Late Pleistocene and Holocene evolutionary history of common (Microtus arvalis) and field (Microtus agrestis) voles

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Introduction

Climate changes that occurred during the Late Pleistocene have profound effects on the distribution of many plant and animal species. The course and mechanisms of responses of species to the past climate changes are now being intensively studied by the use of direct radiocarbon dating and genetic analyses of fossil remains. However, so far large mammals got most of the attention and the studies of small mammals species are underrepresented. Recently, an ancient DNA study of collared lemmings from Eurasian paleontological sites revealed a complicated history of this species with series of continent-wide extinctions and recolonizations that took place during the last 50,000 years [1]. In this project we aim to use similar approach to reconstruct evolutionary histories of two small mammal species M. arvalis (common vole) and M. agrestis (field vole). Both species differ significantly in their environment and habitat preferences. Field voles occupy a range of biotopes, but prefer rather damp environments, like marshes, peat bogs and riverbanks. Common voles prefer dry habitats, like open grasslands and meadows where they inhabit systems of subterraneous burrows. Both species are considered relatively cold-tolerant, have a wide distribution in Europe and are frequent in Late Pleistocene fossil assemblages. Comparison of reconstructed evolutionary histories of two vole species with paleoclimate records may provide important clues on how temperate small mammal species responded to environmental changes. Proposed study design gives also opportunity to compare responses of species with different habitat and environmental preferences to infer what types of habitats were affected most by which kind of climatic factors.

Here we present preliminary results of the project focused mainly on Central Europe

Microtus agrestis

Microtus arvalis

MtDNA diversity of modern European populations of common vole consist of six lineages: Western, Central, Eastern, Italian, Balkan, a lineage sampled in Freiburg, Germany and a sixth Asiatic lineage of Microtus obscurus (Figure 1) [3,4]. It was postulated that beside classical refugias common voles might have survived LGM at high latitudes [4]. Especially Carpathian region was indicated as a possible high latitude glacial refugium for Eastern lineage [5].

To date we obtained cytochrome *b* sequence for 29 specimens from Central Europe (Figure 1C).



5. Martina cave Layer 7 -> 7 ka BP (1 ind)

6. Petenyi -> Holocene (1 ind)



1.Obłazowa (WE) layer III –> **15 ka BP** (10 ind) 2. Cisowa Skala layer III -> **16,5 ka BP** (2 ind) 3.Byci skala layer 8c -> **12,4 ka BP** (1 ind) 4. Bivak cave -> Late Glacial (1 ind) 5.Tucin 1K -> **MIS 3** (1 ind) 6.Zkamenely zamek -> MIS 3 (1 ind)



cytochrome b mtDNA sequences (A), contemporary distribution of field vole mtDNA lineages (from [7] (B), distribution of field vole mtDNA lineages in Pleistocene and Holocene (C)

. Mastlana cave Layer 15 - 16 ka BP (1 ind)

PLEISTOCENE

 $\bigcirc 2$



0.0050





lineages: Western, Central, Eastern and three lineages with limited ranges: one occurs in northwestern France, one in northern Britain and one in southwestern Sweden (Figure 2) [6,7]. Recently the divergence of all mtDNA lineages were dated to ca. 12,000 years BP. This strongly suggests that field voles suffered a severe bottleneck during Younger Dryas cooling and the whole contemporary diversity was formed at the onset of Holocene [7,8].

To date we obtained cytochrome b sequence for 15 specimens from Central and Eastern Europe (Figure 2C).

Materials and Methods

DNA was extracted from molar teeth or mandible fragment using column based method optimized for paleontological material [2]. Fragment of mtDNA cytochrome b (990 bp for common and 1,030 bp for field vole) was amplified in multiplex reactions as a set of 15 (common vole) and 14 (field vole) fragments not exceeding 125 bp. PCR products were transformed into sequencing libraries, poled and sequenced on Illumina MiSeq platform. Sequencing reads were merged in AdapterRemoval, PCR primers were trimmed in Mothur. MtDNA cytochrome b sequences were assembled in SeqMan NGen (DNASTAR). Consensus sequence for each sample was called from two independent replicates. Phylogenies were reconstructed in Beast using HKY + G substitution model with sequence partitioned into three codon positions. Analysis was run for 10,000,000 generations with parameters samples every 1,000 generations. Tracer was used to assess stationarity and convergence.

Pleistocene and Holocene (C).



Conclusions

Altough the project is on an early stage and the amount of data is very limited some preliminary observations and conclusion can be drawn:

1. There was a continuity between Late Glacial and Holocene field vole populations on mtDNA lineages level (however not nececerly on haplotype level)

2. There was a replacement of common vole populations at the time of Younger Dryas (Eastern lineage replced Central lineage)

3. During Late Glacial Balkan lineage of *M. arvalis* was present further to the north than in present.

Presented data suggests that post-glacial recolonization of Central Europe by common and field voles was more complicated than it was inferred from contemporary genetic data.

[1] Palkopoulou, E. et al. Glob. Chang. Biol. 1–12 (2016). doi:10.1111/gcb.13214, [2] Dabney, J. et al.. Proc. Natl. Acad. Sci. U. S. A. 110, 15758–63 (2013) [3] Haynes, S., Jaarola, M. & Searle, J. B. Mol. Ecol. 12, 951–956 (2003) [4] Heckel, G., Burri, R., Fink, S., Desmet, J.-F. & Excoffier, L. Evolution 59, 2231–42 (2005) [5] Stojak, J., Mcdevitt, A. D., Herman, J. S., Searle, J. B. & Wójcik, J. M. Biol. J. Linn. Soc. 115, 927–939 (2015) [6] Jaarola, M. & Searle, J. B. Mol. Ecol. 11, 2613–21 (2002) [7] Herman, J. S. & Searle, J. B. Proc. R. Soc. B Biol. Sci. 278, 3601–3607 (2011) [8] Herman, J. S. et al. PLoS One 9, (2014).

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