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GENETIC VARIABILITY OF PHEASANT (*Phasianus* spp.) IN BREEDING STATION RISTOVACA

ABSTRACT: 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. Due to fact that adaptability is a consequence of genetic structure of the populations, the aim of this paper was the analysis of genetic variability in pheasant population from breeding station Ristovaca using molecular markers. Allozyme variability of 20 putative gene loci was detected by polyacrylamide gel electrophoresis. Polymorphism was revealed in 5 loci: *Est-1*, *Pgd*, *Sod*, *Gpi-2* and *Odh*. The values of genetic variability measures — heterozigosity, polymorphism, fixation indices and H/P ratio indicate low level of genetic variability and possible presence of inbreeding depression within pheasant population.

KEY WORDS: allozyme, electrophoresis, genetic variability, pheasant

INTRODUCTION

Introduction of pheasant (*Phasianus* ssp.) into our area has started in mid fifties, and today it has become common and well adapted in our country, but also in large number of European hunting areas. Unfortunately, in the last decade, a remarkable decline of pheasant population number has been detected. In Central Serbia, population number in spring season has decrease 44%, and this trend continues. In Vojvodina, percentage of decline is lower, but population number in Vojvodina is at constant low level, mostly because of environmental conditions (less forest areas, more agricultural areas). Cause for named phenomenon can be high hunting pressure on the pheasant species in central part of Serbia (C e r a n i c, 2001).

Investigation of wild animal genomes is significant because of implementation of results in planing, hunting economy, conservation biology and lack of results published. Low genetic variability is usually related to inbreeding de-

pression and loss of heterozygosity. This leads to most of characteristics of population phenotype, such as metabolic efficiency, reproductive efficiency, disease resistance etc. (Gilpin and Soule, 1986). In wild animal species, lower genetic variability can have larger consequences, because of small populations and high inbreeding. Loss of certain allele of genotype decrease chances for new better adapted genotypes, in case of environmental condition changes.

One of the most commonly used genetic markers in wild animal species are isozymes, present in different molecular forms, allozymes (Vapa et al., 1999, 2002).

The aim of this paper was estimation of genetic variability of pheasant (*Phasianus* ssp.) bred in breeding station Ristovaca, based on variability of isozymes systems.

MATERIAL AND METHODS

Material: Liver samples of forty three individuals were used in this research. Livers were frozen immediately after death of animal and kept in freezer at –20°C until electrophoresis.

Method: Tissue preparation and vertical polyacrylamide gel electrophoresis (PAGE) were performed according to Grillitsch et al. (1992) and Munstermann (1979). After electrophoresis gels were stained according to Selander et al. (1971).

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); Aspartate aminotransferase (AAT, 2.6.1.1, Aat); 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 evaluation of electrophoretic data was supported by the BIOSYS-1 program of Swofford and Selander (release 1.7, 1989). We used the BIOSYS-1 p.c. package to calculate allele frequencies, average heterozygosity (H_0-observed, H_e-expected), proportion of polymorphic loci (99% criterion) P, mean number of allele per locus (A), exact test of deviation of observed genotypes at polymorphic loci from Hardy-Weinberg expectation, as well as basic parameters of F statistics.

RESULTS AND DISCUSSION

Among 14 isozyme systems, represented by 20 putative loci, five loci were polymorphic. Polymorphism was revealed within loci: *Es-1, Gpi-2, Odh, Pgd* and *Sod*, with two to four alleles per locus (Tab. 1).

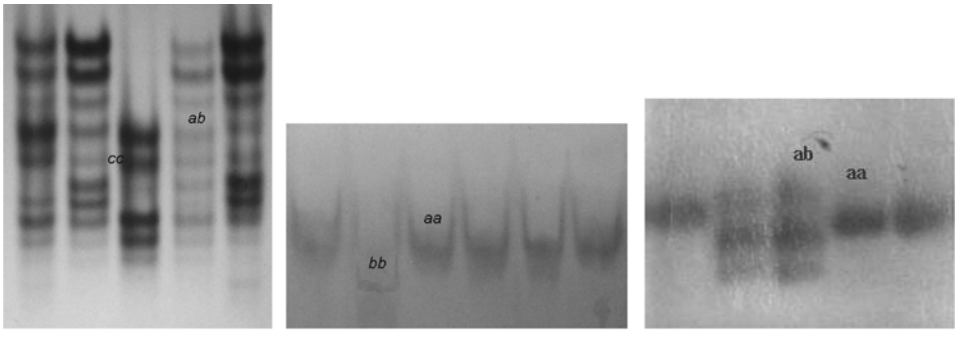
Table 1. Allele frequencies at polymorphic loci and indices of genetic variability in pheasant population from breeding station Ristovača (for acronyms see Material and Methods). H_o — observed population-specific heterozygosity; H_e — expected population specific heterozygosity; $P_{99\%}$ — rate of polymorphism (99% criterion); A — mean number of alleles per locus; n — number of individuals analyzed; F_{IS}, F_{IT} and F_{ST} — fixation indices

Locus	Allele	Ristovača n = 43
Es-1	a	0.174
	b	0.616
	c	0.209
Pgd	a	0.833*
	b	0.188
Gpi-2	a	0.333
	b	0.450
	c	0.200
	d	0.017
Odh	a	0.804*
	b	0.196
Sod	a	0.291
	b	0.570
	c	0.140
H_o		0.045
H_e		0.097
A		1.40
$P_{99\%}$		25%
H/P		0.18
F_{IS}		–0.135
F_{IT}		–0.097
F_{ST}		0.034

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

The mean H_e in various non-endangered bird species and subspecies, as calculated from Table 2. in Evans (1987), 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%. According to H_e = 0.097 and $P_{99\%}$ = 25% in our studied population, it clearly fits the values of genetic variability of non-endangered bird species. Hart and Pucek (1994) proposed the use of H/P ratio index, in order to overcome the differences in sample size and loci number analyzed in different mammal populations. In non-endangered bird species (calculated from Evans, 1987; regarding 53 studies with more than 24 loci analyzed) the mean H/P value was 0.303 and ranged between 0.104 and

0.5. Although with H/P value of 0.18, the pheasant population from breeding station in Ristovaca is slightly above bottom level for non-endangered bird species, it still have conserved some level of genetic variability. The lack of variability at 15 screened protein loci and genotype frequencies deviation in two, *Pgd* and *Odh* loci, 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 population analyzed belongs to low genetic differentiated populations.



a) *Es-1* b) *Odh* c) *Pgd*

Figure 1. Zymogrames of polymorphic loci

The results of allozyme analysis in Golden Eagle population (S u c h e n t r u n k et al., 1999) data showed H_e = 0.034, and $P_{99\%}$ = 10.8%, that reveal lower genetic variability in natural bird population. It can be due to the fact that in their research, variation of 31 isozyme systems represented by 37 putative loci was examined, but only 15 individuals were analyzed and rare alleles are likely to be missed. Number of individuals examined in our research was 43, and this high number of individuals gave us reliable results, because chances to miss rare allele are reduced, comparing with small sample size.

The analysis of breeding population of Common Snipe (Gallinago gallinago) revealed the average values of H_e = 0.461 and $P_{99\%}$ = 80% (P a u l a u s k a s et al., 2002). 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 station (H/P = 0.18) 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. B a r r a t i et al. (2001) reported on genetic variability detected in pheasant breeds by means of microsatellites obtained by heterologous amplification using primers specific to chicken and turkey. 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 (H_o = 0.045

and H_e = 0.097), with generally lower values, 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. Inbreeding could lead to a rapid loss of individual fitness and genetic variability and reduced viability of populations utilized for restocking programs.

In order to be able to estimate influence of genetic variability in breeding program, it is necessary to continue further study of allozymic variability on wide set of isozyme systems, and in several generations of birds. Because of the limited part of genome that can be studied, additional RFLP analysis of nuclear and mitochondrial genome and DNA sequencing, respectively. The analysis of $tRNA^{Glu}$ gene at D-loop region of mtDNA in duck and chicken species (L i u et al., 1996) shows greatest sequence divergence in birds, and it could be relevant marker for estimating pheasant genetic variability.

CONCLUSION

The analysis of allozyme variability of 20 putative gene loci in pheasant population bred in breeding station Ristovaca was detected by polyacrylamide gel electrophoresis (PAGE). Polymorphism was revealed in 5 loci: *Est-1*, *Pgd*, *Sod*, *Gpi-2* and *Odh*. The values of genetic variability measures — heterozigosity (H_o = 0.045 and H_e = 0.097), polymorphism ($P_{99\%}$ = 25%), fixation indices (F_{ST} = 0.034) and H/P = 0.18 ratio indicate low level of genetic variability and possible presence of inbreeding depression within pheasant population.

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ГЕНЕТИЧКА ВАРИЈАБИЛНОСТ ФАЗАНА (*Phasianus* spp.) ИЗ ФАЗАНЕРИЈЕ РИСТОВАЧА

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Резиме

Један од могућих узрока опадања бројности фазана последњих година може бити губитак адаптибилности у популацијама пореклом из фазанерија услед парења у сродству, које води губитку генетичке варијабилности и смањењу хетерозиготности. Због чињенице да је адаптабилност последица генетичке структуре, циљ овог рада био је анализа генетичке варијабилности популације фазана из фазанерије Ристовача применом молекуларних маркера. Алозимска варијабилност 20 генских локуса детектована је полиакриламид гел електрофорезом (PAGE). Полиморфност је регистрована у оквиру 5 локуса: *Est-1*, *Pgd*, *Sod*, *Gpi-2* и *Odh*. Вредности мера генетичке варијабилности — хетерозиготност, полиморфност, индекси фиксације и H/P однос указују на низак ниво генетичке варијабилности и могућег парења у сродству у оквиру популације фазана.