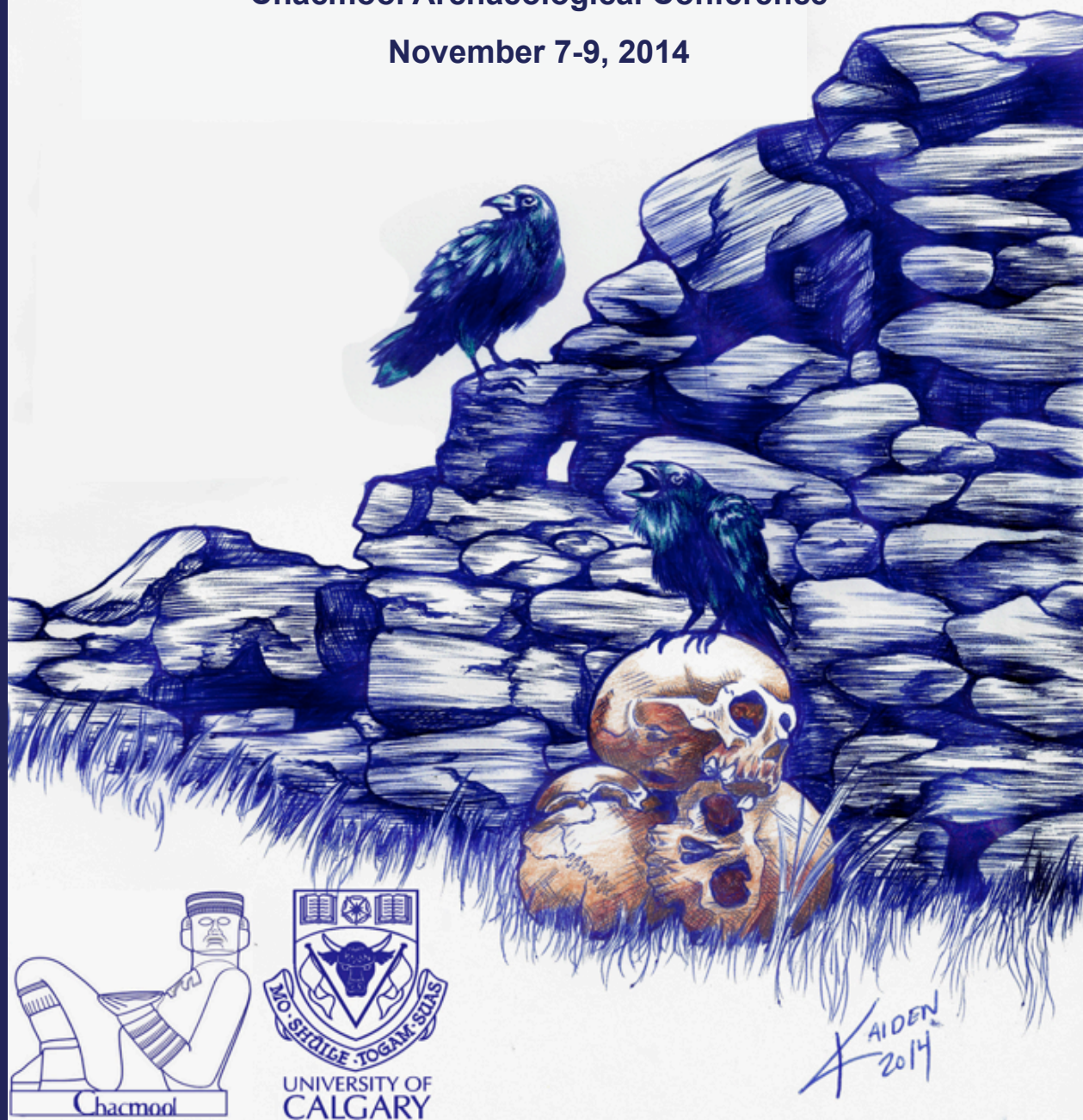


BREAKING BARRIERS

Proceedings of the 47th Annual
Chacmool Archaeological Conference
November 7-9, 2014



edited by

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Chacmool Archaeological Association, 2015

For PDF format: ISBN 978-0-88953-386-8

Cover art by Kaiden Tajiri

Ancient DNA Studies in Mesoamerica: Major Contributions and Limitations

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Abstract (English)

Ancient DNA is a method that many studies have applied in the last decade. Since 1984, the refinement of the method has been challenging due to contamination issues and difficulties for verification of results, although scientists have overcome such problems. In the last few years, the method has been used for answering questions about the dynamics of prehispanic societies in Mesoamerica. The results of these studies have clarified internal social processes suggesting more specific ancestral origins for different ancient populations. Many variables limit the number of studies and the databases are still slowly developing. Here, I explore the major contributions of this method, its more recent technical improvements, and the main challenges that ancient DNA research is facing. Finally, I present the possibilities of this method for future Mesoamerican studies.

Resumen (Spanish)

El ADN antiguo es un método que se ha aplicado a varios estudios durante las últimas décadas. Desde 1984, el refinamiento del método ha sido desafiante debido a los problemas de contaminación y las dificultades para la verificación de resultados; sin embargo los investigadores han logrado encontrar soluciones para sobrellevar muchas de estas dificultades. En los últimos años, el estudio de ADN antiguo se ha utilizado para responder preguntas sobre las dinámicas de sociedades prehispánicas en Mesoamérica. Los resultados de estos estudios han arrojado luz sobre procesos sociales internos, y además han corroborado los orígenes de algunas de estas poblaciones antiguas. Muchas variables limitan el número de estudios paleogenéticos y la construcción de una base de datos genética para la Mesoamérica prehispánica continúa desarrollándose aún lentamente. En este trabajo se presentan las mayores contribuciones del ADN antiguo al conocimiento de sociedades Mesoamericanas, así como las más recientes soluciones y mejoras técnicas, y los mayores retos que aún enfrenta el ADN antiguo como herramienta de investigación para la arqueología. Finalmente, se presentan las futuras posibilidades para estudios paleogenéticos en Mesoamérica.

The genetic history of native populations in the Americas has been widely focused on mitochondrial DNA studies in modern groups, but in recent decades such studies have been enriched by possibilities of conducting ancient DNA studies that have contributed to contrast previous hypothesis about genetic structure of prehistoric populations (Handt et al. 1994; Pääbo et al. 2004; Rasmussen et al. 2014). Most of mitochondrial DNA for native groups in the Americas is found among five haplogroups : A2, B2, C1, D1 and X2, and their derived variants (Torroni et al. 1994; Raff et al. 2011). These findings, in addition to ancient DNA studies, have not only facilitated the understanding of these variants' evolution, but they also relate them to models of colonization and expansion of humans into the Americas, to study local processes of interaction and migration, which are relevant for archaeological research (Jones 2003). These questions

have also been addressed to the ancient populations of Mesoamerica .

Nowadays, 291 Mesoamerican ethnic groups remain in a vast geographic region that covers from Mexico to north Honduras (Gorostiza et al. 2012). The exact prehistoric Mesoamerican population distribution and movements still remain unclear specially at “border” regions (Snow et al. 2011; McCafferty et al. 2012). However, archaeologists and ethnohistorians have hypothesized that at least during the Postclassic period (A.D.1000-1697) Mesoamerican populations extended to northwest Costa Rica and Pacific Nicaragua (Braswell et al. 2002; Carmack and Salgado González 2006).

For this reason ancient DNA studies become even more relevant in revealing insights of genetic structure of past groups despite their limitations with contamination issues and sample bias due to variation of skeletal material preservation. New improvements of sequencing techniques, such as next Generation sequencing and extraction methods are alternative possibilities for Mesoamerica specially in tropical areas (Chatters et al. 2014). Nonetheless, all ancient DNA analyses for human mitochondrial DNA in Mesoamerica can be used to establish a genetic diversity database for future population studies.

TABLE 1. Ancient DNA studies for archaeological sites in Mesoamerica.

Geographic Area	Archaeological Site	# Ind	Period	Reference
Yucatan Peninsula	Hoyo Negro	1	11050 B.C.	Chatters et al., 2014
Mexico City	Tlatelolco	27	1325 A.D.	Kemp et al., 2005
Mexico City	Tlatelolco	30	1350-1400 A.D.	Solórzano, 2006
Mexico City	Tlatelolco	38	1454 A.D.	De la Cruz et al., 2008
Tizayuca, Hidalgo	Tetetzontlilco	36	1531-1600 A.D.	Solórzano, 2006
Apizaco, Tlaxcala	Santa Maria Texcalac	36	1700-1900 A.D.	Solórzano, 2006
Quintana Roo	Xcaret	6	600-800 A.D.	González-Oliver et al., 2001
Quintana Roo	Xcaret	16	900-1521 A.D.	González-Oliver et al., 2001
Quintana Roo	Xcaret	5	No data	González-Oliver et al., 2001
Western Honduras	Copán	9	700-1300 A.D.	Merriwether et al., 2011
Basin of Mexico	Xaltocan	25	1240-1541 A.D.	Mata-Miguez et al., 2012
Oaxaca (Mixteca Alta)	Yucundaa	41	1544 A.D.	Wariner et al., 2012
Total		267		
Non-human samples				
Petén, GU	El Mirador	7	327 B.C-54 A.D.	Thornton et al., 2012
Tamaulipas, MX	Cuevas Ocampo	5	2350-350 B.C.	Jaenicke-Després et al., 2003

Ancient DNA in Mesoamerica

Currently, eight studies represent the total of archaeological human ancient DNA findings in Mesoamerica; two correspond to non-human samples but are associated with domestication of crops (Table 1). This number of studies has slowly increased in frequency over the last ten years possibly due to methodological advances (Kirsanow and Burger 2012). Nonetheless, the number of individuals per site and period varies widely (Table 1). This variation is likely due to paucity and/or absence of human remains, from particular archaeological contexts, and/or the poor state of preservation of the human remains. In addition, it may not be possible to study some ancient remains that do exist because of laws or the unwillingness of the institutions that hold them to allow sampling (LeBlanc et al.

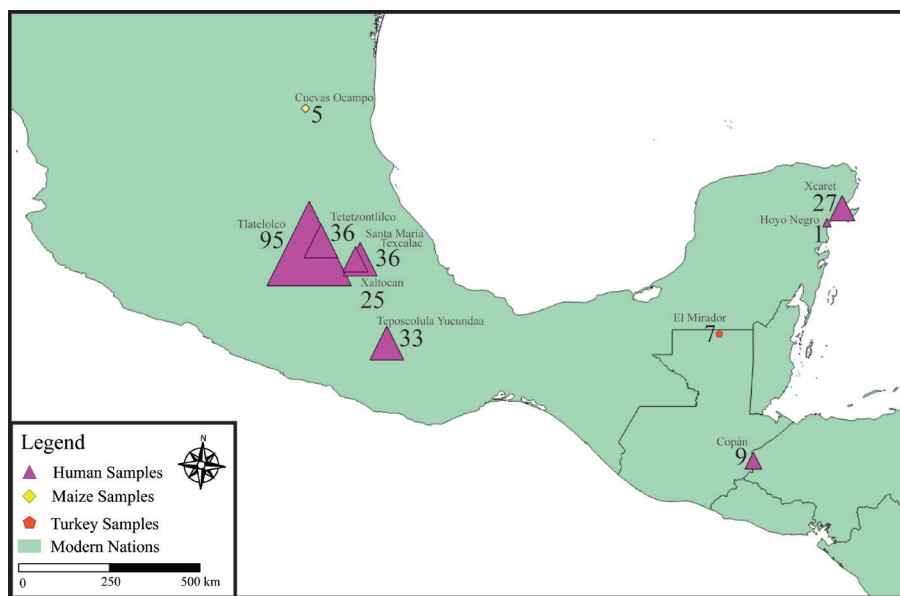
2007).

Yet, even when archaeological material is present and well preserved, that would not fulfill the requirements to conduct DNA analysis; at the end it will depend on the capacity of the researchers to afford it, and also what would be their utility for answering questions around the samples, burials, or sites.

Until now, most of these studies have been limited to mitochondrial DNA diversity, whose molecule is highly abundant in the cell, and present higher probabilities of recovery than nuclear DNA. For example, Merriwether et al. (1997) conducted the earliest ancient analysis in Mesoamerica from skeletons of the Maya site of Copán. In this study, they demonstrated the possibilities to retrieve mtDNA from skeletal remains in tropical regions and also the potential of ancient DNA to suggest genetic discontinuity of past populations. They found a completely different genetic diversity of the past population, showing complete absence of haplogroups A and B, which are the most common in Mesoamerican populations.

Nevertheless, some of the weaknesses of this research include the low number of samples, (only 9) belonging to anytime between 400-700 A.D. to 1200-1400 A.D., certainly a long period of time. The details about the samples are not specified in Merriwether et al. (1997) article, but described by Storey (2006), and Whittington and Reed (2006). All in all, the authors suggested to increased the number of samples for more conclusive statements (Merriwether et al. 2006).

Afterwards, many other ancient DNA studies were conducted for other Post-classic Mesoamerican sites (Figure 1), in the region of Central Mexico (Figure 2). These ancient DNA studies made important contributions to the understanding of the Post-



classic period. For example, Mata-Míguez et al. (2012) showed that the Xaltocan conquest by the Aztecs caused a replacement of mitochondrial lineages, thus demonstrating how such cultural events in the past could alter demography in Mesoamerican populations, and probably more than once, before the arrival of Europeans to the Americas. However, the same did

FIGURE 1. Geographic distribution and number of samples per site analyzed for ancient DNA in Mesoamerica.

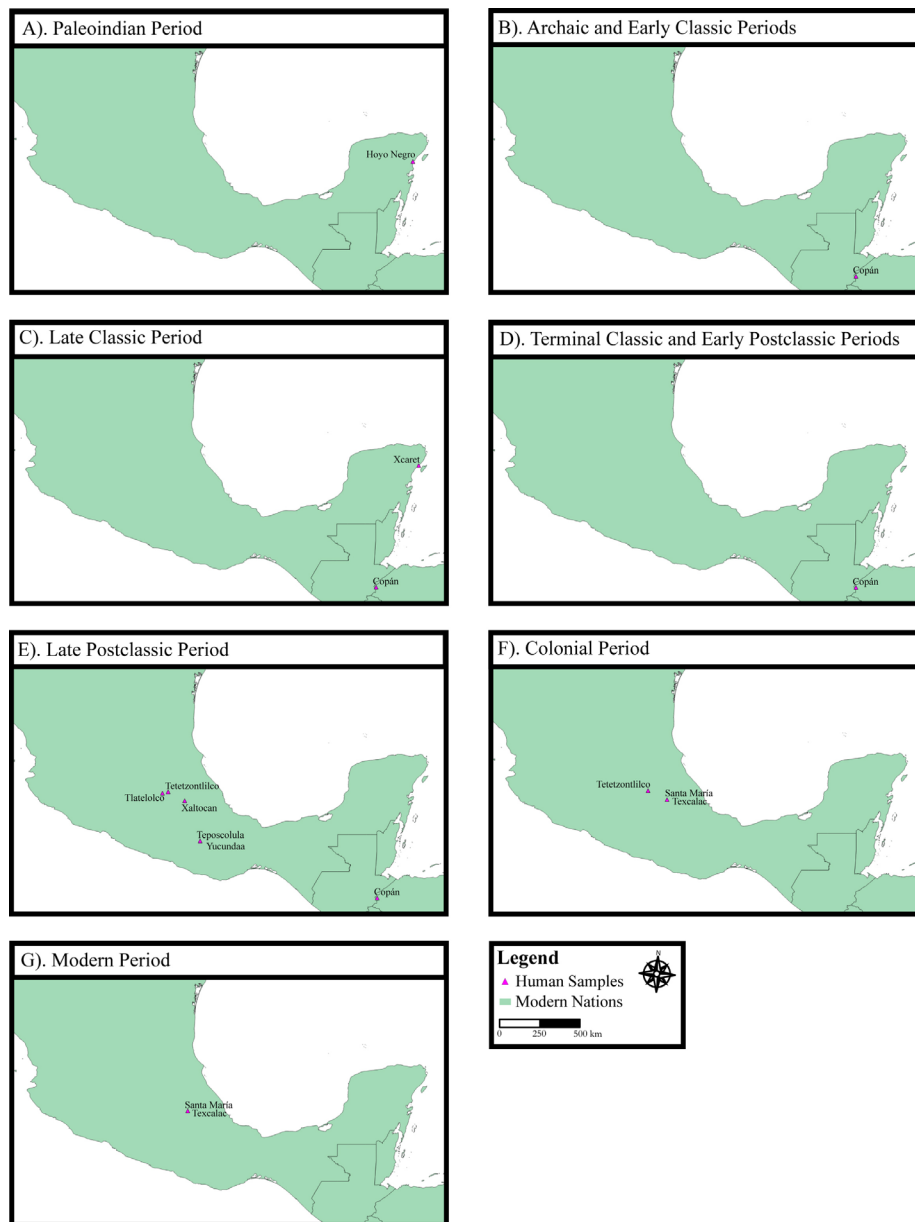


FIGURE 2. Comparative distribution of ancient DNA analysis studies, per archaeological site, and period in Mesoamerica.

not seem to happen with regard to chromosome Y genetic variants, which suggest that the Aztecs used to replace conquered women with their own, while keeping the men (Mata-Míguez et al. 2014).

Another example of specific contribution is sex determination of infants from sacrificial contexts in Tlatelolco where over half of the victims were males (De La Cruz et al. 2008).

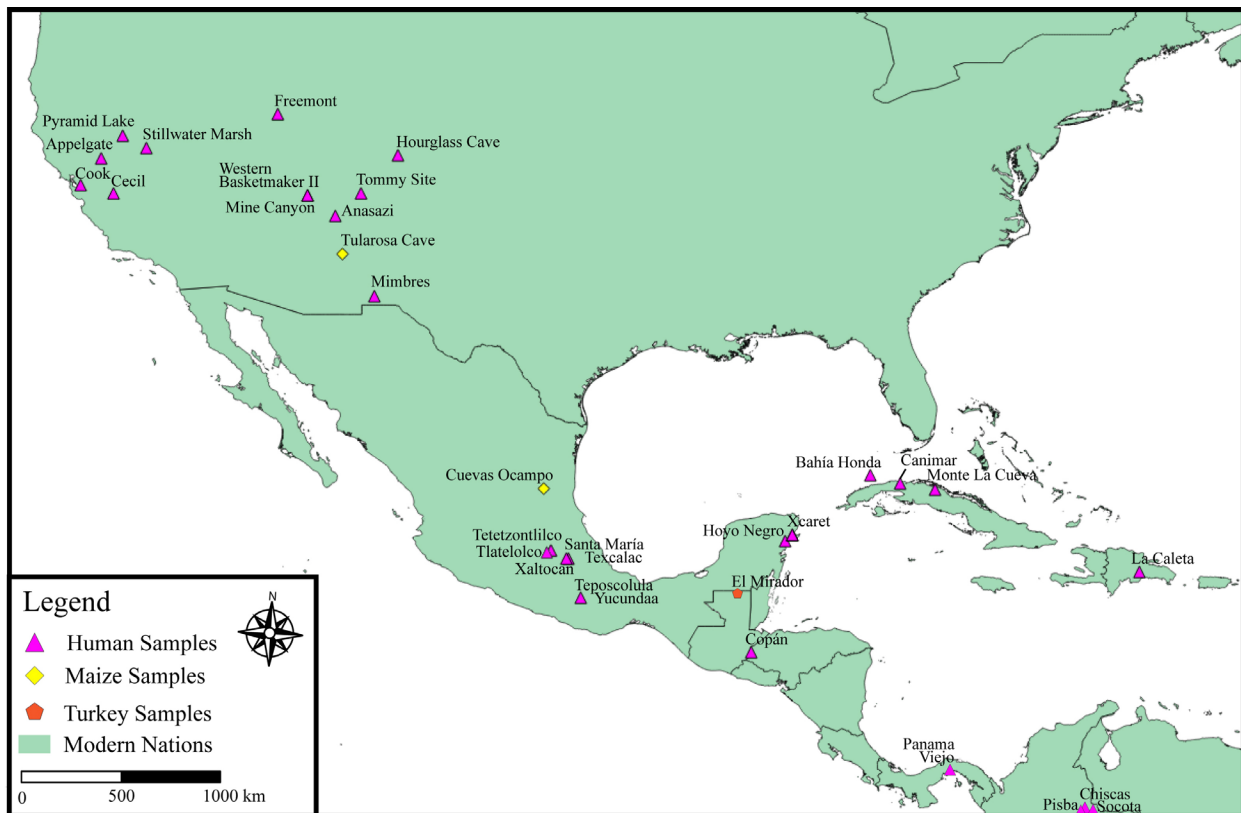


FIGURE 3. Geographic distribution of ancient DNA analyses, for archaeological sites, in Southwestern United States, part of the Caribbean, and Southern Central America.

In addition, the haplogroups composition of sacrificial victims corresponded to what was expected from central Mexican groups, which rejected the possibility that these children were traded from foreign territories.

In general, the Mesoamerican haplogroup frequencies for the Postclassic period show a higher frequency for haplogroups A, followed by B, although this frequency varies from 47 to 65% and 10 to 37% respectively (Table 2). Based on all the ancient DNA analyses in Mesoamerica, haplogroup A show the higher frequency (53%) followed by C with 18%, however this is not the case for the Postclassic period. Probably the average estimate is biased by the findings in Copan, which showed 89% of frequency for haplogroup C. This is not the case for Central Mexico where haplogroup C fluctuates between a 4% and a maximum of 7%.

The haplogroup frequencies could reflect differences on prehistoric populations genetic makeup due to founder effects, but also could be reflecting sampling bias. Only in Tlatelolco the frequency of haplogroup A varies more than 20% for sites analyzed. The distribution of variants per period in Central Mexico is not clear, which would indicate another limitation, such as a limited regional sampling (Figure 3).

With regards to haplogroup B, there is a low frequency for Xcaret, Quintana Roo during the Postclassic that could reflect differentiation within Mesoamerican groups. The possibilities could be explored further as the studies for ancient DNA continue. The

TABLE 2. Frequencies of mitochondrial DNA haplogroups for ancient Mesoamerica.

Geographic Area	Archaeological Site	# Ind	Period	Haplogroup Frequency (%)				Reference
				A	B	C	D	
Yucatan Peninsula	Hoyo Negro	1	11,050 B.C.				D (D1)	Chatters et al., 2014
Basin of Mexico	Xaltocan	25	1,240-1541 A.D.	48	24	4	24	Mata-Míguez et al., 2012
Oaxaca	Yucundaa	41	1,544 A.D.	54	24	17	5	Wariner et al., 2012
Mexico City	Tlatelolco	38	1,454 A.D.	57	21	7	14	De la Cruz et al., 2008
Mexico City	Tlatelolco	30	1,350-1400 A.D.	47	37	6	10	Solórzano, 2006
Tizayuca, Hidalgo	Tetetzontlilco	36	1,531-1,600 A.D.	70	10	16	3	Solórzano, 2006
Apizaco, Tlaxcala	Santa María Texcalac	36	1,700-1,900 A.D.	47	23	10	10	Solórzano, 2006
Mexico City	Tlatelolco	27	1325 A.D.	65	13	4	17	Kemp et al., 2005
Quintana Roo	Xcaret	24	600-1,521 A.D.	88	4	8	0	González-Oliver et al., 2001
Honduras	Copán	9	700-1,300 A.D.	0	0	89	11	Merriwether et al., 1997
Total		267		53	17	18	10	
<i>Non-human samples</i>				Species				
Yucatán Peninsula	Loltún Cave	12	11,000-126,000 B.P.	Ototylomys phyllotis (rodent)				Gutiérrez-García et al., 2014
Guatemala	El Mirador	7	326 B.C.-54 A.D.	Meleagris gallopavo (turkey)				Thornton et al., 2012
Tamaulipas	Cuevas Ocampo	5	2350- 350 B.C.	Zea mays (maize)				Jaenicke-Deprés et al., 2003

results of these studies have clarified internal social processes suggesting more specific ancestral origins for different ancient populations. For instance, a recent publication by Chatters et al. (2014) analyzed only one individual from the Yucatan Peninsula, who actually corresponds to the late Pleistocene. Interestingly, the individual belonged to the haplogroup D, subhaplogroups D1, which presents low or absence frequencies in modern and ancient Mesoamerican groups but occurs in higher frequencies in indigenous people from Chile and Argentina. Further conclusions would need higher numbers of individuals from Pleistocene times which is difficult in special for these types of older remains.

Finally, Gorostiza et al. (2012) have showed that distribution of haplogroups in modern Mesoamerican groups is not very well defined when performing more detailed

geographical studies. They also suggest that genetic origin and differentiation of important populations such as the Nahua, occurred between the late Classic and Postclassic periods. For this reason ancient DNA studies can clarify better where and how these processes took place. Until now, observed tendencies of higher haplogroup A frequency in modern Mesoamerican groups seems to fit the Prehispanic Central Mexican region but is still questionable for the rest of Prehispanic Mesoamerica.

Ancient DNA from non-human samples enlightening human past practices

Considering the ancient DNA studies for crops or animals, such as maize and turkey, it has been clarified that their domestication and spreading occurred before it was thought. For example, ancient DNA analyses of turkey samples in the archaeological site of El Mirador, Guatemala, demonstrated that the turkey was introduced to the Maya region around 200 B.C. (Thornton et al. 2012). On the other hand, before ancient DNA studies, it was thought that maize was domesticated around 6,250 years ago. However, the molecular studies proved that this event occurred around 9,000 years ago in the Balsas River Valley in southern Mexico (Jaenicke-Després et al. 2003). The analysis of ancient DNA for animals and crops could be also be enriched by applying extraction methods to ancient tools and other artifacts that could preserve DNA (LeBlanc et al. 2007). Definitively, DNA analyses from lithic tools present a good potential to better understand past cultural practices.

Future of ancient DNA studies and possibilities for Mesoamerica

The refinement of ancient DNA methods has been challenged by contamination issues, as well as by the difficulties in verification results. However, scientists have overcome such problems thanks to recent developments in sequencing technologies, such as Next Generation Sequencing (NGS) and bioinformatics tools to discriminate contaminant versus endogenous molecules in the final results (Der Sarkissian et al. 2015). Nowadays, ancient DNA is exceptionally valuable to investigate past human groups regarding evolutionary questions, such as: patterns of their genetic diversity, gene flow, reductions of population sizes due to genetic drift, and natural selection, regarding humans versus pathogen response, among other factors across time and space (O'Rourke and Raff 2010; Raff et al. 2011; Devault et al. 2014).

Without a doubt, new sequencing technologies could extend the possibilities to recover DNA from ancient Mesoamerican remains especially in those sites located in tropical latitudes (Chatters et al. 2014). Some ecologists, such as Gutiérrez-García et al. (2014), recovered genetic material from a rodent found in the Loltún Cave, located in the Yucatan Peninsula (Table 2). The same methodology employed by Gutiérrez-García et al. could be applied to other archaeological sites in this region. Additionally, a current project carried out by the University of Calgary focuses on the southern limits of Meso-

america, with the purpose of understanding past migrations from central Mesoamerica to southern Central America, more specifically the Greater Nicoya region. The application of Sanger and Next Generation sequencing are part of the techniques applied in this project.

Acknowledgements: I would like to recognize the assistance of Adam Benfer in producing the maps for this article.

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