

The use of ancient DNA (aDNA) analyzes to reconstruct the phylogeny and evolution of the northern vole population (*Alexandromys oeconomus*)

Abstract

The climatic and environmental changes in the Late Pleistocene (ca. 129-11.7 thousand years ago) and especially during the last glacial period had a great impact on the evolutionary histories of most species. However, small mammals are particularly well suited to studying the impact of climate and environmental fluctuations on population dynamics because, unlike megafauna species, they were little affected by Paleolithic humans and their population dynamics were driven mainly by environmental changes. One of interesting species in this context is the tundra vole *Alexandromys oeconomus* (Pallas, 1776), which is an example of a cold-adapted rodent, widespread in the Palearctic and western Nearctic (Alaska and western Canada). During the last glaciation, *A. oeconomus* was more widespread in Europe than at present, but due to specific habitat requirements, it showed a heterogeneous distribution.

In order to reconstruct the most likely phylogenetic relations and demography in *A. oeconomus* populations since the Pleistocene, 33 mitochondrial genomes (mtDNA) from contemporary specimens and 148 ancient mitochondrial genomes from fossil specimens from 26 paleontological sites were sequenced. The second dataset containing much more samples consisted of newly acquired and deposited in GenBank cytochrome b (cytb) molecular marker sequences. Furthermore, radiocarbon dates to calibrate the molecular clock were obtained from 10 fossil specimens. Based on fossil records, we also applied an approach using the divergence time of *Alexandromys oeconomus* from its closely related species reed vole *Alexandromys fortis*, used as an outgroup.

The comparison of two approaches in molecular clock calibration applied for two data sets (mtDNA and cytb) showed that the calculation of divergence times is less influenced by the sequence set than the calibration methods. The performed analyzes confirm that the populations of the tundra vole can be divided into two main groups, including Asian-American and European specimens, and that Western and Central Asia was the initial region of this species evolution. The presented results solved the existing doubts related to the evolution of the examined rodent. Namely, it has been showed that Northern Europe was inhabited from Central



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and Eastern European refugia, at least three times, and that North America was colonized across the Bering Strait during at the end of the last glacial period, ca. 22-15 thousand years ago, but not much earlier. Based on the divergence times of groups and phylogenetic lineages and their comparison with the MIS periods and the $\delta^{18}\text{O}$ oxygen isotope curve, a good climate proxy, it was shown, e.g. that the separation of Asian and European voles occurred before the last glaciation, and further differentiation took place during the last ice age. The time of the greatest differentiation coincides with the time of interstadials, the Hengelo-Charbon interstadial (43-41 thousand years ago) and the Denekamp-Grand Bois interstadial (36-33 thousand years ago), while the extinction of many European lineages took place during the Last Glacial Maximum (LGM). During the warming of the climate, there were further migrations and colonizations of new areas, e.g. North America and repopulation of Fennoscandia. In addition, the analyzes performed in this work indicate that the increase in the number of phylogenetic lineages through time and the effective population size was associated with climate warming, and their decreases with cooling, the largest reduction of these values occurred in the LGM. The distinguished groups were characterized by different genetic diversity. The greatest variability was shown by long-evolving populations over a large area of Asia, from the Urals to the Central Asia. On the other hand, the least variability was shown by groups that evolved recently and settled new areas, such as Fennoscandia and north-eastern Europe as far as the Urals. The obtained results made it possible to reconstruct the geographic distribution of ancestors of phylogenetic lineages as well as the times and potential routes of migration of *A. oeconomus* populations.