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ALLOZYME VARIABILITY OF PHEASANTS FROM BREEDING STATIONS

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ABSTRACT

The aim of this paper was the analysis of genetic variability in pheasant populations from breeding stations Sombor and Kikinda (Vojvodina). Allozyme variability of 20 putative gene loci was detected by polyacrylamide gel electrophoresis. Polymorphism was revealed in 7 loci: Ldh-1, Mor-1, Mor-2, Est-D, Mod-2, Pgd and Gpi-2.

The values of genetic variability measures – heterozigosity (H_o=0.159 and 0.206), polymorphism ($P_{95\%}$=30% and 35%) and H/P ratio (H/P=0.474 and 0.471) indicate normal level of genetic variability for bird populations. Parameters of F statistics (F_{IS}=-0.135; F_{IT}=-0.096 and F_{ST}=0.034) revealed possible presence of inbreeding depression within pheasant populations.

Comparative analysis of two pheasant populations showed low level of interpopulation variability, with the modified Rogers genetic distance value D=0.106. In further breeding programs special attention must be payed on avoiding inbreeding within one population and importing individuals from more genetically distant populations.

KEY WORDS:
allozyme, electrophoresis, genetic variability, pheasant

1. INTRODUCTION

The investigation of wild animal genomes is significant because of implementation of results in conservation biology and ecological management. The prime goal is to detect genetic variability within and among populations of interest. Low genetic variability is usually related to inbreeding depression and loss of heterozygosity.

Loss of certain allele or genotype decrease chances for new better adapted genotypes, in case of environmental condition changes. This could lead to metabolic defficiency, reproductive defficiency, lack of disease resistance ect. In wild animal species, lower genetic variability can have larger consequences, because of small populations and high

inbreeding. One of the most commonly used genetic markers for determining genetic variability in wild animal species are isozymes, present in different molecular forms, allozymes [4, 7, 8, 10, 11].

The pheasant population number decline was detected during 1970's in many hunting areas of Europe. Main named factors for this phenomenon were implementation of fertilizers and pesticides, as a consequence of improved agriculture developement. Contrary, the decline of population number in past decade could not be connected to environmental conditions. Due to this fact, one of the possible reasons for pheasant population number decline, in past several years, might be loss of adaptability in populations originated from breeding stations, caused by inbreeding depression.

Considering adaptability as a consequence of genetic structure of the populations, the aim of this paper was the analysis of genetic variability in pheasant populations from breeding stations Sombor and Kikinda, based on variability of isozymes systems.

2. MATERIAL AND METHODS

Material: Liver tissue samples of 38 individuals from breeding stations Sombor and Kikinda (Vojvodina), were used in this research. Livers were kept in freezer at -20°C until electrophoresis.

Method: Tissue preparation and vertical polyacrylamide gel electrophoresis (PAGE) were performed [3] and after electrophoresis gels were stained depending on isozyme system.

The following isozymes systems were examined (isozyme/-system, abbreviation, E.C. number and corresponding structural gene loci in parenthesis):

Lactate dehydrogenase (LDH, 1.1.1.27, Ldh-1, -2); Malate dehydrogenase (MOR, 1.1.1.37, Mor-1, -2); Malic enzyme (MOD, 1.1.1.40, Mod-1, -2); 6-phosphogluconate dehydrogenase (PGD, 1.1.1.44, Pgd); Octanol dehydrogenase (ODH, 1.1.1.73, Odh); Superoxid dismutase (SOD, 1.15.1.1, Sod); Glutamate-oxaloacetate transaminase (GOT, 2.6.1.1, Got); Hexokinase (HK, 2.7.1.1, Hk-1, -2); Pyruvate kinase, (PK, 2.7.1.40, Pk); Creatine kinase (CK, 2.7.3.2, Ck-1, -2); Adenylate kinase (AK, 2.7.4.3, Ak-1, -2); Esterases (EST, 3.1.1.1, Es-1); Aldolase (ALDO, 4.2.1.3, Aldo); Glucose-6-phosphate isomerase (GPI, 5.3.1.9, Gpi-2).

Statistical analysis: Statistical analysis of electrophoretic data was performed by the BIOSYS-1 program [9] to calculate allele frequencies, average heterozygosity (H_0-observed, H_e-expected), proportion of polymorphic loci (95% and 99% criterion) P, mean number of allele per locus (A), exact test of deviation of observed genotypes at polymorphic loci from Hardy-Weinberg expectation, basic parameters of F statistics, as well as genetic distancies values according to Nei [6] and Rogers [13].

3. RESULTS AND DISCUSSION

Electrophoretic analysis of 14 isozyme systems, represented by 20 putative loci, revealed polymorphism within seven loci: *Ldh-1, Mor-1, Mor-2, Est-D, Mod-2, Pgd* and *Gpi-2*, with two to six alleles per locus (Tab. 1).

TABLE 1. ALLELE FREQUENCIES AT POLYMORPHIC LOCI IN PHEASANT POPULATIONS FROM BREEDING STATIONS SOMBOR AND KIKINDA (N – NUMBER OF INDIVIDUALS ANALYZED)

LOCUS	Allele	Sombor (n=22)	Kikinda (n=16)
Ldh-1	a	0.591	0.500*
	b	0.409	0.500
Mor-1	a	0.500*	0.500*
	b	0.500	0.500
Mor-2	a	0.500*	0.438
	b	0.455	0.531
	c	0.045	0.031
Es-D	a	0.023*	0.031*
	b	0.205	0.406
	c	0.409	0.500
	d	0.295	0.063
	e	0.045	0.000
	f	0.023	0.000
Mod-2	a	0.476*	0.813*
	b	0.524	0.188
Pgd	a	0.952	0.813
	b	0.048	0.188
Gpi-2	a	0.733	0.611
	b	0.200	0.389
	c	0.067	0.000

** significant deviation of genotype frequencies from Hardy-Weinberg expectation based on seven polymorphic loci and exact Fisher test, criterion p<0.05*

The significant deviations of genotype frequencies from Hardy-Weinberg expectation were detected at four loci of each population (Tab. 1). Loss of heterozygosity was detected at loci *Mor-1, Mor-2, Es-D* and *Mod-2* in individuals from breeding station Sombor, and loci *Ldh-1, Mor-1, Es-D* and *Mod-2* in individuals from breeding station Kikinda. This occurrence could be due to inbreeding present within populations, or possible related individuals included in our samples.

TABLE 2. INDICIES OF GENETIC VARIABILITY IN PHEASANT POPULATIONS FROM BREEDING STATIONS SOMBOR AND KIKINDA

Genetic variability indicies*	Sombor	Kikinda	Mean value
H_o	0.159	0.206	0.182
H_e	0.166	0.165	0.165
A	1.650	1.500	1.575
$P_{95\%}$	35%	35%	35%
$P_{99\%}$	30%	35%	32.5%
H/P	0.474	0.471	0.472

**(H_o – observed population-specific heterozygosity; H_e – expected population specific heterozygosity; $P_{95\%}$ - rate of polymorphism; $P_{99\%}$ - rate of polymorphism;*
A – mean number of alleles per locus; H/P – ratio heterozygosity/polymorphism)

Indicies of genetic variability (H_o – observed population-specific heterozygosity; H_e – expected population specific heterozygosity; $P_{95\%}$ -

rate of polymorphism (95% criterion); $P_{99\%}$ - rate of polymorphism (99% criterion); A – mean number of alleles per locus; H/P – ratio heterozygosity/polymorphism) in pheasant populations from breeding stations Sombor and Kikinda were calculated within each population respectively, and as average value overall both analyzed populations (Tab. 2).

According to H_e=0.166 and H_e=0.206, as well as $P_{99\%}$=30% and $P_{99\%}$=35% in our studied populations Sombor and Kikinda respectively, they belong to non-endangered bird species populations. The mean H_e in various non-endangered bird species and subspecies amounted to 0.06, with a range from 0.0 to 0.158, with more then 25 loci analyzed. The mean $P_{99\%}$ was 22.02%, and ranged between 0 and 54.2% [2]. Also, the mean H/P value was 0.303 and ranged between 0.104 and 0.5. H/P values of 0.474 and 0.472 respectively (Tab. 2), confirm that the pheasant populations from breeding stations Sombor and Kikinda belong to non-endangered bird species, and that they have conserved some level of genetic variability. On the other hand, the lack of variability at 13 screened protein loci and genotype frequencies deviation in four loci in each population, indicate the restricted breeding range. The presence of inbreeding was also proved by negative F_{IS}=-0.135 and F_{IT}=--0.097 values. Considering the F_{ST}=0.034 value, it can be concluded that pheasant populations analyzed belong to low genetic differentiated populations.

According to the previous data, genetic distance between two analyzed populations were calculated. Nei genetic distance was D=0.008, and modified Rogers genetic distance value was D=0.106. Unrooted Wagner dendogram was constructed based on modified Rogers distance (Fig. 1). Together with the values of fixation indicies, genetic distancy values confirmed low genetic differentiation between populations from breeding stations Sombor and Kikinda.

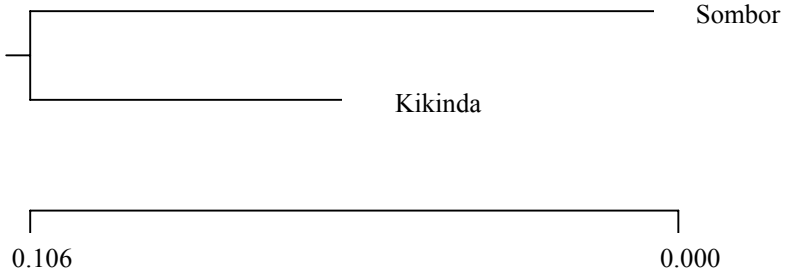

FIGURE 1. UNROOTED WAGNER DENDOGRAM BASED ON MODIFIED ROGERS DISTANCE BETWEEN PHEASANT POPULATIONS FROM BREEDING STATIONS SOMBOR AND KIKINDA

Comparing obtained data with data produced in same laboratory on pheasant population from breeding station Ristovaca (Vojvodina), a higher level of genetic variability is present within pheasant populations from breeding stations Sombor and Kikinda. Pheasants analyzed from breeding station Ristovaca showed H_e=0.097 and $P_{99\%}$=25%, corresponding H/P ratio of 0.180, which indicated lower genetic diversity [12].

In breeding population of Common Snipe (*Gallinago gallinago*) were revealed the average values of H_e=0.461 and $P_{99\%}$=80% [7]. Considering the smaller number of loci analyzed in this research (n=6), we have calculated H/P ratio of 0.576. Comparing with our results for pheasants in breeding stations Sombor and Kikinda (H/P=0.474 and 0.471) it is clear that our pheasant population has lower level of genetic variability, comparing to other species breeding population.

Due to fact that common pheasant is a game species widely and increasingly used for restocking of natural populations depleted by hunting, more effort is done in using highly polymorphic molecular markers, e.g. microsatellites. Genetic variability detected in pheasant breeds by means of microsatellites obtained by heterologous amplification using primers specific to chicken and turkey was done [1]. This analysis showed that actual heterozygosity of the pheasant populations was lower then expected under Hardy-Weinberg equilibrium (H_o=0.191 and H_e=0.271; H_o=0.165 and H_e=0.210). Same phenomenon occurred in our analyzed population from breeding station Sombor (H_o=0.159 and H_e=0.166), even with a less resolution power molecular markers. This was probably the effect of poor genetic management, e.g. the small number of founders, small population size.

In order to be able to estimate influence of genetic variability in breeding programs, it is necessary to continue further study of allozymic variability on wide set of isozyme systems, and in several generations of birds. Furthermore, additional RFLP analysis of nuclear and mitochondrial genome and DNA sequencing would provide more valuable data on genetic variability within and among different bred populations. The analysis of $tRNA^{Glu}$ gene at D-loop region of mtDNA in duck and chicken species [5] shows greatest sequence divergence in birds, and it could be relevant marker for estimating pheasant genetic variability.

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