

**Szent István University**  
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**Phenotypical and genotypical investigation of th Hungarian  
Grey cattle breed**

Thesis of PhD dissertation

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**2010**

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## **1. Introduction**

In this thesis the phenotype and genotype of the autochthonous Hungarian Grey cattle breed were investigated.

With the Video Aided body measurement (VAM) method the current phenotypic description of the present stock were carried out. Furthermore based on mitochondrial DNA the origin and the mitochondrial diversity were investigated. The last study is focusing on a possible genetic sign of heat stress adaptation in the Hungarian Grey breed.

## **2. Video Aided Measurement method for conservation of phenotypical traits of an indigenous cattle breed**

### **Introduction**

The phenotypical characterisation besides monitoring the genetic diversity of endangered breeds is also an important task of conservation management.

The autochthonous beef breeds are frequently kept in extensive technology. Taking of body measurements as one of the most important part of phenotype in these populations with the classical methods (e.g.: stick, tape) due to the often lower level of domestication, and sometimes dangerous horn conformation is not safe. The optometric Video Aided Measurement (VAM) method calculate the measurements from the captured still pictures of recorded video and could be a more safe, practical and faster way of measuring because it stops the motion picture and not the animal. The other aspect of the VAM method is archiving, because the video records and the still pictures of the animals from two perspectives

will be not only “by-products” in the future.

In our work, as a case study, the body measurements of three podolic beef breed were measured and compared. All groups or herds of the breeds are in extensive keeping, and were not measured before. The three podolic breeds have been kept in semi wild, open-pasturing system for centuries.

### **Material and Methods**

In the indigenous Hungarian Grey breed the last comprehensive measuring was made in 1965 with stick and tape. Nowadays, after the bottleneck of 1960's, the breed status is not more endangered, and parallel to the preservation work the commercial selection for beef production has started. In this study, data of 1090 cows from the HG herd of Hortobágy, Bugac, Tiszaigar, Apaj, Sarród were analyzed. These herds are the breeding centres of these cattle. The Hortobágy herd (n=1090) of 1960's was the founder of the surviving HG population.

The autochthonous Maremmana beef breed of Italy is phenotypically very similar to the HG. The Torre Mancina stock is one of the nucleus stock of the breed (n=26).

The Anatolian Grey is autochthonous, small frame cattle breed of Turkey. It is in critical status and only a small nucleus has been involved to the Turkish Gene Preservation programme at Bandirma Gene Preservation Station (n=30).

Cows older than two years were measured in all herds.

A computerised optometric method was developed based on video recordings, which is able to measure the animals on the captured still pictures. The Video Aided Measurement (VAM) method was tested

for the methodical errors, and it has been developed more precise than the classical methods. The VAM measures are exchangeable to the classical measures with correlation functions.

The optometric VAM method enables recording approximately one hundred animals per hour if the leading of animal is continuous at the recording scene. The measurements were taken using the pictures of the digital video recording by two digital cameras from different perspectives. The output, comma separated (.css) file of this software contains the measures in centimetre.

Table 1: The body measurements of Hungarian Grey cows by herds and summarised in cm, standard deviation in brackets

breed	farm	n	Height at withers	Height of back	Rump height	Trunk length	Chest depth	Body length	Width of chest	Ischium width of rump
<b>Hungarian Grey</b>	Apaj	213	133(5,6)	134(6,6)	137(7,0)	148(7,8)	79(3,7)	142(8,0)	44(3,6)	20(2,5)
	Tiszaigar	178	138(4,7)	133(4,7)	134(4,7)	165(9,1)	77(3,3)	162(9,1)	48(3,7)	22(3,4)
	Hortobágy	394	130(4,4)	129(4,6)	131(4,1)	157(6,9)	76(3,5)	151(6,9)	45(3,2)	28(3,3)
	Bugac	192	133(5,0)	130(5,2)	131(5,9)	163(8,8)	78(3,7)	160(8,6)	44(5,9)	20(3,1)
	Sarród	113	133(5,8)	132(5,9)	136(6,5)	154(8,7)	71(4,8)	147(8,8)	47(4,3)	24(3,0)
	<b>total</b>	<b>1090</b>	<b>133(5,7)</b>	<b>131(5,8)</b>	<b>133(6,1)</b>	<b>157(9,8)</b>	<b>76(4,3)</b>	<b>152(10,7)</b>	<b>45(4,3)</b>	<b>24(4,6)</b>

## Results

The means and variances of the body measurements were calculated for all the measured cows and for the herds separately (table 1.)

## **Discussion**

The phenotypical differences among Anatolian Grey and the two European podolic breed were well visible also at the beginning. The harsh climatic and grazing conditions and the lack of classical, organised breeding selection resulted in smaller frame and less productivity. The differences between Hungarian Grey and Maremmana breed are not well expressed. In the Italian breed the selection work for higher meat production has been continuous from the beginning of the last century. Both the body measurements are higher than in the Hungarian Grey and the body proportions are different. The body is longer and accordingly the top-line is softer than in Hungarian Grey Breed. These changes show a greater capacity of meat production in Maremmana Breed. The discriminant function analysis showed that the height at withers and the trunk length have the main factor in discrimination of herds.

The measurements of Hortobágy herd are very close to the total mean, and with the high level of variance, presumably it shows the success of preserving selection.

Based on these results the efficiency of the preservation programme of the HG following the serious bottleneck is calculable on the level of phenotype.

Nowadays the success of conservation programmes of farm animal breeds is measured predominantly on molecular level, maybe due to the fast and precise methods and results.

The phenotypical aspects of conservation needs also easy-to-use methods, and the VAM method could be an alternative and a potential tool for this task.

### **3. Genetic diversity and origins of the Hungarian Grey cattle breed based on mitochondrial D-loop sequence variation**

#### **Introduction**

The Hungarian Grey (HG) is a traditional, long horned, Podolian type cattle breed of Hungary, classified with the primigenius craniological group. The origins of this breed is largely unknown, since archaeozoological research produced no horn cores from long horned cattle before the end of the Late Middle Ages in the Carpathian Basin, although by the early 16<sup>th</sup> century Hungarian cattle were driven on a large scale to urban markets in Southern Germany and Northern Italy. The first explicit reference to long-horned, *magnus cornutus bos Hungaricus*, appears only in a 16<sup>th</sup> century document when the export increased. Large horn core finds, occurring somewhat more frequently by the 18<sup>th</sup> century, are still smaller than those of modern HG cattle. After the “Golden Age” of the HG breed (in 1884, ca. 80% of 4.9 million cattle) a decline began. In 1962, only 200 purebred HG cows and 6 bulls were saved from a systematic policy of slaughter. During its history foreign cattle breeds did not exert any impact on purebred HG. To date, the increasing number of breeding animals (over 7000 cows and 150 bulls) has shown clear signs of recovery and the success of genetic preservation.

The origins of the HG breed are still unknown. Four competing hypotheses have been put forward:

*Theory No. 1:* these cattle came to the Carpathian Basin with the Conquering Hungarians during the 9<sup>th</sup> century;

*Theory No. 2:* they were domesticated from local aurochs

populations;

*Theory No. 3:* they arrived during the latest, 13<sup>th</sup> century waves of eastern, Cumanian immigrations;

*Theory No. 4:* the modern HG breed may be seen as a result of relatively intensive local breeding work during the 17<sup>th</sup>–19<sup>th</sup> century.

The objective of our study was to review and test these theories of origin by calculating the genetic diversity of the HG breed based on mtDNA displacement loop (D-loop) sequences.

## **Materials and Methods**

**Sample Collection.** The surviving HG stock was saved in Hortobágy National Park during the 1960's. The sampling method (founder sampling) focused on the maternal inheritance of mitochondria. In an effort to find as many maternal lines as possible, the studbook database was processed with yED 3.1. network visualization software (yWorks GmbH) to identify matrilineal families. The maternally unrelated samples were assigned on the basis of the separated mitochondrial lines. Of the 200 original founder maternal lines 18 were extinct. Eighty animals from 80 different lines from the remaining stock were selected randomly for further investigation. The average number of generations between founder and sampled individuals was 14.5.

Genetic distances between HG and other breeds and groups of breeds were estimated by comparisons with well-annotated D-loop sequences of *Bos taurus* breeds downloaded from the GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). The groups used in these comparisons were as follows: breeds in the so-called Podolian group: Maremmana, Chianina, Romagnola and Greek Grey, and for



the broader geographical comparison: modern Old World breeds from Europe, Africa, the Near Eastern, and the rest of Asia, Archaic: mtDNA sequences of ancient European and Near Eastern cattle bones.

### **Results**

mtDNA haplotypes in HG: mtDNA D-loop sequences of the 80 founder lines resulted in 36 different haplotypes defined by 43 polymorphic sites. Each haplotype can be assigned to four of the five common haplogroups, which are defined by a 240 bp long track of the mtDNA D-loop: 74 to T3, 3 to T2, 2 to T and 1 to T4 which tends to occur in Asian breeds. The T1 haplogroup, distributed phylogeographically first of all in Africa, was not observed in the HG breed. The Anderson T3 reference sequence was the most common haplotype (29 times), and 7 other haplotypes were also found more than once.

The P and E haplotypes, assigned to the European aurochs, and the hypothesized surviving Q aurochs haplotype were not found in HG. The haplotype diversity (H) was  $0.8595 \pm 0.0364$ , while nucleotide diversity (Pi) was  $0.005758 \pm 0.003543$ .

In the comparison, made on sequences of European cattle bones from the Late Neolithic, the Old World breeds were grouped by continent except for Podolian breeds that were kept as a separate group (Table 2.).

Table 2: Pairwise  $F_{st}$  s (upper diagonal) and the  $F_{st}$  p values (lower diagonal) between “Old World” breeds

	HG	Podolian	European	Archaic	Near-Eastern	African	Asian
HG		0.05229	0.01450	0.11308	0.06995	0.55987	0.13777
Podolian	0.00000		0.01611	0.09911	0.01682	0.37888	0.12233
European	0.00198	0.00198		0.20873	0.04673	0.42557	0.16660
Archaic	0.00000	0.00000	0.00000		0.11734	0.24550	0.15740
Near-Eastern	0.00000	0.00430	0.00000	0.00000		0.32629	0.11641
African	0.00000	0.00000	0.00000	0.00000	0.00000		0.21259
Asian	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	

### Discussion

The method of “founder” sampling seemed to be suitable for estimating the number of different mitochondrial haplotypes in the HG population that survived the bottleneck. The genetic diversity of HG mitochondrial D-loop sequences in founder lines does not show serious consequences of the genetic drift that took place during the 1960s. This may be explained by the success of the decades long HG conservation program and by the luckily diverse surviving breed nucleus.

The grouping of HG haplotypes displays a pattern typical for European breeds, but in contrast to previous results the distribution of T haplotypes is less diverse (Figure 2).

Due to the missing P and Q haplotypes of aurochs from the HG breed and the D-loop haplotypes from aurochs bones from Hungary theory No. 2 of local domestication of HG can be rejected.

The spread of domestic cattle on the European continent from the Near East pre-dated the hypothesized extinction of European

aurochs. This is also confirmed by the high percentage of T3 haplotypes in the present European breeds. One of the mtDNA sequences obtained from Late Neolithic domestic cattle bone was found in one of the HG cow families.

The results of genetic distance calculations, and PCA analysis (Figure 3) between Podolian breeds show the closest relationship between HG and the Romagnola breed.

Due to the short geographical distance between the Carpathian Basin and the traditional breeding centre of Italian breeds (provinces of Forli, Bologna and Ravenna) such a phylogenetic connection would be possible. Surprisingly, the striking similarity in body conformation between the similarly long horned Razza Maremmana and HG is not reflected in these mtDNA results.

In the comparison between Old World breeds grouped by continents, the position of the Podolian group and HG were consistent with the theory of Edwards et al. (2007) concerning the Near Eastern domestication and spread of the Neolithic ancestors of modern European breeds.

Based on the mtDNA Fst values, the HG is closest to the European average. Podolian breeds and Near Eastern cattle as well as aurochs from Hungary and other breeds are increasingly distant from this European point of reference. This phenomenon clearly confirms that the local domestication of female aurochs in the Carpathian Basin may be ruled out as a source of HG.

The second closest group to archaeological sequences after the Near Eastern group is the Podolian breed group. The possible reason for this close relation could be a continuing connection

between eastern Italy and the Near East, possibly resulting from later (e.g. medieval) maritime cattle export.

Hypothetical cattle export could also be the reason behind the small genetic distance between HG and the Podolian group, because dynamic movements of stock related to export drives were common in the Carpathian Basin as well. The calculated genetic distance shows, however, that the theory of a renewed eastern import of domesticated cattle by Cumanian mediation during the Middle Ages (theory No. 3) is also questionable.

The mitochondrial haplotype diversity in HG is very similar to the indices found in other European cattle breeds, but due to the special sampling method, the direct comparison of these values needs further investigation.

Theory No. 4 of the possible development of local cattle into the HG breed is best supported by the results of this study. It is impossible that, as suggested by theory 1, any breed would have survived a period of over 1000 years unchanged since the 9<sup>th</sup> century. On the other hand, a demand for both draught oxen and for a trademark appearance of a long horned beef cattle in foreign markets in the 17<sup>th</sup>–18<sup>th</sup> century may have stimulated the conscious creation of this breed at the time (Matolcsi 1982).

This study offered a look into the varieties of possible origins of HG, and helped excluding local aurochs domestication and Late Medieval imports as possible sources of this breed. For a final, comprehensive answer, however, more studies involving a more refined reference population will be necessary.

#### **4. Possible genetic sign of heat stress adaptation in Hungarian Grey breed**

##### **Introduction and aim of the study**

Global climatic changes pose challenge to the wild and production animals in the near future. Long-term climatic models predict that the present breeds of low heat tolerance face serious risks for the stability in their production. Solymosi and al. have specified the possible regional elevation in the number of heat stress days per annum to quantify environmental stress in the past and in the future in Hungary.

These results emphasize the importance of preparing for the heat stress problems in animal production. Beside changes in management technologies the introduction of heat tolerant populations seems to be the most obvious step in the rapid adaptation to the new climatic condition.

For the microevolution in heat regulation the cell level heat stress adaptation is based on a genetic framework (fixation of heat tolerance genes) and mutations affecting heat tolerance (specific genetic structures, differences in gene expression, differences in mRNA levels, etc.).

Among the above genetic pathways recent studies highlighted the role of HSP70 gene and gene products and differences in its expression level in the cellular regulation of heat tolerance and in heat stress. In cattle, however, the molecular genetic bases of heat tolerance are not completely understood up to the present.

Living organisms respond to stress at the cellular level by a transient but rapid and strong increase in the biosynthesis of various stress

proteins including those of the heat shock protein (HSP70) family (25). In the category of heat shock proteins different molecules with different effects are presented. The investigated HSP70 family frequently constitutes the predominant factor in protein synthesis in stressed cells and plays a central role in proper folding, oligomerisation, and transport of polypeptides across. Expression of inducible HSP70 genes is regulated mainly at the transcription level. The amount of the synthesized protein, although may mean some problems for the organism, due to its high energy content, has a linear relationship to its protective potency. The role of HSP70 genes is manifold and our studies focus only on the functions determining heat tolerance at cellular level. In heat stress HSP associated cell level changes lead to alterations in the level organism as well. HSPs enter the circulation where detectable levels are measured, affect the survival of embryos and in their absence the heat stress may be lethal (3).

The promoter region of the HSP70.2 gene cluster shows polymorphism (wild type and AP2 mutant) in contrast to the conservative coding sequences. The different types of the region associated with regulation basically differ in the amount of mRNA produced by the gene, which determines the amount of the synthesised protein.

According to published data allele variants associated with higher level of mRNA products confer better heat tolerance in cattle than other allele variants.

In this study frequencies of different HSP70 promoter alleles were investigated as a possible indicator of environmental adaptation in an indigenous Hungarian Grey, and in a Norwegian Red cattle

population as a control group.

### Material and Methods

The Hungarian Grey (HG) is a traditional, long horned, Podolic type cattle breed of Hungary, classified with the primigenius craniological group. The origin of this breed is largely unknown, although by the early 16<sup>th</sup> century Hungarian cattle were driven on a large scale to urban markets in Southern Germany and Northern Italy. Its breeding centre is Hortobágy on the Great Hungarian Plane for centuries which was and is one of the warmest regions of the country. 253 HG blood sample were taken randomised, from not closely related cows. The NFR was established in 1935 when they crossed the old Norwegian breeds (Telemark, Trønderfe, Rødkolle) with the Swedish Red (SRB) and the Finnish Ayrshire (FAY). 20 Norwegian Red (NFR) cow samples were used as control from Kysnes-gard dairy farm.

The majority of genotyping was made with PCR-RFLP method.

### Results

Based on results of PCR-RFLP tests the frequencies of wild and AP2 alleles in the Hungarian Grey and NFR populations were calculated (Table 1).

Table 1: Summarized result of PCR-RFLP tests in both breeds

	wt/wt (n)	wt/AP2( n)	AP2/AP2( n)	p(wt)	q(AP2)
Hungarian Grey	201	48	4	0.859419	0.140580
Norwegian Red	6	8	6	0.5	0.5

Both samples were in Hardy–Weinberg equilibrium. In the NFR sample the frequencies of wild and AP2 mutant alleles were the same.

### **Discussion**

Our results showed that the frequencies of the wild type and AP2 alleles of HSP70.2 in HG and breeds are significantly different. In Hungarian Grey breed the frequency of wild type allele is a close-to-fixed value  $p(\text{wt})= 0.859$ .

The genomic and metabolic background of heat stress response is not completely revealed yet. However the Heat Shock Proteins have a very important role at cellular level in adaptation, and protection of metabolism. The quality of the response depends on quantity of these proteins, which is positively related to the quantity of synthesized mRNA.

The investigated two breeds are adapted to their local environmental conditions during centuries of selection. The extensive keeping in the Hungarian warm and arid region during the centuries may have proved to be a selection pressure for heat tolerance.

In Norwegian Red breed the frequencies of the two alleles are nearly the same. In this breed it seems to be less important to give an adaptive response to heat stress comparing to HG breed. The lack of (probably non conscious) selection for heat tolerance in Scandinavian climatic condition is evidence.

Based on our result the close-to-fixed value of wild type allele of HSP 70.2 promoter in Bos Taurus breeds might be an indicator of the long term heat stress adaptation. From the animal breeding point of view this marker could be used for heat tolerant selection.



### **New scientific results**

1. A new easy to use, transportable, safe Video Aided Measurements Method was developed.
2. With the VAM Method body measurements of the five major herd of Hungarian Grey cattle were measured. The photos of the animals were archived in a user friendly database.
3. The body measurements of the Turkish Grey and the Italian Maremmana breed were also measured for the comparison of Podolic breeds.
4. Statistical analysis of the measured body measures were performed by herd and by breed.
5. A new founder based sampling strategy for the mtDNA study was constructed (founder sampling). Identifying of the cow-families in the present stock and founder sampling of HG.
6. Sequences of the 80 investigated mtDNA D-loop were published in the Genbank. The mtDNA diversity parameters were calculated based on our results.
7. The theory of the possible development of local cattle into the Hungarian Grey breed is best supported by the results of this study.
8. Calculation of the genetic distances between the Podolic and other cattle breeds were performed.
9. Calculation of the frequencies of the two common alleles of HSP70 in Hungarian Grey breed were performed.

## Publications

### Full-text papers published in peer-reviewed journals in English

**Maróti-Agóts Á.**, Bodó I., Jávorka L, Gyurmán Alice, Solymosi N., Zenke Petra, Skogseth Marita, Zöldág L.: Possible genetic sign of heat stress adaptation in Hungarian Grey Bos taurus breed.  
Acta Biologica Hungarica submitted for publication

**Maróti-Agóts Á.**, Bodó I., Jávorka L., Gyurmán A., Zenke P., Egyed B., A. M. Choyke, Bartosiewicz L., Zöldág L.: Genetic diversity and origins of the Hungarian Grey cattle breed based on mitochondrial D-loop sequence variation  
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Solymosi N., Kern Anikó, **Maróti-Agóts Á.**, Horváth L. and Erdélyi K.: Tetyn: an easy to use tool for extracting climatic parameters from tyndall data sets  
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**Maróti-Agóts Á.**, Markó A., Zöldág L.: Macskák polycystás vesebetegségének (PKD) meghatározása módosított molekuláris diagnosztikai eljárással  
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**Poster or oral presentation on conferences:**

Solymosi N., **Maróti-Agóts, Á.** Ózsvári L., Könyves L., Horváth L., Kern A.: Region specific heat stress forecast for cattle production based on climate change scenarios,  
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In World Conference on Agricultural Information and IT, Joint Conference of IAALD, AFITA and WCCA, Tokyo, Japan. 2008 poster

Solymosi, N., **Maróti-Agóts, Á.**, Könyves, L., Kern, A., és Berke, O., Climate change scenarios and the projection of geographic risk for heat stress in Hungarian dairy cattle  
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59th Annual Meeting of the European Association for Animal Production, Vilnius, Lithuania 2008 poster

## **Acknowledgments**

I would like to express my gratefulness to my supervisor, Prof. Dr.László Zöldág for his continuous guidance, and generous encouragement, without whom I could never accomplish this thesis. Special thanks have to granted to József Szabó and István Hullár, the former and present head of the Institute of Animal Breeding, Nutrition and Laboratory Animal Science for the continuously support of my work.

I wish to express my sincere gratitude to Prof. Dr. Imre Bodó and Dr. Levente Jávorka for the continuous support of Association of Hungarian Grey Cattle breeders.

I am grateful to Tamás Bakonyi for the enthusiastic help in practical implementation of my experiments at the beginning.

I deeply appreciate the support and assistance of Norbert Solymosi in statistical and methodological questions.

A special thanks has to be granted to my colleagues Petra Zenke, Zsolt Pádár, Balázs Egyed for their great assistance and partnership during the laboratory work.

I wish to thank to the academic staff, co-workers and technicians from the Institute of Animal Breeding, Nutrition and Laboratory Animal Science.