

## Genetic variability of raccoon dogs and their impacts on the environment in Lithuania

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### Abstract

The raccoon dog (*Nyctereutes procyonoides*) is a prominent example of a pest with a wide distribution in Europe and significant ecological impacts. Alien species may have an effect on various levels of biological organisation: genetic, population, community and habitat/ecosystem. Using basic information on abundance, distribution (Bioinvasion Impact/Biopollution Assessment System (BINPAS) and genetic diversity of raccoon dogs, we identified their impact on the environment in Lithuania. Genetic variation of 269 individuals of *N. procyonoides* from Lithuania was analyzed using RAPD and D-loop analysis. RAPD analysis showed that there were 70 polymorphic loci and the number of fragments varied among the primers. The genetic polymorphism was most with ROTH – 180 – 05 and ROTH – 180 – 06 (100%) and least with ROTH – 180 – 10 (25%). Using Rac-1F and Rac-1R primers were amplified D-loop fragments of individuals from different locations in Lithuania. The genetic data suggest that raccoon dogs colonised Lithuania from different neighbouring countries Belarus and Latvia. The raccoon dog impact on native species and communities (C0-C2) were moderately negative for amphibians, mollusks, rodents, birds, insects, and reptiles and for transmission of pathogens.

Keywords: genetics, impacts, invasion, *Nyctereutes procyonoides*, RAPD, raccoon dog

### Introduction

During 1929 to 1955 the raccoon dog (*Nyctereutes procyonoides*) was introduced to 82 locations in the European area of former Soviet Union from the Amur-Ussuri region in far-eastern Russia and has spread from the southern districts to boreal forests, steppe and semi-desert. They were not introduced to Lithuania but were first observed there in 1948, in the eastern part of the country. It is believed that the raccoon dog came from neighboring countries since it was introduced in 1936 to Belarus and Latvia in 1948. Since 2000, raccoon dogs have spread in widely in Europe, observed in 2002 in Macedonia, 2005 in Italy and 2008 in Spain. The raccoon dog population situation in Lithuania can be divided into five periods, during which population size varied. Since 1970 the species has been declared invasive and hunting is permitted throughout the year. Invasive alien species are often a significant threat to ecosystems and, in Lithuania, the raccoon dog impacts on various levels of biological organisation: genetic, population, community and habitat/ecosystem and populations of native rodents, birds, insects, amphibians, and reptiles. In this study we examined raccoon dog genetic variability in relation to the origin of invasion and the consequences of raccoon dog bioinvasion of Lithuania.

### Materials and methods

Between 2007 and 2011 hunters sampled tissue from 269 raccoon dogs from different locations in Lithuania. Genetic variation was analyzed using RAPD and D-loop analysis. Genomic DNA was extracted from frozen liver and muscles using “Genomic DNA Purification Kit#KO512” (Fermentas, Lithuania). PCR for RAPD was performed on a Mastercycler gradient (Eppendorf) in a 21 µl reaction mix and 4 µl template DNA (~25-50 ng). Thermocycling parameters after predenaturation step at 94°C for 4 minutes were 44 cycles with denaturation step at 94°C for 1 minute, annealing step at 49.9 – 60.1°C for 1 minute, elongation step at 72°C for 2 minute and final elongation step at 72°C for 5 minutes.

PCR for D-loop analysis using primers Rac-1F 5'-tcgtgcattaatggcttc-3' and Rac-1R 5'-ccattgactgaatagcaccttg-3' was performed in 20 µl reaction mix and 5 µl template DNA (~50 ng) and thermocycling parameters after predenaturation step at 95°C for 5 minutes were 35 cycles with denaturation step at 94°C for 45 s, annealing step at 55°C for 45 s, elongation step at 72°C for 1 minute and final elongation step at 72°C for 7 minutes. PCR product of D-loop were prepared for sequencing using “GeneJET™ Gel Extraction Kit#K0691” (Fermentas, Lithuania).

RAPD results were analyzed using “TREECON for Windows” (Van de Peer et al., 1994) program. Relationships among individuals were represented in a UPGMA cluster tree and we also calculated degree of polymorphism among individuals. GenBank blastn search algorithm was used (Altschul et al. 1990) to confirm that sequences were from *N. procyonoides*. To determinate haplotypes we used “CLC Sequence Viewer 6.4”. Haplotypes phylogenetic relationships were assessed through haplotypes network using “Network” program.

To evaluate the biopollution level (BPL) of *N. procyonoides* we used the method of Bioinvasion Impact/Biopollution Assessment System (BINPAS), available at <http://www.corpi.ku.lt/databases/binpas> (Olenin et al. 2007). Invasive species impacts were scored at five levels ranging from: no impact (0), weak impact (1), moderate impact (2), strong impact (3), massive impact (4). We similarly assessed raccoon dog impact on native communities (C0–C4), habitats (H0–H4), ecosystem functioning (E0–E4) and biopollution.

## Results

Raccoon dog DNA was amplified with ten primers (ROTH-180-01-ROTH-180-10) and 70 fragments were obtained ranging from 110 to 2000 base pairs. The primer ROTH – 180 – 09 amplified the most polymorphic bands (n = 16). Genetic polymorphism was highest for ROTH – 180 – 05 and ROTH – 180 – 06 (100%) and least for ROTH – 180 – 10 (25%). Using the Rac-1F and Rac-1R primers we successfully amplified D-loop fragments of individuals from different locations in Lithuania.

## Discussion

Following translocation of raccoon dogs by people from Far East to North West European Russia during 1929 to 1955, they began to invade neighbouring areas. However, unlike most other invading species, understanding of the specific dynamics of invasion by raccoon dogs is facilitated by the known date of the European invasion and its geographical source, which is the NW region of the former Soviet Union. Thus, theoretical predictions regarding the general population development seemed fairly simple (Pitra et al., 2010). The genetic data suggest that raccoon dogs colonised Lithuania from the neighbouring countries of Belarus and Latvia, beginning some time after their introduction to Latvia in 1948 and Belarus in 1936) with westward expansion into the wide open ecological niches. However, raccoon dogs were introduced to Latvia and Belarus from different original populations. This situation suggests the likelihood of a second genetic bottleneck because the invaders would only represent a subsample of the originally translocated populations (Pitra et al., 2010).

The raccoon dog impacts on native species and communities (C0-C2) were moderately negative for amphibians, mollusks, rodents, birds, insects, and reptiles, and for transmission of pathogens.

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