

**PRELIMINARY MODELING OF GENETIC VARIABILITY OF
BROWN HARES, *LEPUS EUROPAEUS*, IN THE UKRAINE**

*Suchentrunk F.*¹,

*Delegan I. (†)*²,

*Stefanović M.*³

¹*University of Veterinary Medicine Vienna,*

²*Ukrainian National Forestry University,*

³*University of Novi Sad*

franz.suchentrunk@vetmeduni.ac.at

The high ecological adaptability of hares, genus *Lepus*, may be at least partly caused by their relatively recent adaptive radiation and shallow gene pool divergence among species, which provides still the chance of combining genetic variants by introgressive hybridisation in several species. During the late Pleistocene, particularly the Late Glacial Maximum, brown hares, were absent from northern and central Europe, where only mountain hares roamed the tundra region there. They were, restricted to southeastern and eastern European refugia, Anatolia, and probably other parts of the Middle East. Subfossil records indicate their late Pleistocene presence on the Crimean Peninsula in sympatry with mountain hares, with the latter still occurring today in the north of the Ukraine. The postglacial northward range expansion of brown hares from several of those glacial refugia probably over many generations of sympatry with northward retreating mountain hares and possible frequent (bidirectional?) introgressive hybridisation may have increased the genetic diversity in brown hare populations in the Ukraine.

To determine the level of genetic diversity of brown hares from the Ukraine and to specifically examine, if their allelic composition indicates introgression of mountain hares, even in regions where the latter species is not any longer present for many generations, we used organ tissue samples of 90 brown hares, *Lepus europaeus*, collected in 2009 and 2010 from across the Ukraine and analysed the allelic variation at eleven microsatellite loci. We included data of three mountain hares from Norway to specifically obtain information on mountain hare-specific gene pool elements possibly still existing in Ukrainian brown hares. Norwegian Mountain hares most likely have not had naturally contact with brown hares since the Late Glacial Maximum and may thus represent “pure” mountain hare gene pool characteristics, which cannot be necessarily

expected for mountain hares from the Ukraine or many other parts Europe. We based our individual genotype analyses on georeferenced samples for a first spatial model of gene pool differentiation in the Ukraine (using Geneland) and used STRUCTURE to identify mountain hare-typical gene pool portions in individual brown hares. We also tested for spatial differences of mountain hare-typical gene pool portions, expecting higher levels in the north of the Ukraine, the region of current sympatry with mountain hares.

We found 101 alleles at the eleven studied loci (Sat 2, 8, 12, Sol 8, 28, 30, 33, Lsa 1, 2, 6, 8), with a mean number of alleles per locus of 9,182, an overall observed and expected heterozygosity of 0,577 and 0,665, respectively. One (0,0267 %) of all pairwise comparison of composite individual genotypes revealed identity, which indicated a very high level of individual genotypic resolution. No deviation from linkage disequilibrium was observed but the significant deviation from the Hardy-Weinberg equilibrium and F_{is} values significantly above zero for several loci indicated some spatial structuring (Wahlund effect) rather than (local) inbreeding. The relative and absolute genetic differentiation between the Ukrainian brown hares and the Norwegian mountain hare samples (F_{ST} =0,174; $p < 0,001$; and $d=0,217$ $p < 0,0001$; Cavalli-Sforza & Edwards distance, Genetix) as well as the first three factors of our factorial correspondence analysis indicated significantly distinct gene pools for the brown and mountain hares studied, despite many alleles in common. The unambiguous genetic distinction of the two species as represented by our samples was also confirmed by Bayesian assignment analysis of each individual to either the brown hares or the mountain hares, according to their respective composite genotypes: our Bayesian approach of individual assignment and Monte-Carlo resampling implemented in GeneClass 2.0.h. resulted in 100 % correct classifications, i.e., all individuals were assigned to the species to which they were originally assigned phenotypically without genetic data.

Unexpectedly, our STRUCTURE analysis revealed that basically all individuals show signals of mountain hare-typical gene pool characteristics at variable degrees, but without distinct spatial distribution pattern (Fig. 1).

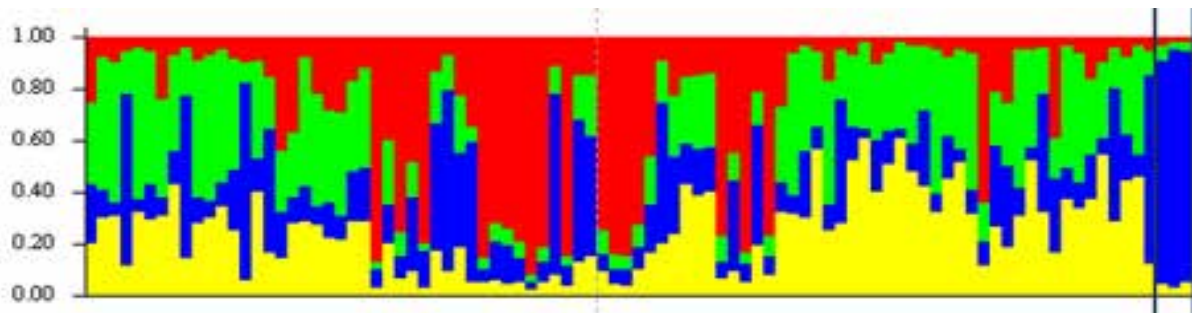


Fig. 1. Example of a STRUCTURE run for K=4, admixture model without population priors. The three rightmost individuals are the three Norwegian mountain hares, and all other individuals are Ukrainian brown hares, with variable portions of mountain hare-typical gene pool portions (i.e., blue-coloured segments).

Our Geneland modeling revealed a significant genetic partitioning into five populations (Fig. 2).

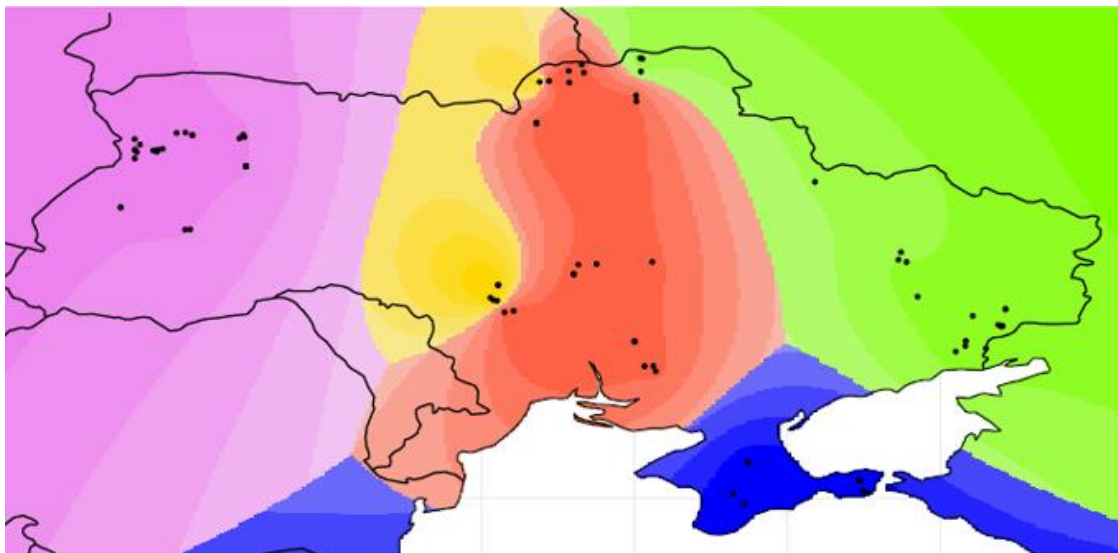


Fig. 2. Spatial model of genetic differentiation of brown hares in the Ukraine (according to Geneland). Black dots are sample locations.

In conclusion, our population genetic results revealed relatively high genetic diversity in the brown hares from the territory of the Ukraine with significant spatial partitioning into five populations with only little but significant differentiation. This pattern of genetic partitioning may be due to the combination of the presence of different autochthonous Late Pleistocene gene pool units and gene flow from different late glacial source populations west, east, and south of the Black Sea. Some elements of gene pool diversity have seemingly been added by long-term historical introgression by historically sympatric mountain hares.



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директор НДІ лісівництва та декоративного садівництва,
кандидат сільськогосподарських наук, доцент О.П. Бала

кандидат технічних наук, доцент О.Ю. Горбачова

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