Ancient Migrations in West Mexico: MtDNA Analyses

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ANCIENT MIGRATIONS IN WEST MEXICO: MTDNA ANALYSES

By

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Thesis
Presented in partial fulfillment of the requirements for the degree of

Master of Arts
In the Department of Anthropology

In the College of Sciences
At the University of Montana
Missoula, Montana

May 4th, 2023

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Abstract

Gutiérrez Ruano, Patricio, M.A. May 2023

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Despite the mounting evidence that suggests The Aztatlán tradition in West Mexico was a major cosmopolitan region during the Postclassic period (AD 900-1521) with connections to the rest of what is now Mexico, archaeologists have characterized items in West Mexico as culturally distinct from the rest of Mesoamerica. Recently, endogenous, and exogenous material culture has been interpreted as movement and exchange of goods and ideas between subregions and surrounding areas, all of which mention physical contact and trade were involved between Aztatlán and elsewhere. This has included interacting with areas as far as the U.S. Southwest, as well as in Southeastern Mexico, through long-distance economic exchanges. Current macroregional population models for this geographic region have focused on these material exchanges. However, the question of geneflow between these regions that accompanied the trade routes has not been addressed. Genetic information from skeletal remains disinterred during excavations in the 1950s offers insights on determining population dynamics and regional connections. This thesis discusses the first use of mtDNA analyses and genetic biological sex estimations of the West Mexico skeletal remains to assess the factors that shape this genetic variation within the Aztatlán Core Region. This research has obtained all permissions necessary from the Mexican government and museum housing the human remains and highlights the benefits of international collaboration.
Acknowledgements

Above all, I would like to thank, infinitely, my committee chair and advisor Dr. Meradeth Snow, not only for her enviable patience, advice, and help, but for her genuine human presence, and because without her, this cross-border anthropological journey could not have been possible.

I would also like to thank my committee member Dr. Randall Skelton for his help, allowing me to better understand statistical and forensic matters, and to have patience with them inside and outside the classrooms. Moreover, I would further thank Dr. James Tuttle for his readiness to be part of my committee.

Along with these lines, I am grateful to all my colleagues, but especially to Ava Godhardt, Hannah Carson, and Allison Mitchell, as well as Lacy Hazelwood, just for being the best, for supporting me and sharing their knowledge and experience.

I express my gratitude to Dr. José Luis Punzo because without him, this would have been just a dream, like many others.

I dedicate this thesis once again to Cristina and Marisa for being always here, there, and everywhere and for being a guide on my ways. Not to mention thankfulness to my family and friends —where I cannot see any difference between either of them.

Finally, I would like to express special thanks to my cats: Chapi and Hernán for being abroad with us and making our lives as blissful as theirs.
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1. **Introduction**

This thesis intends to elucidate ancient migrations and dynamics that occurred in the pre-Hispanic Mesoamerican region of far West Mexico. The goal of this project is to discover the ancient migration patterns of the highlands and coastal plains populations by the analysis of human remains belonging to five main archaeological sites: Amapa, Peñitas A and Peñitas B, that are located in the Aztatlán Region in the coastal plains of Nayarit, and, Tizapán El Alto, and Eztatlán, which are located in the Highlands of Jalisco, México. The five sites are all dated as Mesoamerican Postclassic period (AD 900-1350+) (Mathiowetz 2021, 176).

Three additional West Mexican coastal archaeological sites were also included, although their sample size is smaller: Morrett, Colima; Barra de Navidad, and Revalcito, Jalisco.

This goal will be reached by assessing the genetic variation, coupled with archaeological data, of these archaeological sites that may represent biologically distinct, yet culturally related populations.

1.1. MtDNA lineages in the new World: Inferring West Mexico’s genetic past.

The use of ancient DNA (aDNA) has improved substantially during the past few years allowing further characterization of genetic diversity in past populations and in the use of central aspect of archaeological research and chronology (Sedig et al. 2021).

Mitochondrial DNA haplogroups are defined by a set of mutations that have accumulated over time (much faster and maternally inherited) that represent distinct evolutionary branches of the mtDNA tree. This tree can be seen as a genealogy linking matrilineal lineages in modern human
populations to a common ancestral (homogametic sex XX chromosome, or biological female\(^1\)) individual (Cann, et al. 1987). Theses lineages will eventually coalesce into a single lineage we call the most recent common ancestor (MRCA). On the other hand, haplotypes are specific combinations of mutations within a haplogroup’s main mutational motif that are found in individuals.

The findings of the first reviews of collective mitochondrial DNA data from Native Americans, concluded that the Americas were populated from Asian populations over 30,000 years ago (Wallace, et al. 1985; Schurr et al. 1990; Torroni et al. 1993). This suggested a complex and dynamic history of human migration and settlement in the Americas, which has been further elucidated through subsequent genetic and archaeological research. The use of mitochondrial DNA provides insights into the genetic diversity and ancestry of Native American populations.

Insights on the genetic diversity and ancestry of Native American populations supports the hypothesis of multiple migrations from different Asian populations to the Americas. Early studies of Native American mitochondrial DNA (mtDNA) identified five major haplogroups (A, B, C, D and X respectively) (Eshleman, Malhi, and Smith 2003), and a sixth haplogroup was discovered years later by Malhi et al. (2007).

When geneticists contributed with calculating distances between modern Asians and Native Americans, and since they were part of the same gene pool, estimates confirmed that first inhabitants of the Americas left their Asian relations (Meltzer 2015, 63) at least in the last marine

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\(^1\) Biological sex refers to the category that an individual belongs to base on their genetic or physical characteristics, while gender is a social construct that does not depend fully on genetic makeup, but instead is an expression of experience and identity (Gibb et al. 2020).
entry (Bate and Acosta 2016, 67), when the Last Glacial Maximum (LGM) was occurring about 21 kya. when the LGM was happening (Dyke 2004).

Beringia emerged during the Pleistocene as growing glaciers formed to the point where global sea levels were drawn down by up to 130m (Meltzer 2015, 61). This geographical landscape was subject to a bridge that made it possible for humans to walk inter-continentally without noticing if there was a continental division. The ice sheets opened circa 16 kya from both ends and along the eastern side of the Rockies. Although there is no evidence to prove that the only way to get through was initially via coast or the interior-Great Plains (Meltzer 2015:64).

Raghavan et al. (2015), as well as Scheib et al. (2018) proposed that a single source ancestral population split into two populations: one in northern North America and a southern one that is now dispersed across North and South America represented by a 12,600 yo male infant from Montana (Anzick-1) and by a 11,500yo female from Alaska which lead to the conclusion that this event is considered to have happened 17,500-14,600 ya most likely somewhere south of the Cordilleran Ice Sheet.

Following Scheib et al. (2018), the northern branch of the split is ancestral to populations such as: Algonquians, Na-Dené, Salishan, and Tsimishian speakers of what today is Canada, and the southern branch includes the ancestors of Anzick-a, Mexicans (sic) (Mesoamericans), Central Americans and South Americans. The results showed that the B2a haplogroup arose in North America around 11-13 ka from one of the founder Paleo-Indian B2 mitogenomes, while the A2a haplogroup originated only 4-7 ka in Alaska and contributed to the formation of the Na-Dene gene pool. The study also revealed that most mtDNA variation in North America stems from the first wave from Beringia, followed by a second inland migratory event, and subsequent gene flow and local population dynamics (Scheib et al. 2018).
This is also consistent with an inferred star-like radiation starting around 15kya where genomic analyses indicate two basal lineages: both North Native Americans (NNA, or ANC-B) and South Native Americans (SNA or ANC-A) North and South American continents. All late Pleistocene and early Holocene individuals that have been studied south of the Cordilleran, are part of the SNA group (Potter et al. 2021).

Assemblages from earlier sites such as Cerro Chiquihuite, in México, Pedra Furada, Brazil and others, generally do not cohere with each other, with any antecedent technologies in north-east Asia or with later Paleoindian assemblages (Potter et al. 2021, 2). Moreover, it would seem implausible that these populations that a modern human population that survived for 20 k years went extinct leaving no trace and no DNA has been sampled (Potter et al. 2021).

However, it is thought that Athapascan populations migrated to the US Southwest maintaining a high frequency of haplogroup A. Following Snow et al. (2011, 3215), populations in the Southwest are not characterized on having a high frequency of haplogroup A. This could be due to population dynamics such as genetic drift or gene-flow. Moreover, the distribution of haplogroup A is high in Mesoamerica. (Snow et al. 2011:3215).

The understanding of mtDNA diversity of six haplogroups (A, B, C, D, X and M) (Bisso-Machado and Fagundes 2021a), can explain the earliest mtDNA distributions to characterize haplotypes of individuals or populations who share the same haplogroup.

A comparative analysis of frequencies between haplogroups involved in Mesoamerica, particularly in West Mexico, which, is an important subregion that links to the American Southwest can bring insights in building a greater picture of the genetic dynamics of populations.
The rich, wide, and complex, yet often ignored region of West Mexico has received little attention throughout the history of archaeological investigations. Up until today, no genetic research has been done in Far West Mexican Pre-Hispanic populations.

Moreover, beginning in the 1930s, archaeologists have characterized items in West Mexico as culturally distinct from the rest of Mesoamerica and have been compared to those found in the surrounding areas which have been attributed to be similar to those at West Mexico. More recently, interaction with surrounding areas and subregions in different periods has been proposed, all of which mention possible physical contact that trade would have been involved.

While archaeologists have focused on the local scale of cultural technologies and particularities, endogenous and exogenous migration explained by material culture movement, exchange, and ideas are on the rise. Scholars studying the Aztatlán region are trying to figure out when and where the Aztatlán culture began, if their beliefs and customs originated in coastal or inland locations, and if migration or travel affects how elements of the culture spread. However, no genetic studies have been done in order to complement these hypotheses at the Aztatlán region and this project will emerge as a pioneer in determining population dynamics and offering new insights on the theme.

Can far ranging economic activity, religion, and the advent of new art styles (such as Flower Worlds) (Mathiowetz and Turner 2021), and cultural complexes (similarly, the Aztatlán cultural complex) be related with the actual movement of people (gene flow) scattering across and beyond Mesoamerica during the early Post-Classic Period? Genetic research into these ideas, coupled with archaeological data, can help address these questions.

In order to test these ideas, the following two hypotheses are presented:
Hypothesis 1: Because no highland Aztatlán ceramics are reported at coastal sites (Snow and Mathiowetz 2020), I expect that the populations of the Highlands (Tizapán El Alto and Eztatlán, Jalisco) and the Coastal Region (Amapa, Peñitas A and B, Nayarit; Morrett, Colima; and Barra de Navidad, Jalisco) are genetically distinct.

If populations that share genes are expected to carry similar haplogroup frequency signatures, then the populations in the highlands are expected to possess a higher frequency of those haplogroups that are present in the populations from the coast. However, those haplogroups present in the Highlands will not be found in the coastal sites. This hypothesis can be tested through the identification of haplogroup frequencies.

Hypothesis 2: The populations in the Coastal Plains are more heterogeneous than the populations in the Highlands of Jalisco.

A gradual softening of the identified haplogroups in populations from the Highlands will be expected to be found in the Coastal region, which would indicate migration was occurring from the Highlands to the coast. It would be expected that the Coastal Plains will have more variability (more genetical diversity) than those from the Highlands. This test can be carried out by assessing genetic distances between both subregions.
1.2. Broader impacts

If genetic studies are conducted in order to determine the migration patterns of the coastal and highland populations of the Aztatlán Region and provide comparable and verifiable data of these dynamics, then this new scientific research program would be useful not only to scholars interested within the region, but also outside of it. Moreover, it would function as a critical area to address different anthropological questions such as the understanding of genetic connections between archaeological regions and contemporary indigenous peoples. Identity ties may be reaffirmed to those peoples that are historically tied into their space and genetic studies can bring new insights on the history of migration between historically connected broader regions.

Other archaeological issues would be complemented by recent bioarchaeological information. Such situations may include:

- Chronology building can be complemented and augmented with direct dating of the sample of the individuals tested for ancient DNA (aDNA) and propose new insights into the Aztatlán region.

- Evidence of proposed coastal vs highland corridors from ceramic assemblages (Nance et al. 2013) can be enhanced with biomolecular data.

Wider corridors are also proposed that connect the Aztatlán region to others, which may be better interpreted with comparable interdisciplinary analytical data.
2. **West Mexico**

West Mexico is extensive and has a geography of abrupt varied changes. Located as most of the rest of Mesoamerica within the Tropic of Cancer, the climatic annual cycle is paired with more or less seven dry months (November through May) and five months (June through October) of strong torrential rains that may vary depending on hurricanes and other climatic phenomena, these having an important outcome in temporary streams, bogs, and lakes. Its variable topography includes the Transversal Neo-Volcanic axis, which meets with the Sierra Madre Occidental ranges, the Pacific coastal plain, and the depressed landform of Bajío (Beekman 2010), which is part of the central highlands.

The cultural boundaries of West Mexico vary dynamically depending upon the perspective of different authors. There is no agreement among archaeologists to indicate precise limits to this vast area. Schöndube, however, points out a fundamental issue when differentiating the “Core Zone of West Mexico” including only Colima, Jalisco, and Nayarit, while the rest of the states have Western characteristics and yet share features with other cultural areas (Schöndube 1982). Following Claudia Espejel (2019), it may include the modern Mexican states of Michoacán, Colima, Jalisco, Nayarit, and Sinaloa, to which are sometimes added parts of Guanajuato, Aguascalientes, Zacatecas, Durango, and Guerrero, and in precise cases, a western segment of the State of Mexico (Gutiérrez Ruano 2020). However, for the purpose of this project, I will frame it more narrowly by including the “Far West Mexico’s” Pacific-coastal core zone and the entire Aztatlán region (Mathiowetz 2021) that overlap the states of Colima, Jalisco, and Nayarit.

Archaeologists have centered their studies in emphasizing their local material (endogenous) particularities, but also wealth material goods that came from other places...
(exogenous) conceived as movement and networks. Moreover, religion and ideas are also part of the exchange, and this has been thought to be accompanied by the commutation of peoples. This can be explained by the study of gene flow within the Aztatlán region in order to complement these dynamics in a biological fashion.

A series of displacement dynamics was magnified at the end of the Classic period (ca. 650/800 and 900/1200 AD), when the entire Mesoamerican territory became a huge melting pot where ethnically and culturally distinct populations were merged into great states like Teotihuacán. The loss of power propitiated, among other phenomena, the displacement of large demographic sectors over long distances mainly of artisans and merchants, but also farmers (López Austin and López Luján 1999, 17), which could have contributed to the gene flow between regions.

Some scholars argue that the study of the populations that inhabited the limits of the Western and Central regions is essential to understanding the global dynamics of the Epiclassic (Turner 2017; Aramoni Burguete 2014; Filini 2010). During that time, the Postclassic highland in southern and northwest Mexico (Snow and Mathiowetz 2020; Mathiowetz 2021) was vital for the greater understanding of this region and its connections between Mesoamerica through large-scale interactions.

Geographically, this cultural region of Mexico was well connected in the past. Few major rivers linked these different areas, but some were important for communication. Specifically, the Río Lerma connected central and western Mexico, and the Río Balsas Depression connected south-central Mexico and Oaxaca.

Consequently, the rising and expanding economies in West Mexico and its privileged geographical position between the American Southwest and the rest of Mesoamerica articulated
specific mechanisms of interaction such as coastal trade and inland routes that facilitated communication and migration.

West Mexico has been the hardest to determine what language were spoken, particularly in the Coastal Plains (Beekman 2010, 45). What languages were spoken in the past are hard to figure out because there was not a writing system tied to graphemes, which means that there is not enough evidence to show what language was spoken in the area. Also, Spanish chroniclers, people that wrote things down, outside of Michoacán did not document the native languages. In addition, in historic times, many other languages like Uto-Aztecan were spoken in different areas. Finally, the modern Otomanguean languages were brought into the area after the Spanish arrived, so it is not possible to say that those languages were spoken in the area in the past.

2.1. The Flower Worlds: A Link to The Southwest

Northern Mexico and the American Southwest is undoubtedly an important geographical position for connecting different parts of Mesoamerica and the rest of North America and has been like that since maize agriculture arrived at this region over four millennia ago. It has been a place for recent investigations regarding important archaeological and genetic findings. For instance, by comparing ancient population size and population divergence, García-Ortíz et al. (2021) suggest that Aridoamerican and Mesoamerican populations diverged approximately 4-5kya, around the time that sedentary agricultural societies arose in Mesoamerica. Moreover, this region is critical for the understanding of Aztatlán dynamics to address the US Southwestern cultures connections.

The Flower Worlds encompass a series of archaeological items that refer to a specific religion, iconology, and ideology in Mesoamerica, Northwest Mexico and the US Southwest.
It was widespread throughout Mesoamerica during the Epiclassic and the early Postclassic periods but first expressed in far West Mexico among the Aztatlán culture after A.D. 850/900, and A.D. 1000 and 1150 among the Chaco Canyon and Mimbres cultures (Mathiowetz 2021).

The Flower Worlds in far West Mexico led to significant changes in various aspects of society such as architecture, art, and politics. This period saw the concentration of power among elites within individual provinces. It also involved the adoption of Xochipili solar ritualism and symbolism from highland to central and southern Mexico (Mathiowetz 2021).

Many of the characteristics of The Flower Worlds in Northwest Mexico/US Southwest are associated with depictions of solar, feathered/horned sepent, or maize deities, ancestral cloud and breath imagery, cacao, among others (Mathiowetz 2021).

Di Peso’s excavations at Paquimé (1974) revealed that this entity traded an extraordinary diverse array of wealth goods that characterized the new rising economies in the new Postclassic Mesoamerica, however, he disagrees with the possibility that Pacific coastal West Mexican cultures were influencing the Casas Grandes transformations (Pohl and Mathiowetz 2022).

Wealth goods are named as such by archaeologists because their findings mean the presence of a region-specific scarce material, and hence they assume they are more valuable than other goods because they would have to travel long distances to get it. Such wealth goods that characterize these broad exchanges include turquoise from the Southwest, and copper from West Mexico, and scarlet macaws from the South-East of Mexico to name a few.

Genetic data not only augments archaeological evidence, but it also ensures the mobility and dynamics of what anthropology aims to study: people. The presence of a clinal (geographically gradual) distribution of haplogroups in pre-hispanic populations present in the American
Southwest may reflect a remanent of migration dynamics. This conjecture may be consistent with the haplogroup frequency distribution of the Mimbres, which resembles that of other Southwestern populations. Snow et. al (2011) findings suggest a close relationship between the Mimbres and the neighboring populations and population continuity for a long period of time. However, their relatively low frequency of mtDNA haplogroup A (11% n = 5) is consistent with a clinal distribution of haplogroup A from Mesoamerica to the Southwest.

Recent studies have found that present-day Indigenous populations from Mexico exhibit substantial geographical structure following a northwest-southeast cline coinciding with pre-Hispanic individuals, which is also reflected in the maternal lineages, especially haplogroup A, B, C and D (Villa-Islas et al. 2022).

What is more, the social, cultural, and population structures from over a thousand years ago have left an imprint in the present-day populations of Aridoamerican and Mesoamerican societies that is more complex than previously thought. The authors (Villa-Islas et al. 2022), emphasize that this complexity might have been caused by commercial trade routes, allowing for more movement between populations and the exchange of gene flow between different cultural areas.

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2 Aridoamerica, encompass the geographic region spanning Northern Mexico and the Southwest of The United States(Nabhan 1985), while Mesoamerica is a term coined by Kirchhoff in 1943 to describe the ancient cultural areas of Mexico, and Central America (Kirchhoff 1960).
2.2. The Pacific Coast

Flower symbolism and Aztatlán ceramics were first and most intensively expressed in the coastal core zone of Nayarit and southern Sinaloa. Wealth goods such as cacao use and consumption by elites became prominent in the core zone after 850/900 AD near the pre-Hispanic cacao cultivation zone that ranged from central coastal Nayarit to Banderas Bay, Jalisco. Cotton and weaving industries in the coastal zone further linked together ideologies of cloud making, ancestral rain, and the road of the sun (Mathiowetz 2021). Religious economies followed a Pacific-coastal route through West Mexico to Oaxaca and Chiapas rather than via Gulf Coast route (Gilman, Thompson, and Wyckoff 2014 cfr. Mathiowetz 2021).

Because this region has been proposed as a corridor where prestige goods originated as well as were traded to the Southwest and the rest of Mesoamerica, we would expect people were moving into an area of high gene flow. In order to visualize the dynamics of populations, we can rely on haplogroup frequency signatures and visualize the most common indigenous lineages (A2, B2, C1, D1, X2) and variants (Achilli et al. 2008).

Grosscup (1976) describes how the ceramic analysis at the coastal site of Amapa site was done. The analysis was focused on complete vessels and sherds and may involve individual modes or traits such as design elements. The author states that is convenient to divide the Amapa ceramics in two different periods (Early and Late periods) with more suggested subdivisions (Grosscup 1976, 212). This same author emphasizes that attention must be turned to some of the problematics regarding the definition of the Aztatlán complex.
2.2.1. The Aztatlán Complex

Sauer and Brand (1931) first used the term “Aztatlán” to refer to a culture area on the coastal plain of southern Sinaloa and northern Nayarit (Grosscup 1976). Later, Kelly (1938) used the term “Aztatlán ware” referring to a ceramic complex. We agree with the author’s idea that this was not a complex but a ceramic phase.

Elkholm (1942), hypothesized that the widespread occurrence of the Aztatlán ceramic phase or horizon was due either to migration from the Mixteca-Puebla area or to a religious movement spreading from that area. Grosscup (1976), on the other hand, embraces the diffusionist idea that cultural items were radiating from a common center rather than individuals migrating from the Mixteca-Puebla area. He also points out another important issue, which is the sudden disappearance of Lolandis-Tuxpan ceramic material at 650-900AD (See Table 1). This is interpreted as an abandonment of the coastal plain sites of Amapa, and possibly Peñitas, possibly due to an extensive volcanic activity in the highlands that could have had an effect on the course off the rivers and flooding. The author also hypothesizes that droughts could have been the cause of such abandonment (Grosscup 1976, 252).

Table 1. Ceramic Phases, estimated relative chronologies, and ceramic typologies divided into Late and Early periods adapted from Grosscup (1976).

<table>
<thead>
<tr>
<th>(Grosscup 1976)</th>
<th>Ceramic Phases</th>
<th>Estimated relative chronology</th>
<th>Ceramic typologies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Santiago</td>
<td>1300-1521 A. D.</td>
<td>Santiago White-on-red, Santiago Engraved, Iguanas Polychrome</td>
<td></td>
</tr>
<tr>
<td>Ixcuintla</td>
<td>1100-1350 A. D.</td>
<td>Ixcuintla White-on-orange, Ixcuintla Polychrome</td>
<td></td>
</tr>
<tr>
<td>Cerritos</td>
<td>900-1100 A. D.</td>
<td>Cerritos Polychrome, Botadero Incised, Tuxpan Engraved</td>
<td></td>
</tr>
<tr>
<td>Tuxpan</td>
<td>750-900 A. D.</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
2.2.2. Amapa, Nayarit.

In the coastal site of Amapa, Nayarit, a small synopsis of human skeletal remains was made by Raymond L. Bernor (1976, 184–86). The descriptive analysis lists a small description for each burial that includes the name of the burial, age and sex estimations, and additional information such as pathologies or anomalies. The author consulted with Dr. Walter Coulson of the UCLA Department of Pathology. The names they give to identify the burials coincide in numbers and names. Although burial 51 has successive numbers such as: “51-1, 51-2 until 51-6” and our sample is named after “51-a, 51-b until 51e”, suggested that the teeth used for the sample in this thesis are described in this work (op. cit.), so long as the names have not been changed at some point.

Table 2. Contextual information of the sample data in the site of Amapa. Adapted from Bernor (1976, 182–86).

<table>
<thead>
<tr>
<th>Burial No.</th>
<th>Age</th>
<th>Sex</th>
<th>Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37, 35-45</td>
<td>Male</td>
<td>M1 right maxilla lost pre mortem, M1 mandible present</td>
</tr>
<tr>
<td>2</td>
<td>35, 25-35</td>
<td>Prob. female</td>
<td>M1 and M2 of right maxilla lost pre mortem</td>
</tr>
<tr>
<td>51a</td>
<td>30, 25-35</td>
<td>Prob. male</td>
<td>Loose teeth associated: 10 molars, 8 premolars, 3 incisors, 4 canines</td>
</tr>
<tr>
<td>51b</td>
<td>25-35</td>
<td>unknown</td>
<td>Only present: M1 and M2, associated: 2 incisors, 3 canines, 5 premolars</td>
</tr>
<tr>
<td>51c</td>
<td>25-35</td>
<td>unknown</td>
<td>Pre-mortem loss of M1 and M2, M3 still in its socket. P4 and M1 in situ</td>
</tr>
<tr>
<td>51d</td>
<td>35-45</td>
<td>Prob. female</td>
<td>P3 and P4 present, Pre-mortem loss of M1 and M2. M3 is secure in the mandible only by the end of its dorsal root.</td>
</tr>
<tr>
<td>51e</td>
<td>17-25</td>
<td>unknown</td>
<td>The right maxilla is present with P4, M1, M2 and M3 in their sockets. The left half of the mandible is present with P3, P4, M1 in their sockets.</td>
</tr>
<tr>
<td>67</td>
<td>18-20</td>
<td>unknown</td>
<td>All teeth in situ</td>
</tr>
<tr>
<td>115</td>
<td>mature</td>
<td>probable male</td>
<td>No information about teeth</td>
</tr>
</tbody>
</table>
MtDNA can answer the question of maternal relatedness between these individuals, given that the mitochondrial genome is inherited in its entirety from the mother and therefore bearing identical mitochondrial lineages must belong to the same maternal line.

2.2.3. **Peñitas, Nayarit**

Peñitas is located in the north and south bank of Río San Pedro in the Coastal Plains of West Mexico just in the Core Zone of the Aztatlán Tradition. It encompasses two archaeological sites: Peñitas A and Peñitas B. Peñitas A is located about 500m north of Río San Pedro in a corner formed by the junction of highway 15 with the road leading the west to the village of Realitos about 8 km distant (Bordaz 1926, 23). The total area excavated at Peñitas was 370 m² and 390 m³ of earth (Bordaz 1926, 23). Mound 1, the highest one of all, was first used as a burial ground: “a burial was found (631-632-644) and the remains were left in place”(Bordaz 1926, 32). Mound 4 gave evidence of four looted and scattered burials (Bordaz 1926, 25).

Peñitas B is located 100m north of Highway 15 on the south bank of Río San Pedro. Following the same author. The site had been partially cut down by the river and a total area of 160m² was excavated leading to 230m³ of earth being removed.

In mound 1 (Peñitas A), Bordaz and colleagues recovered twenty-three isolated human remains and the preservation was “extremely poor” (Bordaz 1926, 33). The author explains that the environmental conditions of high moisture plus access to air, and the rise of the San Pedro
River may have played a role in the skeletal conditions. However, burial 150, 151-252 showed "relatively better preservation" (Bordaz 1926, 34).

The author also emphasizes that the remains of calvarium, femora, and teeth, generally molars, were the only remaining skeletal evidence. For specific stratigraphical loci of the skeletal remains, see: Table 3 and 4.

Table 3. Osteological material excavated at the burial Remains at the site of Peñitas (Bordaz 1926, 396)

<table>
<thead>
<tr>
<th></th>
<th></th>
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<td>A=Above Architecture</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
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<td>20 cm.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
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</tr>
<tr>
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<td>N2W2</td>
<td>45</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>621</td>
<td>N3W6</td>
<td>25</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B=In architectural Layer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>N4W6</td>
<td>36 (25-50)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>Mitlan</td>
<td></td>
</tr>
<tr>
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<td>50-75</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>Mitlan</td>
<td></td>
</tr>
<tr>
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<td>N3W3</td>
<td>30 (25-50)</td>
<td>-</td>
<td>-</td>
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<td>+</td>
<td>-</td>
<td></td>
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<td>Mitlan</td>
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</tr>
<tr>
<td>40</td>
<td>N3W4</td>
<td>25-50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>Mitlan</td>
<td></td>
</tr>
<tr>
<td>C=Below Architecture</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
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<td>-</td>
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<td>+</td>
<td></td>
<td></td>
<td>Mitlan</td>
<td>Right of Head</td>
</tr>
<tr>
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<td>N3W6</td>
<td>60 (75-100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Ext. Dors.</td>
<td>NW-SE</td>
<td></td>
<td></td>
<td>Mitlan</td>
<td></td>
</tr>
<tr>
<td>135</td>
<td>&quot;</td>
<td>125 (150)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Ext. Dors.</td>
<td>NW-SE</td>
<td></td>
<td></td>
<td>Mitlan</td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>&quot;</td>
<td>125 (125-250)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Legs Bent</td>
<td>NW-SE</td>
<td></td>
<td></td>
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</tr>
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<td>N3W6-7</td>
<td>75-100</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>NW-SE</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>Legs Bent</td>
<td>NW-SE</td>
<td></td>
<td></td>
<td>Mitlan</td>
<td>&quot;</td>
</tr>
<tr>
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<td>+</td>
<td>-</td>
<td>-</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NW-SE</td>
<td>Right of Foot</td>
<td>Mitlan</td>
<td>&quot;</td>
<td>&quot;</td>
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<td>+</td>
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<td>-</td>
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<td>&quot;</td>
</tr>
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<td>N3W6</td>
<td>100 (130-190)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>Mitlan</td>
<td>&quot;</td>
</tr>
<tr>
<td>123</td>
<td>&quot;</td>
<td>120-175</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>Mitlan</td>
<td>&quot;</td>
</tr>
</tbody>
</table>
2.2.4. Morrett, Colima

The archaeological site of Morrett is located around 3.7 km south east of the modern town of Cihuatlán, Jalisco, on the flanks of low hills that border the coastal plain (Meighan 1972). The Marabasco River runs through the west side of the site. The elevation protected against flooding, however at the time the site was occupied, domestic water may have seeped from springs or perhaps a meander of the Marabasco River which no longer exists (Meighan 1972).

Although Clement W. Meighan emphasizes the “fair amount of disturbance” (Meighan 1972:8) at the site, the chronology and dating includes three kinds: radiocarbon dates, obsidian hydration dates, and ceramic seriation. The first includes a set of 16 dates with one group cluster around 500 A.D. The 115 Obsidian hydration dating technique included chips and flakes that show that most of the obsidian was used in the Late period and the rest in an elapsed time for the different
cultural levels and periods (Meighan 1972:14). Finally, The ceramic seriations, although not ideal, suggest that “the occupation falls within a millennium and the great bulk is probably within a span of 600 years” (Meighan 1972:15).

The author concludes that there were three different occupations, the first (Early) spanning from 300 B.C-A.D.100, the second (Late) from A.D. 150-A.D.750, and a Reoccupation period from A.D. 800-A.D.1000 (Meighan 1972:18).

In Chapter IV (Meighan 1972), the author discusses the burial activity found at the Morrett site. This includes the burial of one dog, as well as 11 human burials. The human remains were in a very poor condition with only their densest bones (such as teeth) were preserved. This means that all of the smaller bones, such as the ribs and vertebrae, were entirely missing. In addition, the smaller long bones also weren’t present in some cases. This also means that only parts of the skulls and some of the long bones are all that remain. Because of this, it's impossible to get a full picture of the burial practices and it is uncertain how many individuals were buried in certain areas.

Meighan (1972) also talks about a burial pit that had four adults, three children or adolescents, and one adult skull, but no infants. The pit was dug in one go and served as a special place to be used as a family crypt. This means that it was noticed on the surface and was easily recognizable for later burials. Although the burial offerings were mostly given to adult males, the richest burial belonged to a child or adolescent of unknown sex. The lack of infant remains was likely due to their delicate nature and would have deteriorated quickly in the burial environment.
2.3. The Aztatlán Highlands

The Neo-Volcanic axis is a west-east mountain range stretching from the Pacific coast to the Gulf of Mexico, which is known for its igneous geology with many sources of obsidian. This includes regions such as Jalisco and Michoacán that have high-quality sources. The Ucareo source in northeast Michoacán is particularly important. Additionally, the Neo-Volcanic axis features enclosed lake basins such as Cuitzeo, Pátzcuaro, Chapala, Sayula, and Magdalena, which provide fish, waterfowl, and land for agriculture. The area is generally lower in elevation than other regions, making it less prone to frost. This area was important for nomadic Paleoindians because of the vast resources it houses, which fueled the development of pre-Hispanic political systems. It is separate from the lower parts of the Western Mexican Highlands that are crossed by the Sierra Madre Occidental. The Sierra Madre Occidental has fewer resources and is less suitable for agriculture and sedentary populations. Despite this, it experienced intense activity during the Epiclassic period. This all makes the Sierra Madre Occidental its own separate cultural subregion of western Mexico.

Scant, or perhaps nonexistent, studies have been completed in the Aztatlán Highlands related to ancient migrations, however, unpublished data from Snow and Mathiowetz (2020) can be helpful guiding a bibliographical review. Because insights in the cultural items such as graphic art representations in the Aztatlán Region which are also shared outside West Mexico (Pohl, 2012), could be evidence to support interaction and probable migration patterns.

Tizapán El Alto, located on the southern shore of Lake Chapala, in Jalisco, saw an influx of material and ideological influence from the coast. Evidence for a lowland-highland corridor is evident in similarities in ceramic assemblages between coastal sites around Amapa and the
21

Etzatlán region nearly at a midpoint between Amapa and Tizapán El Alto (Snow and Mathiowetz 2020). Immigrants to this area have been thought to import ceramics, community and familial organization and religious practices at these highland sites which can be corroborated by DNA data and kinship relationships studies.

2.3.1. Tizapán El Alto, Jalisco

Tizapán El Alto is a site located near the modern city of Tizapán El Alto, which is in the southeastern part of Lake Chapala in Jalisco, México. The site is on a piece of land that sticks out into the lake. The area around the site is full of small mounds of different shapes and sizes (Clement W. Meighan and Foote 1968, 22). Clement W. Meighan and Leonard J. Foote surveyed an area where the water level was constantly changing. It was fluctuating around 3 meters when they surveyed it. They also found a mound with graves at the northwest corner. They excavated 16,000m³ of material from the mound, which included earth, ash, clay floors, domestic refuse, and graves. Out of the 16,000m³, 96m³ were from the burial area (Meighan and Foote 1968, 22–23).

At the North slope of Mound 1, archaeologists dug a trench that went east to west along the northern side of a mound. When they dug, they found burials in the same area. Because of the high number of burials, the archaeologists decided to call this area a cemetery (Clement W. Meighan and Foote 1968, 24). Forty-nine individuals were found in the cemetery, as well as three additional residential burials. Most of the individuals were male adults, aged 18 to 40 years old. However, five of the male individuals showed evidence that they had been decapitated and buried in a specific section of the cemetery. This suggests that the cemetery may have been used for more than just the general population (Meighan and Foote 1968, 54).
As for chronology, one of the radiocarbon determinations from Tizapán showed a date of 4 kyo. However, the cultural remains found at the site were not that old. One possible explanation for this discrepancy is that the object was not originally from the site, brought by intrusive silts brought in by the Río Pasion, which runs along the south bank of the site (Meighan and Foote 1968, 36). The other three dates that were found indicate that the mound was occupied between 1000 and 1220 A.D. The cited authors state that the amount and nature of cultural change at the site suggests that the occupation of the mound did not last very long. Therefore, the 200-year time span is the best estimate of the age of the mound.

The Chapala Complex, based on Lister’s sequence (Lister 1977), is a group of artifacts that were found in a different area at Cojumatlán, Michoacán, and they are believed to be older than the Tizapán collection, but not as old as A.D. 600-900. This means that the Chapala Complex was likely created between A.D. 600 and 900. The researchers believe that Cojumatlán was flourishing and in its fully developed from before the beginning of the Tizapán dates. They estimate that it took about 100 years for the Koumala material to be initiated and developed, plus half of the occupation at Tizapán, which gives the date range of 900-1100 A.D.

Although the Tizapán Complex was not identified by Lister, it is dated to the period between 1100 and 1250 A.D. The mound at the site was abandoned in the first half of the 13th century and never used again. Following Meighan and Foote (1968, 36), it is possible that the abandonment of the site is connected to the spread of the Tarascan state, also known as the Tarascan Empire, or “Irechecua”3. This latter was a powerful polity that at the time of the Spanish

---

3 From the purhépecha language: ruler or governor.
conquest, was the second-largest state in Mesoamerica covering mostly all of what is today the modern state of Michoacán.

As for the condition of the remains found during the excavations, Meighan and Foote (1968: 42, 43) state that most of the skeletal material was in good condition and could be used to determine the sex and age of the individual burials. However, in 11 cases, the bones were too deteriorated to be used for analysis. They suggest that the differences in the condition of the bones were due to the differences in the induration and moisture of the soil in the grave pits and the surrounding area, rather than the age or stratigraphic position of the remains. The best-preserved skeletons were usually found in a matrix of compact, moist clay, while the worst preserved were found in graves with dry, powdery, sandy soil.

The majority of the 48 skeletons found were adults between the ages of 18 and 40, with more males than females. It also mentions that there were very few children younger than 6-8 years old and no infants, which suggests that they may have been buried in a different location or taphonomic changes lead to the decay and complete disintegration of them. The authors also state that there were very few individuals over 40 years old, which may indicate that few of the people living at the site reached an advanced age.

In sum, these authors excavated fifty-two individuals (see: Table 5) from a cemetery and residential mounds. Of those fifty-two individuals, twenty-eight were suitable for the study.

<table>
<thead>
<tr>
<th>Burial Number</th>
<th>Sex</th>
<th>Age</th>
<th>Loc. / Depth (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>Adult</td>
<td>T-26/100</td>
</tr>
<tr>
<td>2</td>
<td>?</td>
<td>06-ago</td>
<td>T/26/75</td>
</tr>
<tr>
<td>3</td>
<td>?</td>
<td>Adult</td>
<td>X-25/100</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>20-25</td>
<td>T-26/98</td>
</tr>
<tr>
<td>4-A</td>
<td>?</td>
<td>dic-15</td>
<td>T-26/98</td>
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Table 5. Summary Data. Adapted from Clement W. Meighan and Foote (1968, 45–48).
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<td>40+</td>
<td>U-26/110</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>18-21</td>
<td>X-25/110</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>25-35</td>
<td>T-26/133</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>25-30</td>
<td>U-26/140</td>
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<td>F</td>
<td>40+</td>
<td>S-25/96</td>
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<tr>
<td>11</td>
<td>M</td>
<td>30-35</td>
<td>V-25/105</td>
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<td>20-30</td>
<td>S-26/115</td>
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<td>M</td>
<td>25-30</td>
<td>S-26/130</td>
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<td>F</td>
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<td>S-26/75</td>
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<td>W-25/110</td>
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<td>M</td>
<td>40+</td>
<td>S-26/105</td>
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<td>F</td>
<td>20-30</td>
<td>T-25/160</td>
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<td>40+</td>
<td>S-26/142</td>
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<td>T-27/90</td>
</tr>
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</tr>
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<td>R-26/115</td>
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<tr>
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<td>-</td>
</tr>
<tr>
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<td>?</td>
<td>10-12</td>
<td>S-25/130</td>
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<tr>
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<td>?</td>
<td>Adult</td>
<td>V-23/182</td>
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2.3.2. Eztatlán, Jalisco

The Eztatlán Basin is located in the westernmost lake basin of the *Mesa Central* or *Altiplano* of Jalisco, México. Situated at an elevation of about 1,400 m (4,593 feet) and about 100 km (62 miles) inland from the coast. There is some evidence that people living in the Eztatlán Basin used resources from the coast (Porcasi 2012, 252; Pollard 1997, 368).

In the late Postclassic period when Spanish people entered the Eztatlán area. Eztatlán had multiple names throughout history, such as Icatlán, Yzatlán, and Izatlán. The Spanish people realized how important this area was and wanted to take advantage of the resources that were available there (Porcasi 2012, 252).

Lake Magdalena was surrounded by many different indigenous communities. These communities were located on the edges of the lake between the modern city of Eztatlán in the south and El Arenal in the north. The lake was a closed drainage basin with no direct outflow about 32 km (20 miles) long and several miles wide. It was used for commercial fishing and also provided habitat for fish and aquatic birds. Unfortunately, the lake was drained in the early twentieth century, but some of the water still remains in the area (Porcasi 2012).

In Nance et al. (2008), the text states that in the city of Eztatlán, there were approximately 600 adult males living there. Weigand (2001) estimated that the city had a population of possibly
several thousand people, and that the city occupied an area of 600 hectares, which is equivalent to 1,483 acres. Large quantities of obsidian were found in the area at Sierra de La Venta. Corn, beans, and cotton were grown in the region and salt and minerals were mined and transported from the area (Porcasi 2012, 252). Weigand (2001) described Etzatlán as a "state" that was powerful enough to defend itself against the Tarascan people, who were trying to conquer it, for almost 50 years before the Spanish arrived.

In 1963, Stanley Long from the University of California at Los Angeles (UCLA) and Michael Glassow explored many areas around Laguna Magdalena. They were working on a project called "Project A" which was led by Clement Meighan and H.B. Nicholson from UCLA. During their exploration, they found three residential communities (Tiana, Anona, and Santiaguito) located south of the lake and one community (Las Cuevas) located on an island in the lake.

Stanley Long was a researcher who was studying the Etzatlán Basin in Mexico. Unfortunately, he passed away before he could finish his research (a description of mortuary artifacts as his dissertation), so we don't know all the details about the area that he was studying. However, he states in his 1963 dissertation draft that radiocarbon dating was completed for shell and bone samples. Although he mentions that human bone samples were dated (unpublished), and fall within the Early Classic Period, it is worth to mention that the other bone samples have not been dated and that burial locations may have been used over extended length of time (p 49).

Glassow (1967) wrote about pottery from a nearby community called Huistla. The Etzatlán project collected materials, including bone, from the area. The materials were kept at UCLA, but no research was done on them until recently.
Although Porcasi (2012) paper refers to the changes in diet that occurred in the city of Etzatlán, from before the Spanish colonization to after the Spanish colonization, occupational phases were assessed in this research. From Las Cuevas, 49 Carbon-14 values were determined. Thirty-five of these values were from before the time of colonization, and 14 were from the colonial period.

3. **mtDNA in aDNA studies.**

Ancient DNA (aDNA) is a term that refers to DNA recovered from old sources, such as deceased organisms, or human remains. In aDNA, the protection of the cellular environment is scant and degraded (Raff 2019, 188). Because the nuclear genome is present only in two copies per cell, it has a lower chance of successful recovery from ancient contexts (Raff 2019, 188). DNA is also found in the mitochondria organelle and often has at least a thousand copies per cell, often allowing for it to be more successfully analyzed from ancient contexts.

DNA can be recovered from a range of biological sources. Depending on the circumstance, it can be recovered from an archaeological context or from a modern mass casualty incident (forensic). However, some sources may be more ideal for the purposes of DNA-based identification than others. Factors such as resistance of the source to degradation or damage due to its natural structure can also affect DNA recovery (Watherston et al., 2018). Different skeletal elements have been found to vary in the way they preserve DNA and consequently yield different amounts of DNA. The general advice is that although spongy bone and cancellous bone can be rich in DNA, preservation is not reliable and the dense cortical bone should not be collected preferentially because DNA can be protected by physical and chemical structures of compact bone within the calcium matrix, which is not present in spongy bones.
Mitochondrial DNA (mtDNA) is DNA located in the mitochondria. It is like the battery of a cell because it converts chemical energy from food into a form that cells can use. There are several advantages of mtDNA. Above all, mutations accumulate much faster than in the nucleus, so it provides a wider view of the diversity of the genome. The fact that it does not recombine, meaning it passes along genetic information from the homogametic sex (XX chromosome), on a matrilineal scale (from the mother’s line of descent), may address questions that are often inferred in the archaeological context, such as: familial ties, residence patterns, and kinship relationships. This latter, following Brown and Brown (2011), is a fundamental contribution that biomolecular research makes to archaeology. Lastly, mtDNA molecules are about $10^{16}$ in length, and they are usually identical to one another (Cann, Stoneking, and Wilson 1987, 31). MtDNA phylogenies are robust, given the time they have been in the literature (Finnilä, Lehtonen, and Majamaa 2001).

With genetic sequencing techniques such as Next Generation Sequencing (NGS), aDNA can be easily amplified with Polymerase Chain Reaction (PCR), together with the implementation of NGS protocols, it is possible to determine the degree of similarity of large blocks of mtDNA (haplotypes) that have passed down from one generation to another and from one mother to another. This peculiar mode of inheritance of mtDNA provide insights on how, when, and where human matriline arose and grew (Cann, Stoneking, and Wilson 1987, 32).
4. **Methodology**

I intend to discover the migration patterns of the coastal and highland populations of the Aztatlán Region by conducting ancient DNA (aDNA) analyses on human remains from the aforementioned archaeological sites through gene flow which can be evaluated through the analysis of mitochondrial haplogroup frequency differences, and genetic distance. This estimation is also frequently valued by the product $Nm$ through the use of $F$-statistics (fixation indices) (Cabana 2019, 140), which may help visualize the correlation between genes in subpopulations.

Another means of looking at the migratory events of populations within West Mexico and between its surrounding areas is the understanding of mtDNA diversity of six haplogroups (A2, B2, C1, C4, D1, and D4) (Achilli et al. 2008; Bisso-Machado and Fagundes 2021b). This is essential to explain the earliest mtDNA distribution of American populations who share the same haplogroup, as well as inferring more recent (1100-900 years ago) population dynamics. Populations would be expected to carry different haplogroup frequencies if they do not share recent common ancestry. The lack of aDNA genomes from Mexico results in the need for a larger sample size, and this study will help increase it and build the foundations for broader studies.

As the environment influences population structure, so it does its geographic distribution. Multivariate statistical methods such as Eigen analysis, principal components, canonical variates, distance, and clustering can help us visualize the distance between populations.
4.1 Teeth

Teeth are a skeletal structure that better preserves DNA over time. Due to their anatomy, with a naturally hard mineral composition and low porosity, teeth are more resistant to contamination and better preserve in archaeological contexts compared to bone (Cafiero et al. 2019). Teeth are effective long-term reservoirs of DNA, especially if the pulp chamber and inner dentine are sampled (Burton and Katzenberg 2019). However, dental calculus seems to be also adequate for storing DNA (Hillson 2019).

Teeth are also key for understanding taphonomy and for preserving DNA. However, they should be approached from a different perspective due to their different composition. A tooth is divided into two primary components (crown and root) and four primary tissue types (enamel, dentine, cementum, and pulp). Teeth enamel contains no living cells and is a highly mineralized tissue with only small amounts of organic material and water. Mature enamel is the hardest tissue in the human body. Dentine is a hard, elastic, avascular, vital, yellowish-white tissue that is less brittle than enamel. It is also not as highly mineralized as enamel (Antoine, Fitzgerald, and Rose 2019).

Bone degradation takes place by two processes: the destruction of the collagen component by bacterial collagenase, and the chemical demineralization of bone apatite. The single most important factor in dissolution of bone mineral is ground water, which acts as the medium for mineral ion exchange between the bone and the immediately surrounding soil, rock, or grave surface.

Diagenesis is an important context to consider. This process is the exchange of ions between bones and their environment which has important implications for research involving
bone chemistry, dating of bone, and isotope-based dietary reconstruction. Teeth are very hard tissue in the human body and are resistant to adverse conditions such as temperature, humidity, and microbial activity (Pilli and Berti 2021). The unique composition of teeth and their location in the jawbone provide additional protection from environmental and physical conditions that accelerate post mortem (PM) decomposition and DNA decay (Watherston et al. 2018). In order to succeed in DNA preservation, we should consider that degradation and scarcity depend on age, temperature, environmental potential of hydrogen (pH), exonucleases/endonucleases, water, and microorganisms.

Sampling teeth is logistically less demanding than other bones, and finally, teeth can be unequivocally assigned to an individual skull. Although teeth provide a valuable source of DNA, little is known about which region is the best in terms of DNA yield (Pilli and Berti 2021, 299).
4.2 Sample, DNA extraction, and analysis

The sample collection (n=75, listed in Table 6) took place at the Fowler Museum in the University of California, Los Angeles (UCLA) to be brought to the University of Montana for the genetic extraction in the ancient DNA laboratory (Snow Lab), which has DNA contamination prevention protocols. aDNA research cannot be done in a regular molecular biology laboratory because contamination is a pervasive problem. This is particularly important for human remains which may have been handled by numerous excavators, archaeologists and osteologists, as well as the laboratory workers (Nieves-Colón and Stone 2019). Cross contamination with aDNA can be solved today with different techniques and methods, these include strict room conditions (UV radiation, bleach treatment, filtered air systems), and adapters to tag molecules that are present at the moment of extraction. After DNA is sequenced, bioinformatic tools remove contaminant reads, estimate the proportion of those reads present in a DNA library, assessing discordant readings, or checking reads that diverge or do not contain signatures consistent with DNA. Patterns of cytosine deamination at the ends of fragments to filter out sequenced reads that do not display this signature are therefore not likely to be ancient (Slatkin and Racimo 2016).

Table 6. Sites and sample numbers.

<table>
<thead>
<tr>
<th>Site</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morrett, Colima</td>
<td>5</td>
</tr>
<tr>
<td>Barra de Navidad, Jalisco</td>
<td>5</td>
</tr>
<tr>
<td>Eztatlán, Jalisco</td>
<td>8</td>
</tr>
<tr>
<td>Revalcito, Jalisco</td>
<td>3</td>
</tr>
<tr>
<td>Tizapán el Alto, Jalisco</td>
<td>28</td>
</tr>
<tr>
<td>Amapa, Nayarit</td>
<td>12</td>
</tr>
<tr>
<td>Peñitas A, Nayarit</td>
<td>10</td>
</tr>
<tr>
<td>Peñitas B, Nayarit</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>75</strong></td>
</tr>
</tbody>
</table>
Prior to any destructive analysis of the teeth, records of their size and shape must be carefully taken. Photographic registry was carried out for every specimen of the sample (n=75) using a Nikon D610 taken in different angles, which were as follows: buccal or labial, occlusal, mesial, and distal views.

3D scanning was completed on the archaeological tooth sample in Ein Scan-SP V3.1.0.1 software with Shining 3D Turntable hardware for further imaging documentation, measurements, and processing at the Missoula Public Library facilities.

Contamination removal protocols of teeth include the irradiation of UV light, soaking the sample in a bleach solution, then rinsing with ultra-pure water (ddH2O) before drilling. After this step, DNA was extracted from teeth using a dental drill to recover dust from the pulp chamber and inner dentine. In order to reach these dental features, the outer layer of the roots must be drilled as well. The dust of this latter will not be wasted as it is expected to increase the yield of DNA extraction, as suggested by Damgaard et al. (2015).

Extraction followed the established protocol following Dabney et al. (2013). Approximately 1.8g of teeth was drilled into a fine powder with the dental drill. Between 85 and 120 mg of teeth powder went into each of 75 extractions using proteinase K digestion, followed by a silica-based spin column with a binding buffer catalyze absorption of DNA to silica particles.

A total of seven single-stranded libraries were prepared by using all sample extracts analyzed on MiSeq Illumina genetic analyzer following established protocols based on Meyer and Kircher (2010).

If we can obtain successful MiSeq runs, the data was put through a pipeline for mitogenome analysis developed in-house to run on the Galaxy Platform. This involves the selection of paired-
end sequences, sorting individual sequences, trimming the ends, and aligning the sequences to the revised Cambridge Reference Sequence (Snow and Mathiowetz 2020).

4.3. Data Analysis.

Pending successful retrieval of data, Principal Components Analysis (PCoA) will be effectuated in order to find distances and similarities on the eigenvalues and eigenvectors of a matrix to locate phylogenetic patterns between populations.

FST calculation will be implemented to group means within genotypic variance in order to identify the highest levels of genetic variability when compared to other populations.

5. Results

Mitogenome analyses were submitted to the MiSeq Illumina genetic analyzer at the Genomics Core facilities at the University of Montana. TapeStation Analysis Software 4.1.1. showed different histograms for DNA size and concentration.

![Figure 1. The sample (A1) in comparison to a known “ladder” of established size (in base-pairs) and concentrations of DNA (EL1).](image)
EL1: Electronic Ladder

Figure 2. The “ladder” of known sizes in length of base-pairs in a linear segment, as well as intensity, which is indicative of the molarity/concentration of the known fragments.

AI: V6P21

Figure 3. Post-Adapter Ligation Histogram analysis performed in TapeStation. The 157 peak is far too high, and due to the length/size of the fragment, is indicative of extensive “primer dimer” or where the adapters used to ligate to the end of the fragments of endogenous DNA have actually annealed to one another. The 265 peak is likely “real” sample DNA, but due to the chemistry of the sequencing process, will not be possible to read, as the primer-dimers are overly expressed.

A peak around 100-200bp is observed in front of the sample in the adapter ligation histograms for DNA size and concentration (See: Figure 2). Adapter Ligation Histograms show that the adapter molecules have self-ligated to each other. They are usually observed around 100-150 bp and are observed as a peak in front of the sample. This could indicate that the adapter
ligation process has not performed optimally. Inefficient adapter ligation process can lead to few consequences for subsequent sequencing steps, which led us to not sequence these samples at this time, as the data would not be useable.

A possible explanation of why the adapter ligation histograms generated a peak in 157 is because of harsh conditions for preservation that lead to a highly fragmented genetic makeup of skeletal remains. For this reason, there is no aDNA currently to report.

6. **Discussion**

Recent studies have found that present-day Indigenous populations from Mexico exhibit a substantial geographical structure following a northwest-southeast cline (García Ortíz et al. 2021) coinciding with pre-Hispanic individuals (Villa-Islas et al. 2022), which is also reflected in the maternal lineages, especially mtDNA haplogroup A, B, C and D. In Mexico, the geographic location of Indigenous populations plays a significant role in their genetic makeup. A study using Principal Component Analysis (PCA) identified distinct genetic groups that align with the North, Center/South, and Southeast regions of Mexico. This suggests that where Indigenous populations are located geographically can impact their genetic diversity (See Fig. 6).
Figure 4. The genetic diversity of Indigenous groups correlates with their geographic distribution. Admixture analysis assuming $K=9$ clusters in Mexican Indigenous populations. Colored bars represent the linguistic family (García-Ortiz, et al. 2021, 4, Fig. 2b and 2c).

Figure 5. Principal Component Analysis of the reference panel of present-day Indigenous Populations. Pre-Hispanic individuals cluster close to present-day central-west Indigenous populations, and Michoacán individuals cluster closely to central-east Indigenous populations (Villa-Islas et al. 2022, 9, Fig. 3a).

In this study, we expect to observe a similar clinal distribution of the genetic diversity of pre-Hispanic individuals, albeit in a smaller scale, focused on West Mexico, and in archaeological samples that would represent a continuity of the Mesoamerican haplogroups that correlate with modern ethnic groups.
7. Conclusions

In this research, Post-Adapter Ligation Histogram analysis performed in TapeStation (See: Fig. 3) showed the formation of adapter-dimers. For this reason, we were unable to proceed to sequence the samples because using fragmented or degraded aDNA for library preparation can contain full-length adapter sequences that can cluster and interfere with the real DNA data.

Ancient DNA (aDNA) possesses unique characteristics that require careful consideration during data analysis. In the double-stranded library preparation protocol for aDNA, it is recommended to decrease the adapter mix concentration when the input DNA quantity is low, as is often the case with degraded samples (Kircher 2012). However, the impact of this adjustment on the conversion rate of template molecules into library molecules and the formation of adapter dimers during amplification is still not well-understood (Henneberger, et al. 2019).

The methodological issues that arise when analyzing data are not the only problems that we face. When thinking of data sampling, theoretical and ethical and anthropological questions should be emphasized. Ancient DNA research has revealed the complex and dynamic history of human populations. The idea of a "pure" population is a myth and aDNA has provided evidence that refutes racist and nationalistic narratives, and genetic data should not be used as a tool for determining group belonging or identity (Alpaslan-Rooßen et al. 2021), but rather as a means of understanding the complex interactions and movements of human populations over time.

Even though individuals from different indigenous groups in Mexico may have had different cultures or spoke different languages, they still mixed and had descendants together. This mixing of genes has lasted throughout history and can still be seen in the genetic structure of populations today. However, a broader understanding of theoretical definitions of ethnicities and linguistic groups is needed. Addressing anthropological questions such as the understanding of genetic connections between archaeological regions and contemporary Indigenous peoples should be carefully considered and emphasized in future studies.

ADNA, can also be seen as another piece of evidence for archaeologists to further understand broader pre-Hispanic interactions. In fact, based on the information gathered through
archaeology, it is difficult to draw conclusions about the modern understanding of ethnicity in social sciences (Chrisomalis et al. 2004) and culture *per se*.

Although it is clear that scientists have to consider that genetic data is not sufficient to provide a clear understanding of culture, by oversimplifying the complexities of living beings (Ingold 2003), genetic studies not only can help support and confirm evidence of population movements that are inferred from archaeological artifacts, but they can also contradict or disprove that evidence. In other words, genetic studies can be used to confirm, challenge, and enhance our understanding of how ancient populations moved and interacted with each other.
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