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Sexing of Roe Deer (*Capreolus capreolus L.*) by PCR amplification reaction

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Introduction

Roe Deer is one of the two deer species that can be considered indigenous in Hungary, the other being the Red Deer. There are two species of Roe Deer, European (*Capreolus capreolus*) and Siberian (*Capreolus pygargus*). In Hungary - as in whole Europe - the European Roe Deer can be found.

Species: Sika Deer (*Cervus Nippon*, Temminck, 1838)

TAXONOMY:

Kingdom:	Animalia
Phylum:	Chordata
Subphylum:	Vertebrata
Class:	Mammalia
Order:	Artiodactyla
Family:	Cervidae
Subfamily:	Cervinae
Genus:	Cervus

Two ecotypes, the larger and heavier field and the forest roe deer. Physical size varies due to age, health and the quality of its habitat. These deer are smaller than Red and Sika standing approximately 60-75 cm at the shoulder. The bucks are taller than does and they wear 6-point antlers. Antlers are shed annually in October-November, and regrow immediately afterwards. Animals generally weigh between 17-28 kg, but bucks are also heavier than does.

Roe Deer are unique amongst Artiodactyls in that process of fertilization after mating is subject to a biological mechanism called *embryonic diapause* or *delayed implantation* (it lasts 5 month). Rutting season is in July and august and kids (generally two per litter) are born in late May and June.

Species: Roe deer (*Capreolus capreolus*; Linné, 1758)

TAXONOMY

Kingdom:	Animalia
Phylum:	Chordata
Subphylum:	Vertebrata
Class:	Mammalia
Order:	Artiodactyla
Family:	Cervidae
Subfamily:	Careolinea - Brookes 1828 (*Odocoileinae- Pocock 1923*)
Genus:	Capreolus

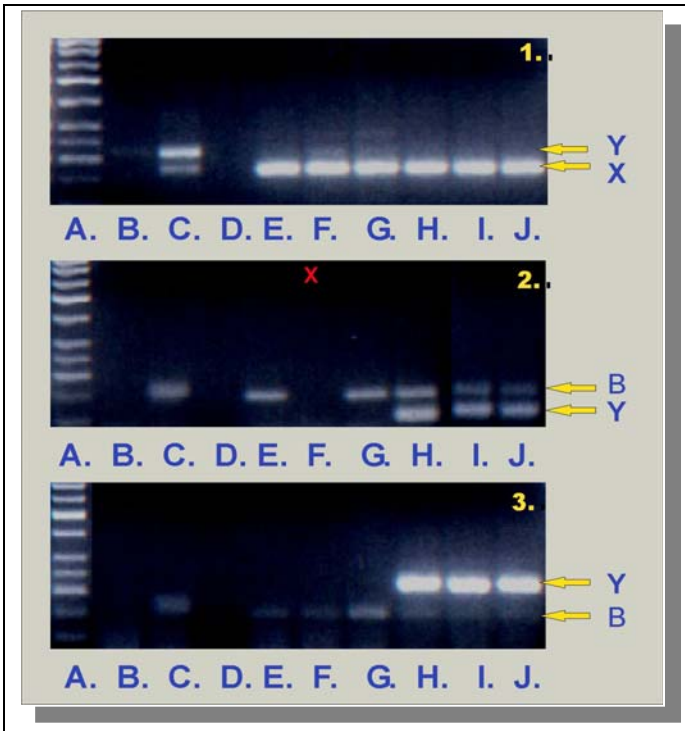
Polymerase Chain Reaction enables us to study the genes of a given species even from a small amount of tissue sample. In a given animal, use of PCR can help in identification of the gender or a trait of economic importance.
In our study, sex-specific sequences of related species published by others were analysed in the hope that, due to interspecies relations, similar sequences could be obtained in Roe Deer.
Sequences of several primers applied in bovine were analysed, but the primers used in Sika Deer by Takashaki et al. (J.Vet.Med.Sci 60(6):713-716, 1998) were found to be most suitable. Using those primers in modified reaction conditions, roes could be sexed successfully.

Results

- Samples were collected and genomic DNA were isolated successfully from shooted deer. The classical mammalian DNA preparing method with Proteinase K digestion, gave a good yield and quality of DNA.
- PCR amplification of genomic Roe deer DNA resulted in valuable fragments with modified method used in Sika deer by Takashaki et al. (J.Vet.Med.Sci 60(6):713-716, 1998)
- MT1 and MT2 primers were amplified to a very similar size of fragment that amplified in Sika deer.
- RG4-RG7 primer pair gave a very high yield of Y-specific fragment with the same size as in Sika deer, but the high yield contributed to a very low yield of autosomal fragment amplification.
- Based on our results the usage of MT1 and MT2 primers together with the bovine autosomal microsatellite specific primers gave us a good and reproducible result in sexing the Roe deer.

- The amplified fragments were isolated from gels and cloned into vectors. Sequence analysis will be performed to make Roe deer-specific PCR primers for sexing the species with high efficiency and accuracy.
- The amplified Y-specific sequence which size is different from that of the Sika Deer fragment is supposed to also be the Sry gene.

Results of PCR amplification



1.
X: bovine X specific primers (XCH) 216 bp
Y: bovine Y specific primers (YCH) 301 bp

2.
B: bovine microsatellite primer (BOVIRBP) 180 bp
Y: sika deer Y chromosome Sry gene specific primer (MT1-MT2) – 104 bp

3.
B: bovine microsatellite primer (BOVIRBP) 180 bp
Y: sika deer Y chromosome Sry gene specific primers (RG4-RG7) – 216 bp

A: molecular weight marker, B: control bovine ♀, C: control bovine ♂, D: negative control, E: deer ♀, F: deer ♀, G: deer ♀, H: deer ♂, I: deer ♂, J: deer ♂

Methods

Muscle and cutis samples were taken from shooted animals at the end of hunting. Samples were put into Eppendorf tubes containing lysis buffer (100 mM TrisHCL, 5mM EDTA, 200 mM NaCl2, 0,2% SDS) and were frozen.

After the samples were delivered into the Laboratory they were thawed and 100 ug/ml Proteinase K was added, and samples were digested O/N at 55 °C. Next day the genomic DNA were prepared by phenol-chloroform elution. The DNA was solubilized in 10 mM Tris-HCL - 20 mM EDTA buffer. Samples were determined by spectrophotometry.

PCR reaction: 10 ng of genomic DNA was added to the PCR mix (10x PCR buffer, 25 mM MgCl2; 10 mM dNTP mix, 25 pM specific primers, 1U *Fermenthas* Taq polimerase, 0.1 % gelatine). The PCR was run on a Perkin Elmer 9600 type thermocycler with the following program:

Predenaturation: 5 min 95°C (1x)
Cycle (35): 30 sec 95°C (denaturation)
60 sec 60°C (annealing)
90 sec 72°C (elongation)
Final elongation: 10 min, 72 °C

The PCR products were analysed by running the samples on 2% agarose (1xTAE) gels, containing 0.5 mg/ml Ethidium-bromide.

Table 1: PCR primer sequences used in the experiments

Primer	Sequence	Amplified fragment (bp)
RG4	5-GGTCAAGCGACCCATGAA(C/T)GCNTT-3	216
RG7	5-GGTCGATACTTATAGTTCGGGTA(C/T)TT-3	
MT1	5-GCTCTAGAGAATCCCCAAATG-3	104
MT2	5-GCCTCCTCAAAGAATGGTCG-3	
BOVIRBP_F	5-TGTATGATCACCTTCTATGCTTC-3	180
BOVIRBP_R	5-GCTTTAGGTAATCATCAGATAGC-3	

Reference:

Takashaki et al. (1998): J.Vet.Med.Sci 60(6):713-716

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