Application of DNA fragment length polymorphism analysis for identification of small rodents

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The DNA fragment length polymorphism (RFLP) analysis is used for determining genetic variations in different species of mouse-like rodents. These methods give a possibility to investigate variations of DNA sequences in a population or species. Genomic DNA isolated from the liver of *Cletrionomys glareolus*, *Microtus agrestis*, *Microtus arvalis*, *Microtus rossiaemeridionalis* and *Microtus oeconomus* was investigated on the basis of restriction fragment length polymorphism (RFLP) analysis. The enzymes *Sca*I and *Xba*I gave restriction patterns that differed among all 5 species analyzed. Using XbaI for DNA cleavage, the polymorphism of fragments was more distinct.

Key words: DNA RFLP, identification of species, voles, *Clethrionomys glareolus*, *Microtus agrestis*, *M. arvalis*, *M. rossiaemeridionalis, M. oeconomus*

INTRODUCTION

The analysis of DNA is an important tool in studing of evolutionary ecology, population genetics, systematics (Avise, 1994). DNA has several advantages over alternatives such as proteins for molecular systematics: the genotype rather than phenotype is analyzed; the methods are common to any type of DNA (Delidow et al., 1997).

One of the methods for DNA research is restriction fragment length polymorphism (RFLP) analysis. This method gives a possibility to investigate variations of DNA sequences in a population or species (Dowling et al., 1996). For population level comparison RFLP analysis of plant chloroplast DNA and animal mitochondrial DNA (Dowling et al., 1996; Watanabe et al., 1985, 1989) is often used. For species or higher-level analysis, specific sequences of genomic DNA could be chosen (Leiro et al., 2000).

In some cases it is difficult to determine the species according to morphological data alone. Individuals of closely related rodent species have a similar morphology (Lietuvos…, 1988; Mažeikytė et al., 1999), they can produce interspecies hybrids (Kaneko et al., 1998), but have different genotypes. In this case DNA RFLP analysis may help. Specific restriction fragments could be species markers.

MATERIALS AND METHODS

Genomic DNA was isolated from fresh liver of 4 individuals of *Clethrionomys glareolus*, 5 – *Microtus arvalis*, and 2 – *M. rossiaemeridionalis*, as well as from frozen liver (it was kept at –20 °C for 6 months) of 3 individuals of *C. glareolus*, 2 – *M. oeconomus* and 4 – *M. agrestis*. About 800 mg of tissue was chopped and digested overnight at 37 °C in 10 ml of lysis buffer (20 ml of 0.05 M Tris–HCl, pH 8.0, 0.585 g of NaCl and 0.75 g of EDTA) to which 1 ml of proteinase K (0.1 mg/ml) and 50 μl of 10% SDS were added. In 10 samples the proteins were precipitated by adding NaCl to lysate (1.5 ml of 4 M NaCl added to 5 ml of lysate) followed by centrifugation at 3000 g for 15 min (Miller et al., 1998). DNA from 20 samples was phenol-extracted with phenol-chloroform-isoamyl alcohol (8 g of phenol dissolved in 24 ml of chloroform, 1 ml of isoamyl alcohol is added) followed by centrifugation at 3000 g for 15 min and precipitated with 1.5 ml of 3 M sodium acetate and 10 ml (2 volumes) of ethanol for 4 h and centrifuged at 3000 g for 10 min (Darbre, 1988).

The extracted DNA was digested at 37 °C for 12 h with an appropriate amount of the restriction enzymes (1 unit of enzyme digests 1 μg of DNA in one hour in 50 μl of solution). In this research, *Sca*I and *Xba*I restriction enzymes were used. For 50 μl of restriction reaction solution up to 20 μg of DNA was taken, 5 μl (1/10 volume) of 10 × restriction buffer was added. Restriction enzymes and 1 μl of BSA were added (Darbre, 1988).

The DNA fragments produced by digestion with restriction endonucleases were sorted according to

their size by electrophoresis in 1% agarose and 5% polyacrylamide gels.

For 1% agarose gel 1 g of agarose was dissolved in 100 ml of 10 × TBE buffer (109.3 g of Tris, 9.3 g of Na EDTA $2H_2O$ and 55.4 g of boric acid were dissolved in water to make 200 ml of solution). The agarose solution was boiled for 2 min (Dowling et al., 1996).

For 5% polyacrylamide gel to 20 ml of 20% AKA solution 8 ml of 10 × TBE buffer and 0.08 ml of TEMED H_2O was added to make 75 ml of solution. 0.08 g of PCA was added (Harwood, 1997).

About 10 µg of the substance was mixed with 1 µl of bromophenol blue and casted on gel. Electrophoresis was run for 4 h at 120 V in agarose gel and for 2 h 30 min at 200 V in polyacrylamide gel. After electrophoresis the gel was stained with 0.1 µg/ml ethidium bromide for 30 min at room temperature (Harwood, 1997). For estimating the size of fragments the molecular weight marker pBR 322/*Alu*I was used.

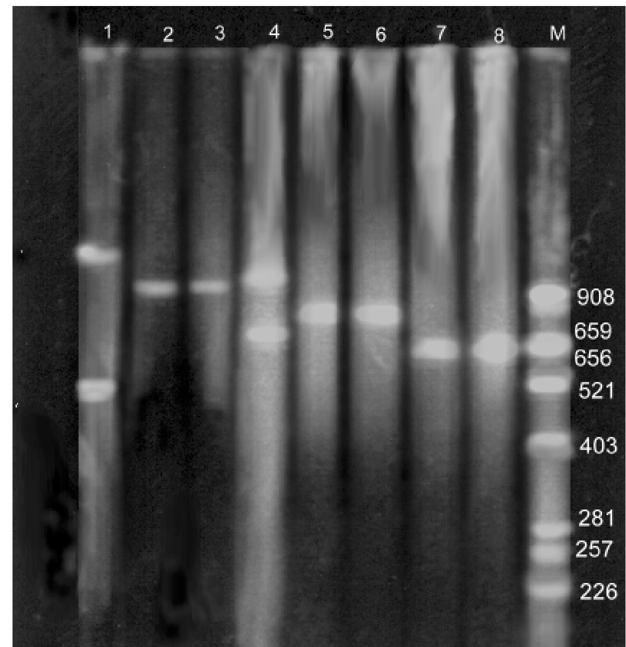

Fig. 1. DNA/*Xba*I restriction patterns in 1% agarose gel among 5 vole species: *1 – C. glareolus; 2, 3 – M. rossiaemeridionalis; 4 – M. agrestis*; *5, 6 – M. arvalis; 7, 8 – M. oeconomus;* M – molecular weight marker pBR 322/*Alu*I

RESULTS AND DISCUSSION

There were no significant differences in DNA isolated from fresh and from frozen tissue. Restriction fragments of *Clethrionomys glareolus* DNA isolated from both fresh and frozen liver were identical. The results showed that the method of DNA extraction was more important. Better results were achieved using phenol-extraction rather than salt-extraction. Using the former method, purer DNA with less protein admixtures was obtained, which is important for RFLP analysis.

DNA digested by *Sca*I enzymes was fractionated by electrophoresis in 5% polyacrylamide gel. DNA treated with *Xba*I was electrophoresed in both 1% agarose (Fig. 1) and 5% polyacrylamide gels (Fig. 2). In both cases the molecular weight marker pBR 322/*Alu*I was used.

After treating *C. glareolus* DNA with *Sca*I, two restriction fragments, 990 ir 670 bp, occurred. One fragment of 940 bp in *M. arvalis*, one fragment of 1000 bp in both *M. oeconomus* and *M. rossiaemeridionalis* were observed. *Sca*I didn't cleave DNA of *M. agrestis.*

In both agarose and polyacrylamide gels, DNA/*Xba*I fragments of one species were identical, but the resolution was better and more fractions were observed in polyacrylamide gel (Table). In both gels, fractions of 1000 and 510 bp in *C. glareolus* were observed. Two additional smaller fragments of 210 and 120 bp occurred in polyacrylamide gel. In both gels two identical fragments of 980 and 620 bp in *M. agrestis*, one fragment of 640 bp in *M. oeconomus*, a fragment of 960 bp in *M. rossiaemeridionalis* and a fragment of 890 bp in *M. arvalis* were observed. Additional fractions of 540 bp in *M. rossiaemeridionalis* and of 220 bp in *M. arvalis* were noted in polyacrylamide gel.

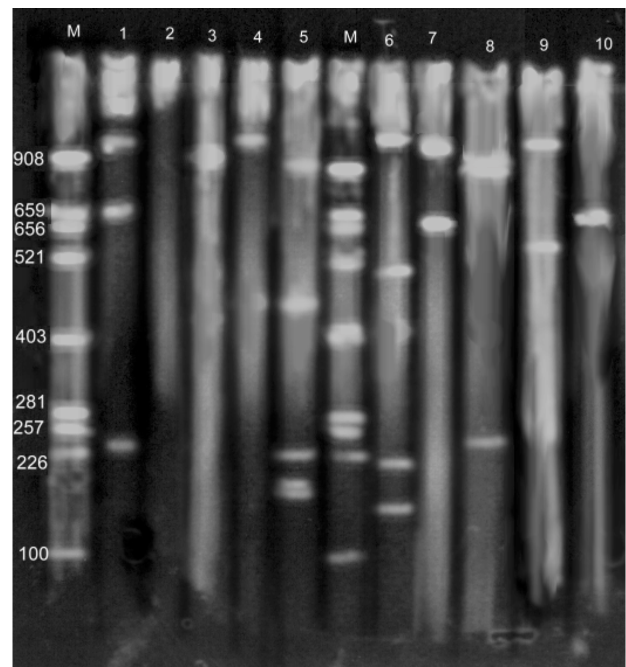

Fig. 2. DNA/*Sca*I (1–5) and DNA/*Xba*I (6–10) restriction patterns in 5% polyacrylamide gel of 5 vole species analyzed: *1, 6 – C. glareolus; 2, 7 – M. agrestis*; *3, 8 – M. arvalis; 4, 9 – M. rossiaemeridionalis; 5, 10 – M. oeconomus;* M – molecular weight marker pBR 322/*Alu*I

The enzymes *Sca*I and *Xba*I gave restriction patterns that differed among all five vole species ana-

Table. **Comparison of vole DNA/*Xba*I restriction fragments**

Species	1% agarose gel		5% polyacrylamide gel	
	Number of fragments	Length of fragments, bp	Number of fragments	Length of fragments, bp
Clethrionomys glareolus	2	1000	4	1000
		510		510
				210
				120
Microtus agrestis	2	980	2	980
		620		620
Microtus arvalis	1	890	2	890
				220
Microtus rossiaemeridionalis	1	960	2	960
				540
Microtus oeconomus	1	640	1	640

lyzed, but using *Xba*I for DNA cleavage the polymorphism of fragments was more distinct. *Xba*I could be used for identification of the vole species.

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References

1. Avise J. C. *Molecular Markers, Natural History and Evolution*. Chapman & Hall, 1994.
2. Carvalho G. R. *Advances in Molecular Ecology*. IOS Press, 1998.
3. Boffey S. A. Restriction endonuclease digestion and agarose gel electrophoresis of DNA. In: *Experiments in Molecular Biology*. Humana Press Inc., 1986. P. 19–27.
4. Darbre P. D. *Introduction to Practical Molecular Biology*. John Wiley & Sons Ltd., 1988.
5. Delidow B. C., Lynch J. P., Peluso J. J., White B. A. Polymerase chain reaction. *Methods in Molecular Biology, Basic DNA and RNA Protocols*. 1997. Vol. 58. P. 275–292.
6. Dowling T. E., Moritz C., Palmer J. D., Rieseberg L. Nucleic acids III: analysis of fragments and restriction sites. *Molecular Systematics*. 1996. P. 249–320.
7. Harwood A. J. Native polyacrylamide gel electrophoresis. *Methods in Molecular Biology, Basic DNA and RNA Protocols*. 1997. Vol. 58. P. 93–103.
8. Kaneko Y., Nakata K., Saitoh T., Stenseth N., Bjornstad O. N. The biology of the vole *Cletrionomys rufocanus*: a review. *Researches on Population Ecology*. 1998. Vol. 40. P. 21–37.
9. Koneshige T., Takagi K., Nakamura S., Hirasawa T., Sada M., Uchida, K. Genetic analysis using fingerial DNA. *Nucleic Acids Research*. 1991. Vol. 20. P. 5489–5490.
10. Leiro J., Siso M. I. G., Parama A., Ubeira F. M., Sanmartin M. L. RFLP analysis of PCR amplified small subunit ribosomal DNA of three fish microsporidian spiecies. *Parasitology*. 2000. Vol. 120. P. 113–119.
11. *Lietuvos fauna. Žinduoliai*. Red. kol.: Kontrimavičius V. ir kt. Vilnius: Mokslas, 1988. P. 134–151.
12. Mažeikytė R., Baranauskas K., Morkūnas V., Mickevičius E. Distribution of the sibling vole (*Microtus rossiaemeridionalis* Ognev, 1924) (Rodentia, Circetidae) in Lithuania. *Acta Zoologica Lituanica*. 1999. Vol. 9. P. 3–13.
13. Miller S. A., Dykes D. D., Polesky H. F. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*. 1988. Vol. 16. P. 1215.
14. Watanabe T., Hayashi Y., Ogasawara N., Tomita T. Polymorphism of mitochondrial DNA in pigs based on restriction endonuclease cleavage patterns. *Biochemical Genetics*. 1985. Vol. 23. P. 105–113.
15. Watanabe T., Masangkay J. S., Wakana S., Saitou N., Tomita T. Mitochondrial DNA polymorphism in native Philippine cattle based on restriction endonuclease cleavage patterns. *Biochemical Genetics*. 1989. Vol. 27. P. 431–438.

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DNR RESTRIKCINIŲ FRAGMENTŲ ILGIO POLIMORFIZMO PANAUDOJIMAS PELINIŲ GRAUŽIKŲ RŪŠIAI NUSTATYTI

S a n t r a u k a

Atlikta Lietuvoje paplitusių Microtinae pošeimio pelėnų *Clethrionomys glareolus, Microtus agrestis, M. arvalis, M. rosiaemeridionalis, M. oeconomus* DNR restrikcinių fragmentų analizė. Skaidant pelėnų DNR restriktaze *Xba*I gaunami polimorfiški specifiniai skirtingoms rūšims priklausančių pelėnų DNR restrikciniai fragmentai. Naudojant šį fermentą atskirų rūšių skirtumai išryškėja geriau nei naudojant restriktazę *Sca*I. Restriktazę *Xba*I galima panaudoti nustatant pelėnų rūšį.

Raktažodžiai: DNR fragmento ilgio polimorfizmas, rūšies identifikavimas, pelėnai, *Clethrionomys glareolus, Microtus agrestis, M. arvalis, M. rossiaemeridionalis, M. oeconomus*