		gain		100kg		layer, mm
Yorkshire	18	3,22 <u>+</u> 0,07	621,0 <u>+</u> 18,2	169,42 <u>+</u> 0,99	128,2 <u>+</u> 2,29	17,8 <u>+</u> 1,80
Duroc	12	3,81 <u>+</u> 0,27	628 <u>+</u> 44,08	164,1 <u>+</u> 1,25	126,9 <u>+</u> 5,11	20,8 <u>+</u> 1,92
Landrace	2	4,69 <u>+0,30</u>	470 <u>+21,20</u>	213,5 <u>+1,45</u>	125,5 <u>+2,06</u>	18,0 <u>+1,25</u>
Hampshire	6	4,38 <u>+</u> 0,26	657,5 <u>+</u> 47,7	152,1 <u>+</u> 1,24	119,3 <u>+</u> 3,21	20,7 <u>+</u> 2,01

# The results of boars' appreciation according to their descendents fattening qualities ( $\overline{X} \pm S\overline{x}$ )

The effect of selection in populations of pigs largely depends on the productive performance of young pigs for replacement animals reforms. The results of youth reproduction appreciation are presented in the table 4.

The day-old progeny included 50% heterozygous males, genotype Bb, with black down and a white spot of variable size on their head and 50% heterozygous females, genotype bB (trade name Robar SL-2001) with black down on the body and head. Day-old chicks sexing by the down color was explained by the existence of a heterosomal epistatic gene located on chromosome W.

Age reaching 100 kg weight is an indicator that characterizes the precocity of animals. The shortest period of time necessary to achieve weight of 100 kg has been recorded at the breed Duroc - 176 days, while at the maternal breeds Yorkshire and Landrace this index was achieved in 212-215 days. The average daily increase at Duroc and Hampshire youth breeds of pigs was 563-529 g, being higher with 58-102 g in comparison with breed animals like Landrace and Yorkshire. The thickness of a bacon fat layer has a value of over 20 mm at youth pigs regardless the breed.

Table 4

<u>1 Touterive performance at young pigs in selection and hybridization <math>(X \pm 5x)</math></u>							
Genotype	Nr.of heads	Body mass, kg	Length crop, cm	Thickness of fat layer, mm	Age reaching 100 kg weight		
Yorkshire	47	102,7 <u>+</u> 1,18	125,0 <u>+</u> 4,29	21,5 <u>+</u> 0,25	215,3 <u>+1,05</u>		
Landrace	36	102,2 <u>+</u> 1,98	124,2 <u>+</u> 1,12	21,2 <u>+</u> 0,66	212,0 <u>+1,16</u>		
Duroc	20	122,0 <u>+</u> 4,48	128,5 <u>+</u> 1,34	27,9 <u>+</u> 0,83	176,0 <u>+1,21</u>		
Hampshire	24	116,2 <u>+</u> 3,51	130,2 <u>+</u> 2,62	24,9 <u>+</u> 1,31	188,7 <u>+1,09</u>		

Productive performance at young pigs in selection and hybridization ( $\overline{X} \pm S\overline{x}$ )

#### 3. CONCLUSIONS

1. The transfer of the technologies and genetic materials with potentially high productivity from the centers of selection and hybridizing to the farms in production will contribute to the increase of pigs' roductivity with 15 - 25%.

2. The implementation of testing young pigs after their performance by the growth control method, contributes to the formation of selection groups with productive capacity, which directly affects the process of improving the populations within each type of breed.

3. In perspective the technological transfer of the biological material in production units, has to be made by the results in testing the sows according to their own performance using the control growing method.

4. Using young pigs will contribute in increasing the productive potential of pig populations, as well in obtaining commercial intensive hybrids type with good carcass quality.

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#### I. CONTRIBUȚII ORIGINALE LA FUNDAMENTAREA ȘTIINȚIFICĂ A TEORIEI GENICE A SEXUALITĂȚII

#### I. ORIGINAL CONTRIBUTIONS TO THE SCIENTIFIC SUBSTANTIATION OF THE GENE THEORY OF SEXUALITY

\*F. PRICOP

Avicola Bucuresti, Str. Jandarmeriei, Nr. 2, RO-013893, Bucharest, Romania, Fax 0040212313114; Phone 0040212300449, pricopflorinro@yahoo.com

Cuvinte cheie: recombinare genetică la păsări; genetica culorii penajului la Galinacee; sexarea puilor de o zi.

**Key words**: poultry genetic recombination; genetics of feather color of Gallinaceae; day-old chick's sex screening.

#### SUMMARY

The genetic determinism of sex and the equal male to female ratio in Galinaceae was explained in literature by the existence of the male sex, homogametic ZZ, and of the female sex, heterogametic ZW, as well as by the existence of genes in chromosome Z, however, with no corresponding genes of it in chromosome W (Morgan, 1919). This paper presents experiments of genetic recombination. The experimental design allows the identification in generation F1 of the dominant sex gene linked to the gene that determines the monitored phenotype, namely the colour of feathers. Both these genes are located in chromosome W. In the same generation the recessive sex gene was identified in chromosome Z. In generation F2, males and females are in equal numbers in every category of genotypes. Three categories of feather colour genotypes were produced: dominant homozygous, heterozygous and recessive homozygous. The experimental results show the presence of two genes in chromosome W, the dominant sex gene and the gene transmitting the colour of the feathers, contrary to the hemizygotic theory. The genes identified during the experiments led to the development of a new theory, named by us "The Gene Theory of Sexuality of Galinaceae", which contradicts the chromosomal mechanism of Morgan.

The increasing demand for high quality protein in the developing world is expected to be one of the most important trends in the future of agriculture (Rosegrant et al 2001). Chicken meat and eggs are likely to be the major contributor to meeting this demand. The chicken is increasingly becoming of great interest as an intermediate evolutionary model organism, ideally placed between mammals and more distant vertebrates as fishes (Groenen et al 2000; International Chicken Genome Sequencing Consortium 2004).

The chicken is also a very good model for studies concerning color patterns, sex chromosomes, sex-determining genes and sex-determination in vertebrates. Sex is determined genetically in birds, having a ZZ-ZW sex chromosome system characterized by female heterogamecy: a large Z chromosome and a smaller W chromosome (Ohno, 1967). Sex is suppose to be determined in this group by the dosage of a Z-linked gene (two in males, one in females) or by a dominant ovary-determining gene carried on the W sex chromosome, or both (Smith, 2007).

Sex and plumage color inheritance of Gallinaceae was explained so far using the two levels of organization of the genetic material: the chromosomal level and the gene level respectively. The chromosomal determinism starts form the premise that the genes responsible for plumage color inheritance are located within the chromosome Z and that they do not have any correspondent genes within the chromosome W (Morgan, 1919). The chromosomal theory of sex determination by heterosomes is still cited, mainly, because of lack of new investigations. Well known scientists in poultry genetics (Groenen et al, 2000, Fridolfsson & Ellegren, 2000, Ellegren, 2001, Kerje et al, 2004) always begin their studies assuming the conclusion of the hemizygotic theory mentioned earlier.

The investigations presented in this paper were started due to the existence of major discrepancies between the experimental data from own research (Pricop, 2005) and the chromosomal theory of sex determination by heterosomes, which is why this paper relates to the paper of Morgan (1919).

#### 1. MATERIALS AND METHODS

We applied the method of the direct and reciprocal cross of Gallinaceae using two different breeds in order to monitor the phenotypic expression of the heterosomal genes responsible for the gene determinism of the plumage color and of the sex. The cross of parent males and females produced generation F1, while the cross of the males and females from generation F1 produced generation F2.

The parents originate from pure homozygous lines for the barred (B) and gold (s) heterosomal genes which are responsible for the plumage color inheritance. The parents belonged to the following breeds: barred Marans and red Rhode-Island characterized as follows: phenotypically, the barred Marans birds have barred plumage and their genotype is homozygous dominant (BB) for the barred gene (B) (Campo 1991); phenotypically, the red Rhode-Island birds have red feathers and their genotype is homozygous recessive (bb) for the gold gene (b). These pure lines are reproduced each year in full pedigree and are used for the production of the commercial layers sexable by the color of the plumage.

The experimental groups consisted of over 3200 individuals which were evaluated in generation F1 whereas over 1500 individuals were evaluated in generation F2. The technological male to female ratio in the parent groups was 1:10. The macroscopic examination of the plumage color and of the sex of the individuals was conducted twice, once for day-old and the second for birds at the age of 18 weeks.

#### 2. RESULTS AND DISCUSSIONS

#### DIRECT CROSS: BARRED MARANS MALE × RED RHODE-ISLAND FEMALE

The cross of barred Marans males with red Rhode-Island females produced in generation F1 a number of 3218 day-old genetic recombinants which were sexed using the cloacal method. The generation F1 contained heterozygous barred males and heterozygous barred females (Figure 1).

The heterozygous barred males received from the father the chromosome Z together with the recessive sex gene (sdw) linked to the barred gene (B) and from the mother the chromosome Z together with the recessive sex gene (sdw) linked to the gold

gene (b). The recessive homozygous genotype sdwsdw determines the male sex, while the heterozygous genotype Bb determines the plumage color.

Table 1

produced in generation F1 ( $\circlearrowleft$ barred Marans × $\bigcirc$ Red Rhode – Island)	Down color and sex repartition of day-old and 18 weeks-old genetic recombinants
	produced in generation F1 ( $\circlearrowleft$ barred Marans × $\bigcirc$ Red Rhode – Island)

Day-old				18 weeks-old		
Phenotype and	Nr. of	%	Sex	Phenotype and genotype	Nr. of	%
genotype	birds				birds	
Black with a white				Barred (Bb)	992	34.9
spot on the head	1601	49.8	Μ	Barred body and red	386	13.6
(Bb)				neck and head (Bb)	300	13.0
Black with a white				Barred (Bb)	1460	51.4
spot on the head	1617	50.2	F	Barred body and red	2	0.1
(Bb)				neck and head (Bb)	2	0.1
Total number of	3218	100		Total number of males	2840	100
males and females	3210	100	-	and females	2040	100



**Figure 1.** 18 weeks-old progeny of generations F1 and F2 produced from the cross of barred Marans males with red Rhode-Island females

P = parent generation	a and b = heterozygous barred males			
F1 = generation 1 progeny	c and d = heterozygous barred females			
F2 = generation 2 progeny	e = homozygous barred females and males			
SDW = dominant sex gene	f = heterozygous barred females and males			
Sdw = recessive sex gene	g = homozygous gold females and males			
B = barred gene	* unexpected subject in generation F2			
b = gold gene	E = epistasis			

The heterozygous barred females received from the father the chromosome Z together with the recessive sex gene (sdw) linked to the barred gene (B) and from the mother the chromosome W together with the dominant sex gene (SDW) linked to the gold gene (b). The heterozygous genotype sdwSDW determines the female sex while the heterozygous genotype Bb determines the plumage color. Table 1 shows the statistic distribution of birds from generation F1.

We examined a number of 1601 heterozygous (Bb) day-old males and 1617 heterozygous (Bb) day-old females. Both sexes had black down with a white spot of variable size on their head.

The day-old chicks were sexed using the cloacal method. The plumage color of a number of 2840 birds of age 18 weeks was examined. From the 1378 males 34.9% had barred plumage and 13.6% had barred plumage on the body with red feathers on the neck and head. From the 1462 females 51.4% had barred plumage and 0.1% had barred plumage on the body with red feathers on their neck and head. The sexually mature genetic recombinants from generation F1 were crossed to produce the generation F2. Table 2 shows the statistic distribution of generation F2.

Table 2

**Down color and sex repartition of day-old and 18 weeks-old genetic recombinants** produced in generation F2 ( $\bigcirc$  barred heterozygous ×  $\bigcirc$  barred heterozygous)

Day-old			18 weeks-old		
Sex, phenotype and genotype	Nr. of birds	%	Sex, phenotype and genotype	Nr. of birds	%
Mixture of males and females with black down and a white spot on the	1158	75	Barred males and females, homozygous (BB)*	307	25.0
head, homozygous (BB) and heterozygous (Bb)*	1156	15	Barred males and females, heterozygous (Bb)*	615	50.1
Males and females with red down, homozygous (bb)*	386	25	Males and females with red down, homozygous (bb)*	305	24.9
Total number of males and females	1544	10 0	Total number of males and females	1227	100

\* In each phenotypic category the ratio males : females was equal to one.

According to the sex chromosomes segregation, the genetic recombinants from generation F1 should contain four expected categories of genotypes in generation F2, differing by sex and plumage color as follows: homozygous barred males (sdwsdw BB), heterozygous barred males (sdwsdw Bb), heterozygous barred females (sdwSDW Bb) and homozygous gold females (sdwSDW bb).

According to our experimental data the progeny of generation F2 (Figure 1) contained two more unexpected genotypes, detected for the first time worldwide, together with the four expected ones: homozygous barred females (sdwSDW BB) and homozygous gold males (sdwsdw bb).

The homozygous barred females should have received the barred gene from the heterozygous barred females of the generation F1 but instead, they displayed the gold gene linked to the dominant sex gene (SDW) rather than the barred gene within the

chromosome W. The homozygous gold males should have received a gold gene from both parents together with chromosome Z. In Figure 1 is shown that the F1 females displayed the barred gene linked to the recessive sex gene (sdw) rather than the gold gene within the chromosome Z.

The observed features in the inheritance of plumage color show that the last two categories of genotypes that were detected should not be present since they do not respect the pattern of sex chromosomes segregation in the genetic recombinants of generation F1 and of their recombination in generation F2.

In a similar experiment, Morgan (1919) obtained in the generation F2 only barred males, whereas half of the females were barred and the other half of females were black. In our experiments we produced in generation F2 three categories of genotypes. The ratio males:females within each category of genotypes was 1:1. These results can be explained as follows: **a**) in generation F1 the gold gene is located within the chromosome W of the heterozygous females (Bb); it determines the red feathers on the neck and on the head in 0.1% of the heterozygous barred females and represents a particular way of action of this gene within the chromosome W; **b**) the barred gene is located within the chromosome Z of all F1 subjects and is transmitted to the progeny as any autosomal dominant gene; **c**) the cross of F1 heterozygous males with F1 heterozygous females shows that plumage color inheritance in generation F2 by the heterosomal genes is similar to the inheritance of the traits determined by genes located within an autosomal locus; **d**) the existence of 25% dominant homozygous females and males, of 50.1% heterozygous females and males and of 24.9% recessive homozygous rather than hemizygous.

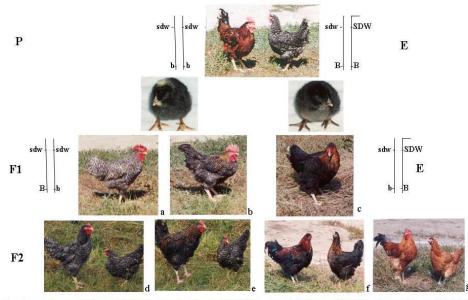
RECIPROCAL CROSS: RED RHODE-ISLAND MALE  $\times$  BARRED MARANS FEMALE

Figure 2 and Table 3 show the results of the reciprocal cross between red Rhode-Island males and barred Marans females.

Table 3

# Down color and sex repartition of day-old and 18 weeks-old genetic recombinants produced in generation F1 (♂Red Rhode – Island × ♀ barred Marans)

Day-old				18 weeks-old		
Phenotype and	Nr. of	%	Sex	Phenotype and genotype	Nr. of	%
genotype	birds				birds	
Black with a white				Barred (Bb)	964	36.6
spot on the head (Bb)	1636	50	М	Barred body and red neck and head (Bb)	378	14.4
Black body and head (bB)	1639	50	F	Black body and reddish- black neck and head (bB)	1291	49.0
Total number of males and females	3275	100	-	Total number of males and females	2633	100



 Genotype: sdwsdw BB\* sdwSDW BB
 sdwsdw Bb sdwSDW Bb\*
 sdwsdw bB\* sdwSDW bB
 sdwsDW bb
 sdwSDW bb

 Frequency (%):
 24.7
 24.7
 25.1
 25.5

**Figure 2.** 18 weeks-old progeny of generations F1 and F2 produced from the cross of red Rhode-Island males with barred Marans females

D					
P = parent generation	a and b = heterozygous barred males				
F1 = generation 1 progeny	c = heterozygous reddish-black females				
F2 = generation 2 progeny	d = homozygous barred females and males				
SDW = dominant sex gene	e = heterozygous barred females and males				
Sdw = recessive sex gene	f = heterozygous reddish-black females and males				
B = barred gene	g = homozygous gold females and males				
b = gold gene	* unexpected subject in generation F2				
E = epistasis					

The day-old progeny included 50% heterozygous males, genotype Bb, with black down and a white spot of variable size on their head and 50% heterozygous females, genotype bB (trade name Robar SL-2001) with black down on the body and head. Day-old chicks sexing by the down color was explained by the existence of a heterosomal epistatic gene located on chromosome W.

A number of 2633 birds of 18-weeks were examined. From the 1342 males 36.6% had barred plumage and 14.4% had barred plumage on the body with red feathers on their neck and head. All 49% heterozygous (bB) reddish-black females had black plumage on the body with reddish-black feathers on their neck and head.

Correlating the results of plumage color inheritance of day-old chicks with the results observed for those of 18-weeks old birds one can notice that the phenotype of the F1 heterozygous females (bB) is different from that of their parents and from that of the heterozygous males (Figure 2). Based on the macroscopic examination Pricop (2005)

reasoned that the heterosomal gene noticed previously within the chromosome W is the dominant sex gene (SDW) which plays two roles: a) as a dominant sex gene in relation to its recessive allele, sdw, located within the chromosome Z; b) as an epistatic gene that interacts with the gene determining plumage color, located within the chromosome W.

The non-allelic interaction (E) of the dominant sex gene of the barred gene occurs only in the W linkage group and modifies the allelic interaction of the genes from the heterozygous genotype that determines the plumage color. The plumage color of the F1 heterozygous reddish-black females was the phenotypic marker that allowed the identification of the dominant sex gene. The cross of a recessive homozygous males sdwsdw with a heterozygous females sdwSDW produced 50% recessive homozygous males sdwsdw and 50% heterozygous females sdwSDW. This explains the genetic determinism of sex inheritance and the equal number of males and females obtained in the cross. Table 4 shows the statistic distribution of generation F2 produced by the cross of heterozygous (Bb) barred males with heterozygous (Bb) reddish-black females.

Table 4

Day-old			18 weeks-old	,	
Sex, phenotype and genotype	Nr. of birds	%	Sex, phenotype and genotype	Nr. of birds	%
Mixture of homozygous (BB) and heterozygous (Bb) males	857	49.6	Homozygous (BB) barred males and females*	345	24. 7
and females with black down and a white spot on the head *	837	49.0	Heterozygous (Bb) barred males and females *	345	24. 7
Heterozygous (bB) males and females with black down*	435	25.2	Heterozygous (bB) reddish- black males and females*	351	25. 1
Homozygous (bb) males and females with red down (bb)*	436	25.2	Homozygous (bb) red males and females*	357	25. 5
Total number of males and females	1728	100	Total number of males and females	1398	100

Down color and sex of day-old and 18 weeks-old genetic recombinants produced in generation F2 (♂ barred heterozygous × ♀ reddish-black heterozygous)

\* In each phenotypic category the ratio males : females was equal to one.

Following sex chromosomes segregation in the genetic recombinants from generation F1, one would have expected four categories of genotypes in generation F2 (Figure 2) differing by sex and plumage color: **a**) homozygous barred females (sdwSDW BB); **b**) heterozygous barred males (sdwsdw Bb); **c**) heterozygous reddish-black females (sdwSDW bB); **d**) homozygous gold males (sdwsdw bb).

In generation F2 we evidenced four more unexpected genotypes, detected for the first time ever, worldwide, together with the four expected ones: **a**) homozygous barred males (sdwsdw BB); **b**) heterozygous barred females (sdwSDW Bb); **c**) heterozygous reddish-black males (sdwsdw bB); **d**) homozygous gold females (sdwSDW bb). The unexpected four genotypes do not respect the pattern of sex chromosomes segregation in the genetic recombinants from generation F1 and of their recombination in generation F2 and thus should not appear.

The homozygous barred males should have received the barred gene from the heterozygous reddish-black females of generation F1 but instead of this they displayed the gold gene linked to the recessive sex gene (sdw) rather than the barred gene within the chromosome Z. The heterozygous barred females should have received the gold gene together with chromosome W from the heterozygous reddish-black females of generation F1 but instead of this they displayed the barred gene linked to the dominant sex gene (SDW) rather than the gold gene on chromosome W.

The heterozygous reddish-black males should have received the barred gene from the heterozygous reddish-black females, but instead of this they displayed the gold gene linked to the recessive sex gene (sdw) rather than the barred gene within the chromosome Z. The homozygous gold females should have received the gold gene from the heterozygous reddish-black females, but instead of this they displayed the barred gene linked to the dominant sex gene (SDW) rather than the gold gene within the chromosome W.

Morgan (1919) crossed the generation F1 progeny resulting from Langshan males  $\times$  barred Plymouth Rock females and obtained two phenotype categories in generation F2: 50% barred males and females and 50% black males and females.

In our experiments we obtained in generation F2 four categories of phenotypes and three categories of genotypes which, together with the ratio males: female equal to one for each category of genotype, reveal the following: a) in generation F1 the barred gene is located within the chromosome W of the heterozygous females (bB); it determines genetically the black plumage of the heterozygous reddish-black females and represents a particular way of action of this gene within the chromosome W; b) the gold gene is located within the chromosome Z of all F1 subjects and is transmitted to the progeny as any autosomal recessive gene. However, the red color appears only on the neck and head due to the modified allelic interaction between it and the barred-hypostatic gene located within the chromosome W; c) the cross of generation F1 heterozygous males with generation F1 heterozygous females shows that the plumage color inheritance in generation F2 by the heterosomal genes is similar to that of the autosomal genes. One exception has to be noticed, namely that the 49.8% heterozygous progeny are displayed in two phenotypic categories of plumage color summarizing (24.7% heterozygous barred females and males and 25.1% heterozygous reddish-black females and males); d) in generation F1 the females are heterozygous and not hemizygous because of the following distribution: 24.7% dominant homozygous females and males, 49.8% heterozygous females and males and 25.5% recessive homozygous females and males.

The existence of the unexpected categories in generation F2 both in the direct and reciprocal cross might be explained by the presence of a pseudoautosomal region in chicken (Berlin & Ellegren 2004, Wahlberg et al 2007), similar with those observed in Mammalian (Petit et al 1988, Henke et al 1993, Rappold 1993, Chandra 1994, Blaschke & Rappold 2006) and fish sex chromosomes (Traut & Winking 2001, Petrescu-Mag 2007), where the genes determining the plumage color inheritance are located. In that pseudoautosomal region the genes are recombining by crossing-over just like in the autosomal regions, although this area is located in the heterosomes.

The hypothetical SDW gene could be HINTW gene, described by Smith (2007) as an intriguing candidate for a dominant female-determining gene on W chromosome. HINTW gene encodes an aberrant form of a hydrolase enzyme. In chicken embryos, HINTW is strongly expressed in the gonads and other tissues of ZW embryos (Smith 2007). In the same paper is indicated that in vitro biochemical data show the fact HINTW gene can interfere with the action of a Z-linked orthologue: HINTZ, which could be our hypothetical sdw gene. The author underlines HINTW is conserved among flying birds, and recent molecular analysis indicates that it has undergone positive selection over evolution.

THE CROSS OF RED RHODE-ISLAND MALES WITH HETEROZYGOUS BARRED FEMALE

The generation F1 heterozygous barred females produced in the direct cross (Figure 1) was crossed with red Rhode-Island males and produced genetic recombinants that can be sexed by the down color when day-old. The heterozygous males' genotype Bb had black down with a white spot of variable size on their head, while the homozygous females' genotype bb (trade name Robar SL-2002) had red plumage, as shown in Table 5.

The color of the heterozygous barred males down is due to the barred and gold genes from the heterozygous genotype Bb. The color of the homozygous gold females is due to the gold gene located both within the chromosome Z and within the chromosome W.

Table 5

Day-old				18 weeks-old		,
Phenotype and genotype	Nr. of birds	%	Sex	Phenotype and genotype	Nr. of birds	%
Black with a white	birds			Barred (Bb)	1784	36.0
spot on the head (Bb)	2702	50.1	М	Barred body and red neck and head (Bb)	701	14.1
Red (bb)	2686	49.9	F	Red (bb)	2473	49.9
Total number of males and females	5388	100	-	Total number of males and females	4958	100

Down color and sex repartition of day-old and 18 weeks-old genetic recombinants produced in generation F1 ( $\partial$  Red Rhode – Island ×  $\circ$  barred heterozygous)

The color differences of Robar SL-2001 and Robar SL-2002 females show that they can be heterozygous, respectively homozygous for this trait. These results are in contradiction with the hemizygotic mechanism: 36% of the heterozygous males have barred plumage and 14.1% of them have red feathers on the neck and head. 49.9% of the homozygous gold females have red plumage and a recessive homozygous genotype (bb). Down color inheritance of day-old Robar SL-2002 chicks is due to the action of the heterosomal genes barred (B) and gold (b). Day-old Robar SL-2002 chicks sexing by the down color is determined by the allelic interaction between the barred and gold genes from the heterozygous genotype Bb that determines the black down color of the heterozygous barred males. They have a white spot of variable size on the head. The recessive homozygous genotype (bb) for the gold gene (b) determines the red down color of the homozygous females that can easily screened from the males.

### **3. CONCLUSIONS**

The identification of the gene that determines the down color linked to the dominant sex gene within the chromosome W and of the recessive sex allele within the chromosome Z allowed, for the first time, a gene approach of sex inheritance and the development of a new theory, "*The Gene Theory of Sexuality of Gallinaceae*". The experimental results obtained by us require the revision and amendment of Morgan's chromosomal theory of sex inheritance through heterosomes. Consequently we propose the following amendments of the heterosome map modified by Hutt (1936), based on the above described experiments:

<u>1. the simplification of the heterosome map</u>; We propose to replace the two loci (barred-nonbarred and silver-gold) by one polyallelic locus, where the silver (S), barred (B) and gold (s/b) genes should be located;

<u>2. the introduction of the polyallelic locus</u> for the genes within the chromosome W, where the genes silver (S), barred (B) and gold (s/b), responsible for the inheritance of the plumage color should be located. This locus should be similar to the polyallelic locus located within the chromosome Z;

<u>3. the introduction in the heterosome map, both in chromosome Z and in chromosome W, of the locus for the gene determining sex inheritance; thus, the dominant sex gene, SDW, has been identified on chromosome W, while its recessive allele, sdw, was identified on chromosome Z.</u>

According to the new theory we conclude that: a) the gene determinism of sex inheritance is explained by the cross of a recessive homozygous male sdwsdw with a heterozygous female sdwSDW which produces 50% recessive homozygous males sdwsdw and 50% heterozygous females sdwSDW; b) the down color inheritance of the day-old hybrid chicks of generation F1 is due to the heterosomal genes barred and gold within a heterozygous genotype, both in the males (Bb) and in the females (bB); c) F1 day-old hybrid chicks sexing by the colour of the plumage is determined by the allelic interaction modified in the females by the epistatic (E) action of the dominant sex gene (SDW) on the barred gene that turns hypostatic and by the non-modified allelic interaction in the males; d) the colors difference of Robar SL-2001 and Robar SL-2002 females indicated the existence of the barred, respectively gold genes within the chromosome W and evidences the universal character of the homo- and heterozygotic mechanism, in contrast to the hemizygotic mechanism; e) the presence of three categories of genotypes in generation F2 demonstrates both the universal character of the homo- and heterozygotic mechanism and the autosomal origin of the heterosomes. The hemizygotic mechanism is not able to explain our experimental results; f) the simplification of the heterosome map from two to just one polyallelic locus is needed. Within this locus the genes silver, barred and gold should be located and should be responsible for the plumage color inheritance; g) the introduction in the heterosome map of the locus determining sex inheritance. Both chromosomes Z and W should have the sex determining genes.

The hypothetical SDW gene could be HINTW gene, described by Smith (2007) as an intriguing candidate for a dominant female-determining gene on W chromosome. HINTW gene encodes an aberrant form of a hydrolase enzyme. In chicken embryos, HINTW is strongly expressed in the gonads and other tissues of ZW embryos (Smith 2007). In the same paper is indicated that in vitro biochemical data show the fact HINTW gene can interfere with the action of a Z-linked orthologue: HINTZ, which could be our hypothetical sdw gene. The author underlines HINTW is conserved among flying birds, and recent molecular analysis indicates that it has undergone positive selection over evolution.

### **ACKNOWLEDGEMENTS**

The continuous assistance and support of Dr Ursula Lucia Bologa, Degussa Romania SRL and the financial support of Degussa AG, department of feed additives are gratefully acknowledged.

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### CÂMPUL BIOELECTROMAGNETIC ÎN DETERMINAREA SEXULUI GENETIC LA EMBRIONI DE TAURINE

### BIO ELECTROMAGNETIC FIELD IN GENETIC SEX DETERMINATION OF BOVINES EMBRRYOS

### I. BENCSIK, N. PACALA, JANA STANCULET, ALENA BENCSIK

**Cuvinte cheie:** sexul genetic, embrioni, câmpul bioelectromagnetic **Key words:** genetic sex, embryos, bio electromagnetic field

### SUMMARY

The genetic sex determination of bovine embryos was measured by the presence of bio electromagnetic field. The bovine embryos which had a circular movement of the pendulum were considered female embryos and those with transversal movements were considered male embryos. The prediction rate for the group M (87.50%) was relatively comparable with that for the group F (81.82%).

Genetic sex is determined by the interaction of gonosomes XY (male) and XX (female) in Drosophila sex type. Some methods employ sex determination in gametes, other in preimplantational embryos. One of the highly used methods in gametes is based on the flow-cytometric separation of X- and Y-chromosome-bearing sperm based on X/Y DNA content difference (2, 4). Skewed sex ratios of 85 to 95% of one sex or the other were achieved in most species. Embryo sexing has been attempted by a variety of methods including cytogenetic analysis, X-linked enzyme activity assays, detection of male specific antigens, use of Y-specific DNA probes and PCR based assays (6). Cytogenetic analysis is pointing out the sex chromosomes from embryonic cells. Methods like karyotiping (1), Barr body staining (3) can be used to predetermine the genetic sex. These methods rely on embryo biopsy which affects the integrity and the viability of embryos. Measurement of X-linked enzyme activity implies micro-surgical drawing of embryonic cells, their development in special media and the assessment of the quality of enzymes coded by the X chromosome (7). Female embryos having double set of X chromosomes are producing doubled quantity of enzymes. Sex prediction accuracy of 60-70% was reported with this method. The use of antibodies (H-Y) against a Y chromosome specific surface antigen leads to elimination of male preimplatational embryos whereas the female embryos are surviving. (9) Using this non-invasive method sex prediction accuracy of 80,9% was reported in mice and bovine (8). Detection of genes known to be presents either on the Y chromosome or on the X chromosomes were adapted for sexing embryo by polymerase chain reaction (5).

### 1. MATERIALS AND METHODS

The experiments were conducted on H-F cows and heifers breaded in a private farm in Giroc. The superovulatorry treatment was made with the following products: FOLLITOPIN- BIONICHE – Ireland (FSH- follicle stimulating hormone obtained from

pituitary extracts from swine) used to induce poliovulations; INTERGONAN- Intervet, Germany (PMSG- pregnant mare serum- seric gonadotropine ) used to induce poliovulations; CRESTAR Intervet – Holland; is a subcutaneous implant and Norgestomet plus Estradiol valeriat (injectable solution) used to reduce ovary reaction variability and stopping the atrezic process of the small and medium follicles; PROLIZ – Pasteur , prostaglandin F2 $\alpha$  (PgF2 $\alpha$ ) used to control the estrus onset; OVOGEST – Intervet – Germany, chorionic gonadotropine used to control ovulation; PROCAINE 2% used for local anesthesia; LIDOCAINE HIDROCLORID 2% used for epidural anesthesia.

The super ovulation induction was made only in cycling females, which manifest regular estrus. Non-surgical embryo recovery consists in flushing the uterine horns, with a special media, which was introduced in the uterine horns with the help of a flexible two ways catheter. The flushing media used was ViGro COMPLETE FLUSH-AB Technology - USA; embryo holding media - EMCARE - COMPLETE ultra ; CURTIS filter – embryo filtration and examination system (75-80u); Millipore filter 0.22µm, used for sterilization; Petri plates 35 mm, 100 mm; binocular loupes; Mini straws 0.25 ml (cm 3). The non-surgical embryo recovery was made as it follows: the donor female was restrained directly in the stable or in special places and an epidural anesthesia was done using 4-5 ml Procaine 2%. The uterine horns were flushed with flushing media one after the other. After 5 successive flushing when the total flushing media used was 450 ml embryo recovery operation was consider finished. The filter coverage was removed and the embryos were washed with cultivation media, also the filter was washed, and embryo searching was made on the filter plate (which has marks) with the help of the binocular loupe. The embryos that were recovered were transfused into a Petri plate in an embryo holding media, they were examined and evaluated to establish the development stage, quality code and the sex.

The preparing of the embryos for freezing was made as it follows: washing the embryos from the recovery media 10 times; washing the embryos 5 times with PBS+ 0.4%BSA; washing the embryos two times with 0.25% Hank's tripsine media without Ca and Mg fore 60-90 seconds; washing the embryos 5 times in PBS+10% FCS; introducing the embryos in freezing media with glycerol 1.4M or ethylene glycol 1.5 M (for direct transfer, one step).

The method is based on bio electromagnetic field generated by the embryonic cells. The generated bio electromagnetic field can move a pendulum indicating by its movement the sex of the bovine embryos.

The genetic sex determination of bovine embryos was done as followed: by means of a pendulum was measured the presence or the absence of bio electromagnetic field and the genetic sex of the embryos. The bovine embryos which had a circular movement of the pendulum were considered female embryos and those with transversal movements were considered male embryos.

### 2. RESULTS AND DISCUSSIONS

After super ovulation from 9 H-F females were obtained 124 embryos out of which only 87 were good for transfer. The results are presented in the table below:

Table 1

Results concerning	the number of embr	vos after superovulator	v treatment
		$v_{05}$ and $supervyurator$	y u caunciii

				01 011101 J			•••	or <b>J u u u u u u u u u u</b>
Number	of	super	Number	of	Number	of	good	% of good embryos
ovulated fe	males		recovered e	mbryos	embryos	for tr	ansfer	
9			124		87			70.16

From the recovered embryos only 70.16% are good embryos for transfer. The mean of recovered embryos was 13.16 embryos/female and 9.67 good embryos for transfer/ female.

At the embryos which were good for transfer was determined the genetic sex using a pendulum and the results are presented in table 2.

Table 2

10	concerning t	ne chibi yos aiter	sex ucter mination	
Total number	Male embryos	%	Female embryos	%
of embryos				
87	46	52.87	41	47.13

Result concerning the embryos after sex determination

From table 2 it can be observed that after the genetic sex determination from 87 embryos 46 embryos were male (M) and 41 female (F). The rapport between the sexes M/F was 1.12.

In table 3 are presented the results obtained after the transfer of frozen embryos with determined sex.

From table 3 can be observed that after the transfer of frozen embryos with determined genetic sex, from 21 transferred male embryos 8 calves are born (38.10% birth rate). From this born calves 7 were males and 1 female, so the sex prediction precision is 87.5% in males. From 26 female transferred embryos 11 calves were born (42.31%) out of which 9 female and 2 males; the sex prediction precision is 81.82% in females.

Table 3

	to obtained a	ter the trui			in accer in		
	No. of	Born	%	Male	%	Female	%
	transferred	calves		calves		calves	
	embryos						
Male	21	8	38.10	7	87.50	1	12.50
embryos							
Female	26	11	42.31	2	18.18	9	81.82
embryos							

Results obtained after the transfer of embryos with determined sex

### **3. CONCLUSIONS**

1. Genetic sex determination using the bio electromagnetic method can be conducted on bovine embryos.

2. The prediction rate for the group M (87.50%) was relatively comparable with that for the group F (81.82%).

3. This method is non-invasive, relatively rapid, simple and inexpensive with application in bovine non surgical embryo transfer.

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### EFECTUL CONSANGVINIZĂRII ASUPRA PARAMETRILOR BIOCHIMICI LA VIERMII DE MĂTASE (BOMBYX MORI L.)

## THE EFFECT OF INBREEDING ON THE BIOCHEMICAL PARAMETERS IN SILKWORMS (BOMBYX MORI L.)

DINIȚĂ GEORGETA \*, DANIEL DEZMIREAN\*\*, CARMEN GEORGETA NICOLAE\*, CARMEN ANTONESCU\*\*\*, ȘERBAN PURDOIU\* \* University of Agricultural Sciences and Veterinary Medicine Bucharest

\*\* University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca \*\*\*Institute of Research and Development for ApicultureBucharest

**Cuvinte cheie**: consangvinizare, vierme de mătase, linie, blastochineză **Key words**: inbreeding, silkworm, line, blastokinesis

### SUMMARY

During six generations period were studied 30 silkworms inbreeding lines following the effect on inbreeding on some biochemical parameters. The results show that inbreeding process doesn't have significant influence on silkworm eggs proteins and lipids content, sericine gland and larvae hemolymph amino acids content as well as sericine and fibroin contents from silk cocoons.

### 1. MATERIALS AND METHODS

The biologic material utilized in the obtaining of inbred lines was represented by two founding races: Alb Băneasa (AB) and Băneasa 75 (B75), both of indigenous origin, representing the main active races used as parents in the obtaining of industrial hybrids. The inbred lines resulted from the two founding races were obtained by related crossings of brother x sister type during six successive generations. The work methods used for determination of: proteins - Lowery; lipids - lipids extraction in ethylic alcohol mix (ethylic ether 3:1), centrifuging, precipitate weighing; amino acids content - chromatographic method of ionic exchange; sericine and fibroin content - method based on the sericine property of dissolving in alkaline and acid solutions, separating from fibroin. The samples size was in accordance with the specific character of the chemical analysis and the requests of the used method.

### 2. RESULTS AND DISCUSSIONS

The inbreeding effect on the quantity of proteins from the silkworm eggs. The study of proteic content of the silkworm eggs resulted from inbred lines, effectuated in two different moments of embryogenesis did not make evident significant quantitative differences in comparison with the non-consanguineous control (table 1). In the determinations effectuated for inbred lines in I<sub>3</sub> in embryo's elongation stage, the quantity of proteins represented 11.91 % at Băneasa 75 group of lines and 12.16 % at Alb Băneasa group of lines. In the next analyzed stage of embryogenesis, blastokinesis respectively,

the quantity of proteins from eggs at the lines in  $I_3$  was of 13.05 % at the lines resulted from Băneasa 75 and of 14.20% at the lines having Alb Băneasa as founding race. The extension of the quantitativ study of proteic content from the silkworm eggs at the lines in  $I_6$ , in the same embryogenesis stages, pointed out a protein content of 12.22% at Băneasa 75 lines and 12.55% at Alb Băneasa lines in the stage of embryo elongation and 13.66% and 14.62 % in blastokinesis stage, respectively.

The inbreeding effect on the quantity of lipids from silkorm eggs. The lipids content in I<sub>3</sub> was of 98.8 mg / g of eggs at the inbred lines Băneasa 75 in the stage of embryo elongation and of 94.2 mg / g of eggs at Alb Băneasa lines in the same stage of embryogenesis (table 1). In both groups the obtained values are superior to the control but the differences don't present statistical significance. In the same inbreeding generation, but in blastokinesis stage, the quantity of lipids is of 80.6 and 86.0 mg/g of eggs respectively, at the inbred lines and of 81.3 and 85.2 of eggs respectively, at the non-consanguineous. In I<sub>6</sub>, as well as in I<sub>3</sub>, it is noticed a difference of the lipids content related to the embryonar stage, this being of 97.3 and 93.7 of eggs respectively, in the stage of maximum elongation, at the inbred lines, with positive differences comprised between 0.6-1.9 mg in comparison with the control. In the blastokinesis stage the quantity of lipids is of 82.2 and 84.8 of eggs respectively, presenting also a small exceeding of the control, without statistical significance.

The inbreeding effect on the content of amino acids from larvae hemolymph. The study of the content of amino acids from silkworm larvae hemolymph belonging to inbred silkworm lines in  $I_6$ , in comparison with the non-consanguineous control, made evident the presence in all analyzed samples of a number of 18 amino acids (table 2). The maximum values comprised between 11.100 - 11.209 x  $10^{-2}$  g/100 g sample, were recorded in the case of leucine, without being noticed any differences between the two groups of inbred lines or between these and control. From quantitative point of view, it is also noticed the content of lysine and valyne, which presents close values between the inbred lines and the non-consanguineous control. The other amino acids, as phenylalanine, hystidine, serine, argynine, treonyne, quoted in the specialty reference as having an important role in hemolymph composition, were also identified in the case of experimental samples, but their values are below the level of those previous quoted.

**The inbreeding effect on the content of amino acids from sericine gland.** Like in the case of hemolymph, in the sericine gland were neade evident 18 amino acids, both at inbred lines and at non-consanguineous pure races (table 2). In all analyzed samples there were found high quantities of glycine, comprised between the limits of 1.801 - 1.885 g/100 g sample at inbred lines and of 1.970 - 2.008 g/100 g sample at the non-consanguineous ones. On the following positions there are situated alanyne, serine, glutamic and aspartic acid, without significant differences between samples.

Alb Dănonen	Sericine ( %	e (%)	Fibroin ( %	in (%)		Sericine (%	ie ( % )	Fibroin ( %	( % )
AIU Dalicasa	$I_3$	$I_6$	$I_3$	$I_6$	Băneasa 75	$I_3$	$I_6$	$I_3$	$I_6$
Line	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\frac{X}{S} \neq X$	Line	$\overline{X} \pm s_{\overline{X}}$	$\overline{X} \pm s_{\overline{X}}$	$\frac{X}{-S} \neq X$	$\overline{X} \pm s_{\overline{X}}$
AB - 1/1	$24,48\pm0,26$	$26,80\pm0.60$	$75,16 \pm 0,14$	$73,\!20\pm0,\!20$	B75 - 2/1	$27,15\pm0,32$	$27,84\pm0,14$	$72,85 \pm 0,66$	$72,16\pm0,30$
AB - 3/2	$22,87\pm0,18$	$23,86 \pm 0,16$	$77,13\pm0,26$	$76,14\pm 0,14*$	B75 - 4/2	$28,40\pm0,16$	$27,90\pm0,22$	$71,16 \pm 0,15$	$72,10\pm0,21$
AB - 4/3	$24,20\pm0.82$	$24,74 \pm 0,24$	$75,80 \pm 0,34$	$75,26 \pm 0,36^{*}$	B75 - 5/3	$25,30 \pm 0,18$	$26,32 \pm 0,86$	$74,70\pm0.82$	$73,68\pm0,14$
AB - 5/4	$23,88 \pm 0,66$	$24,10 \pm 0,36$	$76,12\pm0,08$	$75,90 \pm 0,44^{*}$	B75 - 6/4	$26,40\pm0,60$	$25,60\pm0.62$	$73,60 \pm 0,74$	$74,40\pm0,10$
AB - 7/5	$25,20 \pm 0,44$	$24,70\pm0,10$	$74,80\pm0,10$	$75,30 \pm 0,46^{*}$	B75 - 8/5	$27,30\pm0,12$	$27,10\pm0.58$	$72,70\pm0,15$	$72,90\pm0.30$
AB - 8/6	$26,84\pm0,30$	$25,84\pm0,18$	$73,16\pm0,14$	$74,16\pm0,10$	B75 - 10/6	$28,\!60\pm0,\!30$	$27,90\pm0,10$	$71,40\pm0,12$	$72,10\pm0.62$
AB - 9/7	$24,12\pm0,12$	$24,78\pm0,28$	$75,88\pm0,12$	$75,22 \pm 0,56^{*}$	B75 - 11/7	$26,17\pm0,60$	$26,88\pm0,14$	$73,83 \pm 0,36$	$73,12\pm0,75$
AB - 10/8	$23,68\pm0,10$	$23,87\pm0,44$	$76,32\pm0,16$	$76,13 \pm 0,12^{*}$	B75 - 13/8	$24,14\pm0,30$	$25,10\pm0.32$	$75,86\pm0,14$	$74,90\pm0.14$
AB - 12/9	$24,84\pm0,36$	$23,80\pm0,32$	$75,16\pm0,26$	$76,20 \pm 0,38*$	B75 - 15/9	$26,15\pm0,28$	$26,10\pm0,26$	$73,85\pm0.65$	$73,90\pm0.12$
AB - 14/10	$22,88 \pm 0,44$	$23,10 \pm 0,18$	$77,12 \pm 0,34$	$76,90 \pm 0,26^{*}$	B75 - 16/10	$27,17\pm0,40$	$26,90\pm0,10$	$72,83 \pm 0,12$	$73,10\pm0,28$
AB - 15/11	$23,84\pm0,12$	$24,60\pm0,12$	$76,16\pm0,38$	$75,40 \pm 0,16^{*}$	B75 - 18/11	$25,15\pm0,14$	$25,80\pm0,16$	$74,85\pm0,44$	$74,20\pm0,36$
AB - 18/12	$22,82 \pm 0,08$	$23,68\pm0,10$	$77,18\pm0,46$	$76,32 \pm 0,10^{*}$	B75 - 19/12	$24,17\pm0,22$	$23,79\pm0,18$	$75,83 \pm 0,42$	$76,21\pm0,40$
AB - 20/13	$23,84\pm0,10$	$24,16\pm0,14$	$76,16\pm0,16$	$75,84 \pm 0,30*$	B75 - 21/13	$27,13\pm0,16$	$26,15\pm0,26$	$72,87\pm0,16$	$73,85\pm0,20$
AB - 22/14	$24,68 \pm 0,34$	$25,68\pm0,30$	$75,32\pm0,32$	$74,32 \pm 0,46$	<b>B</b> 75 - 23/14	$28,18\pm0,12$	$27,20\pm0,18$	$71,82\pm0,12$	$72,80\pm0.45$
AB - 25/15	$25,40 \pm 0,36$	$24,90\pm0,20$	$74,60\pm0,16$	$75,10\pm0,16^{*}$	B75 - 24/15	$26,16\pm0,80$	$25,45\pm0,23$	$73,84\pm0,10$	$74,55\pm0,18$
Mean	$24,24\pm0,28$	$24,57\pm0,25$	$75,76 \pm 0,28$	$75,43 \pm 0,25^{*}$	Mean	$26{,}50\pm0{,}36$	$26,40\pm0,30$	$73,50\pm0,36$	$73,60 \pm 0,30$
Control	$24,98 \pm 0,91$	$26,80 \pm 0,83$	$75,02 \pm 0,91$	$73,\!20\pm0.83$	Control	$27,14 \pm 0,39$	$26,23\pm0,47$	$73.86 \pm 0.38$	$73, 77 \pm 0, 47$

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Table I

Table 2

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		Hemolymph	lymph			Sericin	Sericine gland	
Cracification		$(x \ 10^{-2} g / 100 g sample)$	0 g sample)			(g / 100 g sample)	g sample)	
pecification	Inbre	bred (I <sub>6</sub> )	Cor	Control	Inbred (I <sub>6</sub> )	d (I <sub>6</sub> )	Cor	Control
	AB	B75	AB	B75	AB	B75	AB	B75
Dry matter	7,17	7,50	8,83	8,41	32,00	30,98	32,55	33,17
Lysine	7,800	7,535	7,653	7,517	0,447	0,480	0,604	0,597
Methyonine	0,913	0,936	0,885	0,887	0,183	0,167	0,209	0,217
Histidine	5,200	5,208	5,093	4,932	0,575	0,472	$0,\!439$	0,466
Arginine	3,600	3,617	3,585	3,510	0,258	0,206	0,273	0,300
Aspartic acid	0,388	0,473	0,353	0,385	0,700	0,680	0,713	002'0
Threonine	3,600	3,600	3,383	3,605	0,408	0,350	0,337	0,347
Serine	3,708	3,755	3,700	3,773	0.958	0,959	686'0	1,007
Glutamic acid	0,293	0,283	0,205	0,183	0,883	0,753	0,900	0,829
Proline	0,604	0,514	0,509	0,698	0,300	0,311	0,313	0,310
Glycine	3,819	3,883	3,804	3,730	1,885	1,801	1,970	2,008
Alanine	0,281	0,332	0,328	0,218	1,483	1,346	1,513	1,583
Cystine	0,623	0,772	0,553	0,883	0,051	0,027	0,033	0,080
Valine	7,883	7,831	7,830	7,531	0,386	0,339	0,414	0,400
Isoleucine	0,955	0,905	0,815	0,893	0,283	0,225	0,212	0,278
Leucine	11,203	11,209	11,100	11,105	0,449	0,470	0,485	0,488
Tyrosine	2,300	2,201	2,231	2,200	0,525	0,488	0,553	0,501
Phenilalanine	6,000	6,022	5,898	6,123	0,405	0,306	0,414	0,411
Tryptophan	1,010	1,089	1,081	1,000	0,202	0,115	0,219	0,137

Table 3

	Embarronio		Proteins (%)	IS (%)			Lipids (mg	Lipids (mg / g of eggs)	
Race		I		I	9	Ì	3	Ι	ý
	stage	Inbred line	Control	Inbred line	Control	Control Inbred line	Control	Control Inbred line Control	Control
Băneasa 75	Embryo elongation	$11,91 \pm 0,30$	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$12,22 \pm 0,14$	$12,66 \pm 0,36$	$98,8 \pm 2,5$	$97,8 \pm 1,6$	$97, 3 \pm 1, 8$	$96,7 \pm 1,6$
	Blastokinesis	$13,05 \pm 0,66$	$), 66  \boxed{13,34 \pm 0,10}  \boxed{13,66 \pm 0,11}  \boxed{13,82 \pm 0,16}  \boxed{80,6 \pm 2,2}  \boxed{81,3 \pm 1,2}  \boxed{82,2 \pm 1,3}  \boxed{81,3 \pm 1,4}  \boxed{81,3 \pm 1,4} $	$13,66 \pm 0,11$	$13,82 \pm 0,16$	$80,6\pm2,2$	$81,3\pm1,2$	$82,2\pm1,3$	$81,3\pm1,4$
Alb Bămana	Embryo elongation	$12,16 \pm 0,14$	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$12,55\pm0,82$	$13,10 \pm 0.56$	$94,2 \pm 1,4$	$93,6 \pm 2,2$	$93,7 \pm 1,7$	$91,8\pm1,8$
Dallcasa	Blastokinesis	$14,\!20\pm0.40$	$0,40  \boxed{14,68 \pm 0.52}  \boxed{14,62 \pm 0.28}  \boxed{14,46 \pm 0.32}  \boxed{86,0 \pm 2.7}  \boxed{85,2 \pm 1,6}  \boxed{84,8 \pm 2,2}  \boxed{83,6 \pm 0.8}  \boxed{84,8 \pm 2.2}  \boxed{84,8 \pm 2.2}  \boxed{84,8 \pm 2.2}  \boxed{84,8 \pm 0.8}  \boxed{84,8 \pm 2.2}  \boxed{84,8 \pm 2.2} $	$14,62\pm0,28$	$14,46\pm0,32$	$86,0\pm2,7$	$85,2\pm1,6$	$84,8\pm2,2$	$83,6\pm0,8$

# The inbreeding effect on the quantity of protein and lipid from the silkworm eggs

The inbreeding effect on the content of the content of sericine and fibroin from the silk cocoons. The sericine content from the silk cocoons of the inbred lines is situated between 22.82-26.84% (table 3) for Alb Băneasa group of lines in I<sub>3</sub>, being smaller than in the case of the control for a number of 12 lines. However, the differences are not statistical significant. At the same group of lines, but in the sixth generation of inbreeding, the mean value of the sericine content is of 24.57 %, similar with the mean value obtained in  $I_3$ . Analyzing each inbred line in  $I_6$  there are noticed small positive or negative differences in comparison with I<sub>3</sub>, without significance. Correlated with the sericine content, the quantity of fibroin presents a mean value in  $I_3$  of 75.76 %, very close to control, on the whole lines being situated between 73.16-77.18 %. Values of the fibroin content higher than those of the control are recorded in  $I_6$ , the positive differences being statistical significant in the case of 12 lines. In I<sub>6</sub>, the fibroin content is situated between 73.20-76.90 %, without significant differences in comparison with I<sub>3</sub>. At Băneasa 75 group of lines the sericine content in  $I_3$  has a mean value of 26.50 %, value found again in  $I_6$ , in both generations the quantity of sericine being very close to that of the control. At the same group of lines the fibroin is situated between the limits of 71.16 - 75.86 % in I<sub>3</sub> and between 72.10-76.21 % in  $I_{6}$ , without significant differences between the inbred lines and the non-consanguineous control and neither between the generations I<sub>3</sub> and I<sub>6</sub>.

### **3. CONCLUSIONS**

The quantity of proteins from the silkworm eggs was not influenced by the inbreeding process. Some quantitative differences were correlated with the studied stage of embryonic development. The inbred lines present in both inbreeding generations values of the lipids content superior to the control, but the positive differences do not present statistical significance. The amino acids biosynthesis from sericine gland and larvae hemolymph was not influenced by the practice of related crossings of brother x sister type. In all the samples were made evident a number of 18 amino acids and the quantitative differences between samples, in general non-significant, have not been correlated with the consanguineous or non-consanguineous character of the silkworm lines. The content of silk fibroin or sericine presented values comprised between 24.24 - 26.50 %, varying in accordance with the line and the inbreeding generation.

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### THE COMBINED GENOTYPE PaTfAm WITHIN A STOCK OF SWINE, LANDRACE BREED

### IŞFAN NICOLETA, DRĂGOTOIU TOMIȚA, POPA AL. R, GEORGESCU S.E.

Key words : genetic polymorphism, pre-albumin, transferines, amylases, combined genotype, genetic structure

### SUMMARY

The study of genetic markers and identification of new markers make the subject of an increasing number of research projects in various fields such as genetics of immunology, biochemical genetics, molecular genetics, quantitative genetics and the genetic amelioration of animals. The information provided by electrophoresis graphs has been used to determine the frequency of various categories of alleles (for the loci of pre-albumin, transferines and serum amylases), the frequency of various phenotypes and the genetic structure for each and every locus and, simultaneously, for the loci being studied. The discussion over the varieties of serum proteins was carried on for the purpose of using them as genetic markers, in order to appreciate the levels of genetic unity or diversity within the stock of swine that has been studied. A pair of simple alleles has been determined for each of the three loci. When the three loci were studied simultaneously, out of the 27 possible combinations, only 13 have been found. The sample studied has found to be genetically balanced for every of the three loci. However, when the simultaneous study has been applied, the same sample has not been found genetically balanced anymore.

### 1. MATERIALS AND METHODS

In order to determine the types of serum proteins, blood samples were collected from 76 individuals of the Landrace breed.

The technique of vertical electro-phoresys was employed in order to determine the *transferine* and *pre-albumine* types in the analysed samples, using polyacrylamidae as migration support, the same technique used by Meriaux J.C. (1992).

Electrophoresis in starch gel, in a discontinuous system of buffers was employed, in order to emphasize the types of *serum amylases*, as in Smithies (1955).

### 2. RESULTS AND DISCUSSIONS

### The locus of serum pre-albumins

Three categories of individuals have been described within the lot and they are as following: homozygous  $Pa^{A}/Pa^{A}$ , heterozygous  $Pa^{A}/Pa^{B}$  and homozygous  $Pa^{B}/Pa^{B}$ .

The heterozygous individuals  $Pa^{A}/Pa^{B}$  represent approximately one quarter of the sample.

The three genetic categories are genetically determined by the presence, at the serum pre-albumins locus, of two categories of genes,  $Pa^{A}$  and  $Pa^{B}$  (table 1).

Table 1

Distribution	I OI ge	ene and genotype ca	llegories at ser uni pre	-albuiiiii locus
Genotype		The ratio of	The distribution of	of gene categories
categories	Ν	genotype categories	$\mathrm{Pa}^{\mathrm{A}}$	Pa <sup>B</sup>
Pa <sup>A</sup> /Pa <sup>A</sup>	5	6,5		
Pa <sup>A</sup> /Pa <sup>B</sup>	18	23,7	18,4	81,6
Pa <sup>B</sup> /Pa <sup>B</sup>	53	69,8		

### Distribution of gene and genotype categories at serum pre-albumin locus

### The locus of serum transferines

The interpretation of electrophoresis graphs for the 76 individuals has detected three categories of individuals: homozygous for gene  $Tf^A$ , heterozygous  $Tf^A/Tf^B$  and homozygous for gene  $Tf^B$ . The presence of the three genotype categories in the lot proves the presence of two categories of genes,  $Tf^A$  and  $Tf^B$ , identified with different frequency (Table 2).

Table 2

Gonotypo		The ratio of	The distribution of gene categories	
Genotype categories	N	genotype categories	$\mathrm{Tf}^{\mathrm{A}}$	$\mathrm{Tf}^{\mathrm{B}}$
Tf <sup>A</sup> /Tf <sup>A</sup>	3	4		
Tf <sup>A</sup> /Tf <sup>B</sup>	20	26,3	17,2	82,8
Tf <sup>B</sup> /Tf <sup>B</sup>	53	69,7		

Distribution of gene and genotype categories at serum transferines locus

### Locus of serum amylases

The interpretation of electrophoresis graphs led to the identification of three categories of individuals in the sample. They are as following: homozygous  $Am^A/Am^A$ , heterozygous  $Am^A/Am^B$  and homozygous  $Am^B/Am^B$ .

The highest percentage of individuals in the sample was homozygous  $Am^B/Am^B$ . The heterozygous  $Am^A/Am^B$  represents a quarter of it. After calculating the frequency of the genes categories, the gene  $Am^A$  seems to be expressed at a very low frequency (Table 3)

Table 3

Genotype		The ratio of	The distribution of gene categories	
Genotype categories	Ν	genotype categories	Am <sup>A</sup>	Am <sup>B</sup>
Am <sup>A</sup> /Am <sup>A</sup>	3	4		
Am <sup>A</sup> /Am <sup>B</sup>	19	25	16,5	83,5
Am <sup>B</sup> /Am <sup>B</sup>	54	71		

### Distribution of gene and genotype categories at serum amylases locus

Determining the ratios of the genotype categories and, as a result, the genetic structure of the sample in this study allowed building an estimate for the state of genetic equilibrium, for each of the three loci analyzed. The results are presented in Table 4.

equilibrium, for each of the three loci analyzed. The results are presented in Table 4. The analysis of genetic equilibrium was made using  $\chi^2$ , test, and it led to the conclusion that the studied sample express genetic equilibrium for each of the three loci.

Table 4

Genotypes			d²/A		
	observed	expected			
	Serum pre-a	lbumins locus			
Pa <sup>A</sup> / Pa <sup>A</sup>	5	2,573	2,289		
Pa <sup>A</sup> / Pa <sup>B</sup>	18	22,822	1,019		
Pa <sup>B</sup> /Pa <sup>B</sup>	53	50,605	0,113		
Total	76	76	$\chi^2 = 3,421$		
	Serum transferine locus				
Tf <sup>A</sup> / Tf <sup>A</sup>	3	2,248	0,251		
Tf <sup>A</sup> / Tf <sup>B</sup>	20	21,647	0,125		
Tf <sup>B</sup> / Tf <sup>B</sup>	53	52,105	0,015		
Total	76	76	$\chi^2 = 0,391$		
Serum amylases locus					
Am <sup>A</sup> /Am <sup>A</sup>	3	2,069	0,419		
Am <sup>A</sup> /Am <sup>B</sup>	19	20,942	0,180		
Am <sup>B</sup> /Am <sup>B</sup>	54	52,989	0,019		
Total	76	76	$\chi^2 = 0,618$		

The estimate of genetic equilibrium

The analysis of combined genotypes at the loci of pre-albumins, transferines and serum amylases.

The simultaneous analysis of the three loci, pre-albumins, transferines and serum amylases, respectively, reveals the high percentage of individuals that express B type of pre-albumins, B type of transferines and B type of serum amylases, the latter being represented in more than one third of the studied sample.

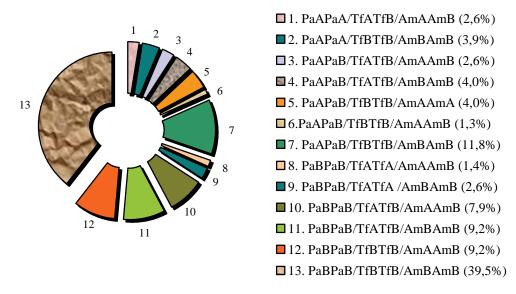


Figure 1. The distribution of the categories of combined genotypes (%)

The individuals who are homozygous for B genes, at the loci for transferines and serum amylases, and are heterozygous for the pre-albumins locus, express the lowest frequency. This frequency is three times lower than that of the individuals with the highest observed frequency.

On the third place, regarding the participation ratio at the genetic structure of the population, we have found individuals that express the aggregate genotypes  $Pa^{B}Pa^{B}/Tf^{B}/Am^{A}Am^{B}$  and  $Pa^{B}Pa^{B}/Tf^{A}Tf^{B}/Am^{B}Am^{B}$ , each with a percentage of 9.20.

Half of the possible combinations amongst the three loci have not been identified in the studied sample. Amongst the missing combinations we have counted the homozygous genotypes  $Pa^{A}Pa^{A}$ ,  $Tf^{A}Tf^{A}$  and  $Am^{A}Am^{A}$ . This is a logic consequence of the low frequency recorded for genes  $Pa^{A}$ ,  $Tf^{A}$  and  $Am^{A}$ .

It is interesting that, even though heterozygous  $Pa^{A}Pa^{B}$  represent 23.7%, homozygous  $Tf^{B}Tf^{B}$  show a frequency of 69,8% and heterozygous  $Am^{A}Am^{A}$  represent a

quarter of the studied lot, the aggregate genotype that results from this combination occurs with the lowest frequency, that is 1.3%.

The determination of the genetic structure allowed the estimate for the genetic equilibrium for the Landrace breed lot. The twenty-seven categories of possible genetic combinations have been lined up to form a matrix with three rows and nine columns, having the following structure:

$$G = \begin{bmatrix} G_{11} & G_{12} & G_{13} & G_{14} & G_{15} & G_{16} & G_{17} & G_{18} & G_{19} \\ G_{21} & G_{22} & G_{23} & G_{24} & G_{25} & G_{26} & G_{27} & G_{28} & G_{29} \\ G_{31} & G_{32} & G_{33} & G_{34} & G_{35} & G_{36} & G_{37} & G_{38} & G_{39} \end{bmatrix}$$

And when replaced with actual values:

$$G = \begin{bmatrix} 0 & 0 & 0 & 0,026 & 0 & 0 & 0,039 \\ 0 & 0 & 0 & 0,026 & 0,040 & 0,040 & 0,013 & 0,118 \\ 0 & 0,014 & 0,026 & 0 & 0,079 & 0,092 & 0 & 0,092 & 0,395 \end{bmatrix}$$

For each locus, at the population level, there are three possible categories of genotypes, enforced by the existence of a pair of simple alleles for each locus. This means that, at population level, considering the categories of genotype combinations previously presented, the following categories of gametes are possible:

Knowing the frequencies of the categories of genotypes we could calculate the gametes pool of the population.

As in the case of genotype categories, the gametes categories were lined up to form a matrix with two rows and four columns, as following:

$$g = \begin{bmatrix} g_{11} & g_{13} & g_{17} & g_{19} \\ g_{31} & g_{33} & g_{37} & g_{39} \end{bmatrix}$$
  
and with values  $g = \begin{bmatrix} 0,010 & 0,020 & 0,033 & 0,121 \\ 0,030 & 0,112 & 0,092 & 0,582 \end{bmatrix}$ 

The condition for equilibrium is met when :

$$(g_{11} x g_{33} x g_{17} x g_{39}) - (g_{31} x g_{13} x g_{37} x g_{19}) = 0$$

For the studied case, the condition of equilibrium is not met because:

$$(0,010 \times 0,112 \times 0,033 \times 0,582) - (0,030 \times 0,020 \times 0,092 \times 0,121) \neq 0$$

It is to be remarked that, for the studied sample, even though for the three loci taken separately, the condition of equilibrium has been met, when working with combined genotypes (pre-albumins, transferines, and amylases) this condition is not achieved.

### **3. CONCLUSIONS**

1. At the locus of serum pre-albumins three genotype categories have been identified,  $Pa^{A}Pa^{A}$ ,  $Pa^{A}Pa^{B}$  and  $Pa^{B}Pa^{B}$ . They are controlled by two categories of genes  $Pa^{A}$  and  $Pa^{B}$ .

2. At the locus of serum transferines we have identified a pair of simple alleles:  $Tf^A$  and  $Tf^B$ . The two gene categories can translate into three types of genotypes  $Tf^ATf^A$ ,  $Tf^ATf^B$  and  $Tf^BTf^B$ .

3. At the locus of serum amylases, the two gene categories ( $Am^A$  and  $Am^B$ ) lead to three categories of genotypes  $Am^A/Am^A$ ,  $Am^A/Am^B$  and  $Am^B/Am^B$ .

4. Out of the 27 possible genotype combinations that can occur at the three loci, only thirteen have been identified in the studied sample. Amongst the missing genotypes we counted the homozygous genotypes  $Pa^{A}Pa^{A}$ ,  $Tf^{A}Tf^{A}$  sau  $Am^{A}Am^{A}$ .

5. The analyzed sample shows a balanced genetic structure for leach locus taken separately. However, when the three loci are taken together, the sample does not meet anymore the condition for genetic equilibrium.

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### CROSSTABULATION MODELS FOR ASSOCIATION OF GENETIC MARKERS WITH HEALTH STATUS IN SMALL RUMINANTS

### MODELE DE CROSSTABULARE PENTRU ASOCIEREA MARKERILOR GENETICI CU STAREA DE SĂNĂTATE LA RUMEGĂTOARELE MICI

GH. HRINCĂ, M GROZA

**Key words:** genetic marker, crosstabulation, mathematical model, sheep, goats **Cuvinte cheie:** marker genetic, crosstabulare, model matematic, ovine, caprine

### SUMMARY

The paper tries to achieve some mathematical models concerning the correlation (association) between genetic markers and health status in small ruminants (sheep and goats). These experimental models are conceived by the crosstabulation method. Different biostatistical parameters and concepts are used to construct these models. The application is explained by some demonstrative examples, both likeness table and diagrammatically.

A very important problem to increase the productivity of sheep and goats is the application of the adequate selection and exploitation technologies in order to assure to the animals proper conditions of feeding, care, maintenance and reproduction to avoid the loss caused by illness and mortality (1). In the world, the pathological problems in sheep represent a critical chapter of productivity in these species. Consequently, all these phenomena induce negative aspects on qualitative, organoleptic, chemical or industrial processing of all small ruminant productions (6). To solve these inconveniences the researches from the different biology fields are very important. The biochemical genetics, immnunogenetics and molecular genetics represent new frontiers of genetics which will lead on long term to essential gains of animal productivity in the ovicaprinae breeding field. The aim of these researches is to identify the marker-genes and their functions in sheep and goats which to be used to achieve rapid genetic gains concerning health, welfare and productivity in these two species (3, 5). But to reach this target the modern husbandry need of supple methods to anticipate these genetic gains using mathematical and informatics concepts (2, 4). Therefore, this study proposes a mathematical methodology of correlation of genetic markers with morbidity entities in small ruminants.

### 1. MATERIALS AND METHODS

### **DESCRIPTION OF THE METHODOLOGY**

The present study proposes the realisation of certain *mathematic models by crosstabulation* concerning the relation of genetic markers (biochemical-genetic, immunogenetic or molecular genetic markers) with different morbid entities which affect the sheep and goat populations. According to the number of variables and the specificity of the dependent variables (blood genotype, pathological entity), the mathematic models can be of two types: a monofactorial analyse model and a polyfactorial analyse one.

For the *monofactorial analyse mathematic model* the dependent variable is represented by a physiologic feature of the animals (e.g. health) with two aspects (healthy animals and ill animals) and the independent variable of a certain blood genotype (with all its expression variants) of a biochemical-genetic, immunogenetic or molecular genetic systems.

For the *mathematic model of polyfactorial analyses* the dependent variable is represented by a physiological feature of animals (e.g. health) with two aspects (healthy or ill animals), and the independent variable of a certain blood genotype (with all its expression variants) of a biochemical-genetic, immunogenetic or molecular genetic systems and of the diseases groups (infectious, parasitical, metabolic, genetic). On their turns, these general features are determined by certain specific characteristics (environmental, technological, experimental factors, etc). The multiplication of these features changes the mathematic model attributes and renders difficult the calculus algorithm but enriches more the information referred to the interdependencies which are settled between these factors.

### 2. RESULTS AND DISCUSSIONS

### **Demonstrative model for crosstabulation**

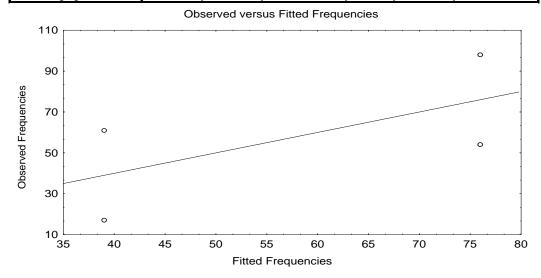
These factorial analyses use as operational levers, the *observed and expected (fitted) frequencies* of different physiological and physiopathological features on each blood genotype (tab. 1). These models can have graphic representations and depending on the point physiognomy and the straight line direction (which can be oblique on the abscissa or parallel to the abscissa) we can prove the existence of influence of some genetic structures on animal health. If the straight line is oblique on the abscissa, it means that there is a relationship between a certain genetic marker and a certain disease, the intensity of the relationship being according to the straight line obliquity. If the straight

line is parallel to the abscissa axis, this relationship would be inexistent (fig. 1).

The observed and fitted frequencies of health status of animals depending on different
blood genotypes

Table 1

	Observed frequency (f <sub>o</sub> )			Fitted frequency (f <sub>f</sub> )		
Specification	Blood g	enotype	Whole	Blood	genotype	Whole
	AA	BB	population	AA	BB	population
Health	98	54	152	76	76	152
Disease	17	61	78	39	39	78
Whole population	115	115	230	115	115	230



*Figure 1.* Observed and estimated frequencies of health status of animals, depending on different blood phenotypes concerning the health status of animals depending on different blood genotypes

The conceptualizations of residual frequencies and standard residual frequencies are very important to approach the factorial analyses by crosstabulation.

The *residual frequency*  $(\mathbf{r}_{ij})$  is the difference between the observed frequency and the fitted one (tab. 1), according to the formula:

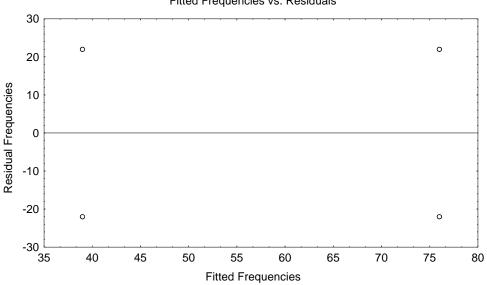
 $r_{ij} = f_{ij} \cdot F_{ij}$  in which, f = observed frequency; F = fitted frequency; i = blood genotype; j = health status.

Table 2

Residual frequencies concerning the health status of animals depending on different
blood genotypes

Specification	Blood g	Whole population	
specification	AA	BB	whole population
Health	22	-22	0
Disease	-22	22	0
Whole population	0	0	0

The graphic configuration shows if there is or not the correctness of the representation model that was chosen. If the straight line is parallel to the abscissa axis, the model is correctly chosen. If the straight line is oblique on the abscissa, regardless of its sense, it means that there is a significant deviation of the experimental data toward to model (fig. 2).



Fitted Frequencies vs. Residuals

*Figure 2.* Graphic representation of dependence of estimated frequencies related to residual frequencies concerning the health status of animals depending on different blood

### genotypes

The *standard residual frequency*  $(s_{ij})$  is the ratio between the residual frequency and the square root of fitted frequency (tab. 3), according to the relation:

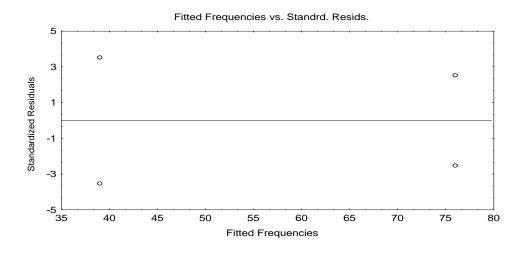
$$s_{ij} = \frac{f_{ij} - F_{ij}}{\sqrt{F_{ij}}}$$

Table 3

# Standard residual frequencies concerning the health status of animals depending on different blood genotypes

Specification	Blood gen	Whole population	
Specification	AA	BB	whole population
Health	2,52357316	-2,52357316	0
Disease	-3,522819281	3,522819281	0
Whole population	-0,99924612	0,99924612	0

The difference between the residual frequencies and the standard residual frequencies is that the standard residual frequencies are obtained only *if the experimental error is normally distributed* (fig. 3).





# *Figure 3.* Graphic representation of dependence of estimated frequencies related to standard residual frequencies concerning the health status of animals depending on different blood genotypes

In order to test if the individual experimental data are adequate for the proposed mathematic model, *the elements of maximum probability (verisimilitude)* ( $c_{ij}$ ) are added into the calculus (tab. 4). These components show the significance degree of the individual experimental data towards the mathematic model. This statistical parameter is calculated by the relation:

$$c_{ij} = 2f_{ij}ln \frac{f_{ij}}{F_{ii}}$$
 in which

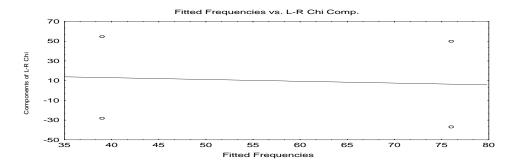
f = observed frequency; F = expected frequency; i = blood genotype; j = health status.

Table 4

# Estimated frequencies related to components of maximal probability concerning the health status of animals depending on different blood genotypes

Specification	Blood	genotype	Whole population
specification	AA	BB	whole population
Health	49,8298912	-36,9089241	12,9209671
Disease	-28,23184204	54,57209015	26,34024811
Whole population	21,59804916	17,66316605	39,26121521

By graphic representation and according to the point physiognomy and the straight line direction, we could show the mathematic model verisimilitude, as well as the aptness degree of individual experimental data (*fitted frequencies*) at the mathematical model (*components of L-R Chi*).). If the straight line is parallel to the abscissa axis, the individual experimental data are suited to the proposed mathematic model. If the straight line is inclined to the abscissa, there are significant deviations of the individual experimental data from the proposed mathematic model (fig. 4).



*Figure 4.* Graphic representation of dependence of estimated frequencies related to components of maximal probability concerning the health status of animals depending on different blood genotypes **3. CONCLUSIONS** 

1. The paper presents a mathematical methodology concerning the correlation (association) of genetic markers (biochemical-genetic, immunogenetic or molecular genetic markers) with health status in sheep and goats, using the crosstabulation.

2. Depending on the number of variables and the specificity of the dependent variables (blood genotype, pathological entity), two mathematical models by crosstabulation are described: the monofactorial analyse model and the polyfactorial analyse model.

3. To validate the functionality of these experimental models some biostatistical parameters (observed frequency, fitted frequency, residual frequency and standard residual frequency) and biostatistical concepts ((the elements of maximum probability or of verisimilitude)) are used.

4. More demonstrative models for crosstabulation are presented, both likeness table and diagrammatically.

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