Genetic diversity analysis of a large cocoa trees collection from the Ecuadorian Amazon safeguarded for local and sustainable cocoa production

Olivier Fouet[1,2], Rey Gaston Loor Solorzano[3], Bénédicte Rhoné[1,2], Cristian Subía[3], Darío Calderón[3], Fabián Fernández[3], Ignacio Sotomayor[3], Ronan Rivallan[1,2], Kelly Colonges[1,2], Hélène Vignes[1,2], Freddy Angamarca[3], Byron Yaguana[3], Pierre Costet[4], Xavier Argout [1,2], Claire Lanaud [1,2]

(1) CIRAD, UMR AGAP Institut, F-34398 Montpellier, France.
(2) UMR AGAP Institut, Univ Montpellier, CIRAD, INRAE, Institut Agro, F-34398 Montpellier, France
(3) Instituto Nacional de Investigaciones Agropecurias, INIAP, Ecuador.
(4) Chocolaterie Valrhona, 14 avenue du Président Roosevelt, F-26600 Tain L'Hermitage, France

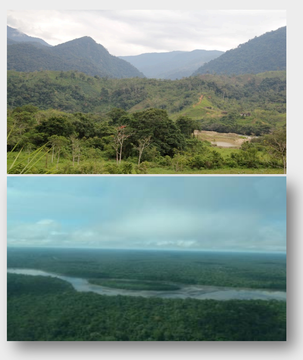
The production of aromatic cocoa has a direct positive impact on the sustainability of the agricultural sector thanks to a significantly higher producer price paid to the farmer. Ecuador is the top cocoa-producing country in South America and the leading exporter of fine and flavor cocoa worldwide. The aromatic Nacional variety, emblematic of Ecuador, is highly sought after by the chocolate industry. Today, the cultivated "modern Nacional" is an hybrid population resulting from genetic mixing of 3 ancestors: Amelonado, Criollo and ancestral Nacional. A long collaboration has been established between INIAP (Instituto Nacional de Investigaciones Agropecuarias) and CIRAD (centre de Coopération Internationale en Recherche Agronomique pour le Développement) to discover the origin of the ancestral Nacional cocoa trees, to collect native cocoa trees in its area of origin (Ecuadorian Amazon), to safeguard and to use them for breeding of new aromatic varieties.

❖ Context

Deforestation of parts of Ecuadorian Amazon for crops cultivation and cow pastures

↓

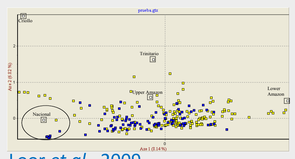
Native cacao trees cut down or severely damaged



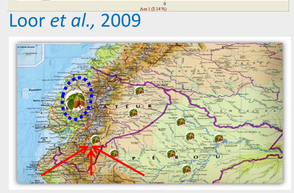
Diversity of habitats: altitudes (250 to 1200 m), temperature, cloud cover, rainfall

↓

Genetic diversity due to the adaptation of cocoa trees to these environmental variations?



Loor *et al.*, 2009



Loor *et al.*, 2012

Modern hybrid cultivated population involving ancestral Nacional, Amelonado and Criollo

↓

Targeting of "Nacional Ancestral" after genetic and geographical analyses of Allen's collections (1988)

❖ Our ambition

- Collect genetic resources related to the ancestral aromatic Nacional variety poorly represented in germplasm collections.
- Safeguard collected trees at experimental stations and schools near the collection sites to evaluate agronomic traits.
- Evaluate the overall genetic diversity of cocoa trees in the Ecuadorian Amazon.

❖ Collections

Four expeditions (2010, 2013, 2017 and 2019) have been organized in the Ecuadorian Amazonian provinces of Zamora-Chinchipe, Morona-Santiago and Pastaza with participation of farmers and Shuar, Achuar and Amazonian Kichwa amerindian communities, to collect native cocoa trees.

Loor *et al.*, 2015

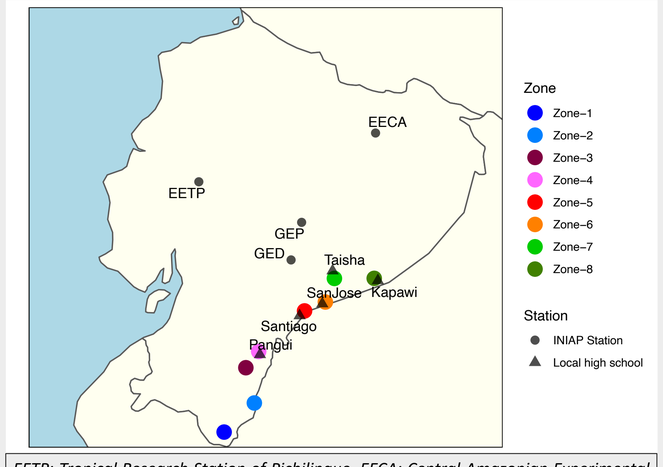
High phenotypic variability found → Sampling:

- Budwoods → Grafting (Ecuador)
- Pods → Sowing and Microfermentation (Ecuador)
- Leaves → Genetic analysis (Montpellier)

283 native cocoa trees were collected in 8 different zones, and the trees or their descendants were planted (with agronomic monitoring) at INIAP's experimental stations and in local communities close to the collection sites.

↓

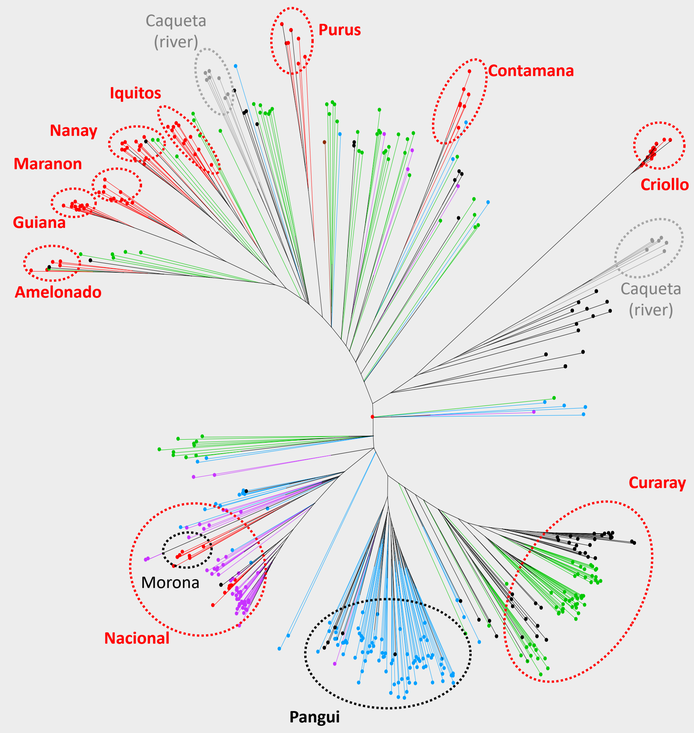
Simplified access to collections for local populations



EETP: Tropical Research Station of Pichilingue, EECA: Central Amazonian Experimental Station located in the province of Orellana, GED: Experimental Farm of Domono, GEP: Experimental Farm of Palora.

❖ Diversity of native cocoa trees in Ecuador

- Collection Zone 1 (2013) and Zone 2 (2010), in south Ecuadorian Amazon
- Collection Zone 3 (2010) and Zone 4 (2010 + 2017)
- Collection Zone 5 et 6 (2017), Zone 7 et 8 (2019)
- 10 control genetic groups (Motamayor *et al.*, 2008)
- Allen's collection (1988) collected in Ecuadorian Amazon
- Allen's collection (1988) collected in south of Colombia (Caqueta river)

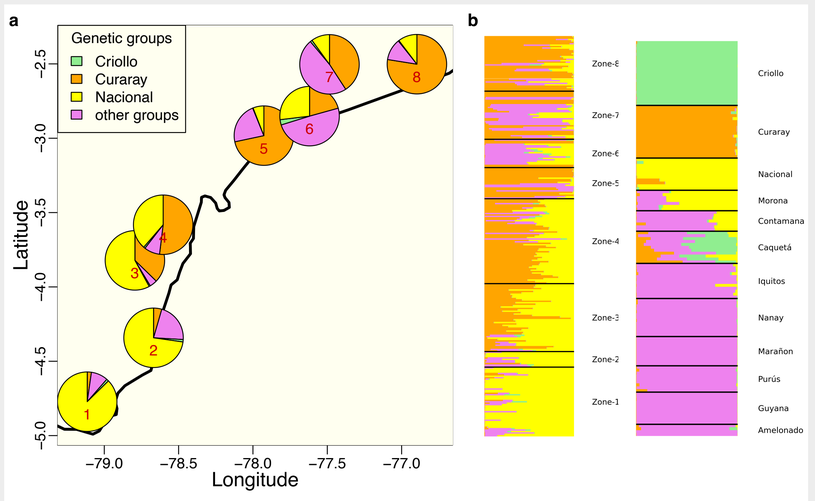


Phylogenetic tree inferred from 48 Simple Sequence Repeats (SSR) markers, 283 cocoa trees from 2010 to 2019 collections and accessions from Allen's collections (1988).

↓

The new resources have greatly enriched the overall diversity of the cocoa tree, particularly around the Nacional and Curaray groups and an intermediate group near the village of El Pangui.

❖ Genetic distribution and origin of Nacional



Structure of the 283 collected accessions genotyped with 48 SSRs with an optimum number of populations at K=4 (Evanno method) a- Map of average ancestry coefficients in the 8 collected areas. b- Bar graph of the population structure of the collected accessions (left panel) and of the accessions of the control population.

Fouet *et al.*, 2022

The Nacional genetic group is predominant in the south and Curaray in the north. In the southernmost "Nacional" zone (1), an archaeological site has been discovered and analyses (including ancient DNA) have shown that the Maya Chinchipe population was consuming cacao 5,000 years ago (Zarillo *et al.*, 2018).

↓

The Mayo Chinchipe population could have been involved in the domestication steps of the Nacional

❖ Conclusion / recommendation

This new collection shows that this region is a hotspot of cocoa diversity that enriches the currently known diversity and improves the knowledge of the global genetic structure of *T. cacao*. Our results also clarify the geographical origin of the Nacional variety. These new resources will be used in breeding programs for the improvement of locally adapted aromatic varieties, and more globally for the selection of new varieties adapted to environmental changes. The collections were replanted near the collection sites so that local communities can easily access them and replant adapted cocoa trees to improve their income.

Allen J.B. (1988). Geographical variation and population biology in wild Theobroma cacao. Ph.D. thesis, University of Edinburgh.
Motamayor J.C. *et al.* (2008). Geographic and Genetic Population Differentiation of the Amazonian Chocolate Tree (Theobroma cacao L). PLOS ONE 3: e3311.
Loor R.G. *et al.* (2009). Tracing the native ancestors of the modern Theobroma cacao L. population in Ecuador. Tree Genetics & Genomes 5: 421–433.
Loor R.G. *et al.* (2012). Insight into the Wild Origin, Migration and Domestication History of the Fine Flavour Nacional Theobroma cacao L. Variety from Ecuador. PlosOne 7: e48438
Loor R.G. *et al.* (2015). Rescue of cacao genetic resources related to the nacional variety: Surveys in the Ecuadorian Amazon (2010-2013). Revista ESPAMCiencia 6: 7–15.
Zarrillo S. *et al.* (2018). The use and domestication of Theobroma cacao during the mid-Holocene in the upper Amazon. Nature Ecology & Evolution 2: 1879–1888.
Fouet *et al.* (2022). Collection of native *Theobroma cacao* L. accessions from the Ecuadorian Amazon highlights a hotspot of cocoa diversity. Plants, People, Planet 4 (6), 618-637

Acknowledgements: *These surveys were carried out thanks to a collaboration between INIAP and CIRAD. We thank the Agropolis Foundation, MUSE (Montpellier Université d'Excellence), Valrhona for their financial support of this project and* CRC (cocoa research center – Trinidad and Tobago) *and CATIE (Centro Agronómico Tropical de Investigación y Enseñanza- Costa Rica)* for providing accessions studied in this work. *This work, part of the MUSE Amazcacao project, was publicly funded through ANR (the French National Research Agency) under the "Investissement d'avenir" program with the reference ANR-16-IDEX-0006. We are very grateful to the local populations, mainly the Shuar, Achuar and Amazonian Kichwa Amerindian communities, as well as to the agricultural colleges of El Pangui, Real Audiencia (San Jose), Raime Roldos (Santiago), Los Angeles (Taïsha) and Tuna (Kapawi) for their invaluable help during these surveys.*

Contact: olivier.fouet@cirad.fr