APPLICATION OF ESTERASES AS GENETIC MARKERS FOR THE DIFFERENTIATION OF GEESE

Sigita Slavėnaitė, Aniolas Sruoga
Institute of Ecology, Akademijos 2, LT-2600, Vilnius, Lithuania tel. 729287, e-mail igl@ekoi.lt

Abstract. The polymorphic systems are widely used in selection processes for observation of the variability of genetic structure in lines. In this connection the objective of our work was the investigation of the polyenzymatic system of esterases and identification of its isoforms (carboxylesterase and cholinesterase) in domestic and wild geese. The blood sera of graylag (*Anser anser*), blue (*Chen caerulescens*), bar-headed (*Anser indicus*) and domestic Pomezhansko and Rheinische geese have been used in our work. The analysis of isoenzymes has been carried out by using the method of double layer vertical electrophoresis in the polyacrilamide gel with the application of specific substrates (1-naphthyl acetate, 2-naphthyl acetate, 1-naphthyl propionate) and inhibitors. The results show that polymorphism has not been detected only in the enzymatic system of cholinesterase from graylag (*Anser anser*) goose. Both enzymatic systems of esterases from the blue (*Chen caerulescens*) and bar-headed (*Anser indicus*) species of geese and also carboxylesterase from graylag (*Anser anser*) showed 1 or 2 polymorphic loci. Maximum 5 isoenzymes fractions of cholinesterase and 10 of carboxylesterase have been observed in the domestic and wild geese. The data obtained show that genetic variability is characteristic to the enzymatic system of carboxylesterase and cholinesterase in the genus *Anser* from the order Anseriformes. Those systems can be used as markers in calculation of genetic distances and identity. Persistent analysis of genetic distances allows monitoring trends of the selection process in a population.
Keywords: Selection, geese, genetic markers, esterases.

ESTERAZIŲ KAIP GENETINIŲ ŽYMENŲ PANAUDOJIMAS ŽĄSŲ DIFERENCIACIJOS NUSTATYMUI

Santrauka. Naminiams gyvūnams būdingas aukštas polimorfizmo laipsnis (aptinkama daug polialelinių ir dialelinių genetinių sistemų). Tai yra sudėtingų kryžminimų bei atrankos rezultatas. Polimorfinės sistemos taip pat plačiai naudojamos nustatyti genetinės struktūros kintamumui linijose. Ryšium su tuo savo darbe vertikalios elektroforezės poliakrilmidiniame gelyje metodu, naudojant specifinius substratus ir inhibitorius tyrėme žąsinių (Anseriformes) būrio *Anser* genties polifermentinę esterazių (F.K. 3.1.1) sistemą. Pagrindinis tikslas buvo identifikuoti karboksilesterazę (F.K. 3.1.1.1) ir cholinesterazę (F.K. 3.1.1.8) naminių bei laukinių žąsų kraujo serumuose. Iš viso buvo ištirti 88 pavyzdžiai, priklausantys Reino ir Pomežansko naminių žąsų veislėms bei laukinėms pilkajai (*Anser anser*), mėlynajai (*Chen caerulescens*) ir kalnų (*Anser indicus*)) žąsims. Polimorfizmas nebuvo nustatytas tik pilkosios žąsies (*Anser anser*) fermentinėje cholinesterazių sistemoje. Kalnų žąsies (*Anser indicus*) ir mėlynosios žąsies (*Chen caerulescens)* fermentinėse abiejų esterazių sistemose bei pilkosios žasies (*Anser anser*) fermentinėje karboksilesterazių sistemoje nustatyta po vieną ar du polimorfinius lokusus. Visų tirtųjų laukinių žąsų bei naminių žąsų veislių fermentinėje cholinesterazių sistemoje nustatyta iki 5 izofermentinių frakcijų, o karboksilesterazių sistemoje - iki 10. Gauti duomenys rodo, kad žąsinių būrio *Anser* genties karboksilesterazės ir cholinesterazės fermentinės sistemos pasižymi genetiniu kintamumu. Jos gali būti naudojamos kaip genetiniai žymenys skaičiuojant genetinių distancijų bei identiškumo indeksus.
Raktažodžiai: selekcija, žąsys, genetiniai žymenis, esterazės

Introduction. High degree of polymorphism (identification of many diallelic and polyallelic systems) is characteristic for domestic animals. This represents the outcome of complex crossbreeding and selection. The polymorphic systems are used in selection processes for observation of the variability of genetic structure in lines. Besides, the analysis of polymorphic systems allows to define the origin of breeds more precisely, to determine the degree of consanguinity, genetic structure of breeds, intrabreed differentiation and also to plan and monitor the process of selection. (Moiseeva *et. al.*, 1992).

Vertebrate esterases (E. C. Subgroup 3.1.1.) feature major intraspecies genetic variability. They are widely used as genetic markers for the purpose of populations investigations in species of many animals (Schnell *et al*., 1981). All the group of esterases splits ether bonds of carbonic acids in artificial substrates. In majority of species the spectrum of esterases is controlled by non-allelic genes the intraspecies homology of which is not clear. The esterases as genetic features are complex and heterogeneous phenomenon. Their heterogeneity varies not only in the levels of order and family but also depending on species. Some scientists assume that esterases are the most variable enzymes of vertebrates (Selander *et. al*.,1973).

For the first time Augustinsson K.B. revealed the variety of esterases in electrophoretic investigations in 1959.. Holmes and Masters established the isozyme status and genetic basis of esterases within a subgroup with the use of specific substrates and inhibitors in 1963 (Koehn *et al*.,1983).

The investigations of blood serum esterases from the Anseriformes order are very limited. The isophorms of this enzyme are investigated in snow geese (*Anser caerulescens hyperboreus*) and in some other species of *Anser* and *Branta* genus (Kuznetsov, 1991; 1995a, 1995b). However, the data are not sufficient, and investigation of esterases in the Anseriformes order are still important.

The purpose of the research was to investigate polyenzymatic complex of esterases (E. C. Subgroup 3.1.1.) in the blood sera of domestic (Pomezhansko and Rheinische) and wild graylag (*Anser anser*), blue (*Chen Caerulescens*) bar-headed (*Anser indicus*) geese with the objective to identify carboxylesterase (E. C. Subgroup 3.1.1.1.) and cholinesterase (E. C. Subgroup 3.1.1.8.).

Materials and methods. Totally 88 samples of blood serum from domestic (Pomezhansko, Rheinische) and wild (graylag (*Anser anser*), blue (*Chen caerulescens*) and bar-headed (*Anser indicus*)) geese have been investigated. Blood samples were obtained from geese and put into the tubes with heparin in order to prevent coagulation. The blood plasma was obtained by centrifugation at a speed of 1500 r/min for 10 minutes and after sedimentation of erythrocytes. Before using the plasma was kept at the temperature of –20˚ C. A double layer vertical electrophoresis in the polyacrylamide gel has been used for investigation of esterases polymorphism. Before detection of isozyme spectrum the undiluted blood sera samples were mixed with 50% sucrose solution at a volume of two parts of each serum and one part of sucrose solution Electrophoresis was performed at a constant 240 V until the tracking dye had migrated to the end of gel. After electrophoresis gels were incubated in the solutions of inhibitors (Harris *et. al*., 1972). For detection of enzymes activity the 1-naphtyl acetate, 2-naphtyl acetate, 1-napthyl propionate were used as substrates (Korochkin, 1977). After staining, the background was destained in 7% acetic acid.

The scientific investigations made following the provisions of Law of Republic of Lithuania № 8-500 on Protection, Keeping and Use of Animals of November 6, 1997 ("Valstybės žinios", №108, 1997.11.28) and of the by - laws, i.e. orders of State Veterinary Service of the Republic of Lithuania: On Breeding, Care, Transportation of Laboratory Animals (№ 4-361, 1998. 12. 31) and Use of Laboratory Animals for Scientific Tests (№ 4-16, 1999. 01. 18).

Results and discussion. Enzymes of carboxylesterase (E.C. Subgroup 3.1.1.1.) and cholinesterase (E.C. Subgroup 3.1.1.8.) belonging to polyenzymatic complex of esterases (E.C. Subgroup 3.1.1.) were identified in all blood sera samples of geese under investigation. The analysis of electrophoregrams of wild (graylag (*Anser anser*), blue (*Chen caerulescens*) and bar-headed (*Anser indicus*)) and domestic (Pomezhansko, Rheinische) breeds of geese has shown that enzymatic systems of carboxylesterase and cholinesterase have several isozyme fractions, which differ in electrophoretic mobility and enzymatic activity. The esterases of the species investigated were located in the zone between albumines and transferines. Polymorphism has not been detected only in the cholinesterase enzymatic system of graylag (*Anser anser*) goose. Other investigated enzymatic systems had one or two polymorphic loci.

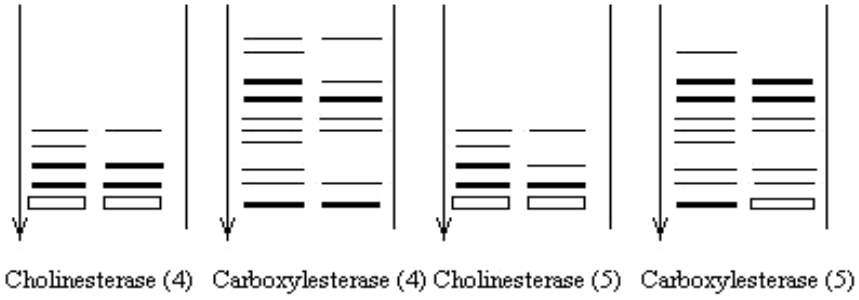

Fig.1. **Diagrammatic representation of electrophoretic profiles of the esterases investigated in domestic Rheinische (4) and Pomezhansko(5) breeds of geese**

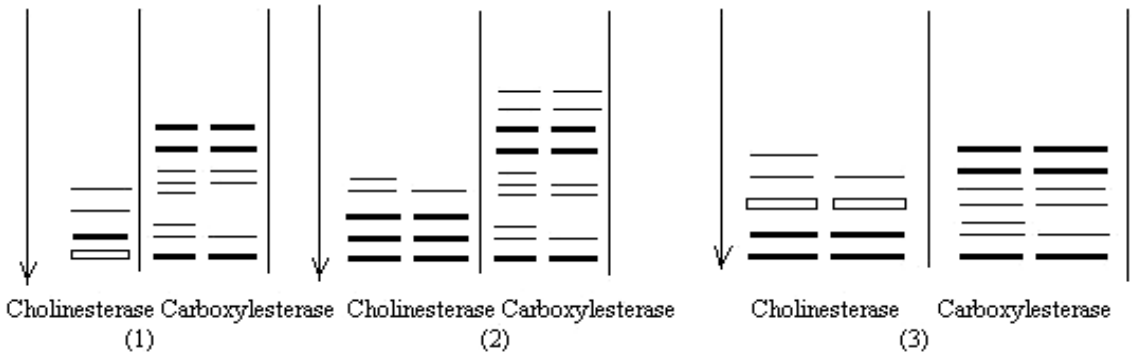

Fig.2. **Diagrammatic representation of electrophoretic profiles of the esterases investigated in graylag *(Anser anser*) -(1), bar-headed (*Anser indicus*) -(2) and blue (*Chen caerrulescens*) -(3) species of geese**

Five fractions of cholinesterase located in four zones of activity were detected in the blood serum of domestic geese. The zone with the fastest migration rate to anode shows the greatest enzymatic activity in Pomezhansko and Rheinische breeds of geese. Polymorphism was indicated in the slowest migration zone to anode also in both breeds. The differences between Pomezhansko and Rheinische geese according to cholinesterase enzymatic spectrum were detected only in the third zone of migration to anode. This zone was more clearly expressed in Rheinische breed (Figure 1).

The enzymatic spectrum of carboxylesterase of domestic geese exhibits up to ten isophorms of Rheinische and up to nine isophorms in Pomezhansko breeds. The isophorms were located in six zones of activity. Polymorphism was detected in the second zone of migration to anode Rheinische geese and in the third zone in both breeds (Fig.1).

Enzymatic spectra of carboxylesterase and cholinesterase of three wild geese species are presented in Figure 2. Five fractions of cholinesterase located in four zones of activity were detected in the blood serum of graylag (*Anser anser*) goose. The zone with the fastest migration rate to anode exhibited the greatest enzymatic activity. The isozymes of the third zone were very faintly expressed. Two isozymes were found in the fourth zone of the slowest migration. Carboxylesterase enzymatic system of the above species showed 8 isophorms located in five zones of activity. Polymorphism was detected in the second third and fifth zones.

The electrophoretic properties of bar-headed (*Anser indicus*) goose were similar to that of graylag (*Anser anser*) goose. Five fractions of cholinesterase located in four zones of activity were also detected. However, the fastest zone represents less enzymatic activity in comparison with graylag (*Anser anser*) goose. The polymorphism was detected in the slowest zone of electrophoretic mobility. Ten isophorms located in five zones of activity were found in the carboxylesterase system of the above species. Polymorphism was detected in the second and third zones of electrophoretic mobility. In the slowest zone of electrophoretic mobility two isophorms were observed.

In the enzymatic spectrum of blue goose (*Chen caerulescens*) five fractions of cholinesterase located in four zones of activity were also detected. However, the enzymatic activity in the third zone of migration to anode was more clearly expressed in comparison with that of graylag (*Anser anser*) goose. Polymorphism was detected in the second zone of electrophoretic mobility.

The obtained data about electrophoretic properties of carboxylesterase and cholinesterase in the blood sera of domestic (Pomezhansko and Rheinische) breeds and wild (graylag (*Anser anser*), blue (*Chen caerulescens*) and bar-headed (*Anser indicus*)) geese have shown that genetic variability is characteristic to the above systems. The observed polymorphism allows to use enzymatic systems of carboxylesterase and cholinesterase as genetic markers of the *Anser* genus. Polymorphism of esterases was also reported by previous authors in the other species (bean goose (*Anser fabalis*), white fronted goose (*Anser albifrons*), swan goose (*Anser cygnoides*), emperor goose (*Anser canagicus*), and snow goose (*Anser caerulescens hyperboreus*) of the *Anser* genus. Therefore, the blood serum of esterases from the *Anser* genus of the order *Anseriformes* as in many other animals can be used for evaluation of the indices of genetic distances and identity. Persistent analysis of genetic distances allows monitoring trends of the selection process in a population.

Conclusion. The isozymes (genetically determined different molecular forms of the same enzyme) of esterases such as carboxylesterase and cholinesterase can be successfully used as genetic markers during the processes of selection of geese, since their determination does not require great quantity of material and many samples can be analysed at a time.

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