Nowadays, European roe deer is one of the most common ungulates and an important game species, which spreads throughout Europe from the Mediterranean to Scandinavia. Across its pan-European distribution, the species faces a wide diversity of environmental and climatic conditions, therefore several factors, including intrinsic ones, shape its life-history traits, and cause significant variability among fitness traits. About 30 % of Poland overlaps forests (Central Statistical Office, 2020) and forest ecotype of roe deer is prevalent on this area. Central part of the country is rarely covered by forest but mainly by agriculture fields, where **field ecotype** of this species developed. Whereas **mosaic** ecotype (forest-field-urbanized) is prevalent e.g. in central Slovenia. Distinct field ecotype of European roe deer is recognised, whereas forest ecotype is not so explicit. Different landscape features affecting availability of resources between the ecotypes lead to inter-ecotype differences in body size and body mass, but also in their distinct ecology and physiology. Roe deer which lives in open landscapes with more severe and variable climatic conditions and limited shelter is in average larger and heavier compared to the forest ecotype. Although migration distances change over the years with roe deer density and seasonally with the reproductive season, it is rather low comparing with other cervids. The experimental material will be collected *post mortem* from three ecotypes of roe deer individuals: forest, field and mosaic. Based on above findings, European roe deer can serve as a model species for studying the long-term effect of environmental factors on biological processes and genetic background.

## General aim: to assess the association between genomic diversity and epigenetic mechanisms of environmental selected parameters of ruminant fitness by using the European roe deer as a model species.

## We hypothesized that:

- 1. Roe deer genetic heterogeneity differs between habitats, and the type of biotope affects the fitness of the individual.
- 2. Susceptibility to stress varies with the environment.
- 3. Body condition and immune status of roe deer differ between biotopes.
- 4. Rumen microbiota depends on the available feeding base in the biotope and correlates with immune status and stress susceptibility.
- 5. The duration of embryonic diapause depends on the biotope of the mother and is regulated by environmental factors, such as feed availability/quality, social relations, and/or exposure to stress.

## Usefulness of the project results:

- Considering how climatic changes and human alterations of the landscape might change habitat availability, it's important to understand the effect of different biotopes on the life history, stress level and welfcare of roe deer. <u>Genetic differentiation</u> between roe deer living in the same climatic conditions, but in different biotopes has not been determined yet. Furthermore, the colonization of different biotopes entails the exposure of individuals to different stress conditions, which will be measured through <u>cortisol and copeptin</u> concentration in faeces, blood, hair and urine. It will allow us to profile and correlate stress parameters immediately prior to death of the individual (blood), over the last 24 h (urine, feces) and over the course of several months (retrospective profile; hair).
- The variety available food varies across habitats and seasons. The feed consumed affects the rumen microflora. Whereas particular feed items might trigger changes in microbiota of rumen and consequently assimilation of nutrition and condition (including immune parameters). Comparison of forage <u>base composition, rumen microbiota, nutrient content, immune parameters</u> in different ecotypes will indicate which habitat is preferred due to availability of forage base for deer and other selected ruminant species.
- So far, no studies have been conducted on the influence of potential maternal factors on the development of blastocysts in roe deer. The process of <u>oocyte vitrification</u>, <u>maturation</u>, <u>in vitro</u> fertilization and <u>culture of blastocysts</u> is innovative, and may be used as reproductive technique in other endangered cervids` species.