

EVIDENCE OF MAIZE (*ZEA MAYS* L.) EXPLOITATION AROUND AN ANCIENT CROSSROAD LINKING DIFFERENT ABORIGINAL AMERICAN CIVILIZATIONS

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RESUMO:

Uma rede de caminhos históricos conhecida como Peabiru foi construída ligando o sul do Brasil com os Andes peruanos. O caminho desapareceu durante a era colombiana, mas foi um importante testemunho da época pré-colombiana brasileira. Visando compreender as migrações humanas durante o período pré-colombiano ao redor Peabiru, foi examinada a relação genética existente entre variedades de milho crioulo moderno e outras arqueológicas, com base no gene ADH2. As sequências DNA derivadas de Peabiru foram agrupadas com espécimes arqueológicos dos Andes, sugerindo uma exploração de milho em torno desse caminho histórico, envolvendo população andina pré-colombiana e nativos Guarany, e endossando o debate sobre a presença de populações humanas pré-colombianas próximas a área de transição em torno de fronteira Brasil-Paraguai.

ABSTRACT:

A historic net of pathways referred to as Peabiru was constructed connecting the southern of Brazil with the Peruvian Andes. The path disappeared during the Colombian era but was an important testimony of the Brazilian pre-Colombian epoch. To understand the human migrations during the pre-Colombian around Peabiru we examined the genetic relationship of modern landraces and archaeological maize considering the ADH2 gene. Peabiru sequences were grouped with archaeological Andine specimens suggesting an early exploitation of maize around this historic path, involving pre-Colombian Andian people and Guarany natives, and endorsing the debate regarding the presence pre-Colombian human populations nearby the transition area around Brazil-Paraguay border.....

1. INTRODUCTION

A trail referred to as Peabiru path was constructed in the pre-Colombian era, connecting the Atlantic and Pacific Oceans, going across forests, rivers and ridges, in an impressive trajectory of approximately 3 thousand kilometers [R. Maack, 1968]. Part of this historic path was tread by important European explorers Aleixo Garcia (1522), Cabeza de Vaca (1542) and Ulrich Schmidl (1553). The main trail began at the coast of the State of São Paulo, crossed the Paraguay Chaco and reached the South of Peru [R. Maack, 1968], but disappeared during the

Colombian era. The engineers of this ambitious task, important testimony of the Brazilian pre-Colombian epoch, remain a mystery, but there are two possibilities to consider: 1. The Guarany indigenous people for cultural, commercial or spiritual purposes (seeking the mythological "Terra sem Mal"- a land without evil) [A. Metraux, 1927]; 2. The Incas as a strategy of empire expansion toward Atlantic Ocean, though this is generally viewed with skepticism in Brazilian territory. Understanding Pre-Colombian history in South America is complex because the conqueror changed a lot the existing landscape and reported tendentious versions. Furthermore, the

lack of financial support, and in some places, the deficit of archaeological data resulting from intrinsic environmental circumstances which destroyed many registers and still the human depredation, has deepened the problem and impaired the conclusion of the true history.

One way to gain insight into past history is to analyze and interpret current genetic patterns. Information about human population resettlement close to the Peabiru may be understood by examining the maize (*Zea mays*) genetic pattern of landraces cultivated around the trail, and can shed light on the human migrations during pre-Colombian America. Why is the maize informative? Maize does not exist in wild form and the domesticated one is dependent on human manipulation, therefore serving as an important marker to guide and identify human presence [Anderson EF and Cutler H, 1942]. Besides, we were stimulated to use maize during our investigations because of its notorious importance for Brazilian indigenous peoples and the fact that it has served as staple food to ancient Andine natives throughout western South America [Burger RL, 1990]. The area studied has been inhabited by different indigenous groups, including Guarany, since the pre-Colombian epoch [B. Meliá, 1988]. Hence, we sampled maize landraces from Guarany communities localized on the historic trajectory described for Peabiru. Additionally, with the purpose of find the historical conductor thread, we included the available samples derived from maize preserved in archaeological sites in Southwest Brazil and around the Andes (Coast and Highlands of Peru and Northern Chile). Previous investigations of the evolution of maize were based on the analysis of diversity of the ADH2 allele sequences [F.O.Freitas et al., 2003, Goloubinoff P et al., 1993] and our molecular evidences support those findings.

MATERIALS AND METHODS

Plant material: We sampled 5 maize landraces obtained in the Germplasm Bank from EMBRAPA (Brazilian Agriculture Research Corporation) that coordinated a program for collection of maize landraces from Brazilian indigenous people in 1978. Data from 07 additional landraces collected elsewhere were included. Details about samples are found in table 1. These samples represent maize maintained in a geographic range associated to Guarany settlements and the Peabiru path. We also included data from Southern Brazil [F.O.Freitas et al., 2003], Highland Peru and Coast Peru [Goloubinoff P et al., 1993] as archaeological specimens. ADH2 sequence from a distant maize relative (*Tripsacum pilosum*) was used as outgroup.

PCR and DNA sequencing. Total DNA was extracted from individual plantlets using a modification of MATAB (Mixed Alyltrimethylammonium Bromide, Sigma) method as previously described [Cardoso MA et al., 1998]. PCR reactions were done in a volume of 25 μ L containing 25 ng of DNA, 200 μ M of DNTPs, 1X PCR buffer containing 2.0 mM MgCl₂, 12 μ g BSA, 20 pmoles of forward primer 5'-CTGTGGATCCTCTCGTGTTCCTTGGAGTGT-3' and the reverse, 5'-CTGTGGATCCTGCGGCTAGAGAGATGCAGCA-3' (Invitrogen) and 1U Taq Platinum (Invitrogen). Amplification of a fragment of ADH2 sequence with about 300 bp was performed in a Mastercycler (Eppendorf) using the following conditions: 94oC for 2.5 min; 40 cycles of 60oC for 1 min, 74oC for 1 min, 94oC for 1 min; 60oC for 1 min; and a final extension at 74oC for 8 min. Cycle sequencing was carried out on 9700 thermal cycler (Applied Biosystems) using BigDye Terminator Cycle Sequencing kit (ABI, v3.1) following the supplied protocol. Sequences run on a 377 Automated DNA Sequencer (Applied Biosystems).

Alignment and Network Analysis: Sequence alignment of 170 bp from the amplified and sequenced region was constructed by ClustalW

and Network was assembled as described before [Polzin T and Daneschmand S V, 2003, Bandelt HJ et al., 1995]. The Network program is available for free from Fluxus Engineering (www.fluxus-engineering.com).

Alleles representation on Network	Reference	Age	Localities	Repeat Type
1	6	Modern	São Paulo / Southern Brazil	GA _n TA
2 / 6*	6	890±50 BP	Minas Gerais / Southern Brazil	GA _n TA/ GA _n TA
3 / 7*	6	630±60 BP	Minas Gerais / Southern Brazil	GA _n TA/ GA _n TA
4*	6	630±60 BP	Minas Gerais / Southern Brazil	GA _n TA
5*	6	940±60 BP	Minas Gerais / Southern Brazil	GA _n TA
8 / 9 / 12*	7	1500±50 BP	Northern / Chile	GA _n TA/GA _n TA/GA _n
10	6	Modern	Paraguay	GA _n
11*	7	440±40 BP	Highlands / Peru	GA _n
13 / 14*	7	4500±500 BP	Coast / Peru	GA _n / GA _n
15	This work	Modern	Mato Grosso do Sul / Central Brazil	GA _n
16/20	This work	Modern	Mato Grosso do Sul / Central Brazil	GA _n /GAAAGA _n
17/19	This work	Modern	Mato Grosso do Sul / Central Brazil	GA _n /GAAAGA _n
18	6	Modern	Paraná / Southern Brazil	GAAAGA _n
21	This work	Modern	Mato Grosso do Sul/ Center Brazil	GAAAGA _n
22/25*	6	570±60 BP	Minas Gerais/ Southern Brazil	GAAAGA _n / GAAAGA _n
23/24*	6	570±60 BP	Minas Gerais/ Southern Brazil	GAAAGA _n / GAAAGA _n
26	<i>Tripsacum pilosum</i> 7	Modern	Jalisco / Mexico	GA _n AACA

Table 1: Summary of plant specimens and dinucleotide repeat types. Multiple allele representation on Network means presence of different allele sequences for unique sample. *Archaeological samples.

RESULTS AND DISCUSSION

Just to provide some background and to support the comprehension of this history: Archaeological samples from Southwest Brazil were probably managed by Central America human migrants [F.O.Freitas et al., 2003]. Samples collected in Coast of Peru with 440-1500 yr B.P. are probably related to maize held by native American cultures evolved in the Highlands, around the Inca epoch [Keatinge RW, 1999]. However, samples from the Highlands of Peru with 4500 yr B.P. brought back to a pre-Inca period [Keatinge RW, 1999]. Over the course of thousand of years, pre-Inca cultures settled along the Peruvian coast and highlands. On of the main was the Kingdom of Chavin, which prospered between 950 B.C. and 450 B.C. Following the decline of the Chavin civilization a sequence of local cultures

emerged and fell during thousand of years. Inca was only the most recent of these developed cultures to evolve in the Andes [Chmyz I and Sauner ZC, 1971]. Comparisons of Guarany landraces and the archaeological specimens revealed three noticeably distinct types of dinucleotide repeat sequences. Some of the Guarany maize cultivated in the border of Brazil-Paraguay Chaco contains a structure of dinucleotide repeat identical to the archaeological maize of 4.500 yr B.P., which was probably maintained by the aboriginal American Indian civilization that evolved in the Andean region. Network Analysis of ADH2 allele sequences from both the modern landraces and the archaeological specimens resulted in two groups, one grouping the samples collected around Peabiru and the oldest maize with 4500 yr B.P. from Coastal Peru, and a second cluster including Peru 440-1500 yr B.P. and Chile

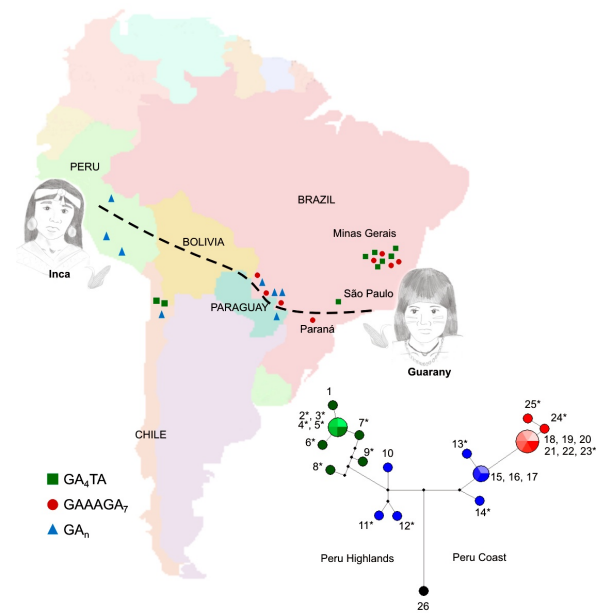


Figure 1: South America showing the area where maize samples investigated have been maintained. The alleles are distributed in three groups of dinucleotide repeat. On the right side the Network analysis. Each disk corresponds to an individual or a group of sequences from different maize landraces. The right group includes Peru Coast and Peabiru samples and the left one represents the cluster with maize of Highlands, Chile and others (details on materials and methods). Outgroup (*Tripsacum pilosum*) is black and the tiny circles represent alleles that were not found in the maize samples studied, though the existence is deduced in the network analysis. The dotted line show one of the possible main Peabiru path.*Archaeological sample

among others (Figure 1). This result is consistent with the idea of the existence of a crossroad linking two different peoples. How does one understand human settlement patterns during the pre-Colombian South America? We reinforce the strategy to investigate cultivated plants to understand human migration histories. The Peabiru-trail endeavor was probably a great undertaking possibly requiring headmen work for generations. The comprehension of the origin of American indigenous people could help to elucidate part of this history. However, the origin of them are still subject of debate among archaeologists and little is known about the migrations of the very antique American culture. A "preliminary Peabiru path" might served for one of the migratory waves and continue with the same intention while its structure was being improved. Besides, we conjecture about the maintenance of maize with a genetic background resembling the ancient samples by a more recent civilization as the Inca empire. Indeed, the Incas are best known for their regular conquest aspirations, always guided by a leader; besides, they were notorious engineers, constructing extensive systems of roads. On the other hand, historic and archaeological data suggest that the Guarany reached a high demographic number that could be mobilized in this venture or in war-related operations [B. Meliá, 1988]. Emphasis on bellicose action is found in a note from the historian and archaeologist Philip A. Means about hostile contacts between indians from Chaco and Incas before 1490 [Nordenskjold BE, 1917]. Every possible explanation may overthrows old speculations about a weak connection between highland and lowland populations during the pre-Colombia period in South America due to the natural obstacle as mountains and forests [Freitas FO et al., 2003].

Archaeological evidence uncovering the Peabiru path during the pre-Colombian period was destroyed by floods and plantations, but it points to the presence of Inca and Brazilian

indigenous people remnants [Chmyz I and Sauner ZC, 1971]. Moreover, Inca and Guarany exchange is suggested by vestiges of cultural influence [A. Metraux, 1946]. From a cultural and economical standpoint, the region around the Brazil-Paraguay border is a transition area among the meadow of Amazonian watershed, Argentine meadow and the subandine zone, and different cultures have been mixed there. Interestingly this transition area includes the Peabiru path, and we are presenting important evidence of population contacts in the pre-Colombian epoch. We expect to promote the debate regarding the human populations that could have existed near that transition area. Although further investigation in a multidisciplinary fashion is required to elucidate the kind of relationship existing then, our study reveals signs of early exploitation of maize around an important pre-European trail, somehow involving the aboriginal American Indian civilizations and the Guarany Indians, in the contemporary Brazilian territory.

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