

## Evidence of positive selection in the mitochondrial DNA lineage of the Mountain Hare (*Lepus timidus*) – Adaptation to cold?

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### Abstract

Hares (*Lepus* spp.) are widely distributed across the globe and adapted to diverse climatic conditions and are therefore an interesting model to investigate adaptive evolution. Mitochondrial DNA (mtDNA) easily crosses species borders among species of hares through introgressive hybridization, which has raised hypotheses of adaptive reticulate evolution in this group. The most intriguing cases of mtDNA introgression among hare species involve the Mountain Hare (*Lepus timidus*), a species with a large range of distribution across the Northern Palaearctic that is well adapted to boreal/arctic climates. Its mitochondrial lineage has been detected in several other hare species from temperate and arctic regions, not only in areas of current interspecific contact but also in regions where *L. timidus* was present during the Pleistocene but from where it went extinct by the end of the last glacial period, such as in the Iberian Peninsula. In Iberia, *L. timidus* mitochondrial DNA reaches high frequencies among *Lepus granatensis* populations of the North, is almost fixed in the Iberian populations of *Lepus europaeus* and completely replaced the mitogenome of the Cantabrian *Lepus castroviejo*. In Southern Europe, *L. timidus* mitochondrial lineage has also completely replaced that of the Italian *Lepus corsicanus*. In addition, *L. timidus* mitochondrial lineage was detected in *L. europaeus* populations of Northern Europe and introgression possibly affected many other species from Eurasia to North America. The ubiquity of mtDNA introgression of *L. timidus* origin raised the question of adaptive reticulation and evolution of *L. timidus* mtDNA, also given the important functions of the genes encoded by mtDNA in thermoregulation and energy production. In this work we test the hypothesis that *L. timidus* mitogenome has evolved under positive selection. We applied codon analyses based on estimates of  $\omega$  (dN/dS) to the nucleotide sequences of the thirteen mitogenomic protein-coding genes of *L. timidus* and eight other hare species representative of different climates and regions, from the Northern to Southern Europe, Africa and North America. We then compared the likelihoods of models of neutral evolution with alternative models of natural selection. Evidence for positive selection in the divergence of *L. timidus* mtDNA was found in one codon of the ATP8 gene, one of the mitochondrial genes that participate directly in energy production by the cell. This finding suggests that *L. timidus* mtDNA has evolved by adaptive evolution, eventually driven by cold climates. It also shows that the mtDNA of *L. timidus* has adaptive value and thus

strengthens the hypothesis that mtDNA reticulation among hares involving this species might have been driven by adaptation.

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