

ABSTRACT

The brown hare (*Lepus europaeus* Pallas, 1778) is widespread throughout Europe, occurring in a variety of environments. It has considerable economic importance because it is one of the most important game species in Europe and especially in the Balkan Peninsula and Greece. The extensive distribution of this species and the fact that its populations respond rapidly to environmental and habitat changes, make this species a suitable model for the study of the behaviour, biology and genetic structure of wild mammals populations.

In this thesis we analysed the population genetics of the brown hare (*Lepus europaeus*) in European, Greek and Anatolian populations, using sequencing, PCR-RFLP and PCR-SSCP methods, performed on mtDNA and Y-DNA genes, in order to:

- assess the genetic differentiation and the phylogenetic status of brown hare populations
- test the hypothesis that this species migrated into central Europe from a number of late glacial refugia, including some in Asia Minor
- estimate the genetic structure of reared populations and the impact of the releases on the native populations genetic structure
- develop molecular markers for the identification of reared and native populations

The conclusions drawn from the study are the following:

- Colonization of large parts of Europe started from only one late glacial/early Holocene source population in the central or south-central Balkans, and it was relatively quick. This contradicts Bilton *et al.*, (1998), who suggested colonization of central Europe by some small mammals from Late Pleistocene refugees in eastern Europe and western Siberia, as an alternative to colonization from Mediterranean refugees.
- So far, mtDNA of non-introgressed brown hares from Iberia have standard central European haplotypes, suggesting no late Pleistocene refuge in Iberia.
- In Bulgaria and northeastern Greece all haplogroups were present, forming a large introgression zone.
- Gene flow from Anatolia to Europe, across the late Pleistocene Bosphorus land bridge, was detected.

- Brown hares from the British Isles had very low mtDNA polymorphism showing that they do not constitute a separate subspecies (*L. e. occidentalis*), as previously suggested. Northern Germany could be a possible source region of the current populations.
- Over hunting and continuous releases of brown hares could explain the massive presence of EU-A haplotypes in central and northern Italy. This practice might have replaced the native and possibly originally widespread SEE haplotypes. Our data contradict the idea of the existence of the subspecies *L. e. meridiei* in the Italian peninsula.
- Although various restocking operations could be partly responsible for the presence of unexpected haplotypes in certain areas, we nevertheless trace a strong phylogeographic signal throughout all regions under study.
- Phylogenetic trees produced by the comparison of mt tRNA genes are similar compared to those that are produced by the standard methods.
- Molecular investigations on mt tRNA could serve as valuable accessory elements for connecting analysis of precise and unimpeded biochemical function in mitochondria with evolutionary and phylogenetic studies.
- We assume that the tRNA genes that were used for the present analysis accumulated mutations in positions that do not seem to affect their role in mitochondrial protein synthesis.
- These tRNA recorded evolutionary changes that can be directly connected with the distinct phylogeographic distribution of *L. europaeus*. The fact that the observed nucleotide substitutions appear in a large portion of the *L. europaeus* individuals indicates that they are tolerated by natural selection pressure.
- Consistent with previous data, phylogenetic analysis of *Cytb* gene corroborates the existence of four different haplogroups with a well defined distribution across Europe and Anatolia.
- The analysis consistently supports the deep separation of Anatolian (Turkey and Israel) and European lineages of *L. europaeus* at the mtDNA level.
- Contrary to other biparental nuclear markers, Y-DNA data underline the existence of two major phylogenetic clades within *L. europaeus* species between Anatolia and Europe.

- Unlike mtDNA, Anatolian Y-DNA extended into central Greece, where it was detected in few individuals. It is very likely, that the tendency toward philopatry of female brown hares makes mtDNA introgression more difficult in comparison with Y-DNA, resulting in different distributional patterns.
- In contrast to natural populations, reared populations showed relatively little genetic variation, as revealed by mtDNA and RAPDs. Greek populations have also higher levels of genetic variation than populations from Central Europe. The only advantage that the released animals could confer is the enrichment of the already rich Greek mtDNA genetic pool, given the absence of the “reared” mtDNA haplotypes from Greece.
- In that case there is still a question of “outbreeding depression” from locally maladapted genes imported with the allocthonous animals. Bulgaria and now, after the releases, north-eastern Greece are the only European regions where all four types of mtDNA profile occur. In both cases either there are no data or it is too early to conclude any possible interaction between the different types of haplotypes. If, in the long run, introgressed foreign genes survive, forming new genotypes with indigenous genes, this would in fact demonstrate that they are successful in terms of competition. As nuclear gene pools are not too divergent between Greek and other European hares, foreign nuclear genes should not be a serious handicap. Hence, in certain situations releasing programs might be tolerated under obeying all other non-genetic strict controls.