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## Introduction

While the genetic structure of many rainforest plants species of West Africa is mainly explained by climatic oscillations, very little is known about savannah species. Savannahs are major biomes both in term of range area and of biodiversity and there is an urgent need to better understand them to propose efficient conservation strategies. Shea tree is of particular interest as being a major component of savannahs in West Africa. It also plays an important social and economical role in these regions as the butter extracted from its kernels is widely used both for food and cosmetics. In this study, we bring new insight into the evolutionary processes affecting West Africa savannah by investigating the genetic diversity and structure of Shea tree (*Vitellaria paradoxa* C. F. Gaertn) in relation to past climate changes.



*V. Paradoxa* tree



*V. Paradoxa* fruits



*V. Paradoxa* nuts

Figure 1 : *Vitellaria paradoxa* (Shea tree or Karité in french)

## Material and Methods

**Sampling.** A total of 790 individuals from 38 locations were sampled in 7 West African countries. Eleven nuclear microsatellites (NucSSR) were analyzed on this whole panel and two chloroplast microsatellite primer pairs (CpSSR) were analyzed on a subset of 370 individuals.

**Bioclimatic data and ecological niche modelling.** Ecological niches of *V. paradoxa* were predicted using BIOCLIM algorithm and DIVA-GIS software (Hijmans et al., 2001). Three different epochs were considered (Fig. 2)

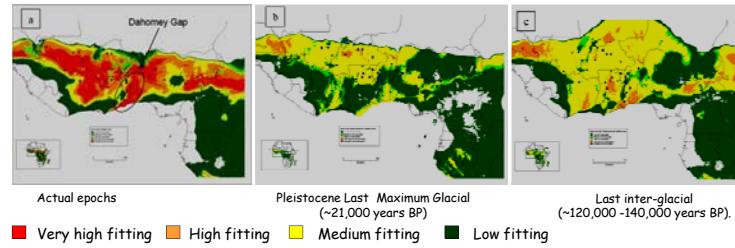


Figure 2 : *V. paradoxa* fitting zones at different epochs in west Africa

**Genetic analyses for nuclear microsatellites.** Diversity indices were calculated and compared among populations associated in d-ray circle centered on each collection site ( $d=2^\circ$  and  $d=4^\circ$ ). Allelic richness ( $R_{nuc}$ ) differences between populations were tested with the Kruskal-Wallis test.  $R_{nuc}$  trends with longitude and latitude were evaluated using Pearson correlation coefficient. We calculated 2 differentiation indices,  $Fst_{nuc}$  and  $Ast_{nuc}$  further used to test for phylogeographic signal (Hardy et al., 2003). Isolation By Distance (IBD) was assessed using Mantel test. STRUCTURE software (Pritchard et al., 2000) was used to detect clusters.

**Genetic analysis for chloroplast microsatellites.** The same methods as for nuclear microsatellites were used.  $R_{cp}$  was calculated according to Petit et al. (2003) method and phylogeographic signal was tested by comparing  $Gst$  and  $Nst$  (Pons & Petit 1996). We build a haplotypic frequency tree with the Neighbor-joining method. Shea tree expansion was assessed using the methods developed by Excoffier (2005). Both pure demographic and spatial expansion models were tested.

## References

- Excoffier, L., G. Laval, and S. Schneider (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1, 47-50.
- Hardy O J, Charbonnel N, Fréville H, Heuertz M (2003) Microsatellite Allele Sizes: A Simple Test to Assess Their Significance on Genetic Differentiation, *Genetics*, 163, 1467-1482.
- Hijmans R.J., Guarino L., Cruz, M. & Rojas, E. (2001) Computer tools for spatial analysis of plant genetic resources data: 1. Diva-gis. *Plant Genetic Resources Newsletter*, 127, 15-19
- Petit RJ, Aguinagalde I, de Beaulieu J-L, et al. (2003) Glacial Refugia: Hotspots But Not Melting Pots of Genetic Diversity. *Science* 300, 1563-1565.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, 155, 945-959.
- Pons O, Petit RJ (1996) Measuring and testing genetic differentiation with ordered vs. Unordered alleles. *Genetics*, 144, 1237-1245.

## Results and discussion

**Genetic diversity and structure based on nuclear microsatellites.** Within population diversity was moderate to high ( $R_{nuc}$  varied from 3.93 to 4.86) with no trend with longitude (Fig. 3) or latitude. Low population differentiation ( $Fst_{nuc} = 0.078$ ) and an IBD pattern were observed but no phylogeographic signal was detected. Structure identified a weak population structure constituted by 3 clusters. (Fig. 4)

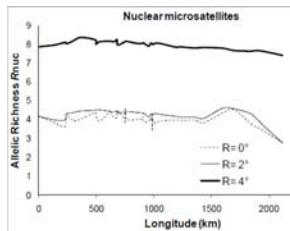


Figure 3 : Trend in  $R_{nuc}$  with the longitude

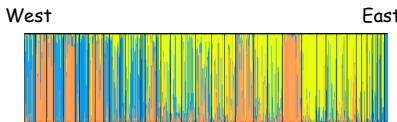


Figure 4 : STRUCTURE genetic clusters

**Genetic diversity and structure based on chloroplast microsatellites.** Chloroplast allelic richness varied greatly among populations ( $R_{cp} = 0.00$  to  $R_{cp} = 3.50$ ) with a higher diversity in West Africa central zone (Fig. 5 & 6). We observed a strong population differentiation ( $Fst_{cp} = 0.567$ ) but no phylogeographic signal. Significant ( $p \geq 0.05$ ) demographic and spatial expansions were observed for most samples except in few populations of the central zone (Fig. 7).

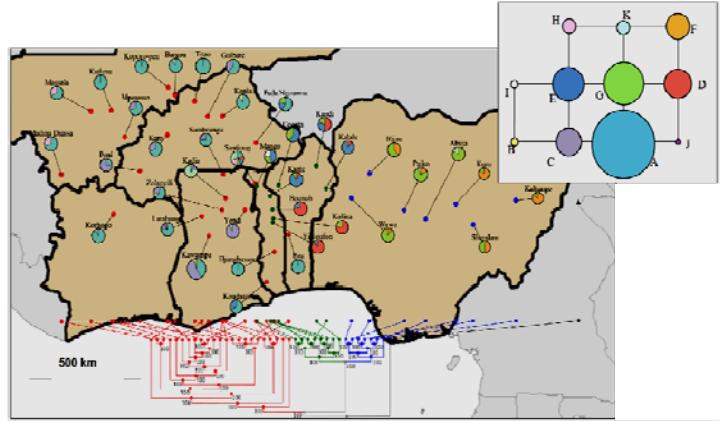


Figure 5 : Chlorotype distribution and NJ tree on cpSSR

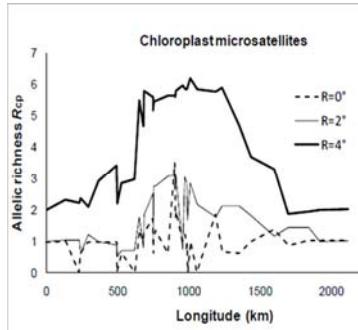


Figure 6 : Trend in  $R_{cp}$  richness with longitude

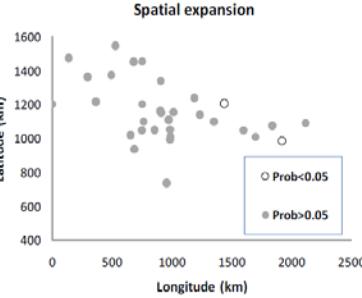


Figure 7 : Spatial expansion

## Conclusions

The combining of molecular data and ENM allows formulating 2 evolutionary scenarios for shea tree in West Africa :

**cpSSR** data suggest that central zone of West Africa was a permanent shea tree refugia. The adjacent zones may have been colonized by migrants from this large central refugia during favorable conditions, especially after Last Maximum Glaciation (LMG). The estimated expansion time is very old (1,875,000 years with mutation rate  $\mu\pi^{-5}$ ).

**nucSSR** data and ENM results suggest that shea tree range was not fragmented after LMG in West Africa. The favorable climatic conditions during late Pleistocene and Holocene have likely allowed the shea tree to reach an equilibrium characterized by IBD phenomenon.

**Agroforestry** may have favored gene flow but it doesn't appear to have impacted shea tree diversity in West Africa yet.