The European ground squirrel (*Spermophilus citellus*) (hereinafter EGS) is under the Law on Nature Protection and is included as a critically endangered into the Red List of Endangered Species. In the Czech Republic, which represents the northwestern border of the species range, EGS currently occurs on 34 localities, which are relatively small and isolated from each other. The spread of this species to central Europe was connected with neolithic deforestation. Disjunctive type of distribution of EGS was caused by strong decline of its abundance during the second half of the 20th century due to changes in field management, fragmentation of landscape and other factors. It can be expected that this process left traces on the genetic structure of EGS populations.

The major objective of this study was a detailed analysis of genetic variability of EGS populations in the Czech Republic and searching for corelations with available data about its biology and demography. It was used 13 microsatellite loci and in total were processed 408 samples from 27 localities in CZ and 3 samples from one Hungarian locality as an outgroup. With aid of various methods, details of substructure and differentiation of individual populations, genetic variability, degree of inbreeding and geographic distribution of genetic variability were revealed. Between studied populations was found significant genetic differentiation, west-east gradient in genetic composition of populations and transition zone between bohemian and moravian populations. Low genetic variability and considerable homogeneity of studied populations EGS in the Czech Republic were confirmed.

Results are further interpreted in relation with demographic history, options of gene flow and other factors. The EGS population in the Czech Republic may show fitness reduction due to inbreeding depression and other phenomena connected with species low abundance and range fragmentation.