Unravelling population structure and recent colonization history of an elusive carnivore, the Eurasian otter (*Lutra lutra*) in western France using microsatellites and Single-Nucleotide-Polymorphism (SNP) markers.

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Introduction

At the dawn of the 20th century, the Eurasian otter (*Lutra lutra*) was widely distributed in France. However, due to intensive trapping and poaching for its meat and fur, the distribution range of the Eurasian otter became highly fragmented. Since the otter received legal protection in the seventies, a recolonization of its former range from refugia has taken place. The present study therefore aims at evaluating the natural recovery of the otter in France through the investigation of the genetic differentiation and diversity of several otter populations originating from Western and Central France, and Northern Spain (Navarre). Indeed, assessing the genetic diversity of endangered species is of major concern in the field of conservation.

Material & methods

To investigate the genetic variation within and among the otter populations, clustering analyses were performed based on 14 polymorphic microsatellite markers (N=205) as well as SNP genotypes (N=19) (Structure v2.3). The GBS library was constructed using the *Pst*I restriction enzyme and the raw Illumina DNA reads data (64bp) was processed through the GBS analysis v2 pipeline, as implemented in Tassel v5.2.15. After filtering, the reads were aligned to the *Mustela putorius furo* reference genome using Bowtie v2.2.7, and about 9,000 SNPs were identified and filtered using Tassel and BayeScan v2.1. The history of otter populations located in West-central France was also investigated through a demographic approach (DIYABC v2.0.4).
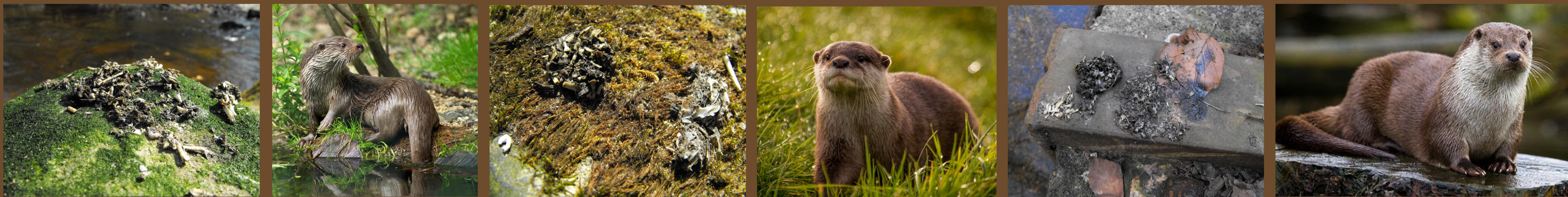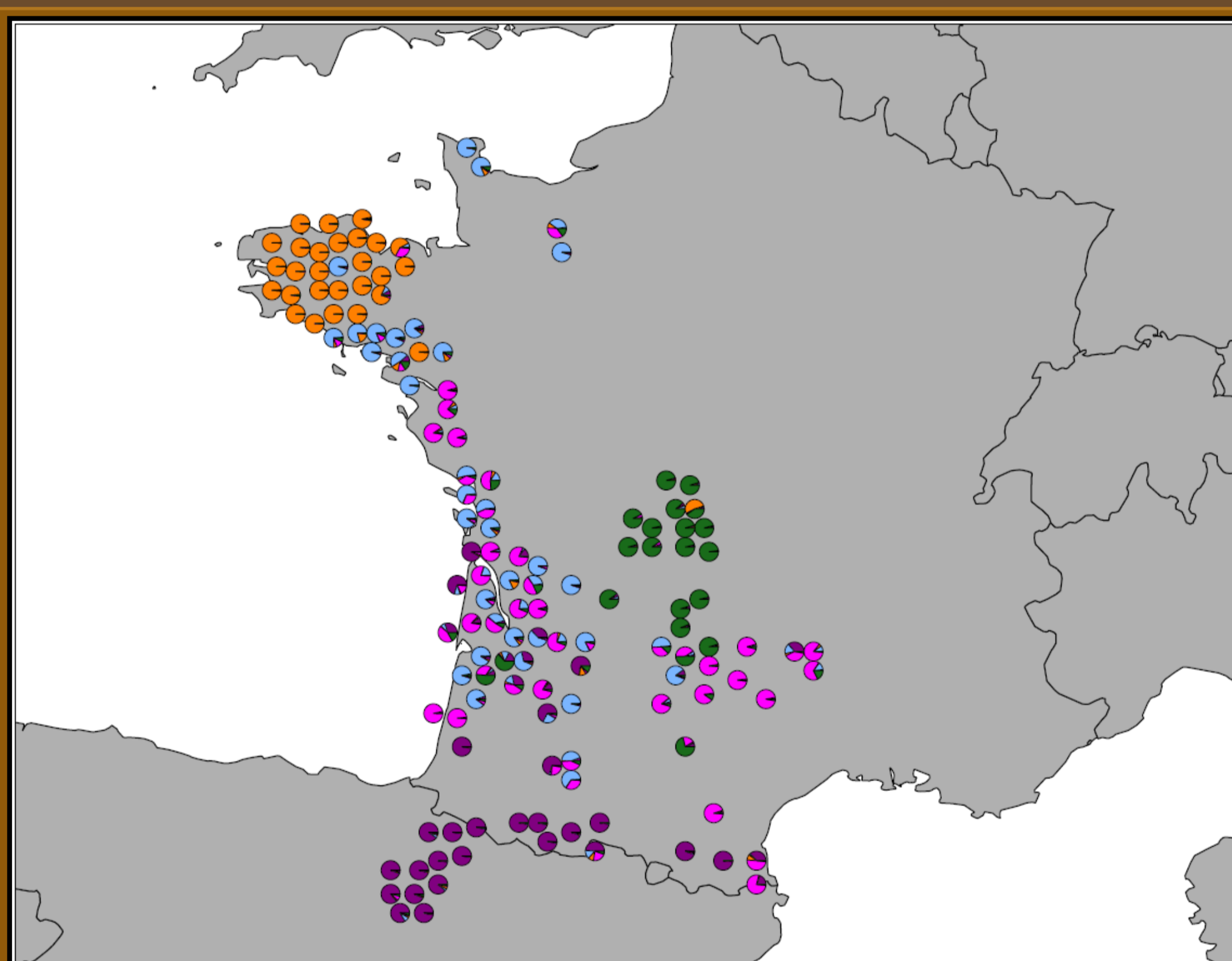

Results

Fig 1. Map representing the sampled individuals and their membership coefficient to each of the 5 inferred clusters in France.
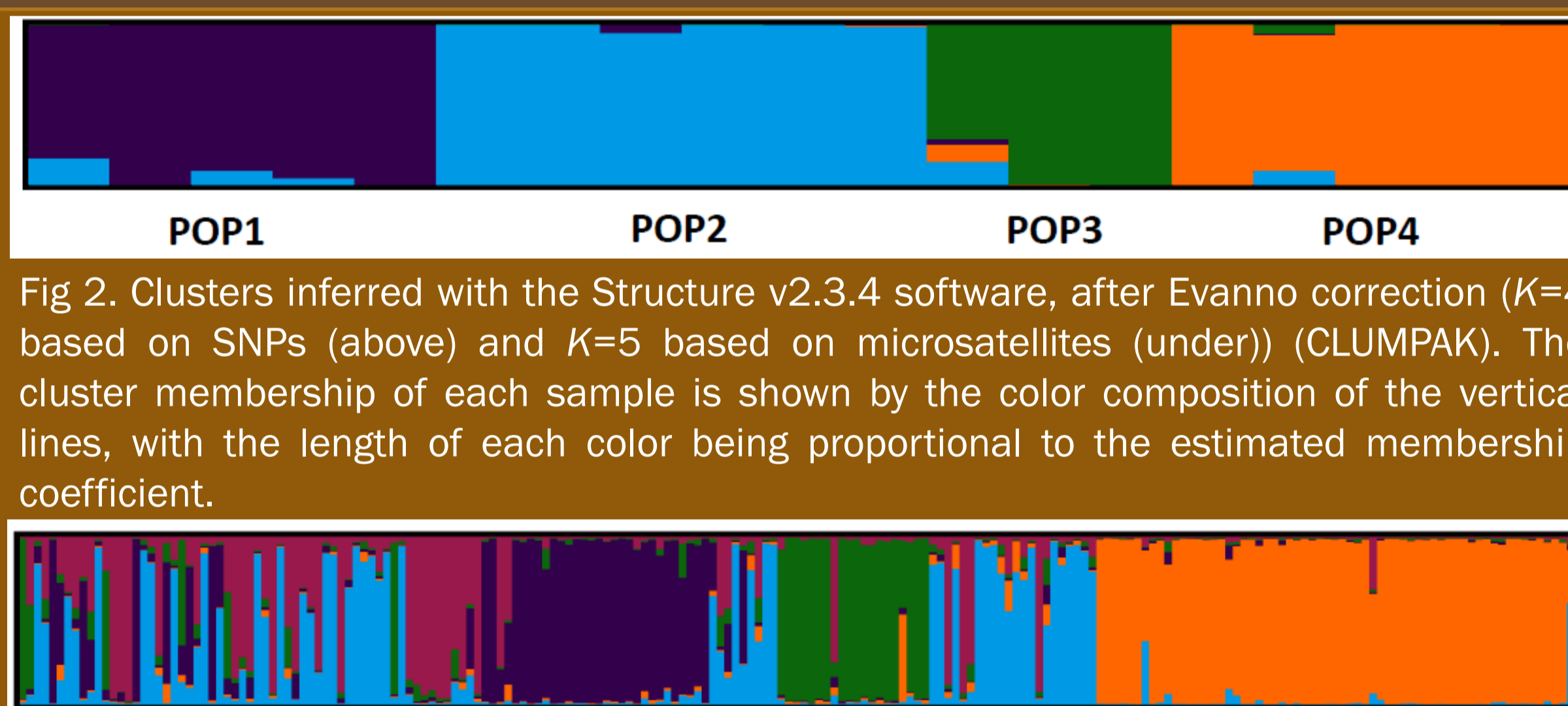

Fig 2. Clusters inferred with the Structure v2.3.4 software, after Evanno correction (*K*=4 based on SNPs (above) and *K*=5 based on microsatellites (under)) (CLUMPAK). The cluster membership of each sample is shown by the color composition of the vertical lines, with the length of each color being proportional to the estimated membership coefficient.

Table 2. Pairwise F_{ST} (above diagonal) and D_{Jost} (below diagonal) estimates calculated for the inferred clusters. Above: based on microsatellites. Under: based on SNPs. PYR=Pyrenees, MC=Massif Central, LIM=Limousin, BRIT=Brittany, ATL=Atlantic.

Usat	PYR	MC	LIM	BRIT	ATL
PYR	-	0.166	0.195	0.301	0.182
MC	0.166	-	0.123	0.233	0.141
LIM	0.183	0.098	-	0.232	0.194
BRIT	0.291	0.222	0.183	-	0.234
ATL	0.150	0.146	0.144	0.182	-

SNP	Pop1	Pop2	Pop3	Pop4
Pop1	0	*	*	*
Pop2	0.153	0	*	*
Pop3	0.230	0.188	0	*
Pop4	0.228	0.183	0.238	0

Scenario 5

PP 0.2814 [0.2106-0.3523]

Ne_Pyr 746
Ne_MC 935
Ne_Lim 659
Ne_Brik 141
Ne_Atl 672
Ne_Nd6 1150
Ne_N7 4400

t1a 94.9
t2a 157
t3a 201
t4a 248

Demographic scenario with highest *PP* (DIYABC).

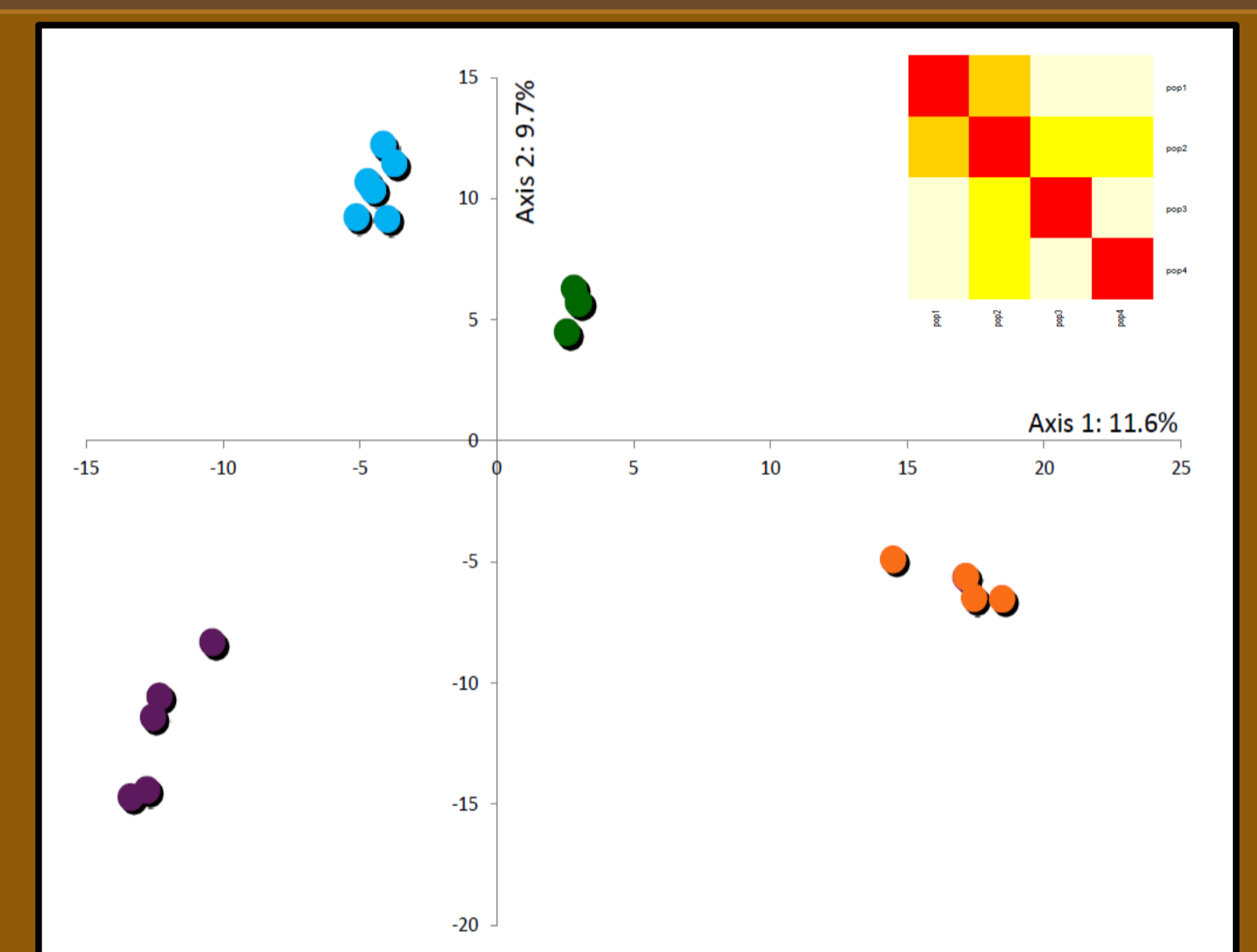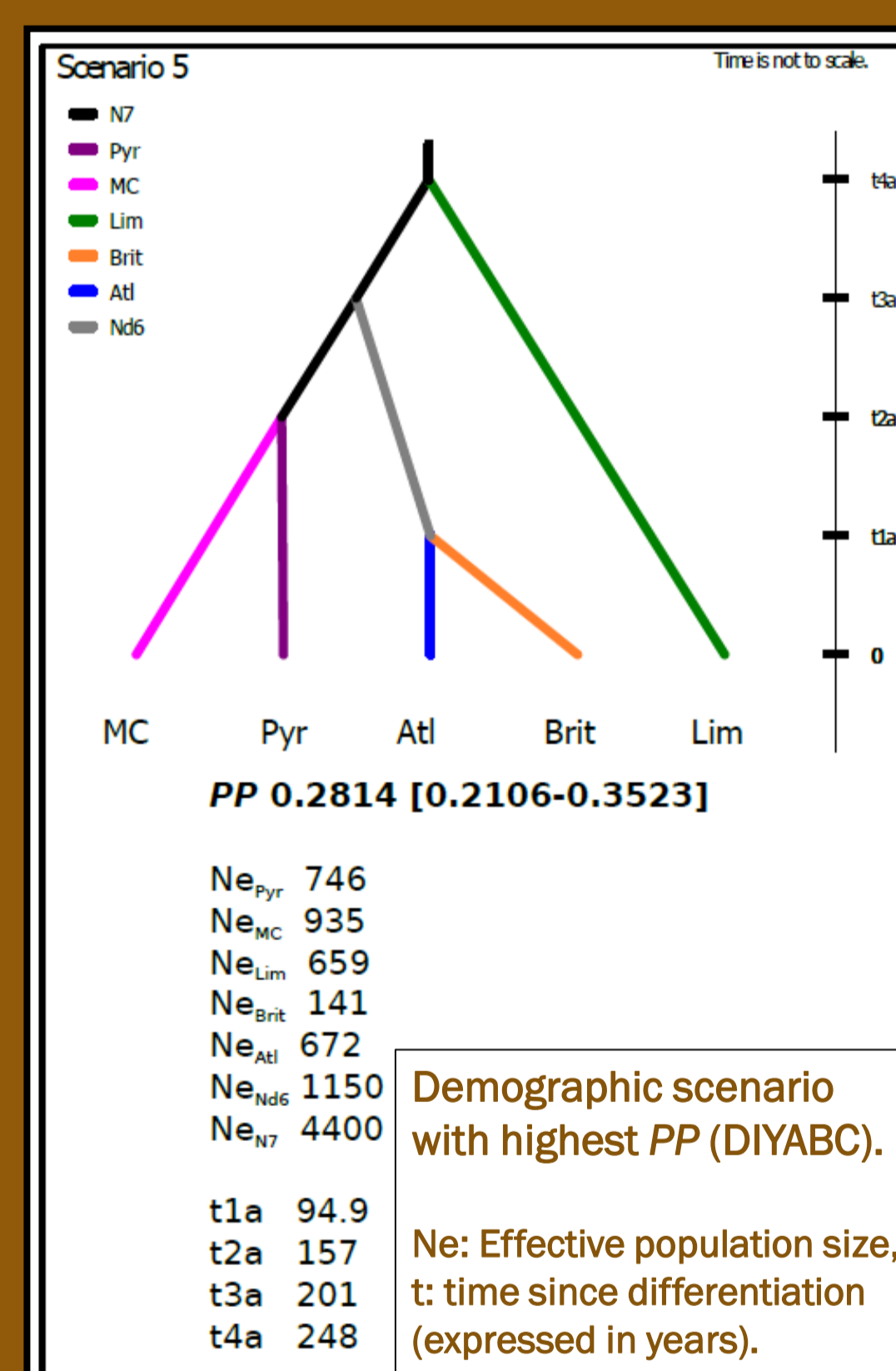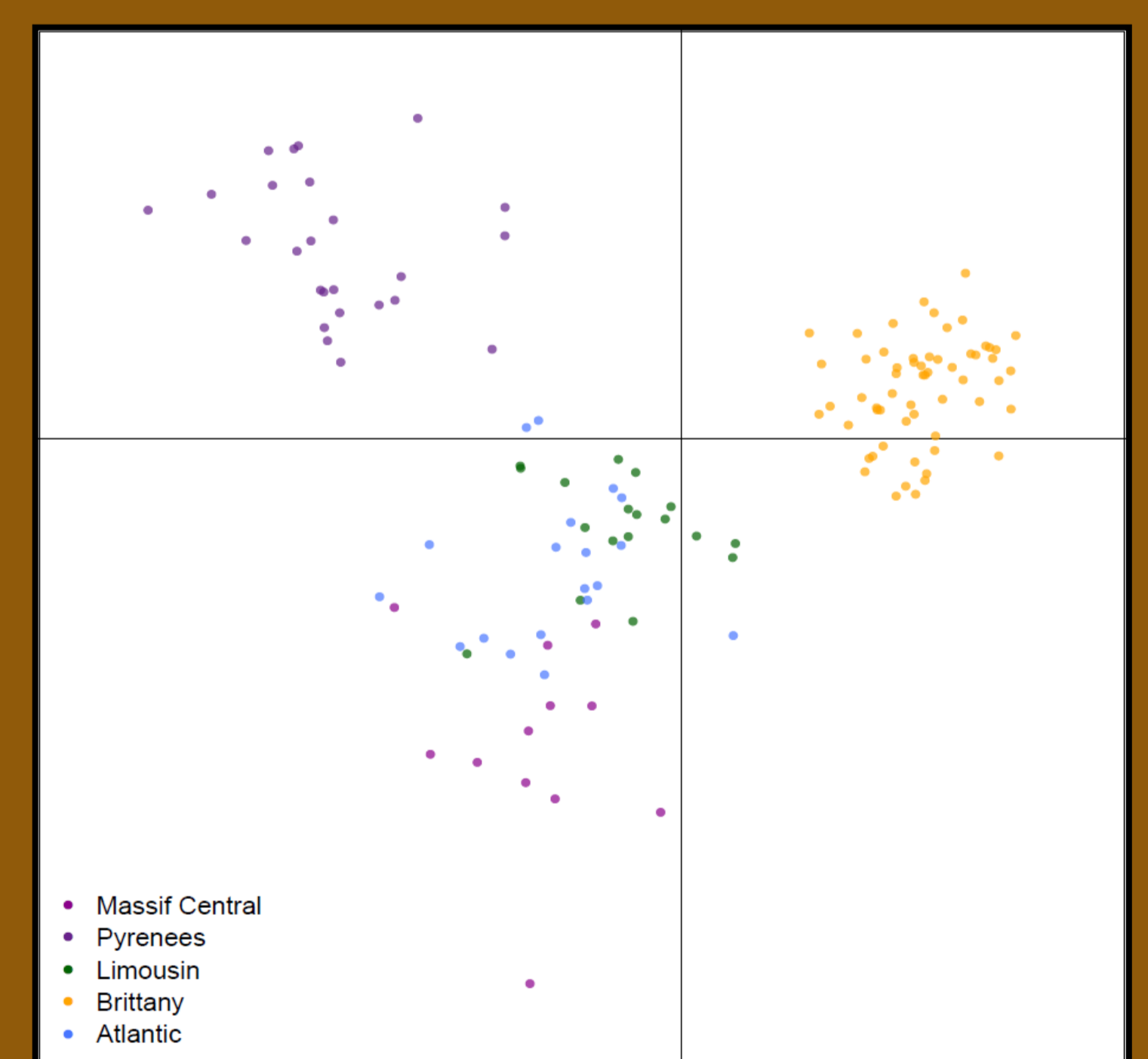Ne: Effective population size, t: time since differentiation (expressed in years).

Fig 3. Principal component analysis (PCA) performed with Tassel and R, respectively SNPs above and microsatellites under.

Table 1. Genetic diversity at the 14 microsatellite loci for the 5 inferred clusters of European otter in France based on microsatellites. F_{IS}=inbreeding coefficient, Ar=mean allelic richness, [CI]=confidence intervals, Ho=observed heterozygosity, He=expected heterozygosity

Cluster	F_{IS} [CI]	Ar [CI]	Ho / He
PYR	0.0451 [-0.05-0.1385]	3.54 [1.95-4.88]	0.52 / 0.55
MC	0.0500 [0.1375-0.2111]	3.70 [2.65-4.85]	0.59 / 0.62
LIM	-0.0683 [-0.1784-0.1324]	3.01 [1.49-4.42]	0.54 / 0.50
BRIT	0.0600 [-0.0098-0.1324]	2.47 [1.97-4.25]	0.45 / 0.48
ATL	-0.0835 [-0.1764-0.003]	3.16 [2.00-4.44]	0.56 / 0.52

Discussion & conclusions

All analyses resulted in the identification of five distinct groups, which appear geographically structured and seem correlated with the respective putative refugia (strongholds where the species may have survived). Indeed, some remnant populations were suggested to have survived along the Atlantic coast and in the Massif Central. Admixture appeared to occur between the genetic lineages, enhancing their genetic diversity and increasing their recolonization dynamics. Demographic approaches allowed to confirm the probable link between ancient human pressures and populations fragmentation, leading to the observed differentiation into several distinct groups.

Implications for its conservation

The French otter is a striking example of natural recovery in mammals. It exhibits a particularly high recolonization potential thanks to its high mobility and its ability to cross many putative physical barriers. The enhanced genetic diversity that might arise from the current ongoing admixture might be beneficial for the future of the Eurasian otter. The risk of outbreeding depression seems negligible because of the historical connectivity and the lack of obvious adaptive divergence. Moreover a reasonable genetic diversity is observed in most otter clusters and the F_{is} estimates have been shown not to be particularly high. Currently, measures to enhance genetic connectivity are considered of upmost importance to counteract inbreeding depression in order to preserve the evolutionary potential and ensure the long-term survival of this endangered species. Restoration of freshwater habitats and reduction of water pollution are still critical for its recovery.