The use of isozymes for ecological analysis of semi-aquatic rodents

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The genetic variability of *Castor fiber* (beaver), *Ondatra zibethicus* (muskrat) and *Arvicola terrestris* (water vole) (which belongs to semi-aquatic rodents) populations were investigated by the vertical polyacrilamide gel electrophoresis (PAGE) method to elucidate ecological differences of these species in different Lithuanian populations. Different numbers of individuals were investigated genetically in various parts of Lithuania. In our investigation, in eight protein systems (EST, G6PD LDH MDH, MDE, SOD, α-GDP and NPR) of different semi-aquatic rodent populations of Lithuania we found 15 loci: *G6pd-1, G6pd-2, Mdh-1, Mdh-2, Mde-1, Ldh-1, Ldh-2, Est-1, Est-2, Est-3, Est-4, Npr-1, Npr-3, Npr-4, Npr-5*. Of them 12 were polymorphic, and *Mdh-2, Npr-1* and *Npr-3* were monomorphic. Relative mobility Rm of esterases and nonspecific protein of the species were different, but in lactate dehydrogenase, malate dehydrogenase, malic enzyme and glucose-6-phosphate dehydrogenase it was the same.

Key words: semi-aquatic rodents, *Castor fiber, Ondatra zibethicus, Arvicola terrestris,* PAGE electrophoresis, isoenzymes

INTRODUCTION

The study and conservation of biological variety is an important scientific problem of zoology. The solution of this problem is possible after a detailed examination of the specific composition of animals. Analysis of the genetic structure of species can give important clues to their structure and ecological relationships. In Lithuania today there are three species of semi-aquatic rodents: *Ondatra zibethicus* (Ондатра..., 1993), *Castor fiber* (Ulevičius, 1997) and *Arvicolla terrestris* (Lietuvos…, 1998). However, only *Castor fiber* was explored by traditional morphometric methods and genetically in Lithuania (Paulauskas, Ulevičius, 2001; Ulevičius, Balčiauskas, 2000). Following natural immigration and reintroduction at the beginning of the 1940s to 1967, the expansion stage of the beaver population in Lithuania ended in about 1975 (Ulevičius, Balčiauskas, 2000). Today, a high population density has become established in various water bodies and areas. The formation of the beaver population in Lithuania from many different centers of spreading has considerably influenced its present genetic structure. The first muskrats were brought to Europe from Canada, Finland and England. There were three centers of their spreading: Czech (from 1905), Finland (from 1916–1919) and France (from 1919) (Ондатра..., 1993). the muskrat was brought to Lithuania from Archangelsk (in 1954) and from Kazakhstan (in 1956). There are 16 subspecies of muskrat in the world, but nobody knows from what places muskrats were taken to spreading centers; today it isn't clear what subspecies are there in Europe. Literature data (Водяная..., 2001) indicate that there are two species of *A. terrestris* in Europe, but in Lithuania there are no data what species we have for today. So, the taxonomy, genetic structure and ecological relationships among these species are still problematic.

The aim of the present study was to determine the polymorphic systems of three semi-aquatic rodent species: *O. zibethicus, C. fiber* and *A. terrestris* and to estimate the genetic variation of these species.

MATERIALS AND METHODS

The material was collected in 2001–2002 from various parts of Lithuania. We examined genetically 42 individuals: 35 *Castor fiber*, 5 *Ondatra zibethicus* and 2 *Arvicolla terrestris*. There were studied 7 enzymes (non-specific esterase (EST, E. C. 3.1.1.), lactate dehydrogenase (LDH, E. C 1. 1. 1. 27), malate dehydrogenase (MDH, E. C. 1.1.1.37), malic enzyme (MDE, E. C. 1.1.1.40), glucose-6-phosphate dehydrogenase (G6PD, E. C. 1.1.1.49), α-glycerosephosphate dehydrogenase (α-GPD, E. C 1.1.1.8), super-

oxidismutase (SOD, E. C 1.15.1.1) and 1 nonspecific-protein (NPR) systems in the populations of these rodents, using enzyme electrophoresis in PAGE.

Approximately 5 g of individual frozen sample liver tissue was crushed in 5 ml of homogenate buffer (0.2 M Tris-HCl pH 8.1, 2% Triton X-100, 2.5 nM $MgCl_2$, 0.02 g NADP) with a glass homogenizer and used for electrophoretic analysis. The homogenate was centrifuged for 5 min at 1500 rpm. The supernatant was collected in 200 µl microtubes and frozen at –20 °C. Five microlitres of homogenate was analyzed by electrophoresis in polyaclrylamide gel (PAAG).

Electrophoresis was performed on a 2.5/7.5%, and 5% PAGE based on the enzyme system under investigation according to established protocols (Maypep, 1971) with some modifications (Paulauskas, Ulevičius, 2001). For G6PDH, MDH, a single-layer 5% PAGE was used; for the EST, MDE systems, a 2.5–7.5% PAGE was used. The electrode buffer was changed depending on the isoenzymes under investigation. A Tris-glycin buffer (pH ~8.9) system was used in separating EST, MDE, NPR and a Tris-EDTA-Borat buffer (pH~8.3–8.4) in separating MDH and G6PDH. Protein mobility was tracked with bromophenol blue. The electrophoresis mode (current and voltage) was set according to the established protocols for enzyme investigation. To expose the isoenzyme and non-enzyme systems, stain mixtures were prepared by several methods (Корочкин и др., 1977). After electrophoresis, the gels were incubated in the stains for 30 min to several hours at 37 °C or at room temperature. At the end of incubation, the gels were fixed with a 7% acetic acid solution, in which they were stored for some time.

RESULTS AND DISCUSSION

Esterases are enzymes that belong to the class of hydrolases. We have found 5 zones of *A. terrestris* and 4 zones of *O. zibethicus* and *C. fiber* in electrophoregrams of this enzyme:

1. The fastest polymorphic zone was Est-1.
2. Polymorphic zone Est-2 was slower than Est-1.
3. The polymorphic zone Est-3 was slower than Est-2, but faster than Est-4.
4. The polymorphic zone Est-4 was faster than Est-5.
5. The polymorphic zone Est-5 was the fastest one.

Thr relative mobility (Rm) of esterase zones in the species studied is diverse. In the first, second and third zones the Rm is faster than the ones of beavers, but the fourth zone of beavers is faster than of muskrat. The Rm of the first zone of *A. terrestris* is faster than of beaver, but it is by far slower than the Rm of muskrat (Fig. 1).

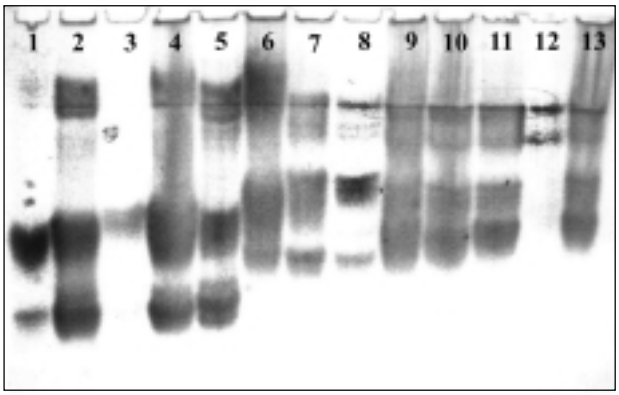

Fig. 1. Esterases spectrum: *O. zibethicus* (1–5), *A. terrestis* (7–8) and *C. fiber* (9–13)

The Est-1, Est-2, Est-3 and Est-5 zones of *A. terrestris* are monomorphic, and only the Est-4 zone is polymorphic. Only the Est-3 zone of *O. zibethicus* was monomorphic; 3 ($Est\text{-}2^A$, $Est\text{-}2^B$, $Est\text{-}2^C$) alleles were found in Est-2 zone and 2 ($Est\text{-}1^A$, $Est\text{-}1^B$) alleles in the Est-1 and Est-4 ($Est\text{-}4^A$, $Est\text{-}4^B$) zones. All zones of *C. fiber* were polymorphic, 3 ($Est\text{-}3^A$, $Est\text{-}3^B$, $Est\text{-}3^C$) alleles were in the third zone, where the Rm of A fraction was the fastest and of C the slowest. We have found a rare allele, $Est\text{-}2^D$, in the second zone of *C. fiber*, in which the Rm is slower than in allele $Est\text{-}2^C$.

Lactate dehydrogenase is a tetramer. It is coded by two independent loci. The results of electrophoresis showed that 5 fractions were cleared in Ldh electrophoregrams, which were geneticaly coded by two loci, *Ldh-1* and *Ldh-2*. The relative mobility of the species studied was found to be the same (Fig. 2).

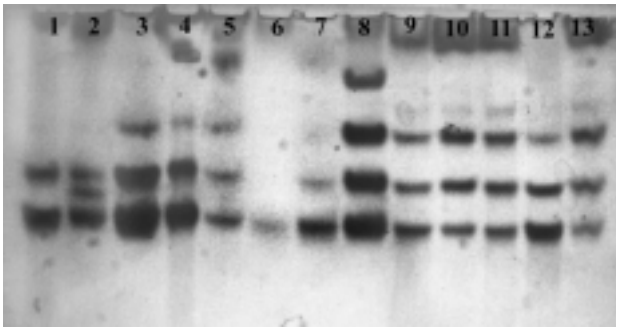

Fig. 2. LDH spectrum: *O. zibethicus* (1–5), *A. terrestis* (7–8) and *C. fiber* (9–13)

Malate dehydrogenase is a dimer. The following stain is for NAD-depended malate dehydrogenase.

The Rm of the first zone of muskrat and water vole is the same and faster than of beavers. The Rm of the second zone is the same in all species. We noticed less allele in the first zone of muskrat (4 alleles) than in the same zone of water vole and

beaver (3 alleles). The second zone was monomorphic in all individuals; the others were polymorphic (Fig. 3).

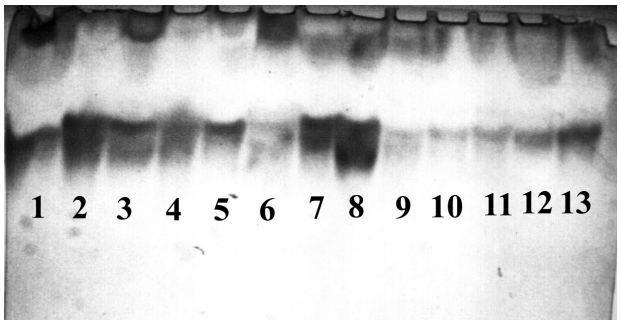

Fig. 3. MDH spectrum: *O. zibethicus* (1–5), *A. terrestis* (7–8) and *C. fiber* (9–13)

Malic enzyme is a tetramer. The following stain is for NADP-depended malate dehydrogenase.

The Rm of the first zone is the same in all species. The Mde-1 zone in water vole and muskrat was monomorphic and in beavers polymorphic. Mde-2 was found only in muskrat electrophoregrams; the Rm of this zone was faster than of Mde-1; both zones were monomorphic (Fig. 4).

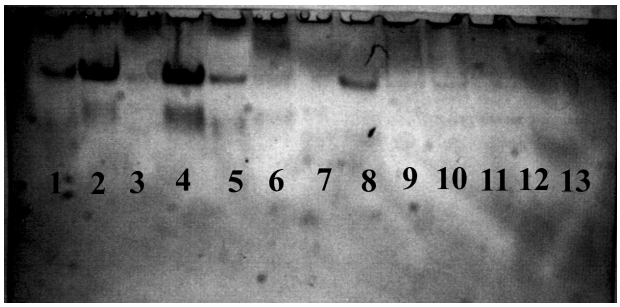

Fig. 4. MDE spectrum: *O. zibethicus* (1–5), *A. terrestis* (7–8) and *C. fiber* (9–13)

The Rm of the first zone of **glucose-6-phosphate dehydrogenase** in beavers is the fastest; it is slower in water vole and slowest in muskrat. In muskrat and water vole the Gdp-1 and Gdp-2 zones are monomorphic, but in beavers they are polymorphic (Fig. 5).

Superoxide dismutase is a dimer and a tetramer. We have found 5 zones of this enzyme. The Rm of these zones was the same in all individuals (Fig. 6).

α-Glycerosephosphate dehydrogenase is a dimer. The activity of this enzyme was found only in few beavers. We have not found it in the spectra of muskrat and water vole (Fig. 6).

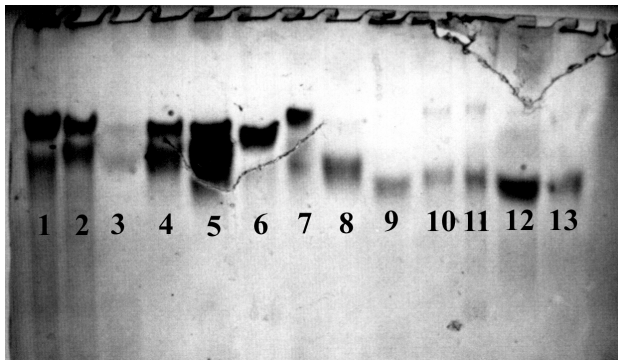

Fig. 5. G6PD spectrum: *O. zibethicus* (1–5), *A. terrestis* (7–8) and *C. fiber* (9–13)

The Rm of the zones of **nonspecific protein** in beavers was the fastest, in water vole slower and in muskrat the slowest. We have found much more zones in the spectrum of the muskrat than of other animals (Fig. 7).

Fig. 6. SOD and GPD spectrum: *O. zibethicus* (1–5), *A. terrestis* (7–8) and *C. fiber* (9–13)

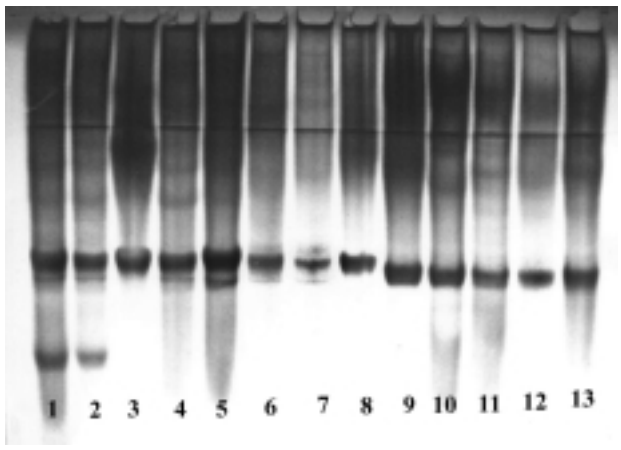

Fig. 7. NPR spectrum: *O. zibethicus* (1–5), *A. terrestis* (7–8) and *C. fiber* (9–13)

CONCLUSIONS

The ecology of introduced muskrats, reintroduced European beaver and native water vole is quite similar: the same biotopes, competition for food and space. Maybe these reasons could explain why the numbers of muskrat and water vole in Lithuania are so low. Many ecological characteristics confirm a high vitality and adaptation possibilities in the present Lithuanian beaver population. We analyzed the isoenzyme spectra of water vole and compared them to those of beavers and muskrats because of lack of information on semi-aquatic rodent species isoenzymes and their genetic structure.

In our investigation, in eight protein systems (EST, G6PD, LDH, MDH, MDE, SOD, α-GPD and NPR) of different semi-aquatic rodent populations of Lithuania were found 15 loci: *G6pd-1, G6pd-2, Mdh-1, Mdh-2, Mde-1, Ldh-1, Ldh-2, Est-1, Est-2, Est-3, Est-4, Npr-1, Npr-3, Npr-4, Npr-5*. Of them 12 were polymorphic and *Mdh-2, Npr-1* and *Npr-3* were monomorphic. Relative mobility in esterases and nonspecific protein of the species was different, but lactatedehydrogenase, malatedehydrogenase, malic enzyme and glucose-6-phosphatedehydrogenase relative mobility was the same in all species. Isoenzyme analysis is suitable not only for evaluating differences of populations from various biotopes, but also for differentiating separate species and subspecies.

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IZOFERMENTŲ PANAUDOJIMAS PUSIAU VANDENS GRAUŽIKŲ EKOLOGINIUOSE TYRIMUOSE

Santrauka

Introdukuotų į Lietuvą ondatrų ir reintrodukuotų europinių bebrų bei Lietuvoje gyvenančių vandeninių pelėnų ekologija panaši, todėl žvėreliai konkuruoja dėl maisto ir gyvenamosios erdvės, o tai gali sąlygoti nedidelę vandeninių pelėnų gausą Lietuvoje. Išanalizavus 42 individus: 5 ondatrų (*Ondatra zibethicus*), 35 europinių bebrų (*Castor fiber*) ir 2 vandeninių pelėnų (*Arvicola terrestris*) 7 fermentines ir vieną nefermentinę baltymų sistemas, nustatyta, kad skirtingų pusiau vandens graužikų rūšių jos yra skirtingos: elektroforeogramose esterazių ondatrų zonų santykinis paslankumas R_m yra greičiausias, vandeninio pelėno lėtesnis, o bebrų lėčiausias, ir, atvirkščiai, nespecifinio baltymo sistemos NPR, bebrų zonų R_m buvo greičiausias, vandeninio pelėno lėtesnis, o ondatrų lėčiausias. Be to, palyginti su kitomis dviem rūšimis, pastebėta kur kas daugiau lokusų ondatrų NPR sistemoje. Nustatytos izofermentinės sistemos (EST, G6PD LDH MDH, MDE, SOD, GPD ir NPR) yra specifinės atskiroms rūšims ir tinkamos įvertinti ne tik genetinius rūšių, bet ir populiacijų iš skirtingų biotopų skirtumus.

Raktažodžiai: pusiau vandens graužikai, *Castor fiber, Ondatra zibethicus, Arvicola terrestris,* PAG elektroforezė, izofermentai