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Mitochondrial DNA Gene Diversity and Morphological Variability of the Common Vole (*Microtus arvalis*) in France

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Introduction

The common vole (*Microtus arvalis*, Pallas 1778) is widespread throughout western Europe, from Atlantic coast of France to central Russia with isolated Iberian populations (Mitchell-Jones *et al.*, 2002; Le Louarn & Quéré, 2003). Genetic structure of this species and colonization processes in Europe were examined by analysing mitochondrial DNA sequences from cytochrome b gene, control region and nuclear microsatellite loci (Haynes *et al.*, 2003; Fink *et al.*, 2004; Heckel *et al.*, 2005). Five main evolutionary lineages in Europe were defined: Western (W), Central (C), Eastern (E), Italian (I) and Freiburg (F). Haynes *et al.* (2003) suggest that there were several glacial refugia, in Iberia (W), Italy (I) and the Balkans (C and E). On the other hand, according to Fink *et al.* (2004) and Heckel *et al.* (2005), the genetic diversity in populations suggests a glacial survival of the common vole outside the classical refugial areas and a potentially more ancient colonization from the northeast to the southwest of Europe.

We investigated the genetic and morphological structure of common vole populations in France from molecular (mitochondrial sequences from the 5' peripheral domain of the control region) and morphometrical (global morphological analyses from teeth) dataset in order to clarify the evolutionary history of the western lineage and its hypothetical implication in postglacial recolonization of western Europe.

Dataset

Our sampling includes 22 *M. arvalis* from 8 populations for molecular analyses and 429 first lower molars (m1) covering 10 populations for morphometrical analyses (Fig. 1). In molecular analyses, other *Microtus* species (*M. rossiaemeridionalis*, *M. (T.) subterraneus* and *M. (T.) multiplex*) were added for phylogenetic reconstructions, as well as *Chionomys nivalis* and *Clethrionomys glareolus* used as outgroups.

Results

Molecular Analyses

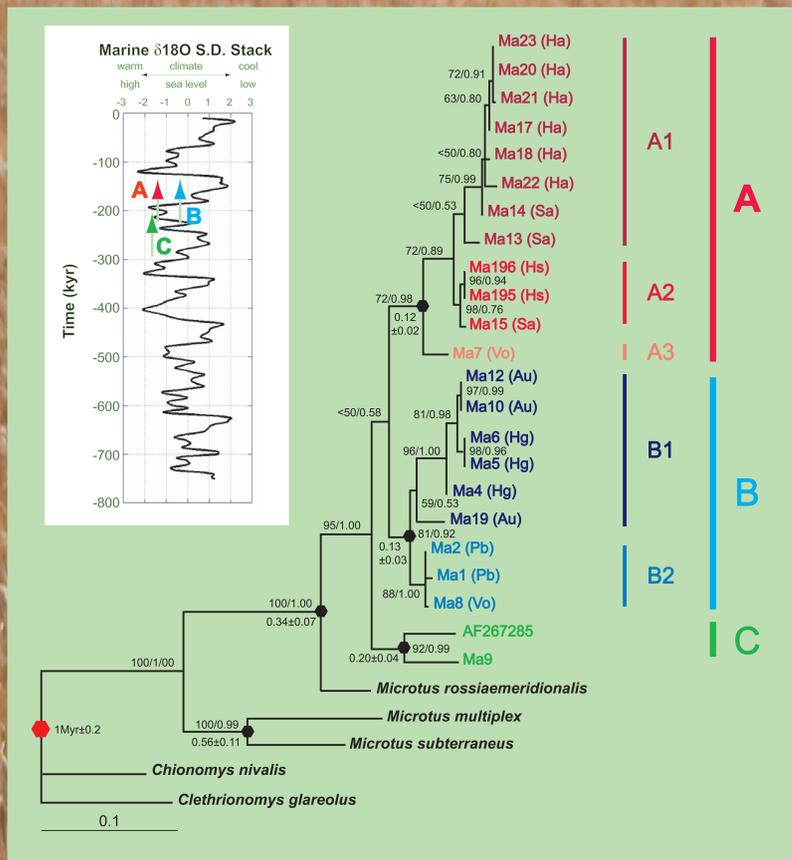


Fig. 2: Maximum-likelihood tree from a fragment (5' peripheral domain; 371 bp) of the control region (GTR+I+Γ8; InL=1745.8923; I=0.2661; α=0.5220). Bayesian approach yielded the same tree topology. Numbers above branches or at nodes refer to (from left to right) bootstraps (1000 replications) in maximum-likelihood (PhyML) and Bayesian posterior probabilities (MrBayes). The molecular clock hypothesis (Puzzle) was not rejected when tested by likelihood ratio test (2 ΔlnL=27.49<χ²=37.65; df=25; p=0.05). Numbers below branches are divergence dates estimated for some population split (black hexagons), calibrated on the split between *Chionomys* and *Microtus* lineages at 1±0.2 Myr (red hexagon; Chaline, 1987; Nadachowski, 1991). In box, composite stack of many δ18O deep sea core (Imbrie *et al.*, 1984).

High supports are observed for the *Microtus arvalis* clade. Three groups are highly or moderately supported: sub-groups (A1, A2 and A3);

- the group B is more geographically structured. It groups together the populations Hg, Au, Pb and also one Vp sample. Two sub-groups (B1 and B2) could be identified; B1 (Hg+Au) is moderately supported, whereas B2 (Pb+Vo) is highly supported;

- the group C encompasses AF267285 (Austria) and the third Vo sample.

Populations from Austria belong to the central lineage, whereas populations from France should be included in the western lineage. Samples from Vo are distributed in groups A, B and C as well.

Discussion

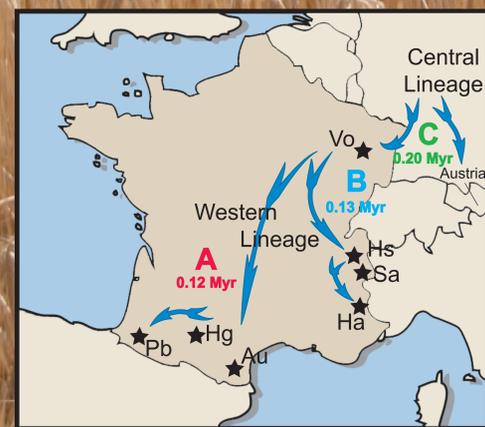


Fig. 4: Proposed colonization scenarios for *Microtus arvalis* in France before and after the penultimate glaciation. Arrows indicate the possible ways of migration. Stars are for the populations mentioned in this study (Vo; Vosges; Hs; Haute Savoie; Sa; Savoie; Ha; Hautes Alpes; Pb; Pays Basque; Hg; Haute Garonne; Au; Aude).

Prospects

After this study, at least two prospects of investigation can be conceivable: 1) the 5' peripheral domain of the control region is sufficiently variable to extend this parameter in ancient DNA study and 2) biometric variables, associated with modularity variations, can be used on fossil record of vole m1 to underline shape variations. Both approaches will complete temporally and spatially our understanding of *Microtus arvalis* migrations and evolutionary history during the last Quaternary glaciations.



Fig. 1: Distribution of the common vole in France (dark green) and sampling locations (Yv: Yvelines; Vo: Vosges; Do: Doubs; Hs: Haute Savoie; Sa: Savoie; Ha: Hautes Alpes; Au: Aude; Hg: Haute Garonne; Pb: Pays Basque; Vi: Vienne; Ve: Vendée). Open stars are for morphometric data, whereas filled stars (with sample labels) are for molecular data.

Morphometrical Analyses

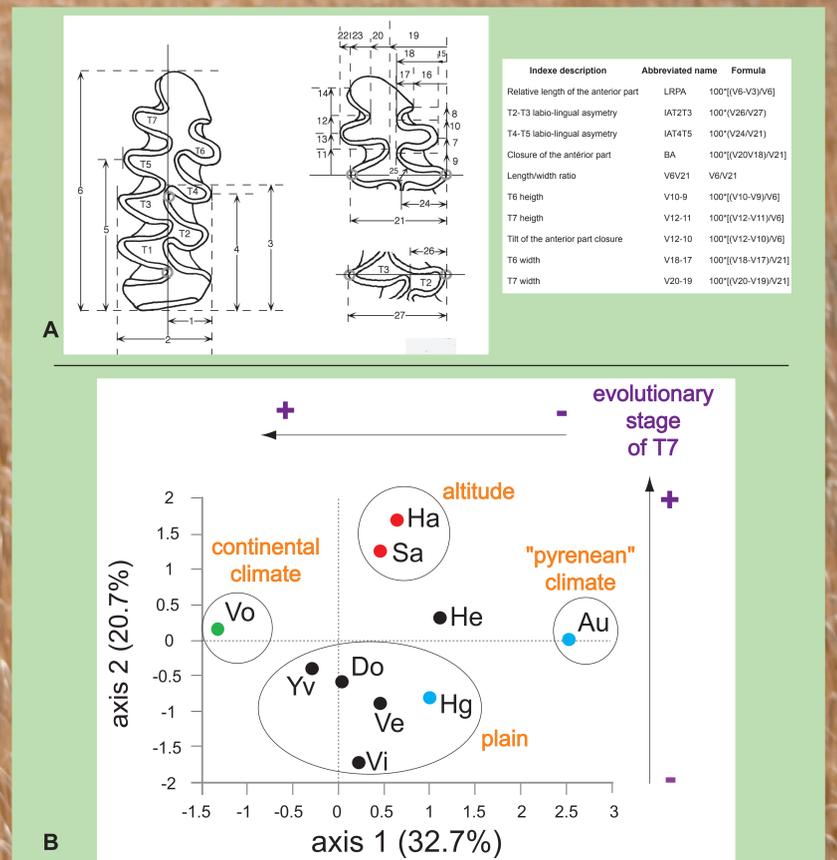


Fig. 3: Morphometry of the first lower molar of *Microtus arvalis*. A: with the 27 measurements (occlusal view) and the morphometric index description, name and formula (in box; Brunet-Lecomte, 1988; Laplana *et al.*, 2000); B: centroid projection of 10 populations onto the first two canonical discriminant axes. Circles, surrounding some populations, have no statistical value but are here for your information on population clusters.

Statistical tests performed on our morphometrical dataset are congruent with the clusters presented on Fig. 3:

- Mahalanobis distances between populations are all significant (p<0.05). Weakest values are for the Ha/Sa (5.51829, p=0) and Hg/Vi/Do (from 4.94289 to 5.80034, 0.000001<p<0.001641) clusters. Highest values oppose 1) the population Ve to those of Ha (14.902216, p=0) and Sa (10.92477, p=0) and 2) the population Au to those of Vo (15.9544, p=0) and Yv (16.27632, p=0);
- the classification matrix enables the reclassifying of populations at more than 60%.

The morphometric analyses display a morphological structure for m1. It seems that this morphological structure for some populations is influenced by a climatic gradient (continental climate vs "pyrenean" climate and altitude vs plain). The discriminant factor for these populations seem to be the evolutionary stage of the T7 triangle (more or less derived).

The influence of Quaternary cold periods on composition and distribution of European fauna and flora is unquestioned. Some general trends of postglacial expansions have been identified for several species of plants and animals: 1) postglacial recolonization of northern regions generally from southern peninsulas (Iberia and the Balkans), acting as refugia; 2) isolation of the Italian lineage due to the Alpine barrier; 3) occurrence of 4 main suture-zones where populations from different refugia meet (Taberlet *et al.*, 1998; Hewitt, 2004). In the case of the common vole, Haynes *et al.* (2003) agree with these general trends of colonization, whereas Fink *et al.* (2004) and Heckel *et al.* (2005) bring an other vision of its evolutionary history in western Europe.

Our study brings to the fore a genetic and morphological structure of common vole populations in France. The Vo population looks particularly like a part of the second suture-zone as defined by Taberlet *et al.* (1998), between the central lineage (C) and the western lineage (A and B), but also as a spreading centre for the French populations after the penultimate glaciation (around 0.13 Myr and 0.12 Myr; Fig. 2 and 4). Morphological variability reflects the evolutionary stage of the T7 triangle of the m1. In fact, this variability seems more related to an adaptation of the common vole to some "colder" habitats (altitude, "pyrenean" climate) and, especially a diet with more abrasive plants as Graminae (Fig.3). This trend was already mentioned for others vole species (Brunet-Lecomte, 1988).

In conclusion, our study corroborates the hypothesis of an ancient colonization from northeast to southwest of Europe (before 0.20 Myr) and the occurrence of other glacial refugia such as the Vosges area (Vo) in France. The glacial survival of the common vole outside the classical refugial areas is maybe due to its power of adaptation to colder environments.

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