

*Past domestication of *T. cacao* in Latin America revealed by paleogenomics and analyses of methylxanthines*

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FRANCE

EQUATEUR

COLOMBIE

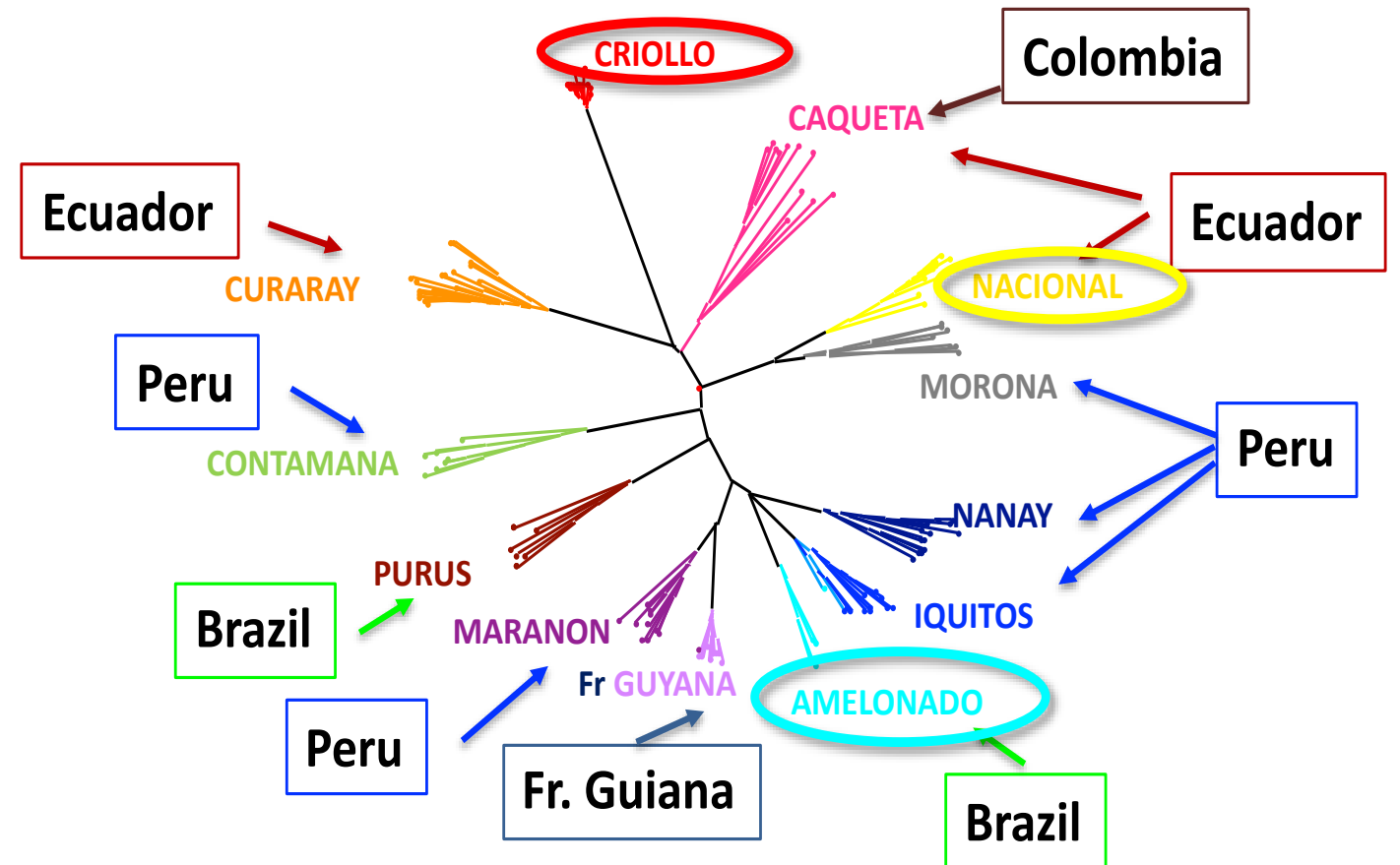
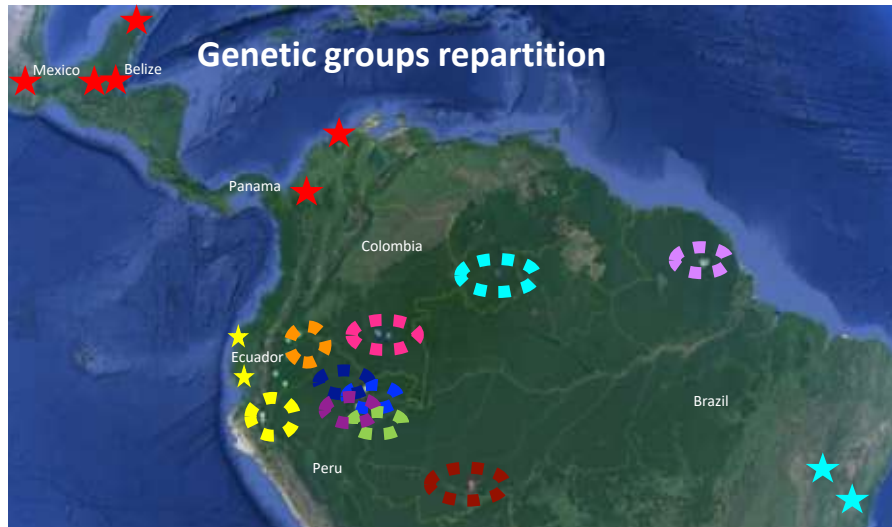
USA

CANADA

MEXICO

Diversity and classification of *T. cacao*

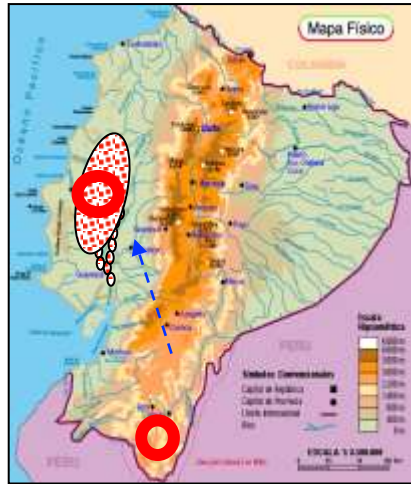
Phylogenetic tree constructed with 54 SSR



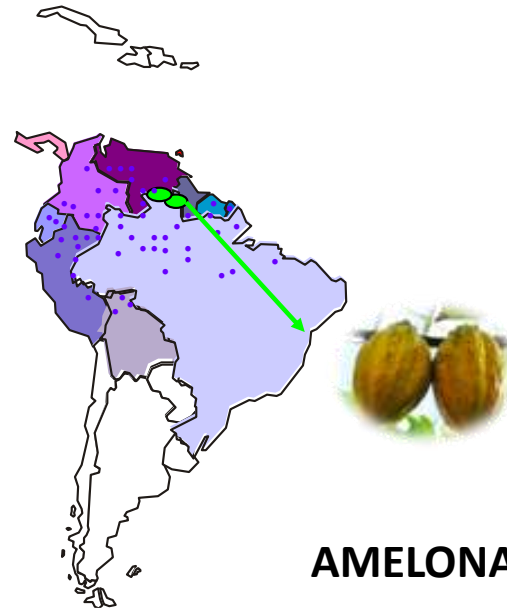
Hypotheses made on the origin of oldest varieties domestication

1) Introduction of Nacional genotypes from South Ecuadorian Amazonia

2) Trinitario (Criollo x Amelonado) introduced from Venezuela one century ago ?



NACIONAL



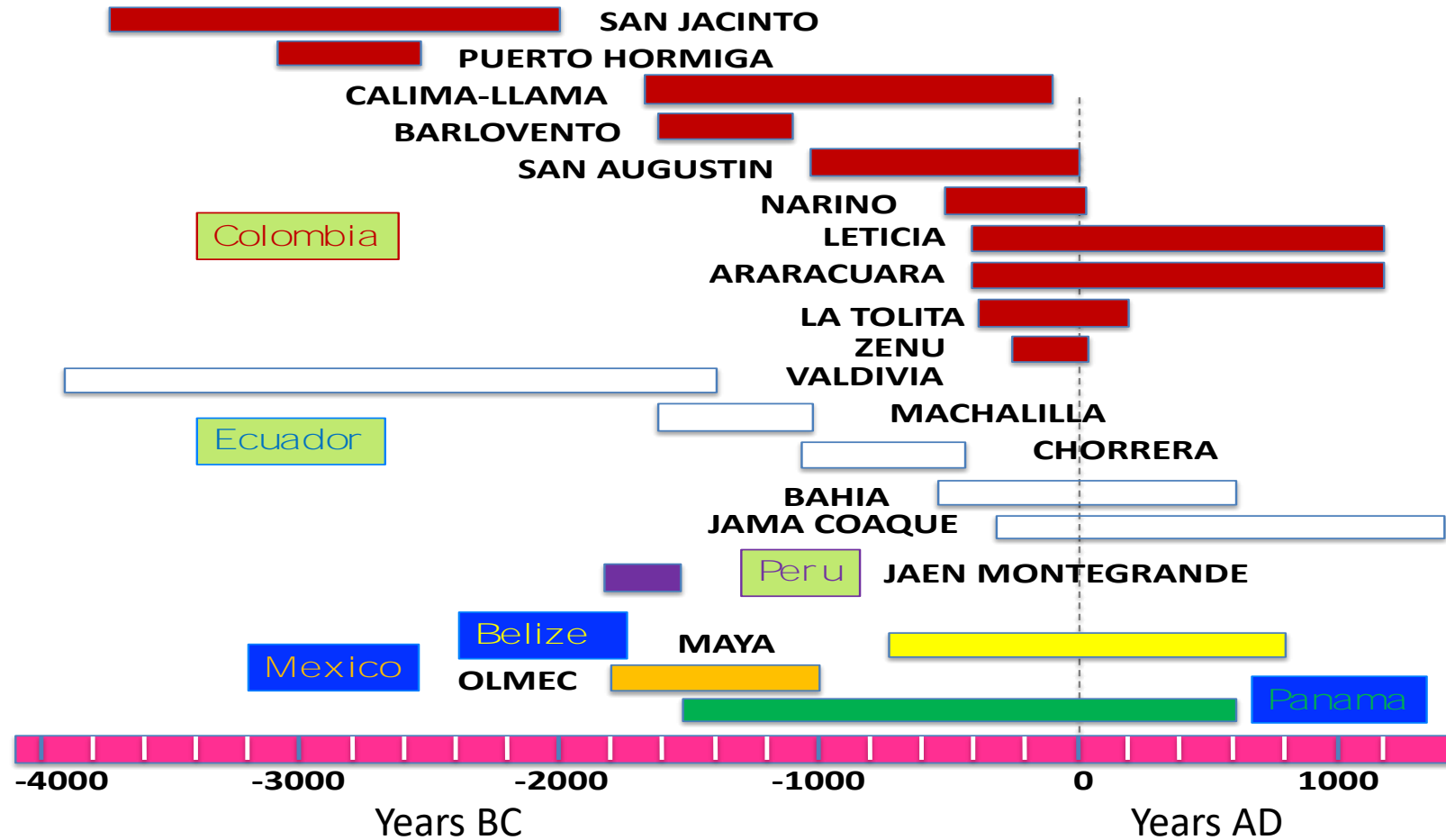
AMELONADO



CRIOLLO

Chronology of ceramics from different cultures

→ collaborations established with archaeologists and anthropologists from different Latin American countries and biochemists to analyse **ceramic food residues**



379 ceramic items analysed from 19 different cultures:

- directly excavated
- in the museum storage rooms

→ Methylxanthines
→ Ancient DNA

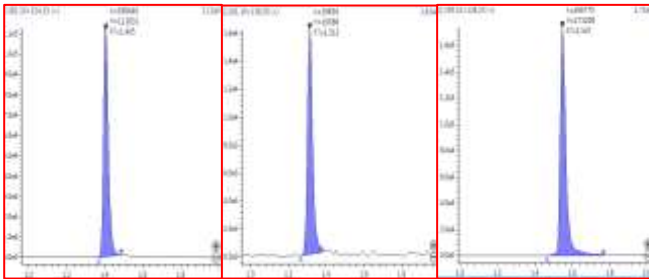
Methylxanthine Analyses

332 analysed samples from South America

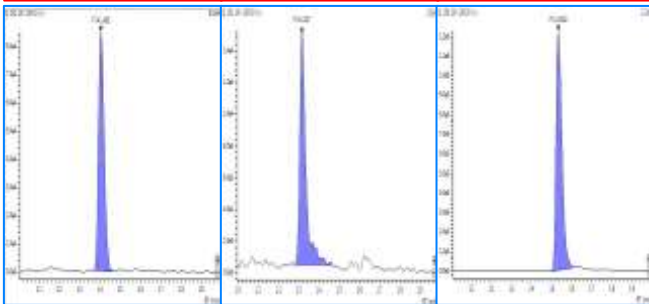
- 121 are positive for theobromine
- 129 are positive to Caffeine
- 25 are positive for theophylline

→ Positive for methylxanthine in 15 cultures, and among them the oldest (Valdivia-San Jacinto)

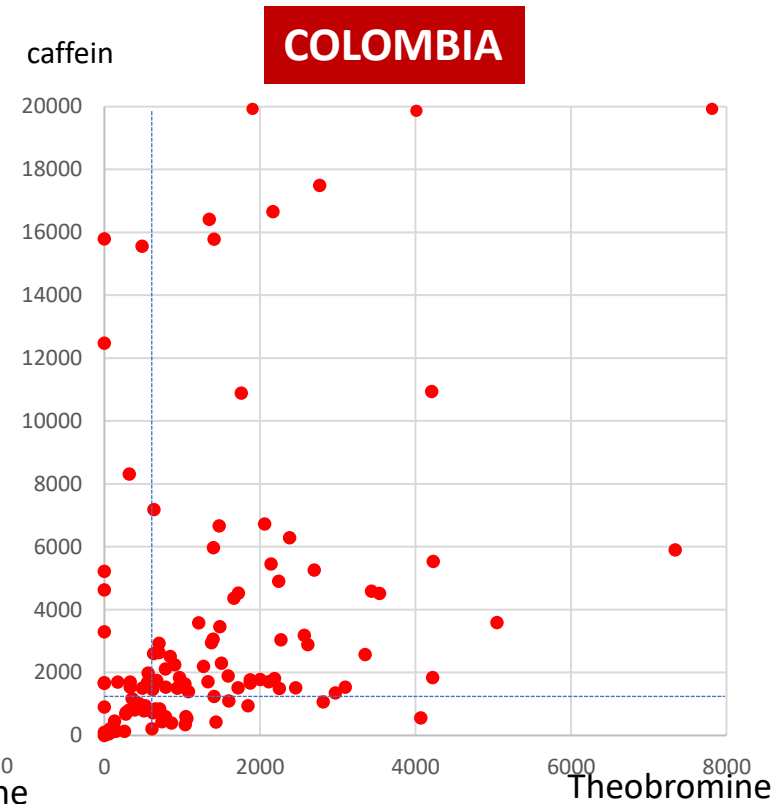
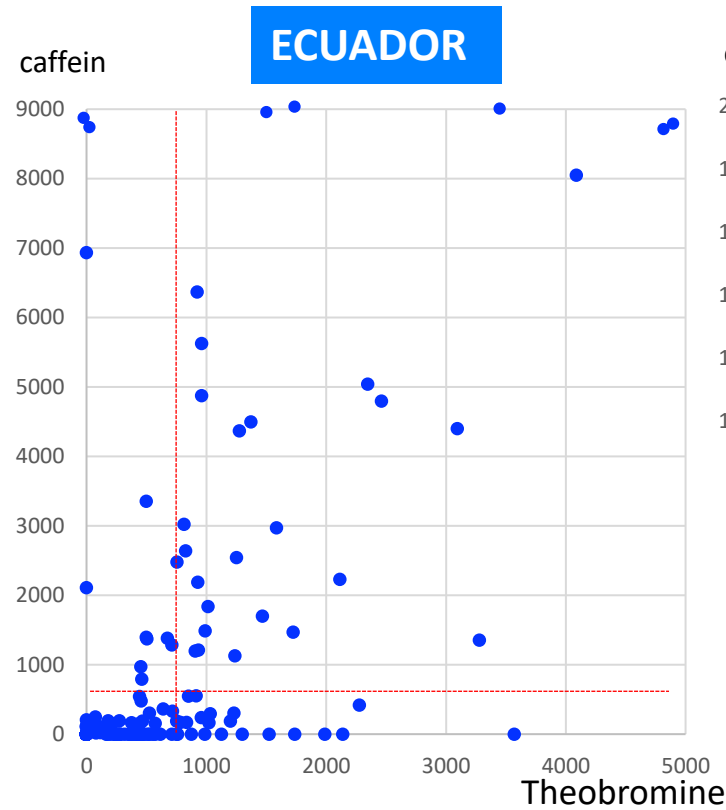
THEOPHYLLINE THEOBROMINE CAFFEINE



Standard
15ng/ml



Valdivia
sample



Use of molecular tools to access cocoa ancient DNA (aDNA)

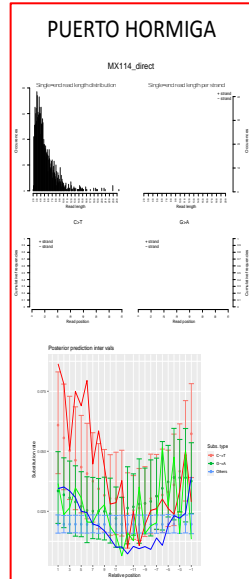
1) **COLLECT** of ceramic residues (archéo. excavations, museums)



2) **EXTRACTION** of ancient DNA (very degraded) – libraries construction of 170 items

Experimentations made in a white room to avoid external contamination by modern DNA. (MNHN)

3) **SEQUENCING** and **BIOINFORMATIC** analyses (mapping on the cocoa genome and blast against international databases (NCBI))



Signature of aDNA

4) **GENETIC analyses** → Identification of SNP markers in the aDNA sequences, based on the SNP pool obtained after resequencing 185 modern cocoa genotypes (Pangenome project)

→ Comparison with a ref. collection of 81 individuals representing the diversity of the *T. cacao* species; Structure and genetic distance.

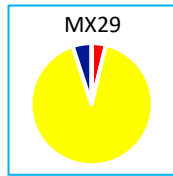
On 170 analysed samples:

- 92 are positive for cocoa aDNA
- 56 allowed genetic comparisons

Structure analysis of cocoa residues in Ecuador and Peru

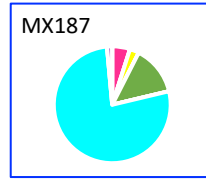
VALDIVIA

FIIa-5.300 - 5.100 BP (Before Present)

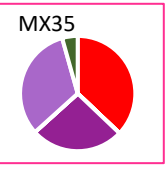
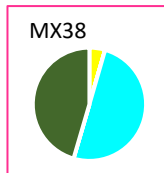
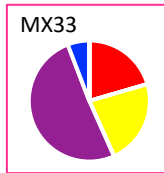
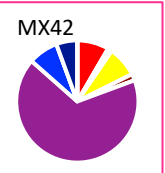
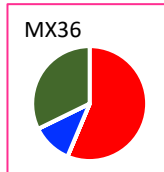
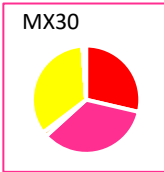
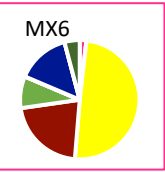


MACHALILLA

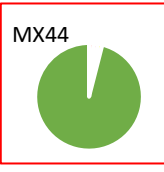
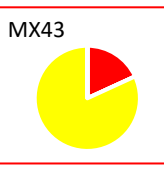
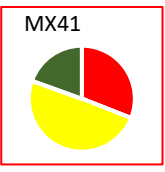
3.600 - 3.000 BP



FIII-4.950 - 4.600 BP

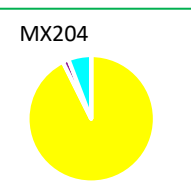
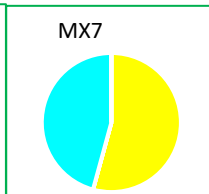
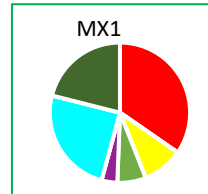


F8-3800 - 3400 BP

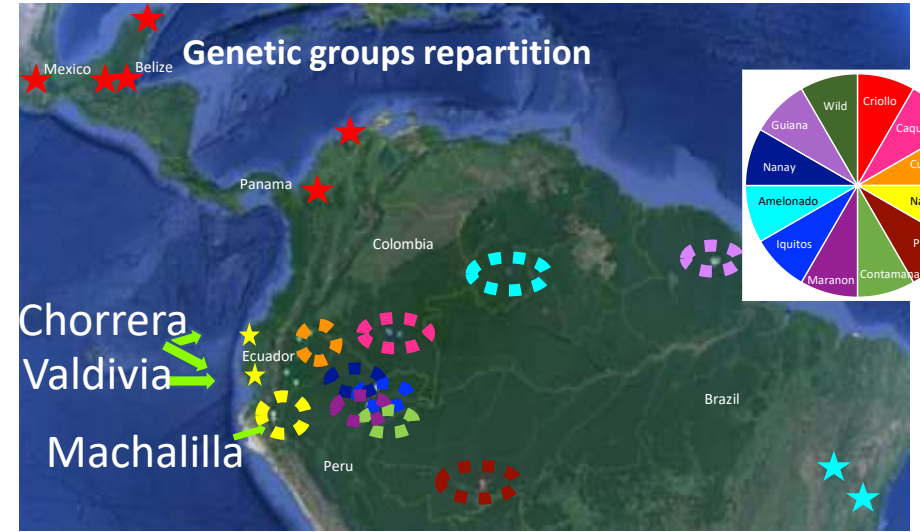


CHORRERA

3000 - 2500 BP

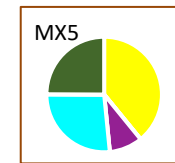


Genotype membership proportion



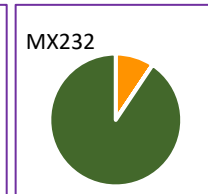
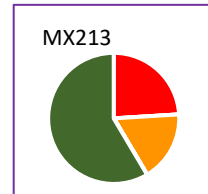
LA TOLITA

2600 BP - 1600 BP



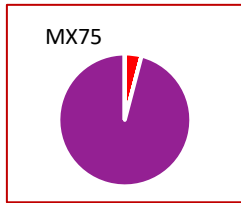
JAEN-PERU

3830 BP - 3590 BP



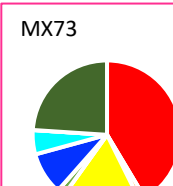
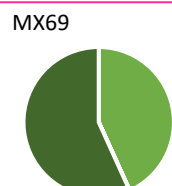
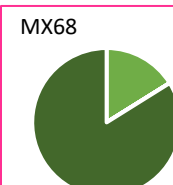
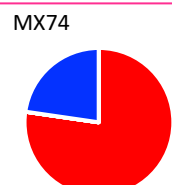
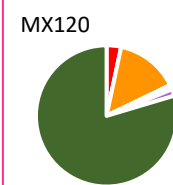
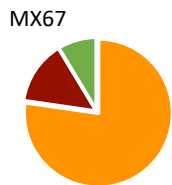
SAN JACINTO

5750 à 4000 BP



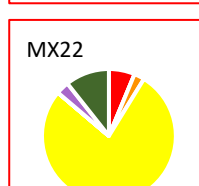
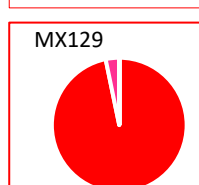
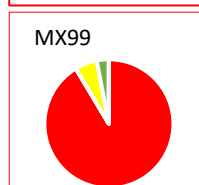
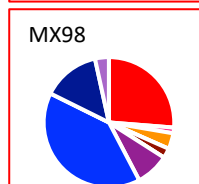
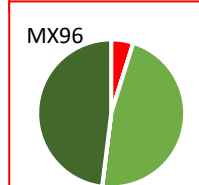
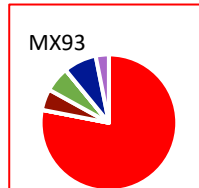
PUERTO HORMIGA

5100 à 4550 BP



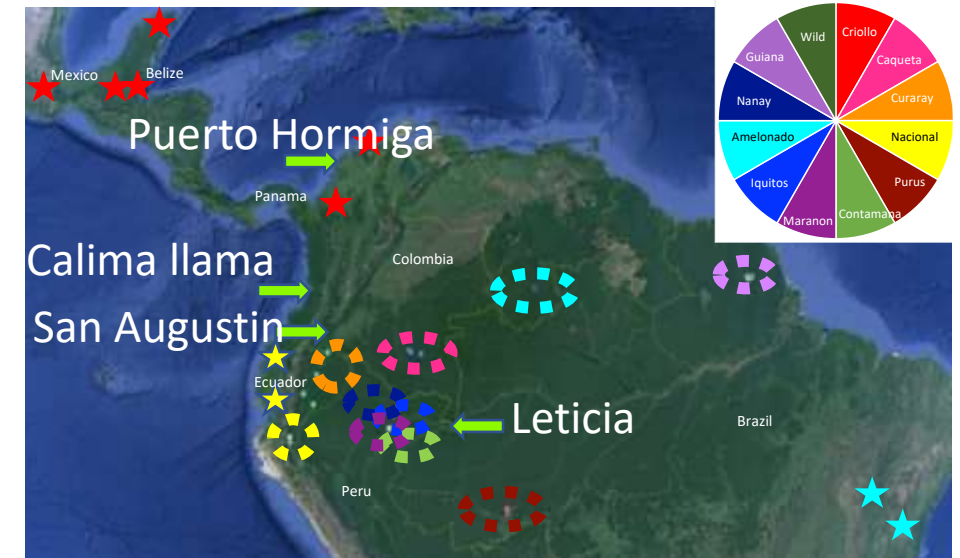
CALIMA LLAMA

3600 à 2100 BP



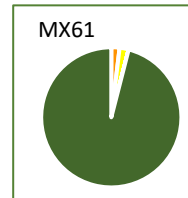
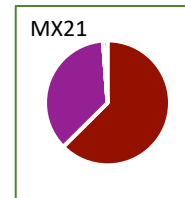
Colombia

Genotype membership proportion

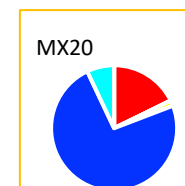


SAN AUGUTIN

3000 à 2000 BP

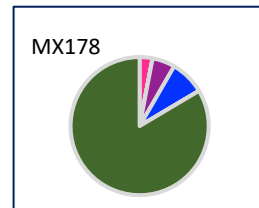
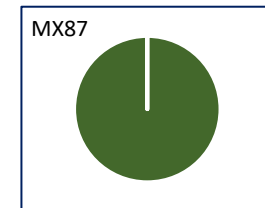


ZENU 2200 à 1900 BP



LETICIA

2350 à 800 BP



Central America

Mexico

Belize

Genotype membership proportion



OLMEC

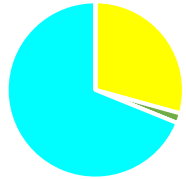
3800-3000 BP

MAYA

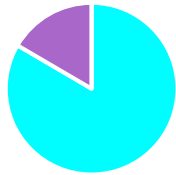
2600-2300 BP



MX8



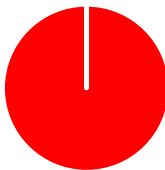
MX9



MX225



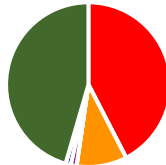
MX224



MX151

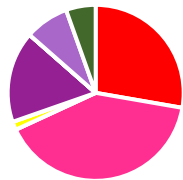


MX156

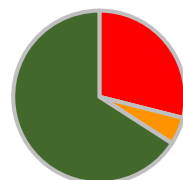


2300 - 1750 BP

MX144



MX198



Panama

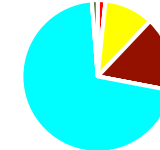
Gran Chiriqui

3500 - 1600 BP

MX92



MX91



Conclusion



Archaeological sites positive for cocoa

- **Widespread use of cocoa in South America** for over 5000 years, in Ecuador as well as in Colombia, evidenced by ancient DNA and methylxanthine presence.

- **Large diversity and genetic mixing** of introduced cocoa trees from different origins, sometimes distant, that reflects the numerous interactions between populations

- During Olmec and Maya occupation, **Criollo was not the unique cultivated variety**, but genotypes related to Amelonado, Nacional and other genetic groups were also present, suggesting possible interactions with Ecuador where these genotypes existed , and with Colombia where genotypes close to Criollo already existed for 5000 years .

Conclusion

- Thus, **paleogenomic approaches have strongly challenged our first hypotheses** on cocoa domestication and help us to better understand and manage the genetic resources available.
- All these results show the **complex domestication history of cocoa varieties** probably linked to different migratory waves from the beginning of the Holocene and to many trading exchanges within the Amazon and with the Pacific Coast.
- These movements and activities were **associated to direct or undirect multiple cocoa introductions from different origins**, leading to hybrid forms, favorable to the domestication of productive varieties adapted to new environment , and now at the basis of the current cocoa varieties

Archaeologists-anthropologists

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- Sonia Zarillo
- Camilla Speller

University of Calgary - (Canada)

- Scott Raymond

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- Natalia Angarita
- Francisco Romano

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- Terry Powis

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- Ann Cypher

ASICAMPE - (Peru)

- Quirino Olivera Nuñez

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- UM (France) - Gilles Valette

- Steroidomics (USA)- Nilesh Gaikwad

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VALRHONA



**Thank you for
your attention**

