

Ancient DNA clarifies the evolution of the field vole species complex



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Introduction

The field vole is a complex of three cryptic species. The short-tailed field vole (*Microtus agrestis*) is present over much of Eurasia, the Mediterranean field vole (*Microtus lavernedii*) is found in southern Europe, and the Portuguese field vole (*Microtus rozianus*) is limited to western Spain and Portugal [1]. Previous studies showed discordance of mitochondrial (mtDNA) and nuclear gene trees and suggested that the Portuguese field voles diverged from Mediterranean and short-tailed field voles ca. 70 thousand years (ka) ago, while the two latter separated about 25 ka ago during the Last Glacial Maximum (LGM) [2–3]. The latter was reinforced by the Ecological Niche Modeling which predicted three potential glacial refugial areas during the LGM [3].

Previous reconstructions of demographic history of the short-tailed field vole showed a species-wide bottleneck around the Younger Dryas (12.5–11.7 ka ago) [4–5]. However, little is known about earlier population dynamics during the Late Pleistocene.

Here, we decided to reassess the previous divergence estimates and to provide new information on evolutionary history of this species complex using mitochondrial and genomic data from ancient and modern field vole specimens.

Workflow and dataset

- Target enrichment of vole mtDNA used to generate mitogenomes of **75** ancient and **47** modern specimens ranging in age between 0 and ca. 70 ka (**Fig. 1A**).
- Ten ancient specimens directly radiocarbon dated to calibrate the molecular clock - collagen pretreatment in MPI EVA + AMS in ETH and Aix-en-Provence.
- Phylogenetic analyses, molecular age estimation of not dated specimens and demographic reconstruction performed using BEAST 1.10.4 [6].
- Shotgun sequencing of **11** ancient (coverage: 0.3–2.57×; median: 0.97×) and **14** museum and modern genomes (coverage: 0.73–36.8×; median: 8.8×).

Mitochondrial phylogeny

- We detected three previously recognised field vole mtDNA lineages and a new one (Italian) confined to the Late Pleistocene specimens from Italian Peninsula.
- Divergence of main mtDNA lineages estimated to Marine Isotope Stage (MIS) 7 and MIS 5 (**Fig 1B**) surprisingly similar to the divergence of main mtDNA lineages in some cold adapted small mammals: narrow-headed voles, collared lemmings and common voles [7].
- Short-tailed field vole suffered a bottleneck around LGM and and post-LGM recovery (**Fig. 1C**) earlier than previously estimated, but mtDNA diversity was higher during Boling-Allerod (14.7–12.5 ka ago) than today.

Genomic data

- MDS revealed three clusters corresponding to main mtDNA lineages. The oldest specimens (ca. 70 and 40ka old) are only slightly shifted towards the centre of the plot providing *terminus ante quem* for the divergence of field vole species (**Fig. 2B**).
- Both PSMC and hPSMC support the divergence of Portuguese field vole ca. 200 ka ago and subsequent separation of Mediterranean and short-tailed field voles about 100 ka ago (**Fig. 2**).
- The Portuguese field-vole had overall the lowest Ne (**Fig. 2C**).
- Short-tailed and Mediterranean field voles suffered a bottleneck around LGM and a recovery around Pleistocene-Holocene transition (**Fig. 2C**).

Conclusions

- The divergence for field-vole species took place much earlier than previously estimated around 200ka (MIS7) and 100ka (MIS5) periods recently suggested to be important hotspots for differentiation [7].
- Discordance of mtDNA and genomic phylogenies - evidenced by different order of divergence - the case of ancient mtDNA capture?
- Distinct mtDNA lineage on Italian peninsula - conforms with theory of Mediterranean peninsulas as a source of endemism [7].
- Both mtDNA and genomic data supports a bottleneck in short-tailed field vole around LGM - where there two bottlenecks around LGM and YD?

To be done

- Use D-statistics to investigate potential ancient introgression
- Use modeling on the genomic data to estimate the timing of bottleneck more precisely.
- Check for the genomic signatures of adaptation of field-vole species to different ecological niches.

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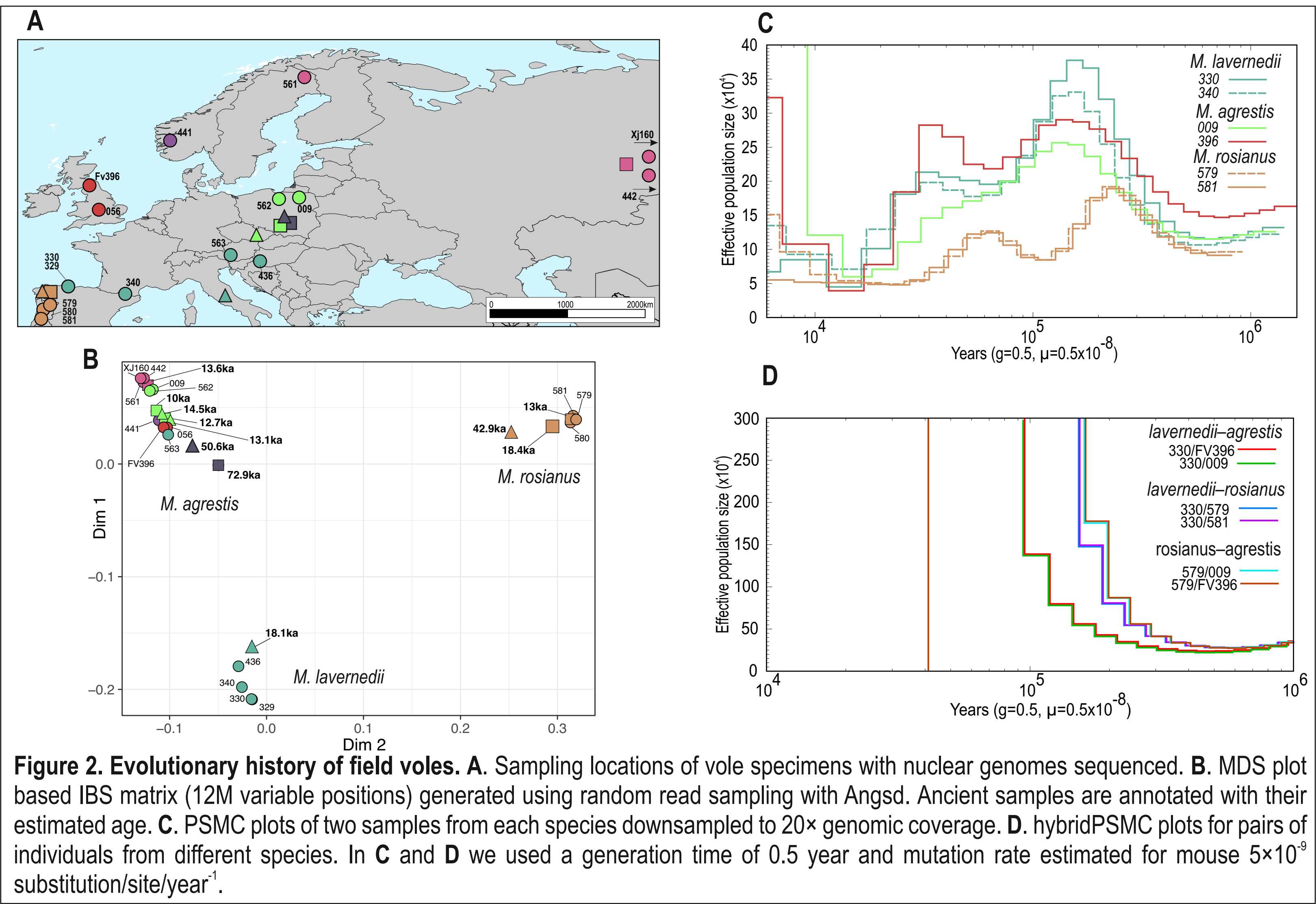


Figure 2. Evolutionary history of field voles. **A.** Sampling locations of vole specimens with nuclear genomes sequenced. **B.** MDS plot based IBS matrix (12M variable positions) generated using random read sampling with Angsd. Ancient samples are annotated with their estimated age. **C.** PSMC plots of two samples from each species downsampled to 20× genomic coverage. **D.** hybridPSMC plots for pairs of individuals from different species. In **C** and **D** we used a generation time of 0.5 year and mutation rate estimated for mouse 5×10^{-9} substitution/site/year⁻¹.

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