Ethnicity, Linguistics, and Genetic Diversity in Native Mexicans: Reconstructing the Population History of Mesoamerica

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Papá, Máma Lucha, Cris y Rocío Vargas

In Memoriam

"Los indígenas no representan solo muestras biológicas, son los portadores esenciales de la historia de la humanidad"

Andrés Aubry 1927 – 2007

"Indigenous populations don't just represent samples; they are essential contributors to the understanding of human history"

Agradecimientos

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Abstract

Mesoamerica is one of the main centers of New World civilization. It represents today a large geographical area exhibiting one of the highest genetic, cultural, and archeological diversity in the Americas. Moreover, its geographic position has been a key factor for acting as a natural corridor between North and Central-South America, thus becoming a direct witness not only of the initial and subsequent human migration waves but also of the many civilizations that flourished later on. Therefore, Mesoamerica deserves special attention in the study of American history. Following a molecular anthropological approach, this thesis evaluates the genetic diversity of a representative sample of the extant Native American gene pool within Mexico, and by constructing continental datasets, it also intends to contribute to the reconstruction of Mesoamerican history and the peopling of the Americas. For that purpose, this work focuses on the study of uniparental markers located in the human mitochondrial DNA and Y-chromosome, which constitutes the main part of the analyses. Additionally, autosomal STR variation, linguistic diversity, and ethnographic data were also investigated. Our results, based on both mtDNA and Y-chromosome, show a clear differentiation of the Native Mexican groups that belong to Mesoamerica, suggesting that population dynamcs occurring within this cultural area were unique during the America's colonization process and thus uniquely shaped the native Mexican genome.

Resumen

Mesoamerica merece especial atención dentro del estudio de la historia del Nuevo Mundo debido a que es una de las principales áreas geográficas con mayor diversidad genética, cultural y arqueológica en América. Un factor clave es su posición geográfica, ya que ha actuado como un corredor natural de unión entre Norte y Centro-Suramérica, convirtiéndose en testigo directo no solo de las primeras y subsecuentes oleadas migratorias, sino también del posterior florecimiento de grandes civilizaciones mesoamericanas. Siguiendo un enfoque antropomolecular, la presente tesis doctoral evalúa la diversidad genética de una muestra representativa del pool genético actual de las poblaciones nativas de México. Asi mismo, por medio de la construcción de bases de datos a nivel continental, pretende contribuir a la reconstrucción de la historia Mesoamericana y del Poblamiento de América. Con este objetivo, se analizaron marcadores uniparentales localizados en el ADN mitocondrial y el cromosoma Y, lo cual constituye el principal componente del trabajo. Complementarianente, también se analizó la variabilidad observada a nivel de STRs autosómicos, clasificacion lingüística y caracterisitcas etnográficas, lo cual aporta un enfoque multidisciplinario a la investigación. Nuestros resultados, basados tanto en ADNmt como en cromosoma Y, muestran una clara diferenciacion de los grupos nativos pertenecientes a Mesoamerica en comparacion con el resto, sugieriendo la presencia de una dinámica poblacional única y enfatizando la relevancia de esta área cultural en el proceso de colonización de América.

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Abreviations

mtDNA: Mitochondrial DNA
RFLPs: Restriction fragment length polymorphisms
SNP: Single nucleotide polymorphism
STR: Short tandem repeat
Ybp: years before present
Kb: Kilo bases
Kya: Thousand years ago
Mb: Megabases
Kyr: Thousand years
Mya: Million years ago
AD: *Anno Domini*
BC: before Christ
LGM: Last Glacial Maximum
DHPLC: Denaturing high-performance liquid chromatography
YCC: Y-chromosome consortium
TMRCA: Time of the most recent common ancestor
NRY: Non-recombining region of Y-chromosome
MSY: Male-specific region of Y-chromosome
HVRI/II: Hypervariable Region I and II
PCR: Polymerase chain reaction
CRS: Cambridge Reference Sequence
Fig: Figure
e.g.: for example
i.e.: that is
km: kilometer(s)

INTRODUCTION

1 The Peopling of the Americas

The peopling of the Americas is one of the most fascinating questions to be solved in human history, and despite it has been largely studied by different disciplines and research approaches, it remains one of the most controversial issues in modern anthropology. Researchers have focused much of their attention in solving particular questions such as:

- When did humans first enter the Americas and where did they come from?
- How many migrations were there and which geographic routes were taken into the Americas by migrant peoples?
- What was the size of the founding populations?
- To which extent the record of the original colonization is retained in Native American genetic variation?
- What is the resulting genetic structure of extant Native American populations?

There is general agreement among the scientific community that the Americas was the last continent to be colonized by modern humans through the Bering Strait (see Fig. 1), but there is considerable uncertainty regarding the timing and pattern of human migration from Asia to the Americas (Ray et al. 2010). It must be established that there are at least two relevant dates, the migration out of Asia and the entry into the Americas. The first date is generally based on the initial diversification of the New World-specific haplogroups. For example, mitochondrial DNA (mtDNA) data support a date of ~30,000-40,000 years ago (Bonatto and Salzano 1997a), reflecting the initial diversification of the New World genetic variation as the populations diverged from ancestral Asians but prior to their entry into the Americas (Mulligan et al. 2004; 2008). The time of entry into the Americas is more debated and dates generally fall into two periods that are pre and post last glacial maximum (LGM). Different dates are frequently based on similar genetic datasets but use different substitution rates, for example the mtDNA offer two substitution rates: fast (e.g. ~1.7 x 10-8 substitution/site/year) supports a post-LGM entry, and slow (e.g. ~1.26 x 10-8 substitution/site/year) supports a pre-LGM (Endicott et al. 2009). Actually two possible routes of colonization into the Americas have been proposed, by land or along the coast.

Since the last three decades until recent days there has been a remarkable increase on the interest to propose complex theories based not only on genetic data but also organic remains, morphological, and archaeological data. The addressed questions may remain the same as the first classical studies, but as new technologies have been available, some approaches have changed. Here, I start by briefly reviewing the main sources of evidence to tackle the long-standing and interesting question about how the American continent was colonized by modern humans.

Figure 1: Colonization of the Americas: possible routes followed by founder populations (red arrows) and main archeological sites (black dots) (from Stringer and Andrews 2005)

1.1 Fossil and Archeological Evidence

Human fossils provide a strong evidence for the presence of modern humans in the Americas. This data associated with other sources could give accurate information about the timing of entry and morphological traits of the first colonizers. The number of early fossils is small, here I mention some of the most remarkable specimens (Jobling et al. 2004). *"Luzia"* skull and some additional bones were found in 1975 in the Lapa Vermelha rock shelter in Minas Gerais, Brazil, dated 13.5 cal Kya (Neves 2000; van Vark et al. 2003). *"Buhl Woman"* was found in a quarry near to Bulh, Idaho in 1989 with artifacts including an obsidian biface and bone needle, suggesting an intentional burial, dating of bone collagen gave ~12.9 cal Kya (Green et al. 1998) . *"Prince of Wales Island Man"* discovered in 1996 is presented by a lower jaw, vertebra and pelvis found with a stone point in a cave near to the coast of Prince of Wales, Island, Alaska; The jaw was date to ~11 cal Kya, providing support to the coastal migration route (Jobling et al. 2004). *"Spirit Cave Man"* is represented by a burial of a short man aged 40-45 years with sings of dental abscess and several injuries, partially mummified by the dry conditions, providing unusual insights into ancient fossils; was found in 1940 in Nevada, dated ~10.6 cal Kya (Jantz and Owsley 2001). *"Kennewick Man"* was discovered 1996 eroding from the banks of Colombian River in Washington State, is represented by almost complete skeleton of 40-45 man; The radiocarbon date was ~8.4 Kya, corresponding to 9.3-9.5 cal Kya (van Vark et al. 2003). A controversy was generated focused on his European skeletal morphology, was he truly ancestral to modern Native Americans? (Dalton 2005), many attempts to amplify DNA by several labs have been unsuccessful (http://www.nps.gov/history/aad/Kennewick/#dna).

The Clovis culture was the first archeological evidence of modern humans in the Americas, dated around 13.5 Kya. There have been many claims for the Pre-Clovis remains, now widely accepted. The Clovis cultural complex was named after the town of Clovis in New Mexico located near to the Blackwater Draw (the first site) was studied. It is characterized by Clovis Points, fluted (grooved) projectile points constructed from a variety of stone types. The earliest Clovis remains dated from around ~13.5 Kya, and the culture appear to have spread over munch of the nonglaciated part of North America within a few hundred years, but was soon replaced by other styles of points, The Folson (~12.9-12,000 ybp), distinguished by their small

size and longer flutes. The Clovis people (called Paleoindians) were big game hunters, their points are found associated with bones of large animals like mammoths (Waters and Stafford 2007). Clovis points are not found outside North America, but Paleoindians remains are also know from many sites in South America. Some contemporaneous of Clovis or Folsom, are those from the *Caverna da Pedra Pintada* near to Monte Alegre in Amazonian Brazil (Roosevelt et al. 1996).

The discovery of archaeological sites predating Clovis (Adovasio and Pedler 1997; Dillehay 2000; Dillehay et al. 2008) has been particularly influential in stirring debates around the antiquity of the initial settlement of America. Also, the analyses of genetic data have generally been found to be consistent with a pre-Clovis settlement (Bortolini et al. 2003; Bourgeois et al. 2009; Fagundes et al. 2008; Fuselli et al. 2003; Tamm et al. 2007; Wang et al. 2007; Zegura et al. 2004). Archeological evidence has a range of 11-33,000 ybp for the first settlements in Beringia and the New World (Hoffecker et al. 1993) and (Szathmary 1993). Some current studies have new consensual ideas: archaeological data shows evidence of human presence in the Yana RHS site in Siberia before LGM (Pitulko et al. 2004), and the first colonizers enter through Bering around 16,000 ybp, leaving footprints of their presence in southern sites like Monte Verde 14,600 ybp (Dillehay 2000; Dillehay et al. 2008); Monte Verde II 14,200 and 13,800 ybp in Chile and Pedra Pintada 12,000 ybp in Brazil (Dillehay 2000; Dixon 1999).

1.2 Morphological Evidence

The studies of craniometrical variation in American human remains have shown morphological differences between the earliest settlers of the continent and some of the later Amerindian populations and the causes of this variation among human populations have been subject of controversy (Perez et al. 2009; Perez and Monteiro 2009). Since the beginning of the 90's the anthropologists Neves and Pucciarelli started to study the morphological affinities and differences, supporting a multiwave colonization of the Americas by the comparison of cranial traits of early South American remains with worldwide human remains from the Late Pleistocene and Holocene concluded that the Americas were occupied by undifferentiated premongoloid colonizers, and suggested that their results called for more detailed investigations about human micro-evolution in

the Americas (Neves and Pucciarelli 1991). Some years after Neves and Hubbe (Neves and Hubbe 2005) compared the largest sample of early American skulls ever studied with worldwide data sets representing global morphological variation in humans, through three different multivariable analyses. Their results show a close morphological affinity between South American Paleoindians and the extant Australo-Melanesianas groups, supporting the hypothesis that two distinct biological populations could have colonized the Americas in the Pleistocene/Holocen transition (Neves and Hubbe 2005).

Some studies have been based on the comparison between Cranial and DNA data. Perez et al. (2009) comparing craniometric and mtDNA data of diachronic samples from East Central Argentina dated from 8,000 to 400 ybp, showed that even when the oldest individuals display traits attributable to Paleoamerican crania, they present the same mtDNA haplogroups as later populations with Amerindian morphology. A possible explanation for these results could be that the craniofacial differentiation was a local phenomenon resulting from random (i.e. genetic drift) and non-random factors (e.g. selection and plasticity). Local processes of morphological differentiation in America are a probable scenario if rapid peopling and the great ecological diversity of America continent is taking in consideration (Perez et al. 2009). Similar studies not only based on cranial data but also in dental morphometric data have been recently done by Bernal and (Bernal et al. 2009a; 2009b), in order to investigate the possible factors for dental variation among human populations from South America, the results shown that the ecological factors as the dominant factor on dental size diversification in this region, while evolutionary relationships account for variation in dental shape (Bernal et al. 2009a).

Other studies have been based on geometric morphology analysis of late Plesitocene/early Holocene remains and modern skulls. González-José et al. (2008) proposed an interdisciplinary view of the settlement dived in four arbitrary periods indicate evolutionary changes across time, suggesting that recent contact among Asian and American circum-Arctic populations took place during the Holocene. The geometric morphology studies made in particular areas like Mesoamerica, allows making inferences about the cultural transitions in particular times, like replacement at the Classic-Postclassic transition in Mexico (Gonzalez-Jose et al. 2008). The Spanish-Amerindian contact can be considered a good scenario to explain the morphological

outcome in human cranial samples shown by Martínez-Abadías et al. (2006), their results were in accordance with the previous molecular and historical interpretations, providing evidence that admixture is a main microevolutionary agent influencing modern Mexican gene pool (Martinez-Abadias et al. 2006).

1.3 Linguistic Evidence: The Greenberg theory and beyond

In (1986) Greenberg, Turner, and Zegura, proposed one of the first interdisciplinary models based on linguistic, morphological and genetic data. They postulated that the ancestors of the present day Native American populations came from Asia via the Bering Strait in three different migratory waves and times. According to this theory, the first migration wave occurred approximately 11,000 ybp. The modern descendants of this migration speak languages that belong to the Amerind family, which has representatives widely dispersed throughout North and South America (Fig. 2). The second wave occurred about 9,000 ybp; its modern descendants include speakers of Na-Dene languages. Na-Dene speaking populations are concentrated in Alaska and northwest Canada. Na-Dene speaking populations are also found sporadically throughout the Pacific Northwest, and include the large Apache and Navajo populations in the Southwest. The last proposed migration into the Americas was more recent, about 4,000 ybp. Its modern descendants include speakers of Eskimo-Aleut languages who live in the arctic and are distributed from Alaska to Greenland. The three-migration theory provided a provocative hook to frame a flood of molecular genetic studies that began in the 1990s (Torroni et al. 1992).

The three-migration theory has important mistakes, the support from three distinct lines of evidence: linguistics, dental morphology, and classical genetic markers (Greenberg et al. 1986) nowadays is refutable. Researchers recognized that not only the genetics were the weakest support for the theory but also the linguistic classification (Bolnick et al. 2004). The major weakness was that very few genetic data existed, and the power of these data to resolve competing hypotheses was weak. The principal genetic line of supporting evidence was drawn from the *Gm* locus, which showed that allele frequency differences among populations paralleled membership in the three language families

(Williams et al. 1985). However, these differences among populations were also compatible with a single migration followed by geographic isolation and allele frequency change by genetic drift (Szathmary 1993). The majority of linguistics provided the best evidence favoring the three-migration theory, but the issue was still debated. Although most linguists in the mid-1980s would have agreed with Boas (Boas 1973 (1933)) original assessment that the Americas had not been settled long enough for the vast linguistic diversity to evolve in situ, they did not all endorse Greenberg's language classification or model for the peopling of the Americas (Campbell 1997). It is difficult to place precise times on the origin and divergence of languages because the modes and rates of change in language are not well understood. Recently, the perceived need for substantial time to generate linguistic diversity was challenged. A new hypothesis suggests that diversity in a linguistic stock explodes as small colonizing groups fill new niches, but this linguistic diversity collapses as population density increases and isolated groups reconnect (Nettle 1999). The interpretation of dental morphology was open at the outset. Laughlin (Greenberg et al. 1986) commented that "The dental evidence carries no hint of a triple division but rather is eloquent evidence of a single migration". Meltzer (1993) noted that Aleuts are more similar dentally to Na-Dene than to Eskimos, contradicting the linguistic affinities. Nonetheless, the three-migration theory gained steam and remains one of the main tenets against which questions are formulated and data are interpreted today. In summary, many populations throughout the Americas share a limited set of Y-chromosome and mtDNA haplogroups. These haplogroups are also found in Asian populations, but rarely. The scarcity of the lineages in Asia makes it improbable that different migrating populations would draw the same set independently. Therefore, the genetic data lend no particular support to the three-migration hypothesis. Instead, a single migration to the Americas that carried all founding lineages simultaneously is a more parsimonious and preferable explanation (Mulligan et al. 2004).

One of the main contributions of the tripartite model was to provide a new vision (interdisciplinary) to the researches, in which Greenberg and colleagues tried to integrated different evidences to reconstruct the peopling of the Americas. Many other studies, based on linguistic, genetic data and archeological data, have not supported the tripartite model (Bolnick et al. 2004; Campbell 1977; Campbell 1997; Chakraborty and Weiss 1991; Karafet et al. 1999; Laughlin 1988; Spencer et al. 1977b; Szathmary et al.

1983; Wallace et al. 1985). Most archaeological evidence points to an initial migration around 12,000 year ago and this has been taken by Greenberg to be the time of the proto-Amerind migration. More recently, this date has been questioned because seemingly older archaeological sites have been found, particularly in South America (Dillehay et al. 2008). However, there is disagreement among archaeologists as to the reliability of the dating of these pre-Clovis sites (Cavalli-Sforza et al. 1994; Crawford 1998b; Fiedel 1992).

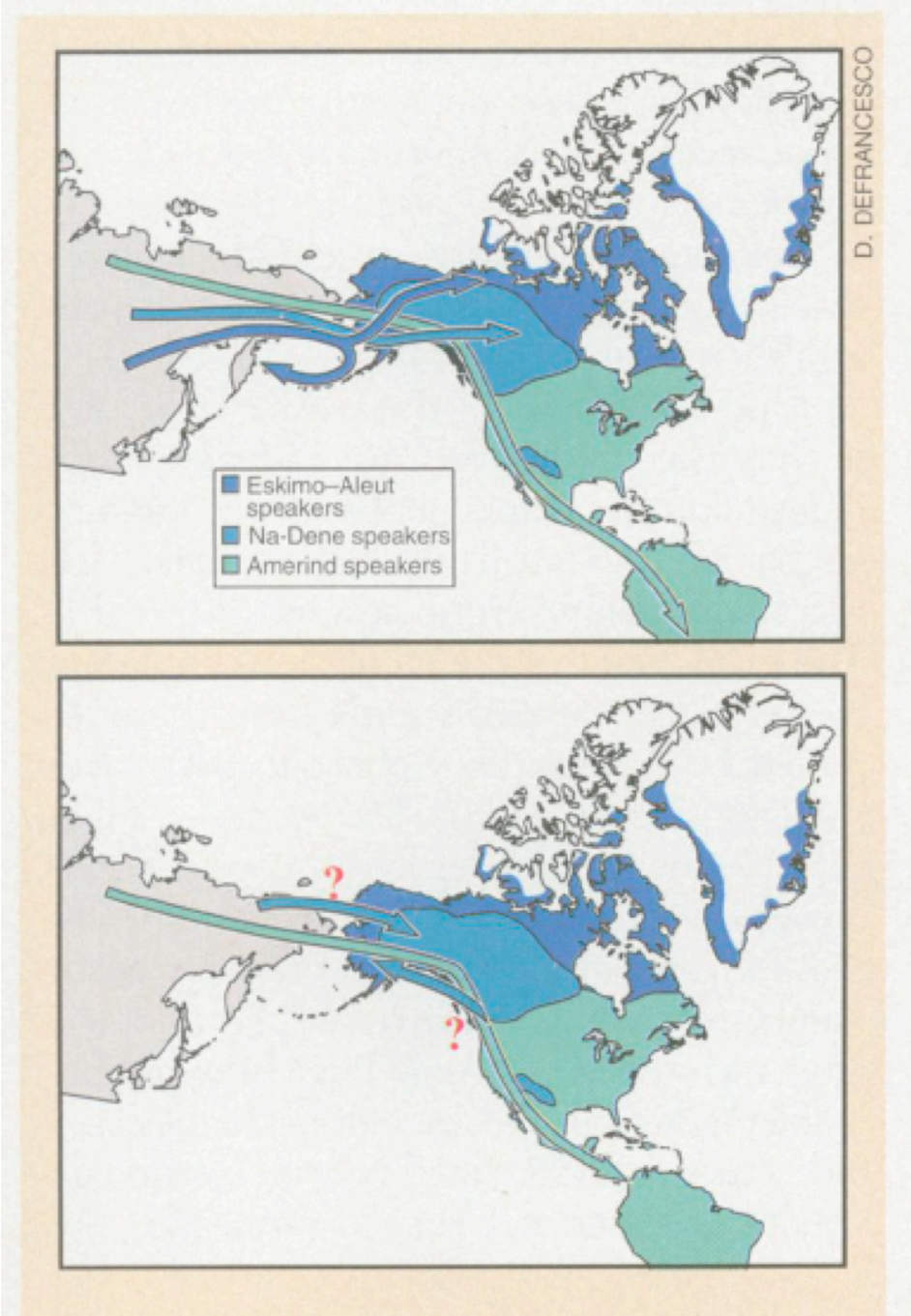

Figure 2: In the top, Tripartite Greenberg Model; below, possible migration routes about 12,000 ybp (from Greenberg 1996)

1.4 Genetic Evidence: Molecular Anthropology

Molecular anthropology is the field of anthropology that makes inferences on human evolution analyzing biological variation within and among species at the molecular level. The term "molecular anthropology" was first introduced by Emile Zuckerkandl in 1962 at the Burg Wartenstein Symposium "Classification and human evolution" (Goodman et al. 1976), where he stated that molecules can offer us as much information on phylogeny as fossil data, and possibly even more (Dietrich 1998).

However, the research in this area began much earlier than its baptism. In fact, it was between 1900 and 1902 that Landsteiner discovered the blood group system (Lewontin 1982). The studies on immunological reactions, due to the interaction between different types of blood, were the first ones to highlight the existence of a molecular variation among individuals, following a Mendelian way of inheritance. The first ABO population study was published in 1919 by Hirszfeld and Hirszfeld (Mourant 1961). The subsequent identification of other blood Group protein markers, such as MNS and Rh, expanded the range of polymorphisms that could be analyzed by the use of antibodies. Immunological methods remained the only technique used to detect genetic variation until 1949, when Pauling and introduced electrophoresis to separate different variants of hemoglobin (Pauling et al. 1949). This innovation paved the way to the analysis of electrophoretical variation in several blood proteins. Thanks to these studies, allelic frequencies started to be considered and description of populations in terms of these variables began. In 1954 the first book on allele frequencies in human populations was published (Mourant 1954).

In the 1960s other important innovations in the field of evolutionary biology occurred. It was in these years when Cavalli-Sforza and Edwards formulated the concept of genetic distance. These authors started to quantify the differences between populations in terms of differences between their allelic frequencies. In 1964 they published the first evolutionary tree obtained through the analysis of five blood group systems in fifteen populations, three per continent (Fig. 3 Cavalli-Sforza et al. 1994).

It is interesting to notice that, already in this first attempt to reconstruct the phylogeny of human species, a good agreement between genetic and geographical distances was observed: the three populations from each continent clustered together. Furthermore, the

presence in the same branch of American Indians and Asiatic populations confirmed the hypothesis of the Mongolic origin of the former and that they passed through Bering strait to reach the American continent (Cavalli-Sforza et al. 1994).

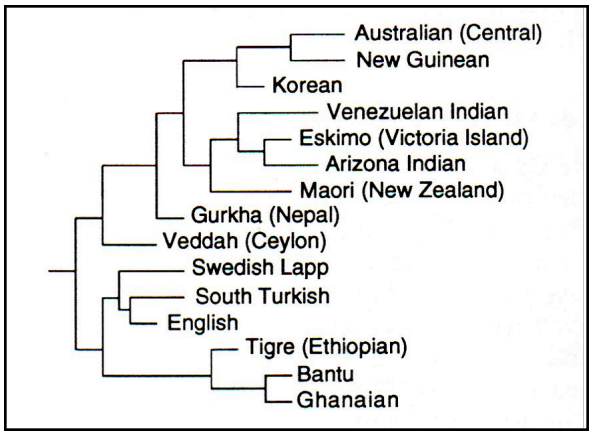

Figure 3: Evolutionary tree of 15 human populations reconstructed on the basis of 20 alleles from five blood groups systems (from Cavalli-Sforza et al., 1994)

Zuckerkandl and Pauling, on one hand, and Margoliash, on the other, had independently introduced a few years before the concept of molecular clock: "if the elapsed time is the principal variable that determines the number of accumulated substitutions, it should be possible to estimate approximately in which period two lineages that led to two different species diverged" (Dietrich 1998). In this definition, the neutrality of the region under consideration was assumed; in fact, if this is not the case, we could not consider the evolutionary rate to be constant in time and among species or populations. Consequently, we could not use aminoacidic substitutions to estimate the divergence time between species. It was time to understand that the analysis of neutral polymorphic genomic regions was necessary for the study of evolution.

The importance of the neutrality of the character under study had already been highlighted by Cavalli-Sforza and Edwards in 1964 with the publication, together with the tree presented above, of a phylogenetic tree based on anthropometric characters, such as measures of body dimensions and skin color. In this case fifteen populations from different continents were included, but the tree showed a topology which was completely different from the one of the first tree. In this case the populations clustered on the basis of the climatic conditions rather than the geographic distances (Fig. 4 Cavalli-Sforza et al. 1994).

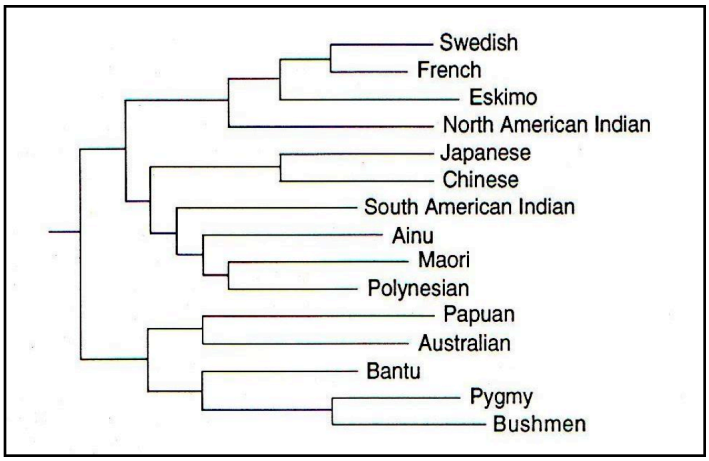

Figure 4: Evolutionary tree of 15 human populations reconstructed on the basis of anthropometric characters (from Cavalli-Sforza et al., 1994)

The topology of these first phylogenetic reconstructions was early reassessed by the work of Nei (1978). He examined a greater number of markers in a still limited number of populations representing the three major ethnic groups (Europeans, Africans and Asiatics). This tree showed a different topology with Europeans closer to Asiatics rather than Africans. This conclusion was later confirmed by successive works of the same group, and of the group of Cavalli-Sforza, with a higher number of populations (Fig. 5 Cavalli-Sforza et al. 1994).

Until then, studies on molecular variation had been focused on protein polymorphisms, through both electrophoretical and immunological techniques. Since the 1970's the attention moved to the DNA molecule itself, thanks to the progress in the recombinant DNA techniques and the discovery of a particular class of enzymes, the restriction endonucleases. These are bacterial enzymes that can bind to specific sequences on the double stranded DNA and cut it. If a mutation occurs in the target sequence, the enzyme cannot bind to it, and, consequently, cannot cut the DNA molecule. This offered a new tool to investigate molecular variation: applying the same set of enzymes to different individuals it is in fact possible to detect differences in the number and in the length of the fragments produced, which depend on nucleotide variation in the target sequences. This new family of polymorphisms was called RFLPs (restriction fragment length polymorphisms). The direct analysis of the DNA molecule allowed the obtaining of a

greater amount of information, overcoming the loss of information due to the maturation of the mRNA and the genetic code degeneracy. Furthermore, looking at the DNA level, it was possible to study non-coding regions, possibly more appropriate for phylogenetic reconstructions.

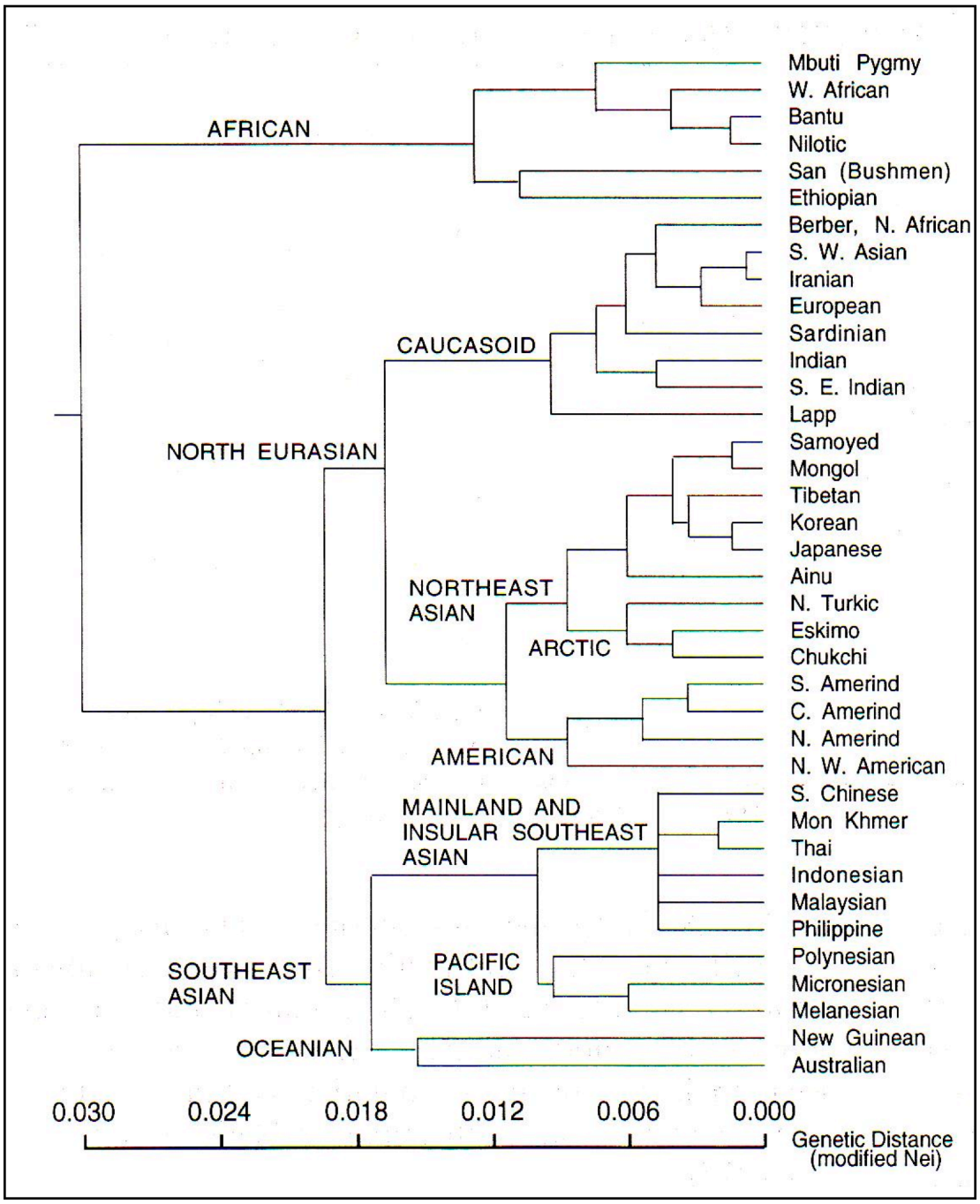

Figure 5: Evolutionary tree of 42 human populations reconstructed on the basis of 12 alleles (from Cavalli-Sforza et al., 1994)

However, the effective turning point for the molecular analysis of DNA came in 1987 when Kary Mullis developed the Polymerase Chain Reaction (PCR) (Mullis and Faloona 1987). This is an *in vitro* technique to isolate and amplify a specific fragment of DNA, faster and cheaper than the recombinant DNA techniques. Furthermore, its introduction had important consequences on the sequencing techniques previously developed, like the Sanger method (Sanger et al. 1977), which found an easier application.

The advances in molecular biology increased considerably the number of studies in the field of molecular anthropology, and the increased level of resolution of the molecular analyses allowed for a more detailed investigation. The first genomic region to be taken into account was the mitochondrial DNA (mtDNA), due to some key advantages: it is small, present in numerous copies in the cell, does not recombine, and has a region of approximately 1000 base pairs without functional constraints. The role of this molecule in molecular anthropology will be explained in section 2.1, and subsequent sections will review some of the mtDNA studies that have shed light to the understanding of the genetic diversity in the Americas. Likewise, since the mid 1990s a second region of the genome attracted considerable interest, the Y-chromosome, which is the natural counterpart of the mitochondrial DNA. It contains a non-recombining region and a lower mutation rate than mtDNA, making it a potentially more reliable system for phylogenetic reconstructions. The role of Y-chromosome in the study of human genetic variation will be discussed in section 2.2, and will be extended in subsequent sections with an emphasis on the implications for the study of the population history of the Americas.

2 Genetic Studies in the Americas based on uniparental Markers

2.1 Mitochondrial DNA and main features

The human mitochondrial DNA (mtDNA) is a circular double-stranded molecule, consisting of 16,569 base pairs (Anderson et al. 1981). It is located inside the mitochondria and it is present in numerous copies in the cell. In fact, each mitochondrion contains between 2 to 10 copies of mtDNA and each cell contains as much as 1000 mitochondria. Its two strands are biased in their base content, with the purine-rich and the pyrimidine-rich strands named “heavy” and “light”, respectively (Chinnery 2006).

The mtDNA is a haploid genome and codes for 13 subunits of the oxidative phosphorylation system, 2 ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs). A fragment of approximately 1kb is the only non-coding region, and it is involved in the regulation of transcription and replication of the molecule. It is therefore named control region (Chinnery 2006; Pakendorf and Stoneking 2005) (see Fig. 6).

The entire sequence of a human mtDNA was published at the beginning of the 1980s by Anderson and (1981), and from then on it has been known as the Cambridge Reference Sequence (CRS). This has been more recently revised by Andrews and (1999). Given its unique proprieties, mtDNA is one of the suitable genetic markers for the study of human population history and evolution. It is present in high copy number in human cells; this property makes it easier to obtain mtDNA for genetic analyses. It is a haploid system with apparent lack of recombination, high mutation rate, only inherited maternally, which allows tracing back female lineages. The high number of copies of mtDNA in each cell makes this genomic region particularly easy to extract and analyze, this property is especially important when studying DNA extracted from fossils or degraded samples.

Although one case of paternal inheritance was observed in man (Schwartz and Vissing 2002), exceptions to maternal inheritance are assumed to be extraordinary and non-

significant for evolutionary inference (Pakendorf and Stoneking 2005). The lack of recombination has been recently questioned by four studies that claimed its existence. However, it has been observed that most of these cases were more likely to result from sequencing errors or caveats in the statistical analyses than from recombination itself (Pakendorf and Stoneking 2005). Only one case was effectively observed and it is the same as paternal inheritance (Schwartz and Vissing 2002). This seems to be still a very rare phenomenon, and not to affect population studies.

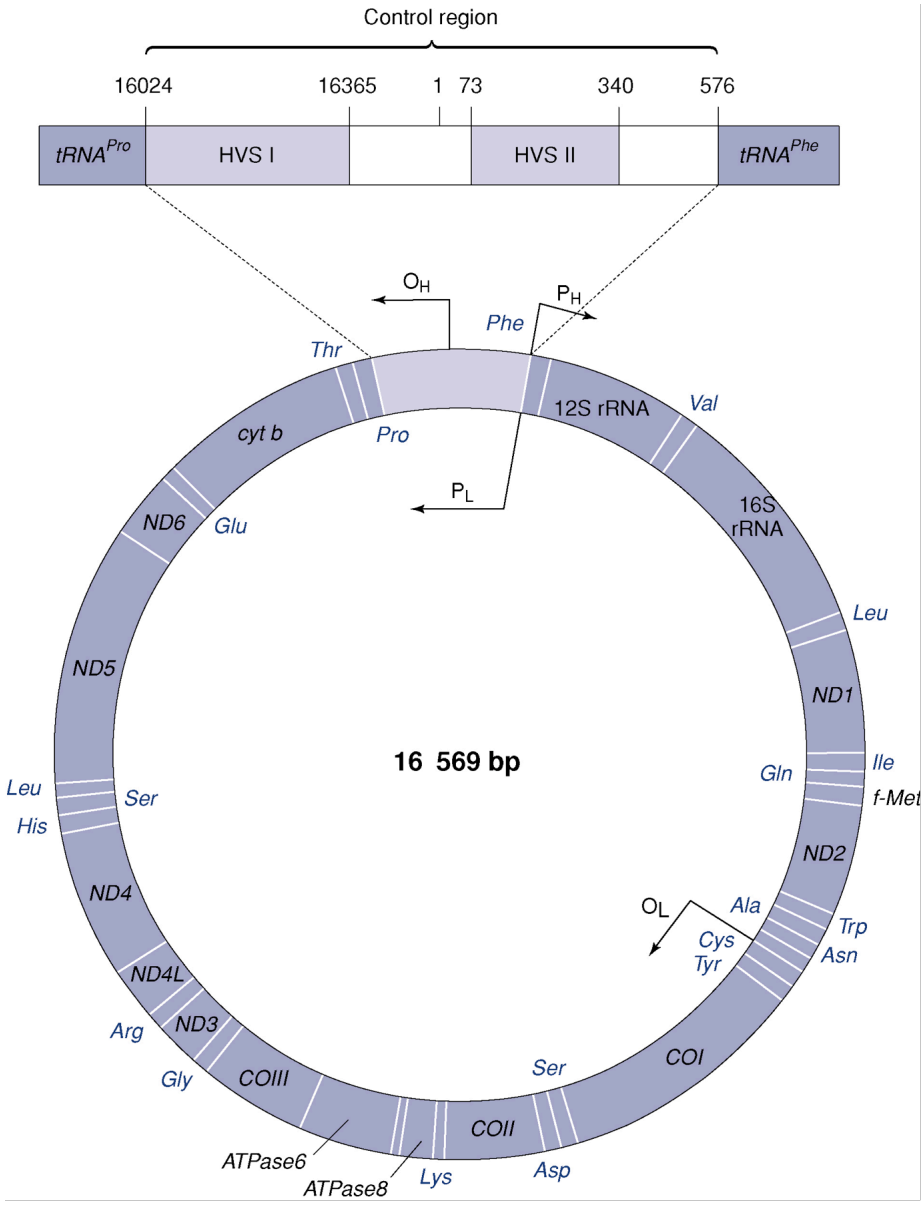

Figure 6: Human mitochondrial DNA molecule (from Jobling et al. 2004)

Concerning the mutation rate, this is several orders of magnitude higher than that of nuclear genes, with an estimated rate of 0.017 x 10-6 substitutions per site per year, without considering the control region (Ingman et al. 2000). In the control region the rate is even higher, even if the exact rate is still a matter of controversy. In fact, phylogenetic estimates yielded a rate of 0.075-0.165 x 10-6 substitutions per site per year, while pedigree estimates yielded an average rate of 0.47 x 10-6 substitutions per site per year, significantly higher than the first ones. However, at present, the phylogenetic estimates are preferred for population studies (Howell et al. 2003). A recent study offered a new mutation rates for the whole mtDNA molecule (Soares et al. 2009).

2.1.1 mtDNA Phylogeny and Phylogeography

Pioneer studies on mtDNA as a molecular marker started in the late 1970s by digestion of the molecule with a single restriction enzyme in a large number of samples (Denaro et al. 1981), or with several enzymes in fewer samples (Brown 1980). Subsequent studies were based on a greater number of enzymes (5 or 6) (Johnson et al. 1983; Santachiara Benerecetti et al. 1988; Scozzari et al. 1988). The resulting phylogeny was starlike, with a single central haplotype, shared among populations worldwide, and originating several lineages, some of which population specific. This gave support to the “multiregional” hypothesis for the origin of modern humans, since it seemed that all populations could have shared a common evolutionary history for a long time (Richards and Macaulay 2001).

Subsequently, the resolution of the analyses was increased to 12 restriction enzymes and the first study published was the famous work by Cann and in 1987. In this paper the authors obtained a more detailed phylogeny than the one seen previously. It was no longer starlike and it showed a deep split between Africans and non-Africans, with the deepest branches leading exclusively to African haplotypes (Fig. 7). Such a result was interpreted as an evidence for the Out of Africa model for the origin of modern humans, and gave rise to the popularized concept that the “mitochondrial Eve” was African (Cann et al. 1987).

At the beginning of the 1990s, when sequencing techniques became available for population studies, a work by Vigilant and was published. This study focused on the variation of hypervariable regions I and II (HVR-I, II) in the control region of mtDNA (Vigilant et al. 1991). In the phylogeny presented, the authors observed that the first 21 branches led to African haplotypes, while in the rest of the tree both African and non-African haplotypes were found. They interpreted this result as evidence favoring the OOA model (Out of Africa). However, since the region under study was more variable than the coding one analyzed before, the number of possible parsimonious tree topologies was very high, and several of them did not show an African root. This gave rise on one hand to several criticisms to the conclusions reached by Vigilant and, while on the other it stimulated the development of new tools for reconstructing phylogenies (Bandelt et al. 1995; Templeton 1992).

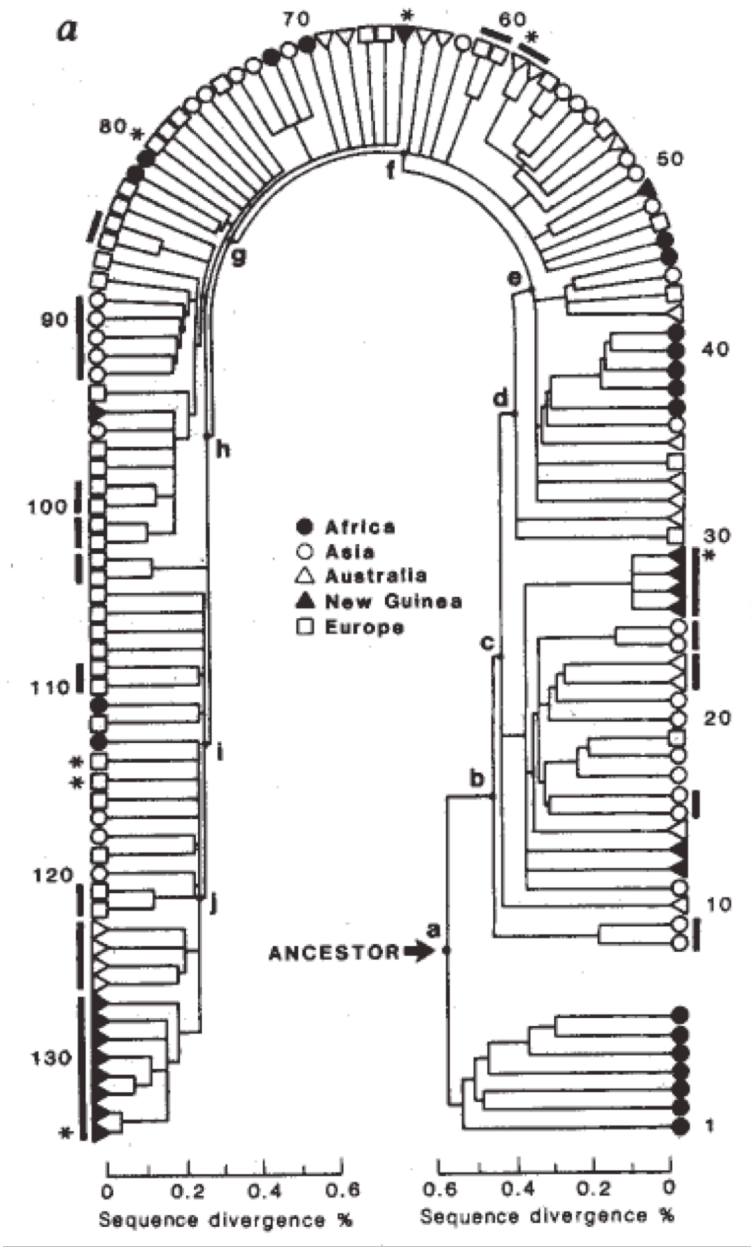

Figure 7: Phylogenetic tree presented by Cann et al. (1987) showing the deep split between Africans and non-Africans

It was in this context that the network approach was developed, and it was in 1997 a study by Watson and colleagues in which it was implemented. Their results supported the ones presented by Vigilant and colleagues, and introduced new interesting findings, like the affiliation of all Eurasian mtDNA to a single African clade. This was interpreted as a signature of a founder effect during the migration out of Africa (Watson et al. 1997). In subsequent years the analysis of high-resolution restriction enzymes started to be coupled to the analysis of HVR-I, combining the more stable information of the

coding region with the variation observed at the control region. This led to a great amount of information accumulated and increased the reliability of inferences, both at regional and worldwide level. The phylogeographic approach started to be applied to the study of human evolution, dispersal and colonizations. The topology of the phylogeny presented by Watson and was subsequently confirmed by a work by Quintana-Murci and , published in (1999) and by the first study on complete mitochondrial genome variation in a Worldwide sample (Ingman et al. 2000). In both papers, the deepest branches of the phylogeny led to African haplotypes, while two different clades seemed to have survived the out of Africa event (Fig. 8). The results of these studies became useful tools for the interpretation of the pattern of human spreading across the world (Horai et al. 1995; Ingman et al. 2000; Kivisild et al. 1999; Macaulay et al. 2005; Mishmar et al. 2003; Plaza et al. 2003; Plaza et al. 2004; Quintana-Murci et al. 2004; Salas et al. 2002; Schurr et al. 1990; Torroni et al. 1993a).

In more recent years, the role of selection acting on mtDNA has been considered. Some authors claimed climate may be a selective pressure on human mtDNA (Mishmar et al. 2003; Ruiz-Pesini et al. 2004). They found a positive correlation between the ratio of non-synonymous versus synonymous substitutions in specific lineages and their geographical distribution. However, these first studies were subsequently criticized since it has been observed that the excess of non-synonymous mutations is significant in the external versus internal branches of the global mtDNA tree, more than on geographical basis. This, together with the application of inappropriate statistical tests, could have biased the conclusions by Mishmar and colleagues (2003) and Ruiz-Pesini and (2004) (Elson et al. 2004; Kivisild et al. 2004). Moreover, the phylogeny of the human mtDNA has been exhaustively defined and a detailed phylogeography has been constructed using complete mtDNA sequences (Herrnstadt et al. 2002; Ingman et al. 2000), establishing the correlation of ethnic origin and geographical location (Fig. 9).

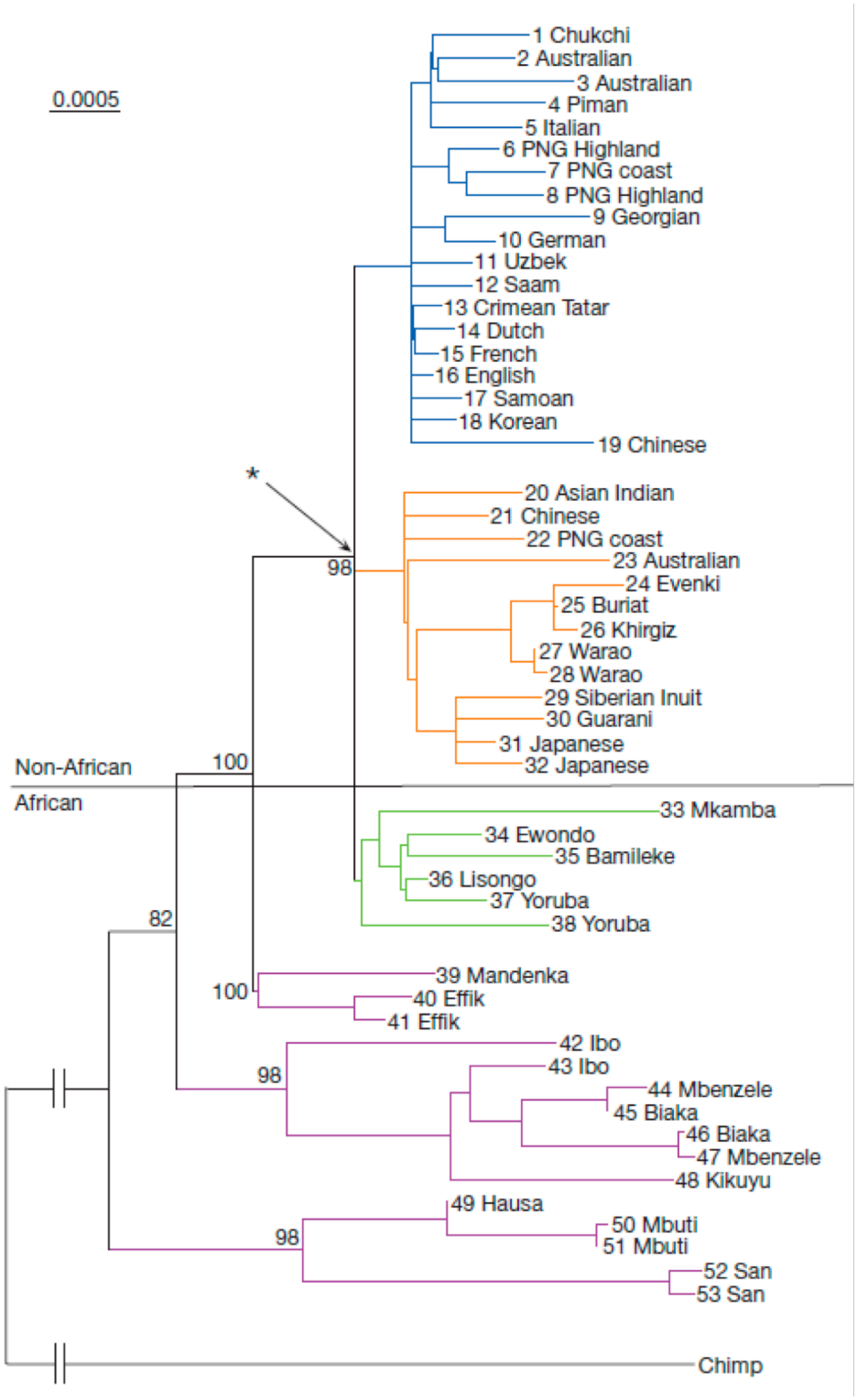

Figure 8: Neighbour-joinig phylogram presented by Ingman et al. (2000)

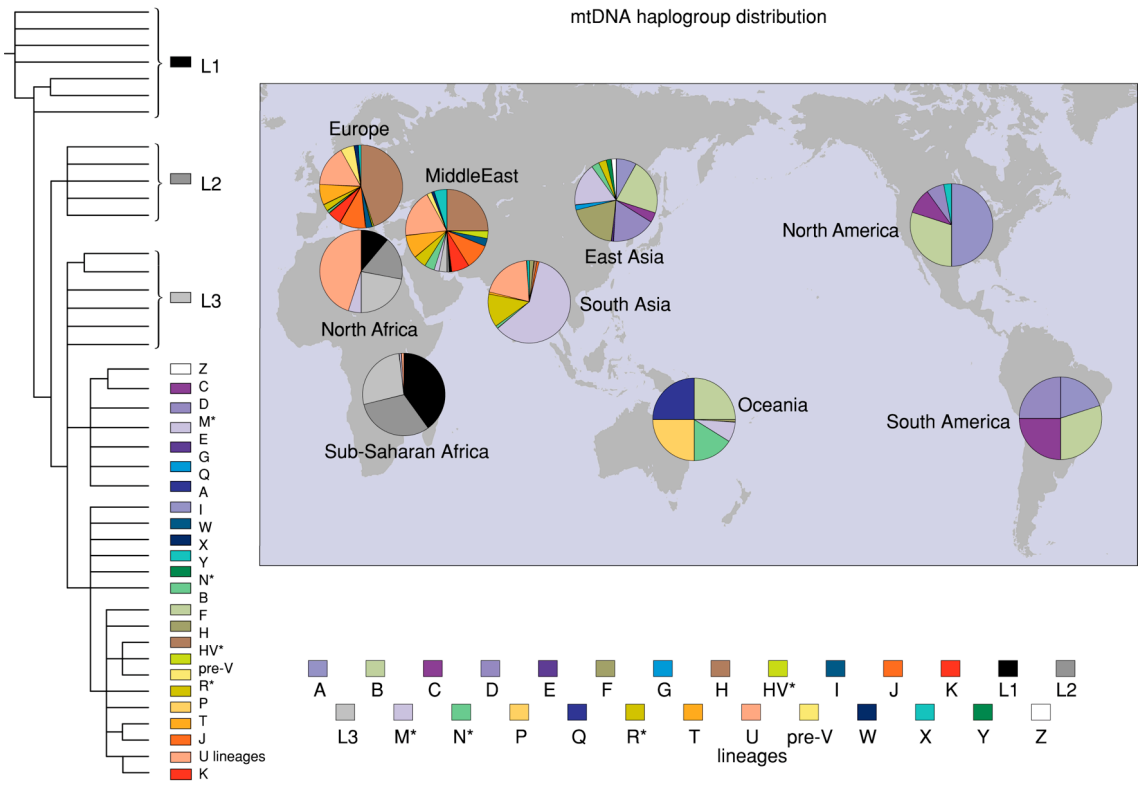

Figure 9: Geographical distribution of the major mtDNA clades (from Jobling et al. 2004)

2.1.2 Mitochondrial DNA diversity in America

The majority of molecular genetic studies of Native American populations have utilized the maternally inherited mitochondrial genome. Early research on mitochondrial diversity identified that, all extant Native American mtDNAs, if not of recent admixture, descend from five founder haplogroups: A, B, C, D (Forster et al. 1996; Schurr et al. 1990; Torroni et al. 1993a; Torroni et al. 1992), and X (Brown et al. 1998). The earliest study that could be traced of mtDNA of Amerindians was one by Johnson et al. (1983). Using five restriction enzymes, which can cut the DNA (or not), the types which could be established on this basis were investigated in 200 individuals from six different ethnic extraction; including 30 Warao Indians from Venezuela. Wallace and collaborators started a systematic evaluation of these polymorphisms in Amerindians. Initially, six restriction enzymes were used (Wallace et al. 1985), but afterwards a set of 14 enzymes was employed (Torroni et al. 1992). Sequencing mtDNA control region was also used to investigate Amerindian population variability (Ward et al. 1991), and in many of the following papers the two techniques had been used (Torroni et al. 1993a; 1993b). Soon it was realized that, depending of the DNA construction in specific sites,

the haplotypes and sequences could be grouped in four main sets (A, B, C, and D haplogroups), that would have been present in the earlier colonizers of the Americas (Salzano 2002).

Each of these maternal lineages is distinguished by a unique combination of restriction fragment length polymorphisms (RFLPs), a 9-base deletion, and direct sequencing of the first hypervariable segment of the non-coding D-loop (HVSI) (Brown et al. 1998; Forster et al. 1996; Torroni et al. 1993a), together, they encompass 96%–100% of the mitochondrial haplotypes in modern indigenous populations of the New World (Schurr and Wallace 2002). Recently, there has been an increasing emphasis on the analysis of entire mitochondrial genomes (Achilli et al. 2008; Fagundes et al. 2007; Kitchen et al. 2008; Perego et al. 2009), facilitating the identification of numerous sub-lineages. Similar diversity values have been found for all haplogroups, with a number of exclusively American polymorphisms indicative of a signature of recent population expansion (Fagundes et al. 2008).

At a continental level, the five founding haplogroups are differentially distributed in the New World. Among Amerindians, haplogroup A decreases in frequency from north to south, whereas haplogroups C and D generally increase in the same direction. However, haplogroup B shows no similar clinal distribution, other than being virtually absent in northern North America (Lorenz and Smith 1996; Schurr et al. 1990; Torroni et al. 1994a; Torroni et al. 1994c; Torroni et al. 1993a; Torroni et al. 1992). Haplogroup B does appear at high frequencies in both the Southwest United States and the Andean region, probably because of recent population expansions (Malhi et al. 2001; Merriwether et al. 1994, 1995). By contrast, haplogroup X is found nearly exclusively in North America (Lorenz and Smith 1994, 1996; Schurr et al. 1990; Torroni et al. 1994a; Torroni et al. 1994c; Torroni et al. 1993a; Torroni et al. 1992), with only trace frequencies of this mtDNA lineage possibly being seen elsewhere (Ribetio-dos-Santos et al. 1996). These distributions probably reflect the original pattern of settlement of the Americas, as well as the subsequent genetic differentiation of populations within certain geographic regions (Fig. 10 Schurr 2004).

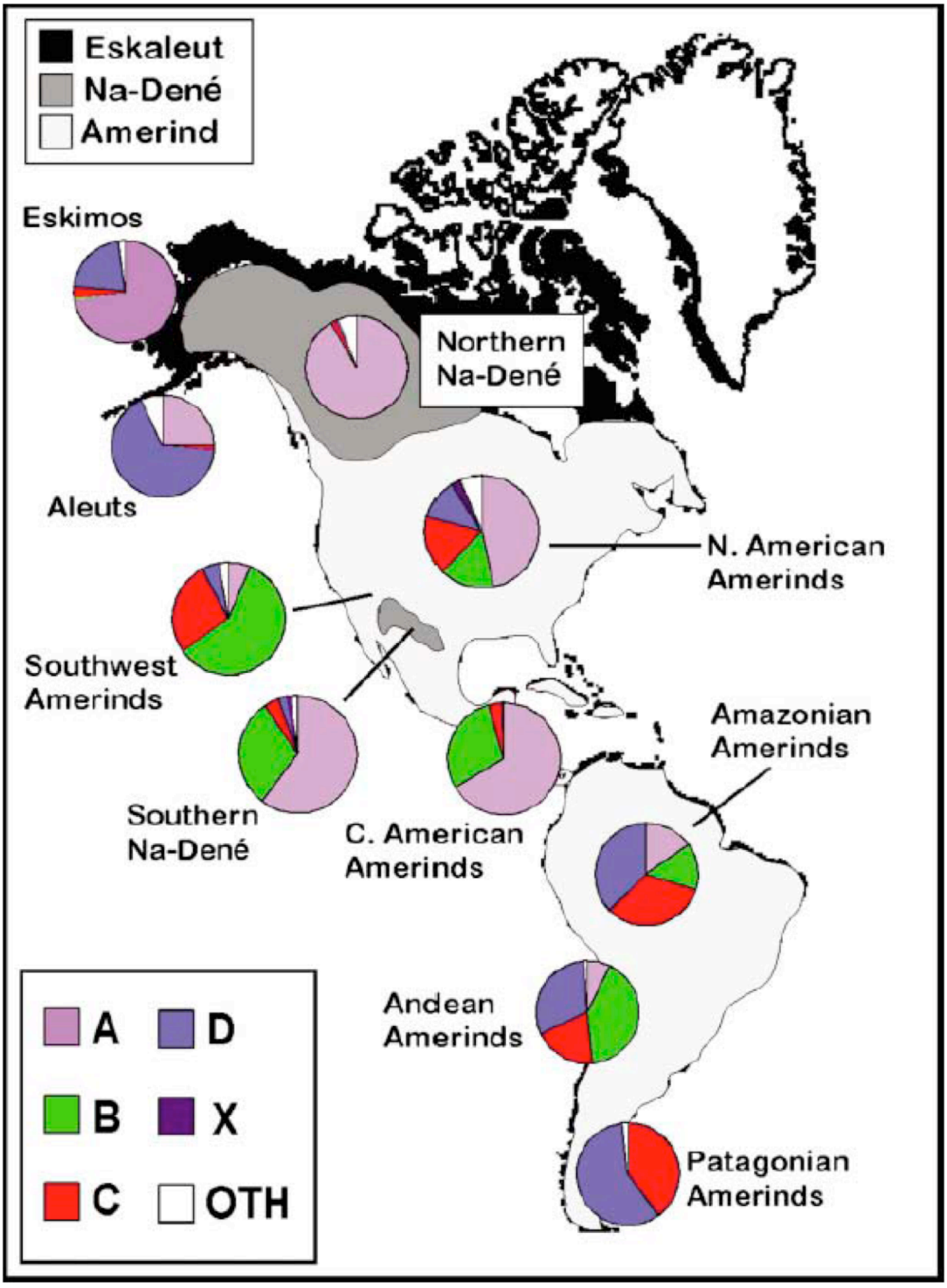

Figure 10: Distribution of mtDNA haplogroups in Native American populations (from Schurr 2004)

There are also several major geographic trends in the distribution of the founding mtDNA lineages in the Americas. Populations speaking Amerind languages typically carry members of three to four of the A, B, C, and D haplogroups. By contrast, northern

Na-Dene speakers carry only haplogroup A. Na-Dene-speaking populations in the southwest United States possess haplogroups B and C, as well as haplogroup A, although the presence of haplogroups B and C is likely a result of genetic exchange with Amerind-speaking neighbors. Haplogroups B and C reach their highest frequencies in Amerind-speaking populations of the Southwest, where the southern Na-Dene live. Mitochondrial DNA haplotypes in Eskimo-Aleut speakers belong predominantly to haplogroup A (Rubicz et al. 2003; Saillard et al. 2000; Shields et al. 1993; Starikovskaya et al. 1998; Torroni et al. 1993a; Torroni et al. 1992; Ward et al. 1993). The mtDNA haplogroups that are shared by all indigenous American populations are rare outside of the Americas (Kolman et al. 1996; Merriwether and Ferrell 1996). They are found in many Asian populations, but the four principal lineages usually do not occur together in the same population (Mulligan et al. 2004). Some interesting exceptions are Siberian populations, who harbor A, C, and D together at relatively high frequencies (Torroni et al. 1993b), and Mongolian, Tibetan, and central Chinese populations, who harbor all four (Ballinger et al. 1992; Kolman et al. 1996; Torroni et al. 1994b). Various studies have also revealed a high frequency of "private haplotypes" in individual populations or groups of related Amerindian tribes (e.g. Lorenz and Smith 1997; Malhi et al. 2001; Torroni et al. 1993a). These patterns reflect the role that genetic drift and founder effects have played in the stochastic extinction and fixation of mtDNA haplotypes in Native American populations. By the mid-1990s, researchers proposed that a single migration could parsimoniously explain the distribution of the four principal mtDNA haplogroups and should be the favored scenario for the peopling of the Americas (Bonatto and Salzano 1997b; Kolman et al. 1996; Merriwether et al. 1994, 1995; Stone and Stoneking 1998).

A number of haplotypes not clearly belonging to these five maternal lineages have been also detected in different Native American groups (Bailliet et al. 1994; Easton et al. 1996; Lorenz and Smith 1996, 1997; Merriwether et al. 1994, 1995; Ribetio-dos-Santos et al. 1996; Rickards et al. 1999; Santos et al. 1996a; Torroni et al. 1993a; Ward et al. 1991). These "other" mtDNAs have often been considered additional founding haplotypes or haplogroups in New World populations. However, most have since been shown to be derivatives of haplogroups A–D that have lost diagnostic mutations (Schurr 2004; Schurr et al. 1999; Schurr and Wallace 2002). The remainder appears to have been contributed to indigenous groups through nonnative admixture. In addition, the

"other" mtDNAs detected in archeological samples (e.g., Ribetio-dos-Santos et al. 1996) may have resulted from contamination with modern mtDNAs, or were insufficiently analyzed to make a determination of their haplogroup status (Schurr 2004). Nonetheless, the number of haplogroups found in Native America is but a subset of those commonly found in central and northeast Asia, clearly reflecting a reduction in mtDNA diversity in the Americas.

The fifth founding mtDNA haplogroup, designated Haplogroup X in Native Americans was originally attributed to recent European admixture because several non-A, -B, -C, and -D haplogroups co-occur in the Native American population where X was first identified (Torroni et al. 1993a). The suggestion that haplogroup X was introduced by an ancient migration from Europe caused a stir (Brown et al. 1998). This interpretation was supported by the presence of distantly related members of haplogroup X in Europeans, but Asian populations had not been studied for the X-defining variant (Brown et al. 1998). The need for a European migration has since been abandoned owing to the observation of haplogroup X in the Siberian Altai (Derenko et al. 2003). Haplogroup X now appears to be an old and complex lineage. Two major branches, X1 and X2, each with worldwide distribution, were recently identified in a parsimony analysis of complete DNA sequences (Reidla et al. 2003). Members of haplogroup X in Native Americans belong to a distinct clade (X2a) that has no close relatives in the Old World. All members of haplogroup X in the Siberian Altai coalesced about 6700 years ago (Reidla et al. 2003) which substantially postdates the peopling of the Americas, and argues against a close relationship between the Altai and modern Native Americans.

Based on mtDNA diversity, Bortolini and Salzano (1996) performed an extensive analysis of the mtDNA variability of Amerindians, comparing it with those of other groups, and reached the following conclusions: (a) Total diversity, either considering characteristic haplogroups or a given set of haplotypes defined by 14 restriction enzymes, is of the same order of magnitude as those observed in other ethnic groups. Moreover, Amerindians present a degree of interpopulation variability that is higher than those found elsewhere; (b) Distinctive features were the low variability of the Na-Dene, and the high interpopulation diversity observed in Central Amerindians; and (c) The total diversity found in A, B, C, and D haplogroups is about one-third of that observed for the African L1 and L2 haplogroups, and the share of this variability that is

due to the interhaplogroup diversity is much more important (2x higher) in Amerindians than in Africans (Bortolini and Salzano 1996).

2.1.3 Routes to America: mtDNA lineages

Greenberg's theory (see section 1.3) gave rise to many discussions about the importance of the interpretation of different evidences for the settlement of the New World and possible colonization routes. This fact boosted genetic studies in Native American populations based mainly on the mtDNA uniparental marker. During the 90s some authors supported Greenberg's theory in some aspects and others refuted it. Schurr (1990) was one of the first authors proposing that the genetic variability in the Americas could be traced through four founding lineages (A, B, C and D). Throughout this decade there were some shared arguments between the different peopling hypotheses like the existence of an ancestral population or populations leaving from northeast Asia during the Last Maximum Glaciation (LMG, defined as an interval centered on 21,000 ybp) (Clark and Mix, 2002), and crossing through the Bering Strait bearing the five founding lineages. It is likely that they remained isolated enough time to generate more genetic diversity within the founding lineages. There is also remarkable consensus in the necessity of studying native populations from Siberia, Alaska and Pacific in order to have a better accuracy in the historical reconstruction of the peopling of the Americas.

2.1.3.1 A single and early migration and "Out of Beringia"

During the 90's decade, simultaneously to the description of the native genetic diversity in the Americas, different theories about the possible colonization routes started to arose. When the first four founding haplogroups (A-D) were identified in the three linguistic families-groups-waves described by Greenberg et al. (1986), the investigators interpreted these findings as indicating that all four mtDNA lineages were present in the original migration(s) to the New World (Forster et al. 1996; Kolman et al. 1996; Merriwether et al. 1994, 1995; Stone and Stoneking 1998). This mtDNA evidence has resulted in the conjecture that all Native American populations trace to a single ancestral founder population that lived in the region of Mongolia/North China (Kolman

et al. 1995; Merriwether et al. 1995). A monophyletic colonization from Asia was accepted, supported the hypothesis of only a single and early migration entered into the New Word crossing Bering carried out with multiple variants of each of the four founding lineages, peopling the rest of the continent.

Using measures of mtDNA diversity and other population genetic parameters Bonatto and Salzano (1997a, b) proposed the called ‘‘out of Beringia’’ model of the continent’s colonization. The picture suggested is that some time after Beringia had been peopled (60,000-11,000 ybp) the population expanded and crossed the Alberta ice-free corridor that connected this region to the south of North America or, alternatively, followed a coastal route. The collapse of ice sheets 14,000-20,000 ybp isolated Beringia from the rest of the continent during some time (2,000-6,000 years) generated more and genetic variability, and it was there that the Na-Dene and Eskimo diverged biologically. Amerind differentiation occurred as the groups that were in North America migrated south. Therefore, there would have been just one major migration wave, which would have started 30,000-40,000 ybp.

Beringia is assumed to be the place where Native American ancestors differentiated before migrating into the New World with a founding time of 22-55,000 ybp (Bonatto and Salzano 1997b). Out of Asia Siberia is considered to be the geographic region of Native American populations, and Beringia is give the role of a corridor, with a founding time of 20-25,000 ybp (Forster et al. 1996). Both hypotheses coincided in suggesting that, after an early colonization event the Bering corridor was destroyed by the coalescence of the ice glaciers (18-12,000 ybp) producing isolation of the migrants and producing genetic and linguistic differentiation. The scarcity of the lineages in Asia makes it improbable that different migrating populations would draw the same set independently. Therefore, the genetic data lend no particular support to the three-migration hypothesis. Instead, a single migration to the Americas that carried all founding lineages simultaneously is a more parsimonious and preferable explanation (Mulligan et al. 2004).

2.1.3.2 Two independent migrations

Based on mtDNA restriction polymorphism (RFLPs) genetic diversity from Amerinds and Na-Dene speakers, Torroni and (1992; 1993b) suggested that genetic differences between these groups indicate that populations derivate from two independent migrations at different times, supporting the three migration Greenberg's theory. However some linguistic and genetic data that does not support the hypothesis of the three migratory waves (Campbell and Mithum 1979; Chakraborty and Weiss 1991; Laughlin 1988; Spencer et al. 1977a; Szathmary et al. 1983; Wallace et al. 1985). Amerinds derived from an ancestral Asiatic population, performed a several sample size and variability reduction during the Americas migration, they carried out five founder haplotypes around 21,000-42,000 ybp. Later the Na-Dene populations derived from two or three founder haplotypes ~5,250-16,000 ybp. In summary, the Americas was colonized in a period of time around 13,000-14,000 ybp by two independent migration waves (Torroni et al. 1992).

2.1.3.3 Multiple migrations

Horai el al., (1993) suggested the "multiple migration hypothesis" in which the four founding lineages derived from four independent and separated migration waves at different times between 14,000-21,000 ybp. The ancestral populations did not experiment a bottleneck and rapid expansion in the sample size. The Amerinds were the fist migration, Na-Dene and Aleut-Eskimo arrived in posterior migrations. The polymorphisms shared within the three populations supported genetic proximity; indicating that the major mtDNA lineages have contributed to the peopling of the Americas. The mtDNA results have been interpreted to support a range of models with as many as four major migrational waves (Horai et al. 1993; Lorenz and Smith 1994; Shields et al. 1993; Szathmary 1993; Torroni et al. 1994a; 1993a; 1992).

2.1.3.4 BIM: Beringian Incubation Model

Tamm and colleagues (2007) analyzed a total of 623 complete mtDNA sequences from Asia and the Americas. Their results described more genetic diversity within the fonder population that previously was reported. The Native Americans derive from a small

number of Asian founders who likely arrived to the Americas through Bering Strait; their phylogenetic structure suggests that the ancestors of Native Americans paused when they reached Beringia, during which time New World founder lineages differentiated from their Asian sister-clades. This pause in movement was followed by a swift migration southward that distributed the founder types all the way to South America. The data also suggest more recent bi-directional gene flow between Siberia and the North American Arctic. The new founders' haplotypes do not showed a specific structure from north to south into the continent, implying a fast migration instead of gradual (see Fig.11 Tamm et al. 2007). These results coincided with the archeological evidence; the remains found in Yana River site were dated ~30,000 ybp, suggesting that likely humans were isolated in Bering more than 15,000 ybp (Dillehay 2000; Volodko et al. 2008).

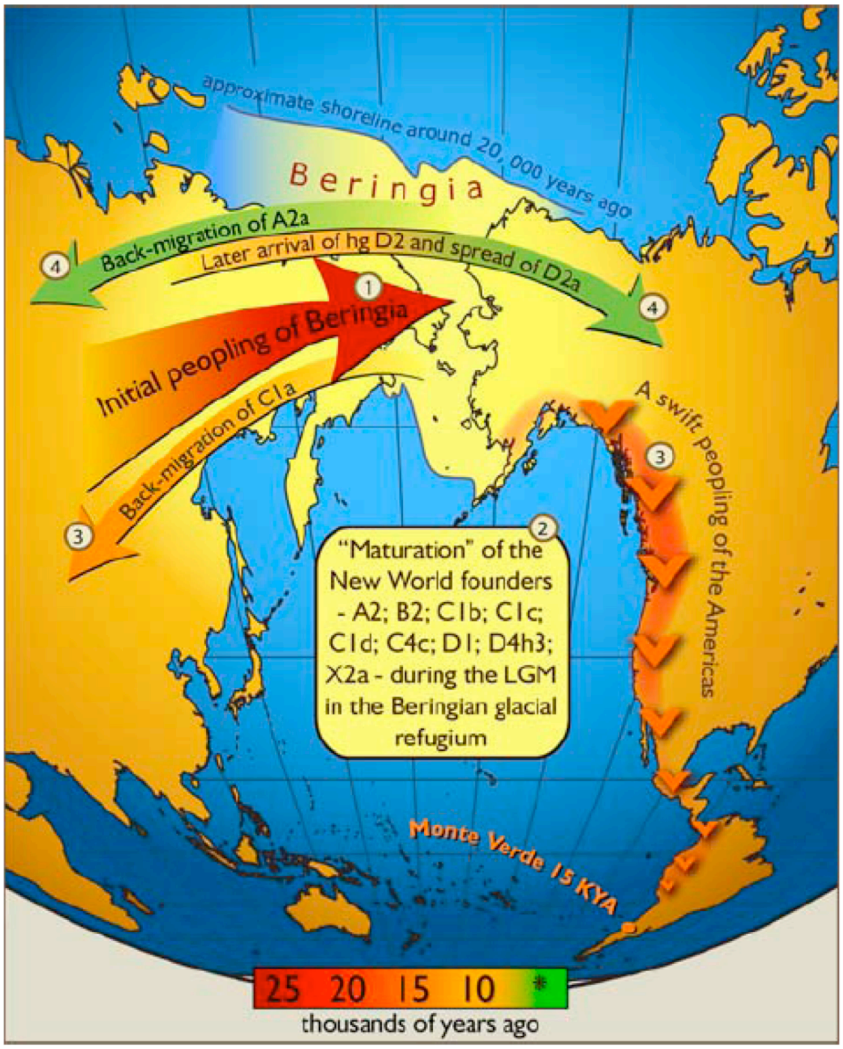

Figure 11: Schematic illustration of maternal geneflow in and out of Beringia (Tamm et al., 2007)

2.1.3.5 Pacific Coastal Model

A detailed demographic history of the mtDNA sequences (86 complete mitochondrial genomes, analyzed) estimated with a Bayesian coalescent method indicates a complex model for the peopling of the Americas (Fagundes et al. 2008). The authors support the existences of a Pre-Clovis occupation of the Americas, followed by rapid settlement, of the continent along a Pacific coastal route. Native American populations exhibit almost exclusively five mtDNA haplogroups (A–D and X). Haplogroups A–D are also frequent in Asia, suggesting a northeastern Asian origin of these lineages. However, the differential pattern of distribution and frequency of haplogroup X led some to suggest that it may represent an independent migration to the Americas. In their study it was show that all Native American haplogroups, including haplogroup X, were part of a single founding population, thereby refuting multiple-migration models. The initial differentiation from Asian populations ended with a moderate bottleneck in Beringia during the last glacial maximum (LGM), around ~23,000 to ~19,000 years ago. Toward the end of the LGM, a strong population expansion started ~18,000 and finished ~15,000 years ago, suffering an important reduction sample size (Fagundes et al. 2008). This theory supports that the existence of sites localized along the coast like Monte Verde (14,500 ybp) were older than others from the interior. Probably when humans colonized the entire continent from north to south, the sea levels rose producing a reduction of the natural resources; therefore, they migrated to the inlands changing technologies and life style (Fagundes et al. 2008).

2.1.3.6 Three-Stage Model

Kitchen et al. (2008) proposed a three-stage colonization process for the peopling of the New World, with a specific focus on the dating and magnitude of the Amerind population expansions, based on the analysis of Native American (mtDNA) coding genomes plus non-coding control region sequences as well as a combined nuclear and mitochondrial coding DNA dataset from New World and Asian populations. Studying complex colonization scenarios, the interpretation of genetic data can benefit substantially from the incorporation of non-genetic material evidence, for these reason they incorporate archeological, geological and paleoecological data in the analysis. The

goal was to provide a comprehensive model for the initial settlement of the Americas that generates new testable hypotheses and had high predictive power for the inclusion of new datasets. They propose a three-stage model in which a recent, rapid expansion into the Americas was preceded by a long period of population stability in greater Beringia by the Paleoindian population after divergence and expansion from their ancestral Asian population. These results support a model for the peopling of the New World in which Amerind ancestors diverged from the Asian gene pool prior to 40,000 years ago and experienced a gradual population expansion as they moved into Beringia. After a long period of little change in population size in greater Beringia, Amerinds rapidly expanded into the Americas ~15,000 years ago either through an interior ice-free corridor or along the coast. This rapid colonization of the New World was achieved by a founder group with an effective population size of ~1,000–5,400 individuals. The model presents a detailed scenario for the timing and scale of the initial migration to the Americas, substantially refines the estimate of New World founders, and provides a unified theory for testing with future datasets and analytic methods (see Fig.12 Kitchen et al. 2008). Few months after, the same authors (Mulligan et al. 2008) reassess their model by giving new data, which support that the period of population isolation required for the generation of New World mitochondrial founder haplogroup-defining genetic variants makes the existence of three stages of colonization a logical conclusion. Thus, their three stage model remains an important and useful working hypothesis for researchers interested in the peopling of the Americas and the processes of colonization (Mulligan et al. 2008).

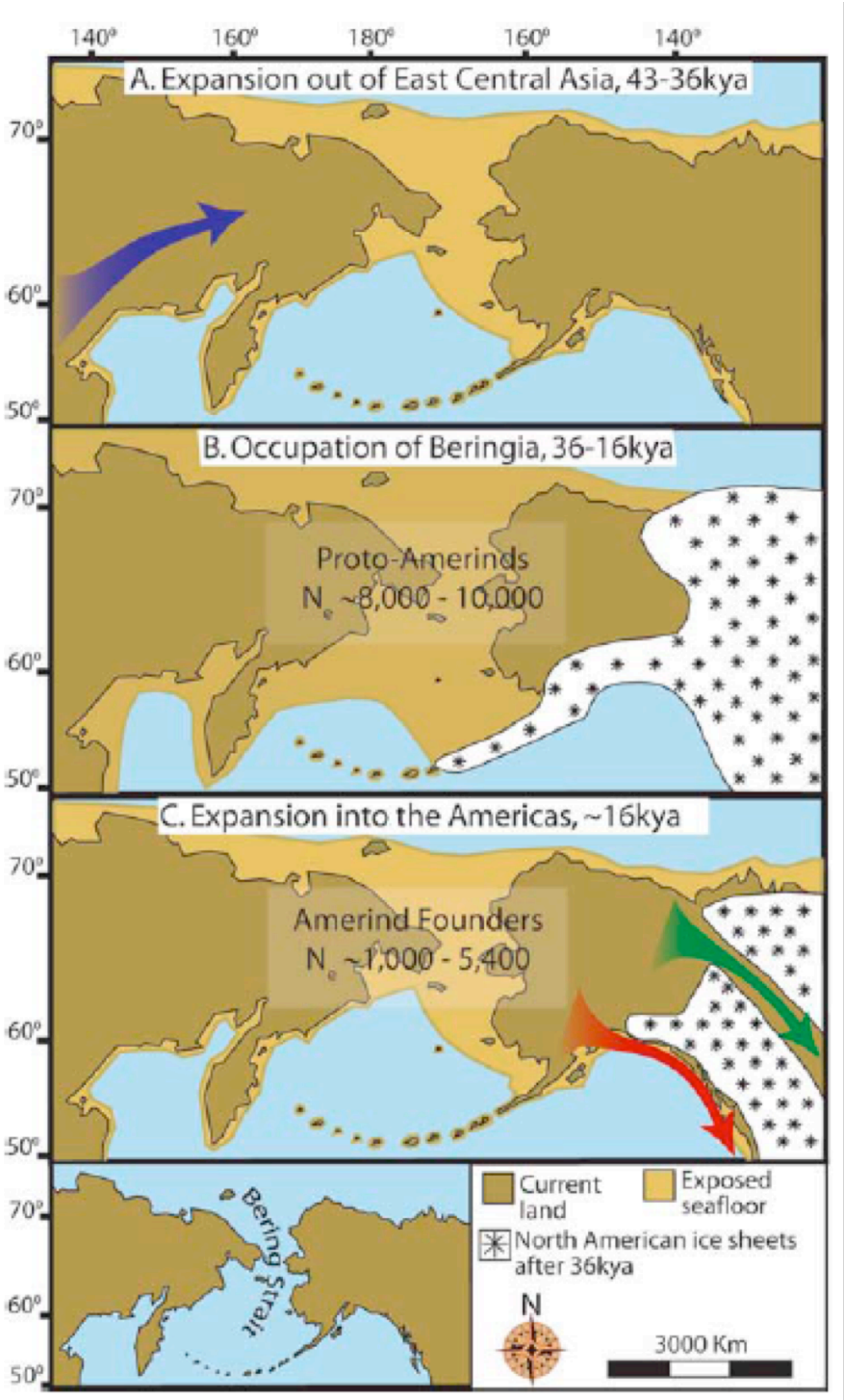

Figure 12: Maps describing each phase of the three-step colonization model for the Peopling of the Americas according to Kitchen et al. (2008)

2.2 Y chromosome and main features

The Y-chromosome is the second smallest human chromosome, after chromosome 21, with an average size of 60 megabases (Mb). It is located in the nucleus and it contains the SRY (Sex-determining Region Y) gene, which encodes for the testis determining factor. The male specific segment of the Y-chromosome in mammals has no homologous counterpart and thus does not recombine; it has all its genes in linkage disequilibrium, and is paternally transmitted. The mutation rate is much lower than mtDNA (Bianchi et al. 1998).

It is constituted by two pseudo-autosomal regions at the extremities, which recombine with its homologous regions in the X chromosome, and by a large non-recombining region (NRY), that accounts for up to 95% of its length. The NRY has been recently renamed MSY, or male-specific region, due to the observation of multiple Y-Y gene conversion events per generation (Skaletsky et al. 2003). The MSY includes a heterochromatic region of variable size that consists of long homogeneous tandem arrays of non-functional DNA, and a euchromatic region of approximately 23 Mb, that is a mosaic of complex and interrelated sequences. The second contains 27 distinct protein-coding genes or gene families, 12 of which are expressed ubiquitously throughout the body, whereas 11 are expressed exclusively or predominantly in testes (Skaletsky et al. 2003).

The euchromatic sequences fall into three classes:

- the X-transported, comprising 10-15% of the MSY and resulting from a massive X to Y transposition that occurred about 3-4 million years ago; these sequences still present 99% homology to their counterparts in X chromosome;
- the X-degenerate, comprising 20 % of the MSY and consisting of a class of sequences that are less closely related to the X chromosome, being remnants of ancient autosomes from which modern X and Y-chromosome derive;
- the remainder comprises the amplicon class, which includes large regions where sequences exhibit marked similarity to other sequences in the MSY; these sequences are located in seven segments scattered across the long and proximal short arms.

Curiously, all 12 ubiquitously expressed MSY genes reside in X-degenerate regions, while all 11 MSY genes expressed exclusively in the testis reside in the amplicon class, except for SRY which is in an X-degenerate region (Skaletsky et al. 2003). The Y-chromosome attracted special attention for human population genetic studies due to some important features, which include haploidy, paternal inheritance, apparent lack of recombination and low mutation rate (Bosch et al. 1999; Jobling and Tyler-Smith 1995, 2003).

Haploidy allows avoiding for the confounding effect of heterozygosity. Paternal inheritance, together with the lack of recombination, makes the Y-chromosome particularly suitable for evolutionary studies, as a natural counterpart for the studies based on mtDNA. The two markers together can give a reasonable idea of the evolutionary history of human populations, even if the processes shaping their diversity can be differently influenced by demographic and cultural factors (Bolnick et al. 2006; Carvajal-Carmona et al. 2000; Destro-Bisol et al. 2004; Mesa et al. 2000).

The lack of recombination has been only recently questioned by the observation of Y-Y gene conversion (Skaletsky et al. 2003), but this is still not taken into account when analyzing phylogenetic relationships. The low mutation rate is both an advantage and disadvantage: on one hand, the use of stable Single Nucleotide Polymorphisms (SNPs) to reconstruct the phylogeny of Y-chromosome makes the tree more reliable, on the other; it decreases the level of variation observable at intra-specific level. But the presence of Short Tandem Repeats (STRs) polymorphisms in the Y-chromosome, characterized by a higher mutation rate than SNPs, allows intra and inter-population/lineage diversity to be observed and estimated.

The non-recombining portion of the human Y-chromosome has been of great interest in reconstructing human phylogeny, it's also evolving through the accumulation of mutations. Y-specific polymorphisms have been successfully used like markers which allow constructing informative current haplotypes that are specific to geographic regions and provide insights on male migration and admixture, allowing make inferences about the possible historic worldwide population movements; these markers are probably the best genetic tool to study early human migrations (Su et al. 1999).

2.2.1 Y-chromosome Phylogeny and Phylogeography

The complete sequence of the euchromatic region of MSY has been published only recently (Skaletsky et al. 2003), but the analysis of the Y-chromosome polymorphisms for population genetic purposes started well before. The discovery of the first polymorphic marker in 1985 (Casanova et al. 1985) was followed by a period in which the search with molecular probes for conventional Restriction Fragment Length Polymorphism (RFLPs) failed to find significant variation in the Y-chromosome (Jakubiczka et al. 1989; Malaspina et al. 1990). The first single nucleotide polymorphism to be identified on the Y chromosome was described only in (1994) by Seielstad and colleagues. Later, Hammer (1995) and Whitfield and colleagues (1995) studied with more accurate techniques the detection of more Y-chromosome polymorphism. The researchers recognized the power of Y-chromosome markers in resolving the migratory patterns of modern humans (Jobling and Tyler-Smith 1995). The first global survey of human Y-chromosome haplotype variation was done in (1995) by Jobling and Tyler-Smith also presents new compound haplotype data, a better refine study was done in (2003, see Fig. 13). Hammer (1995) performed a sequencing survey of 16 geographically diverse humans for a 2.6-kb region (referred to as the *"YAP"* region) on the long arm of the human Y-chromosome and constructed a tree with five Y-chromosome haplotypes.

The introduction of denaturing high-performance liquid chromatography (DHPLC) increased considerably the number of Y-chromosome markers known (Underhill et al. 1997), since that moment the global Y-chromosome tree started to be reconstructed with a fine resolution. Simultaneously the diversity of microsatellites started not only to test the statistical significance of the observed excess of Y-chromosome microsatellites diversity in African populations regarding Europeans and Asians (Jorde et al. 1997) but also to examine the ability of them to discriminate between the predictions of theories about modern human origins (Seielstad et al. 1999). In fact the DHPLC method was used to discover more than 200 SNPs and small indels on the MSY (Hammer et al. 2001; Shen et al. 2000; Underhill et al. 2000). Whit this results the association of two or more markers defined haplotypes correlated with ethnic origin of the populations to describe the genetic diversity found and establish a worldwide phylogeography. A clear picture of the geographic distribution of Y-chromosomal variation has yet to emerge

and started to be rigorously tested for many researches (Capelli et al. 2001; Hammer et al. 2001; Jobling and Tyler-Smith 2000; Karafet et al. 2001; Semino et al. 2000; Su et al. 1999; Underhill et al. 2000). However, one of the main problems in the initial attempts to reconstruct the worldwide phylogeography by different research groups was that they did not use the same combination of genetic markers in their studies; therefore, the emergence of several unrelated and nonsystematic nomenclatures for Y-chromosomal binary haplogroups was an increasing source of confusion. In 2002, the Y-chromosome consortium (YCC) published a paper with two principal aims: to reconstruct a highly resolved tree of MSY and to describe a new nomenclature system (Consortium 2002) moreover contribute to solve the main problem.

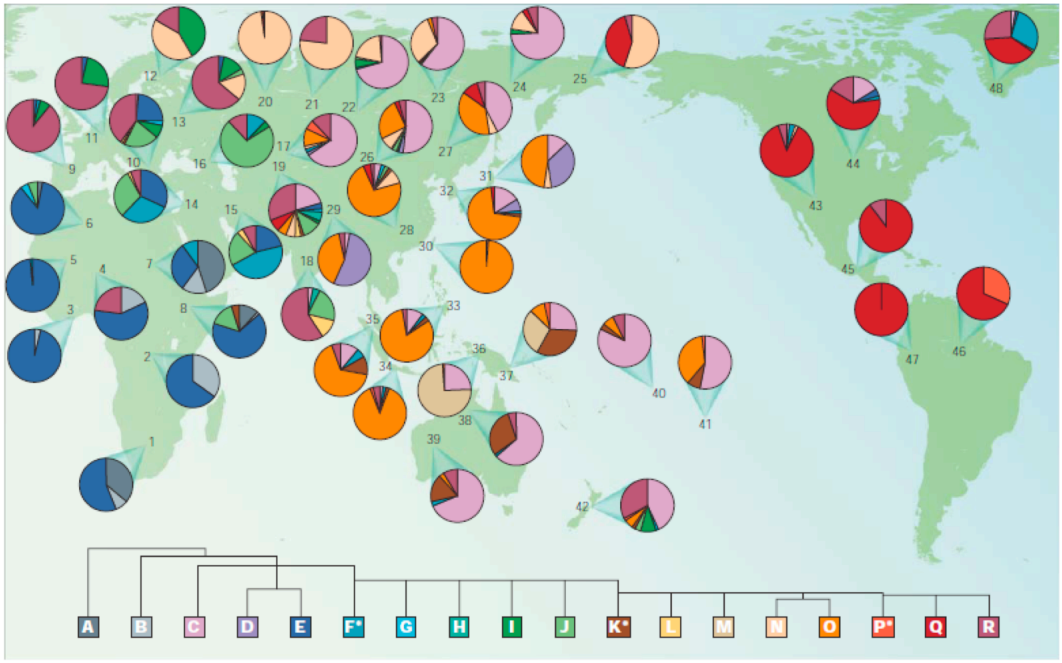

Figure 13: Global distribution of Y-haplogroups (from Jobling and Tyler-Smith 2003)

The methods for dating Y-chromosome haplogroups have employed the SNPs that define them, but are not sufficient to estimate when they evolved in a particular lineage using only this kind of data; and the STRs in which we can observe the internal diversity within each haplogroup. Underhill et al. (2000) estimated an average rate estimated (one per every 6,900 years) from SNP variation in three Y-chromosome genes and date the branches (haplogroups) of their phylogeny (Underhill et al. 2001). With

this mutation rate, was possible to give a date of the origins of the major branches of the phylogeny, as well as other points of SNP haplotype diversification. Other method to date the ages of Y-chromosome haplogroups is to analyze variation in the faster evolving STRs loci that occur on each SNP haplotype.

The contribution of the global Y-chromosome tree to the debate on the origin of modern humans soon became clear. In fact, like the mtDNA, the Y-chromosome tree rooted in Africa, with haplogroups A and B restricted to sub-Saharan Africa (Underhill et al. 2001). The haplogroups that are found in the rest of the world all derive from the B sister-clade. This suggested an African origin for modern human populations, and supported the Out of Africa hypothesis. However, the estimates of the Time of the Most Recent Common Ancestor (TMRCA) were particularly recent, being 46 (16-126) kya when using eight STRs (Pritchard et al. 1999) and 59 (40-140) kya when using SNPs (Thomson et al. 2000). Both these estimates result particularly recent when compared to the ones by mtDNA 177 kya, (Ingman et al. 2000). These two loci are in fact supposed to present the same effective population size, the main factor influencing the TMRCA estimates under neutrality. However, the effective population size of Y-chromosome has been hypothesized to be lower than mtDNA due to different possible factors: a higher variance in male reproductive success, natural selection, higher variance in mtDNA mutation rates, stochasticity in the evolutionary process, or questionable assumptions on generation time (Garrigan and Hammer 2006; Jobling and Tyler-Smith 2003).

Concerning natural selection, there have been several attempts to apply neutrality tests based on nucleotide diversity to the Y-chromosome variation, but it was very difficult to distinguish between signals of population expansion and selection (Jobling and Tyler-Smith 2000, 2003). Although a few examples of consistent natural selection have been observed, the pattern of Y-chromosome haplogroup distribution, compared to other markers, does not seem to have been influenced by selection. For this reason the topology of the tree is still considered reliable (Jobling and Tyler-Smith 2003).

2.2.2 Y-chromosome diversity in America

In 1997 Hammer and colleagues have observed greater Y-haplotypic diversity in Africa for five biallelic polymorphisms and greater Asian diversity in some Y-chromosome lineages (Hammer et al. 1998; 1997). The genetic diversity observed in Asian populations stimulated the researchers to development this kind of studies in human populations distributing in worldwide geographical areas. The identification of founder haplotypes is a key element in the use of genetic data to explore the peopling of the New World. Properties of the distributions of these haplotypes should help evaluate the number of migratory waves and their time of occurrence, and could locate the places in Asia where the migrant populations might have originated. In recent years, most molecular-genetic studies of Native Americans have focused on the clarification of aspects of their Asian origins, by comparing the genetic structure of the two continental groups (Tarazona-Santos et al. 2001). Although genetic approaches to reconstruct the population history of Native Americans have mostly used either autosomal or mtDNA markers, a number of recent analyses have begun to exploit Y-chromosome-specific variation (Ruiz-Linares et al. 1999).

Intensive efforts to clarify the earliest origin of Native Americans, have been produce molecular-genetic studies at more local geographic scales, such as those examining North, Central, or South America, continue been scanty (Tarazona-Santos et al. 2001). The efforts began with the search for useful markers whom where present only in Siberian, Asian and Native American populations, many studies started to appear describing the Y-chromosome genetic diversity in the Americas. Initial studies found high frequencies of ‘‘allele’’ 18 [a complex restriction fragment length polymorphism (RFLP)] pattern defined at locus DYS1 by probe 49-ayf in populations from North and South America (Torroni et al. 1994a). Subsequently a single predominant Y haplotype was detected in North and South Amerinds by combining heteroduplex analysis of sequence variants in repetitive alphoid subunits (called; ah ‘‘allele’’ II) and the tetranucleotide repeat locus DYS394 (allele 186) (Pena et al. 1995; Santos et al. 1996b), these showed the existence of a major northern-Asian founder haplotype, likely migrated from central Siberia (Karafet et al. 1999; Santos et al. 1999). This conclusion was later verified by the discovery of two Y-chromosome SNPs known as “M3” and “M45”, a C-T transition at the DYS199 locus now referred to as “marker M3”

(Underhill et al. 1996; 2000). Extant Native American Y-chromosomes in Siberia and Asia have revealed that M3 lineage was found only on the Chukotka peninsula of far northeastern Siberia, among the Chukchi and the Siberian Eskimos (Karafet et al. 1997; Lell et al. 1997). The most recent ancestors of the M3 lineage have been traced to central southern Siberia (Karafet et al. 1999; Santos et al. 1999). The M3-DYS199 polymorphism occurred after the first populations entered to the Americas (Seielstad et al. 2003), these various polymorphisms have been shown to be associated (Bianchi et al. 1997); thus alleles DYS1 18, ah II, DYS394 186, and DYS199 T could define a major, perhaps single, founder haplotype for all Native American populations (Karafet et al. 1997).

DYS199 know as M3 has been shown to delineate a major Native American founder haplotype (Bianchi et al. 1997; 1998; Lell et al. 1997), in addition to M3 Y-chromosomes revealing a second founding haplotype. The microsatellite variation observed in 11 Y-chromosome-specific polymorphic markers of five Native Colombian and non-Amerindian populations showed that most DYS199-C chromosomes seen in Native Colombians were autochthonous, suggesting that at least two founder haplotypes existed among the initial Native American settlers. These data indicate that the DYS199-T lineage was about 9,000–11,000 years old. The age of the DYS199-C lineage was more difficult to establish but probably fell in the range of 5,000–18,000 years (Ruiz-Linares et al. 1999).

A third Native American haplotype, defined by the RPS4Y/C-T (SNP), was identified in northern Amerind and Na-Dene–speaking populations (Bergen et al. 1999). This haplotype has not been detected in South American natives, with the exception of two Wayus from Colombia (Karafet et al. 1999). The Siberian RPS4Y-T haplogroup has been located in the Lake Baikal region, east of M3 and its progenitors. This has been interpreted as indicating that these Y-chromosome lineages came to the Americas in distinct migrations (Karafet et al. 1999). Finally, Y-chromosomes harboring the Tat polymorphism (haplogroup Tat-C) were found dispersed between native populations of central Asia and northern Europe (Santos et al. 1999), supporting a relatively recent link between these populations (Zerjal et al. 1997). This pattern of limited haplotype diversity has been also observed in mtDNA (Torroni et al. 1994a).

Seielstad et al. (2003), found a novel Y-chromosome SNP that could be reliably dated and occurred before, but sufficiently close in time to the initial human radiation into the Americas, so as to provide a meaningful upper bound on the time of entry. The polymorphism, was call "M242", this mutation arose after the M45 and M74 mutations but before M3, occurred before the first migration into the Americas. M242-T haplotype entered to the Americas very soon after it arose, which indicates a rather more recent entry into the Americas ~15-18,000 ybp, suggested by previous genetic studies, placed the DNA evidence more in line with archeological data, which is characterized by a clear scarcity of credibly sites dated beyond 14,000 ybp. These results do not contradict earlier studies of mtDNA (Torroni et al. 1994a) and the autosomes (Cavalli-Sforza et al. 1994). All findings described above, were a useful markers for the identification of Y-haplotypes originating after the first migration to the Americas or Beringia, however more informative ancient-markers are needed to trace the origin of these Y-chromosomes within Asia and the Americas (Santos et al. 1999).

To continue with the refinement of Native American phylogeny, the ages of others several Y-chromosome lineages present in Siberia and the Americas have also been estimated. F-89 is the oldest SNP in the Eurasian branch of the Y-chromosome phylogeny dates to ~62,000 ybp (Schurr and Sherry 2004) and predates the occurrence of the K-M9 lineage, since it appears in all haplotypes bearing the latter mutation. F-89 is an important SNP because it marks the initial diversification and spread of non-African Y-chromosome lineages into the rest of the world. One of the older lineages in Siberia, K-M9, has been dated at >50,000 ybp (Karafet et al. 2002; Underhill et al. 2000). The antiquity of this lineage is consistent with the presence of this SNP in a considerable majority of Siberian Y-chromosomes (Karafet et al. 1999; Lell et al. 2002). Native American Y-chromosome haplotypes mostly derived from a subsamples of haplogroups present in Siberia like; Q-M3, R1a1-M17, P-M45, F-M89, and C-M130 (Fig. 14 Schurr 2004). Two of them, Q-M3 and P-M45, represent the majority of Native American Y-chromosomes. (Bianchi et al. 1997; Bianchi et al. 1998; Lell et al. 1997; Lell et al. 2002; Santos et al. 1999; Underhill et al. 1996).

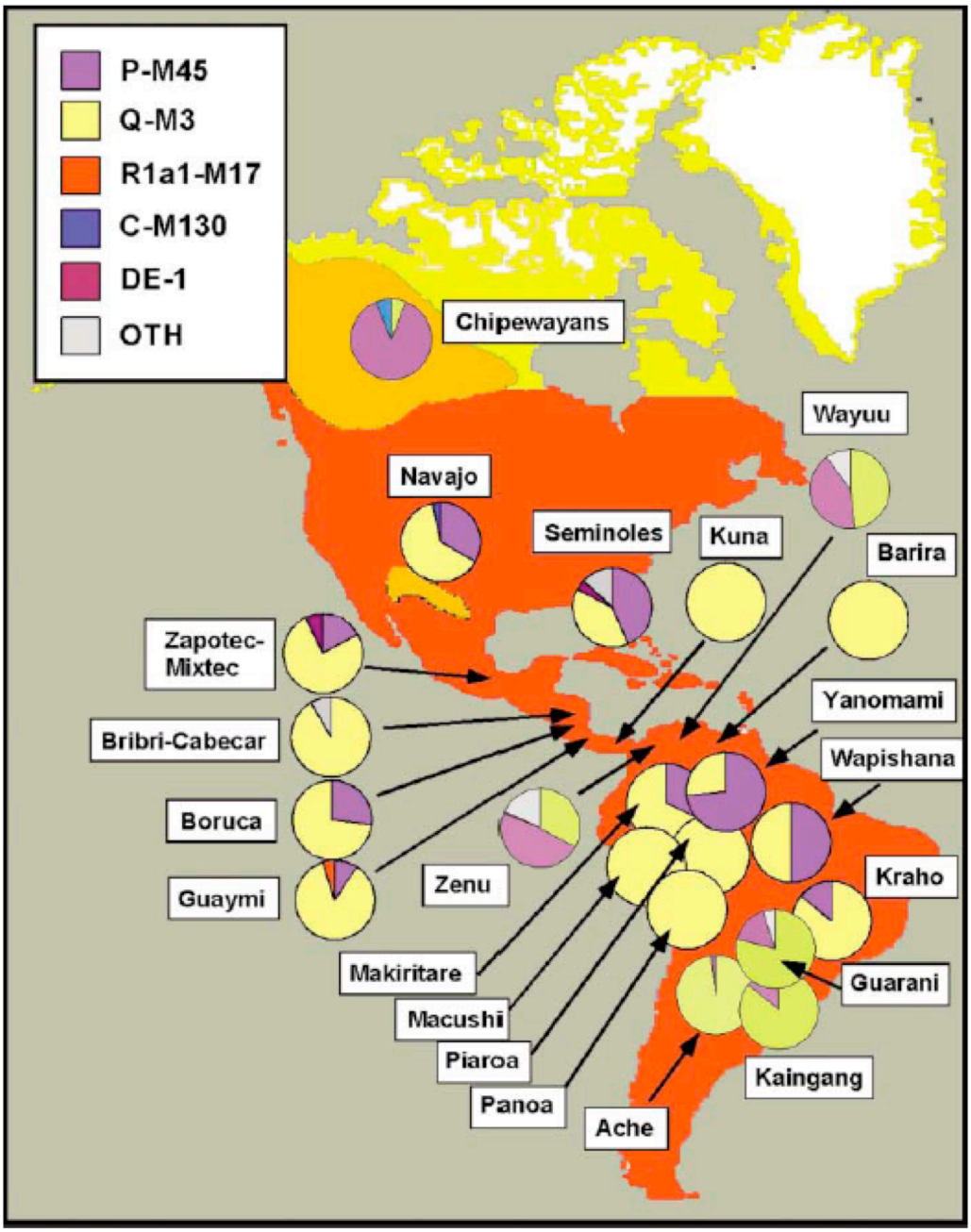

Figure 14: Distribution of Y-chromosome haplogroups in Native American populations (from Schurr et al., 2004) (OTH: other haplogroup)

SNP analysis indicated that three major haplogroups denoted as C, Q, and R, accounted for nearly 96% of Native American Y-chromosomes. Haplogroups C and Q were considered to represent early Native American founding Y-chromosome lineages, however most haplogroup R lineages present in Native Americans most likely came from recent admixture with Europeans. Phylogenetic analyses of STR variation within haplogroups C and Q traced both lineages to a probable ancestral homeland in the

vicinity territory of the Altai Mountains in Southwest Siberia. Divergence dates between the Altai plus North Asians versus the Native American population system ranged from 10,100 to 17,200 years for all lineages, excluding a very early entry into the Americas (Zegura et al. 2004).

The P-M45 lineage is considerably older than the Q-M3 lineage, which derives from it. According with Underhill et al. (2000) SNP mutation rate, P-M45 haplotypes is estimated to be at least 30,000 years old. This degree of antiquity is also reflected by their widespread distribution in Siberia and Eurasia (Lell et al. 2002; Underhill et al. 2000). In addition, phylogenetic analysis showed that this lineage has been present in Siberia long enough to diversify, supported by the presence of two different types of Y-chromosome haplotypes has revealed two distinct sets of P-M45 haplotypes in Native American populations. The first of these (M45a) is more broadly distributed in populations from North, Central and South America, whereas the second (M45b) appears only in North and Central American groups (Bortolini et al. 2003; Lell et al. 2002). Haplogroup P-M45 is also widely distributed among Native American populations and represents 30% of their Y chromosome haplotypes (Bortolini et al. 2002; 2003; Lell et al. 2002; Ruiz-Linares et al. 1999).

Q-M3 haplotypes appear at high frequencies in most Native American populations and are distributed in an increasing north-to-south cline within the continent. In addition the STR data from Q-M3 haplotypes also reveal significant differences in haplotype distributions between North-Central and South American populations, suggesting different population histories in the two major continental regions (Bianchi et al. 1997; Bianchi et al. 1998; Lell et al. 1997; Lell et al. 2002; Santos et al. 1999; Underhill et al. 1996). A pattern also seen in the gamma globulin (GM), major histocompatibility locus antigen (HLA), and nuclear genetic data from Native American groups (Erlich et al. 1997; Rothhammer et al. 1997; Schanfield 1992). Many efforts have been made to estimate the age of Q-M3 haplogroup, given that it appears to signal the initial entry of ancestral populations into the New World. According with the SNP rate mutation proposed by Underhill and colleagues (2000) the age for this haplogroup was ~13,800 ybp (Schurr and Sherry 2004). The estimates made with STR mutation rates have ranged from 30,000–7,600 ybp (Bianchi et al. 1997; Hammer et al. 1998; Karafet et al. 1999; Underhill et al. 1996). Examples of some proposals dating; 30,000-2,100 ybp

(Underhill et al. 1996); 11,000–9,000 ybp (Ruiz-Linares et al. 1999); 7,650±5,000 ybp (Karafet et al. 1999); and Bianchi et al. (1998) whom estimated an average age of 22,770 ybp. The mutational age of Q-P36*, the marker which define the entire Q lineage, is 17,700±4,820 ybp (Hammer and Zegura 2002).

Haplogroup Q is present in about 76% of Native Americans (Zegura et al. 2004), similar to the situation with mitochondrial haplogroups, the Y-chromosome haplogroups (C and Q) common in the Americas are uncommon elsewhere, with the exception of northern Asia, where they reach frequencies of 28% and 18%, respectively (Mulligan et al. 2004). Since the identification of the SNP (Q-M242), the efforts to fine newly Native American haplogroups and date them were continued increased. The Q-M242 marker occurred within haplogroup P-M45 in Central Asia prior to the emergence of the Q-M3 SNP and the expansion of its haplotypes into the Americas. Q-M242 marker may better define the initial entry into the Americas than Q-M3. Base on STR mutation rates, the age of M242 haplotypes in the Americas was calculated at ~18,000–15,000 ybp (Bortolini et al. 2003; Seielstad et al. 1999). Whit the increase of genetic studies focus in particular areas of the Americas, now is possible to identify the microsatellite diversity and distribution of a Y-lineage specific, such is the case of Q-M19 identified only in South America, indicates that certain Native American populations have been isolated since the initial colonization of the region, suggesting an early onset for tribalization of this populations (Bortolini et al. 2003; Ruiz-Linares et al. 1999).

Most of the remaining Y-chromosome haplotypes belong to one of several different haplogroups, and comprise only 5% of Native American Y-chromosomes. In general, these haplotypes have limited distributions in the Americas. C-M130 haplotypes have only been detected in the Na-Dene-speaking Tanana, Navajo, and Chipewyan, and the Amerindian Cheyenne (Bergen et al. 1999; Bortolini et al. 2002; Bortolini et al. 2003; Karafet et al. 1999). C-M130 lineage is younger than the F-M89 or K-M9 lineages, having been dated at ~30,000–25,000 ybp (Karafet et al. 1999; Underhill et al. 2000). Its age is generally consistent with its broad distribution in East and Southeast Asia, in which it appears to have originated, and its haplotypic diversity in eastern Siberian and Asian populations (Lell et al. 2002). Age estimates for the entire C lineage and the

Native American-specific C-P39 are 27,500±10,100 and 2,550±1910 ybp respectively (Hammer and Zegura 2002; Karafet et al. 2002).

R1a1-M17 haplotypes have only been observed in the Guaymi (Ngöbe) a tribe from Costa Rica (Lell et al. 2002) neither of these haplogroups has been detected in South Native American populations. These haplotypes constitute a distinct branch within R1a, and are not especially common in Siberian populations, although occurring across a broad geographic area (Lell et al. 2002). The estimated age of R1a1-M17 haplogroup is rather intriguing, using the Underhill et al. (2000) SNP evolution rate, an age of 13,800 ybp was obtained to this lineage, falling into the very end of the Last Glacial Maximum (Schurr and Sherry 2004). The data suggest that R1a1-M17 haplotypes did not emerge in Siberia until after the Americas had already been colonized and were brought to the Americas through a secondary expansion of ancient Asian populations, along with C-M130 and P-M45b haplotypes (Lell et al. 2002). The limited distribution of these minor haplogroups suggests that they were brought to the New World as part of a secondary expansion of ancient Asian populations (Schurr 2004).

Some studies about the phylogeny, phylogeography of Native American populations, which have provided important insights to the reconstruction of the Peopling of the Americas include: (Bianchi et al. 1997; 1998; Bortolini et al. 2002; 2003; Bravi et al. 1997; Hammer 1995; 1997; Karafet et al. 1997; 2002; 1999; Lell et al. 1997; 2002; Mesa et al. 2000; Pena et al. 1995; Rothhammer and Moraga 2001; Ruiz-Linares et al. 1999; 1996a; Santos 1995; 1999; 1996b; Scozzari et al. 1997; Seielstad et al. 2003; Su et al. 1999; Tarazona-Santos and Santos 2002; Underhill et al. 1997; 1996; 2001; Vieira et al. 2002; Wang et al. 2007; Zegura et al. 2004).

2.2.3 Routes to America: Y-chromosome

To characterize NRY variation in Native Americans, researchers have employed a number of different SNPs and STRs loci to define the paternal lineages present within them (Bianchi et al. 1997; 1998; Hammer et al. 1997; Karafet et al. 1997; 1999; Lell et al. 1997; 2002; Pena et al. 1995; Underhill et al. 1997; 1996; 2001; 2000). However, these research groups have not consistently used the same combination of genetic markers in their studies, which lead to alternative and sometimes confusing

nomenclatures for NRY haplotypes and haplogroups. The synthesis of these data has resulted in a consensus nomenclature based on known SNPs Y Chromosome Consortium (YCC) (2002).

From the recompilation of the aforementioned studies they have focused on the Y-chromosome as an independent source of genetic information about the peopling of the Americas (Mulligan et al. 2004). To ascertain the genetic structure of the Asian and Native American populations have been necessary to make inferences about; 1) the population size of the first colonists (Pena et al. 1995; Schurr et al. 1990; Ward et al. 1991); 2) place(s) of origin; and 3) number of migration "waves" (Karafet et al. 1999; Merriwether et al. 1995; Santos et al. 1999; Torroni et al. 1993a), are issues debated by geneticists, archaeologists, biological anthropologists, and historical linguists. Moreover, they have attempted to date the Most Recent Common Ancestors of Asian-Amerindian molecular lineages (Forster et al. 1996; Torroni et al. 1993b) and the migration waves by dating of the Native American–specific lineages (Underhill et al. 1996) or of the population expansion following the colonization of the new continent (Bonatto and Salzano 1997b). However, these inferences concerning the aforementioned genetic or demographic events are often not consistent among themselves or with available linguistic, archeological, and paleoanthropological data (Crawford 1998a; Powell and Neves 1999).

The first studies based on Y-chromosome diversity have shown a consistent pattern of scarce haplotypic diversity in Native American populations (Ruiz-Linares et al. 1999), also the first findings which suggested almost no or very restricted variability were contradicted by more recent studies which documented more variation, although it is generally less marked than those found in the mtDNA or autosome regions (Salzano 2002).

2.2.3.1 Place of origin and data entry to the Americas

A variety of genetic dating techniques, including mutational ages, mismatch distribution expansion dates, coalescence ages, and population divergence dates, have been employed to estimate the date of colonization. Cavalli-Sforza et al. (1994) used autosomal data as a basis for their estimate of 32,000 ybp for the divergence of the

Native American population system. Stone and Stoneking (1998) discussed a number of studies based on mtDNA that favored colonization dates before 20,000 ybp and presented their own evidence for a 23,000–37,000 population expansion. Although mtDNA haplogroup lineages A, C, and D have generally yielded dates earlier than 20,000 ybp, Schurr (2000) gave a restriction fragment length polymorphism (RFLP) derived date of 17,700–13,500 years ago for haplogroup B, an estimate consistent with the earlier claims of Torroni et al. (1994a) and Wallace (1997) that haplogroup B in the Americas was considerably younger than the other three lineages. A possible dispersal from Eurasia to the Americas has also been dated based on haplogroup X. Brown et al. (1998) proposed that this range expansion took place either between 36,000 and 23,000 ybp or 17,000 and 12,000 ybp. Earlier dating attempts using Y chromosome data have lacked precision (Zegura et al. 2004).

One of the first attempts to reconstruct ancient migration patterns within Asia region was carried out by Su and collaborators (1999); their results based on a set of Y-chromosome biallelic and microsatellite markers shown that southern populations in eastern Asia were much more polymorphic than northern populations, which have only a subset of the southern haplotypes. This pattern indicates that the first settlement of modern humans in eastern Asia occurred in mainland Southeast Asia during the last Ice Age, coinciding with the absence of human fossils in eastern Asia, 50,000–100,000 ybp. After the initial peopling, a great northward migration extended into northern China and Siberia (Su et al. 1999).

Santos and colleagues (1999) studied in 306 worldwide men sample Y-chromosomal polymorphisms to give more insights about Pleistocene male migrations to the Americas. With the obtaining of 32 haplotypes constructed thought the variation found in 30 distinct polymorphic sites, was possible to traced back the major Y haplotype present in most Native American in recent ancestors common with Siberians, namely, the Kets and Altaians from the Yenissey River Basin and Altai Mountains, respectively. The next common ancestor gave rise also to Caucasoid Y chromosomes, probably from the central Eurasian region. These authors suggests a predominantly central Siberian origin for Native American paternal lineages for those who could have migrated to the Americas during the Upper Pleistocene (Santos et al. 1999). This study traces the major Native American Y-chromosome haplotype to the immediate ancestor shared with

present-day Siberians and to an older common ancestor shared with Caucasoids (Europeans and Indians).

The Americas was the last continent to be settled by humans, but many details of the earliest occupation remain poorly understood. Proposals for the date of first entry fall into two ranges, one suggesting a very early occupation ~30,000–40,000 ybp, and the other favoring dates ~13,000 ybp, when the polar climate was again hospitable. Seielstad et al. (2003) present Y-chromosomal data that support strongly the latter dates, probably closer to 15,000-18,000 ybp, which was congruent with the paucity of sites and skeletal material credibly dated to 114,000 ybp has been a consistent puzzle for those who would posit an extremely ancient history of human occupation in the Americas (Dillehay 2000).

2.2.3.2 Single founding haplotype and single wave

Studies based on the nonrecombining portion of the Y-chromosome have revealed a single major paternal haplotype at relatively high frequencies in Native American populations from Alaska to Argentina (Bianchi et al. 1997; Karafet et al. 1997; Lell et al. 1997; Santos et al. 1996a; Santos et al. 1999; Tarazona-Santos and Santos 2002; Underhill et al. 1996). These findings generally have been interpreted to support a single origin for the three major New World linguistic groups (Karafet et al. 1997; Underhill et al. 1996). Bianchi et al. (1998) made one of the first proposals suggested that the old Native populations derived from an ancestral founder haplotype dated at 22,770 ybp been in good agreement with mtDNA estimates. On the other hand Karafet and (1997) suggested that because of the paucity of Y-chromosome polymorphisms sampled in Native American and Asian populations, it was premature to infer a single paternal founder lineage and to conclude that this haploid system unequivocally supports a single-origin model for Native Americans. The first available data was interpreted as indicating that the colonizers of the New World carried a single founder haplotype, however these early studies have been based on a few, mostly complex polymorphisms of insufficient resolution to determine whether observed diversity stems from admixture or diversity among the colonizers (Carvalho-Silva et al. 1999).

Zegura et al. (2004) provide a more fine-grained paternal genetics perspective in order to resolve important questions about the American peopling. These authors used a larger Y-chromosome database including more microsatellite and SNPs markers to refine their previous analyses (Karafet et al. 1997; 1999) of founder versus admixture-derived lineages in the Americas, to give insights about the most probable area of the postulated Asian geographic source of Native American Y-chromosomes; estimate the time of divergence between the Native American population system(s) and various Asian population systems; and address the most likely number of migrations detected so far by Y chromosome data from Native Americans. Their conclusions were similar to recent mtDNA studies; finding Y-chromosome support for a single-migration model, with a potential common source for all major Native American Y-chromosome and mtDNA founding lineages in the Altai Mountains of Southwest Siberia. Because none of their population divergence date estimates exceed 17, 200 ybp, they favored a late entry model (i.e., 20,000 ybp) that postdates the Last Glacial Maximum (LGM). Finally, it has primarily been the interaction of genetic drift and gene flow both on Beringia and in the Americas that has produced the suite of contemporary Native American Y-chromosome haplogroup frequencies found in their survey (Zegura et al. 2004).

2.2.3.3 Two founding haplotypes and two waves

The suggestion of a single founding haplotype for the Americas (see, among others; Bianchi et al. 1997) has been substituted by the notion that at least two Y-chromosome lineages contributed to the early peopling of the Americas (Salzano 2002). Some authors support this new approach; (Bortolini et al. 2003; Karafet et al. 1999; Lell et al. 2002; Rodriguez-Delfin et al. 1997; Ruiz-Linares et al. 1999).

Ruiz-Linares and collaborators (1999) suggested that the available data indicated that most of the constituent alleles of the putative single Y founder haplotype (DYS199) were either absent or seen at very low frequencies in Asia. Their results based on 11 Y-chromosome-specific polymorphic markers in five Native Colombian populations, and the comparisons with non-Amerind Y-chromosomes showed that most DYS199-C chromosomes seen in Native Colombians were autochthonous, suggesting at least two founder haplotype existed among the initial Native American settlers, indicating that the

DYS199-T lineage was about 9,000–11,000 years old. The age of the DYS199-C lineage was more difficult to establish but probably lies in the range of 5,000–18,000 years, the polymorphisms M19-A was detected only in two South American populations, considerable haplotypic diversity associated with this marker was observed, suggesting a wider population distribution in the South region (Ruiz-Linares et al. 1999).

The expansion of Karafet et al. (1999) database lead them to a different conclusion, postulated that there was at least two major New World paternal founder haplotypes arriving to the New World via separate migratory processes, for this reason more than single migration could happen. The observed contrasting distribution patterns of the two major candidate founder haplotypes in Asia and the New World, as well as the results of a nested cladistic analysis, suggested the possibility of more than one paternal migration from the general region of Lake Baikal to the Americas (Karafet et al. 1999). These new results favored a two-migration scenario, a proposal later supported by Lell et al. (2002) whom surveyed Y-chromosome SNP and microsatellite variation in a large sample of native populations of Siberia and the Americas geographically dispersed. In this analysis was accepted that two major male migrations peopled the Americas: one starting from southern Middle Siberia, giving rise to haplogroups M3 and M45a in North, Central, and South America, and a second migration from eastern Siberia, which brought Y-chromosome lineages RPS4Y-T and M45b to the Na-Dene and Amerinds of North and Central America. These results were correlate well with previous conclusions about the maternal migrations that brought mtDNA haplogroups A, B, C, and D to the Americas, suggesting that both males and females came to the New World in at least two coherent waves of migration, the first arising in southern Middle Siberia and the second arising later from southeastern Siberia (Lell et al. 2002).

With the development of the “two migratory waves” approach others authors give more support to the “single migration wave”, like Tarazona-Santos and Santos (2002) argued that the claim of a second major migration was not well grounded and was not necessary to explain the distribution of the haplogroups S4Y and M45b in Native American populations like previously proposed by Lell et al. (2002), otherwise the first wave coincides with their previous findings (Santos et al. 1999) and that of Karafet et al. (1999). These authors thought that Lell et al. (2002) have not provided any solid

evidence about the existence of a second "major migration" and think that the simplest way to reconcile the current available molecular genetic data, which was mainly derived from Y-chromosomes and mtDNA (Bonatto and Salzano 1997a, b), was to assume a single major migration from Siberia, contributing to the gene pool of Native American populations (Tarazona-Santos and Santos 2002).

In the study performed by Bortolini and (2003), based on the Y-chromosome diversity of eight SNPs and six STRs of Native American and Mongolian populations; The distribution, relatedness, and diversity of Y lineages in Native Americans indicate a differentiated male ancestry for populations from North and South America, strongly supporting a diverse demographic history for populations from these areas. These data was consistent with the occurrence of two major male migrations from southern-central Siberia to the Americas (with the second migration being restricted to North America) and a shared ancestry in central Asia for some of the initial migrants to Europe and the Americas. The age estimates based on this genetic diversity placed the initial settlement of the American continent at ~14,000 ybp, in relative agreement with the age of well-established archaeological evidence (Bortolini et al. 2003).

2.2.3.4 Other models

Coastal route: Recent genomic studies have produced detailed genome wide descriptions of genetic diversity and population structure for a wide variety of human populations, both at the global level (Prugnolle et al. 2005; Rosenberg et al. 2002; Shriver et al. 2005; Witherspoon et al. 2006) and for individual geographic regions, including East Asia (Kim et al. 2005), Europe (Belle et al. 2006; Seldin et al. 2006), and India (Rosenberg et al. 2006). The first such analysis of indigenous populations from the American continent landmass, using 678 microsatellites genotyped in 530 individuals from 29 Native American populations sampled from North, Central, and South America and analyzed them jointly with similar data available in 54 other indigenous populations worldwide was carried out by Wang and collaborators (2007). In this study the researches reported evidence of: (1) a higher level of diversity and lower level of population structure in western South America compared to eastern South America, (2) a relative lack of differentiation between Mesoamerican and Andean

populations, (3) a scenario in which coastal routes were easier for migrating peoples to traverse in comparison with inland routes, and (4) a partial agreement on a local scale between genetic similarity and the linguistic classification of populations. These findings offer new insights into the process of population dispersal and differentiation during the peopling of the Americas (Wang et al. 2007).

Recurrent gen flow: To offer an alternative approach Ray et al. (2010) performed a statistical assessment making some simulations in which the probability of alternative migration scenarios and to estimate key demographic parameters associated with them, were used an approximate Bayesian computation framework to analyze a data set of 401 autosomal microsatellite loci typed in 29 native American populations. The results showed that a single, discrete, wave of colonization was highly inconsistent with observed levels of genetic diversity and a scenario with two discrete migration waves was also not supported by the data. The current genetic diversity of Amerindian populations was best explained by a third model involving recurrent gene flow between Asia and America, after initial colonization (see Fig. 15). They estimate that this colonization involved about 100 individuals and occurred some 13,000 ybp, in agreement with well established archeological data (Ray et al. 2010). Similar studies were previously performed in order to explore the complex demographic history typical of natural populations (e.g., Fagundes et al. 2007; Miller et al. 2005).

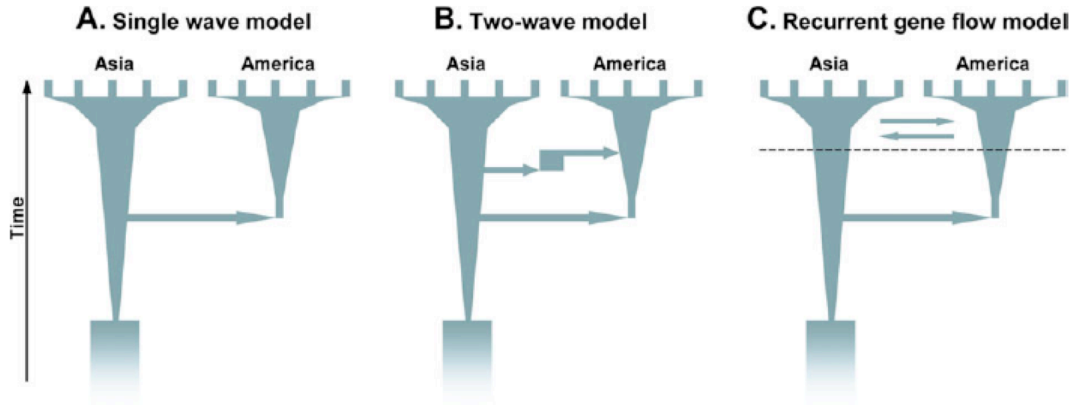

Figure 15: Alternative models for the colonization of the Americas by Ray et al. (2010)

2.2.4 Local Diversity: Y-chromosome studies in Mexican populations

The Y-chromosome genetic diversity has been studied at a local level, to see if the distribution patters are similar or correspond to different demographic and historical movements of the old Native American populations. Like Tarazona-Santos et al. (2001) described a different pattern of the genetic variability in southern populations; their results showed that; 1) the Andean populations exhibit significantly higher levels of within-population variability than do the eastern populations of South America; (2) the spatial-autocorrelation analysis suggests a significant geographic structure of Y-chromosome genetic variability in South America, although a typical evolutionary pattern could not be categorically identified; and (3) genetic-distance analyses and the analysis of molecular variance suggested greater homogeneity between Andean populations than between non-Andean ones. They proposed a model for the evolution of the male lineages of South Amerindians that involves differential patterns of genetic drift and gene flow. In the western part of the continent (Andean area), populations have relatively large effective sizes and gene-flow levels among them, which has created a trend toward homogenization of the gene pool. On the other hand, eastern populations (settled in the Amazonian region, the central Brazilian plateau, and the Chaco region) have exhibited higher rates of genetic drift and lower levels of gene flow, with a resulting trend toward genetic differentiation. This model was consistent with the linguistic and cultural diversity of South Amerindians, the environmental heterogeneity of the continent, and the available paleoecological data (Tarazona-Santos et al. 2001).

The study of genetic significant points which contribute on the understanding of the local diversity, to facilitate the historical reconstruction of the Americas has been development in populations of the three main areas of the continent: North (Barrot et al. 2007; Budowle et al. 2005; Malhi et al. 2008; Zlojutro et al. 2009), Central (Ascunce et al. 2008; Ruiz-Narvaez et al. 2005) and South (Altuna et al. 2006; Bailliet et al. 2009; Bravi et al. 1997; Crossetti et al. 2008; Demarchi and Mitchell 2004; Dos Santos et al. 2009; Goncalves et al. 2008; Gonzalez-Andrade et al. 2009; 2007; 2008; Hunemeier et al. 2007; Leite et al. 2008; Marrero et al. 2007a; 2007b). Local studies not only describe the current genetic diversity of an specific place, but also represents a excellent tool to corroborate some historical or migration hypothesis, like the study carried out by Malhi and collaborators (2008) in which was analyzed the geographic distribution of Y-

chromosomes among Native North Americans to test the Southern Athapaskan migration hypothesis. They found consistent with studies of other genetic systems (mtDNA), identifying close relationships among Y chromosomes in Athapaskans from the Subarctic and the Southwest, suggesting that a small number of proto-Apachean migrants from the Subarctic founded the Southwest Athapaskan populations. Their results suggest that the Y-chromosome population structure of Native North America was significantly altered by European admixture, resulted in a decreasing gradient of haplogroup R from Northeastern to Southwestern North America (Malhi et al. 2008).

To analyze the origins and dispersion of modern humans from Africa to the rest of the new continents, the knowledge of genetic structure of the current native populations has been one of the main sources of information. However, there are large areas of the world for which such genetic information is unavailable. One of the most striking cases is that of cultural area called Mesoamerica (for definition, see section 3). There have been few molecular studies specifically addressing Mesoamerican groups in terms of populations. Most have centered on autosomal markers (Bonilla et al. 2005; Buentello-Malo et al. 2003; Ibarra-Rivera et al. 2008; Rangel-Villalobos et al. 2000) and mitochondrial DNA (Green et al. 2000; Peñaloza-Espinosa et al. 2007; Sandoval et al. 2009; Torroni et al. 1994a), although some studies have recently been published on the Y-chromosome (Gorostiza et al. 2007; Paez-Riberos et al. 2006; Rangel-Villalobos et al. 2008).

The studies of Y-chromosome diversity in Mexican populations have been predominated by studies on Mestizo populations. Over the last 500 years, admixture among (mainly) Natives, Europeans, and Africans, has come to shape the present-day gene pool of Mexicans, particularly Mestizos (Cerda-Flores et al. 2002b; Felix-Lopez et al. 2006; Gorostiza et al. 2007; Gutierrez-Alarcon et al. 2007; Hernandez-Gutierrez et al. 2005; Licea-Cadena et al. 2006; Luna-Vazquez et al. 2008; 2005; 2003; Rangel-Villalobos et al. 2001a; 2001b; 2000; Rubi-Castellanos et al. 2009a; 2009b; Salazar-Flores et al. 2009; Torres-Rodriguez et al. 2006), who represent about 93% of the total Mexican population (Rubi-Castellanos et al. 2009b). The National Institute of Anthropology defines a Mestizo as a person born in Mexico, who has a Spanish derived last name, and has a family with Mexican ancestors who can be traced back to the third generation (Sanchéz-Serrano 1996). In 1570, the Mestizos constituted <0.5%, but in

1810 they comprised nearly 40% of the total Mexican population (Aguirre-Beltrán 1989) and currently make up about 93% of the total population, in addition to Mexican ethnic groups (INEGI). One of the most accepted models for describing the current genetic diversity of Mexican Mestizos is the trihybrid model of admixture, which presents different admixture components depending on the geographical area (Lisker et al. 1996); this model is represented in a tripolar-diagram in which the edges are very narrow, indicating that the number of individuals who are ‘‘genetically pure’’ for any of the three racial components is insignificant (Gorodezky et al. 2001).

Fortunately the above landscape began to change, since 1980’s some researches started to make some inferences about the genetic diversity within the Native Americans (Barrot et al. 2005; 2007; Felix-Lopez et al. 2006; Gonzalez-Andrade et al. 2008; Licea-Cadena et al. 2006; Paez-Riberos et al. 2006; Rangel-Villalobos et al. 2000), due to the difficulty to carry out samplings with indigenous populations, the majority of first studies were focus on Mestizos. One of the main proposes was to describe the current genetic variation in Mexican populations, in order to answer questions about demographic history and migration process. Cerda-Flores and started to work with classical genetic markers in Mestizo populations from the northern part of Mexico (Cerda-Flores and Garza-Chapa 1989; Cerda-Flores et al. 1987). Subsequently the used of Y-chromosome markers like STRs were incorporated to the genetic studies, to describe the maximum likelihood estimate admixture in Mexican Mestizo populations (Cerda-Flores et al. 2002a) and the genetic admixture in the same populations (Cerda-Flores et al. 2002b). Other studies based on STRs Y-chromosome markers, have been done in Mexican Mestizo only with forensic interests, mainly in the metropolis (Luna-Vazquez et al. 2008; Luna-Vazquez et al. 2005; Luna-Vazquez et al. 2003), and other cities (Barrot et al. 2005; Barrot et al. 2007; Gutierrez-Alarcon et al. 2007; Licea-Cadena et al. 2006; Martinez-Gonzalez et al. 2005).

3 Mesoamerica

The term Mesoamerica refers to a geographical area occupied by a variety of ancient cultures that shared religious beliefs, art, architecture, and technology that made them unique in the Americas for three thousand years–from about 1500 B.C. to 1519 A.D.– the time of European contact (Kirchhoff 1943; López-Austin and López- Luján 2001). The region spans from the current central-south Mexico to some areas of Central America. The prehistory of the region inferred from human and archeological remains supports the existence in time and space of a continuous human presence for at least 11,000 years. Mesoamerica is one of our planet's six cradles of early civilization. Many aspects of the ancient cultures of Central-South Mexico, Guatemala, Belize, Honduras, El Salvador, Nicaragua and Costa Rica continue to the present and several of these cultural inventions and traits have spread throughout the world (Kirchhoff 1943).

3.1 General background

Paul Kirchhoff was the first who introduced the term “Mesoamerica,” he described it as a cultural zone where the indigenous inhabitants spoke as many as sixty different languages, but were united by a common history and shared a specific set of cultural traits that made their civilization unique in the world. Among the most significant was the development of both pictographic and hieroglyphic writing as well as the production of books constructed from animal hide or *amate paper*. A divinatory calendar of 20 x 13 days *(tonalpohualli)*, calculated together with a solar calendar of 365 days, is widely regarded as being more accurate than those of many other ancient civilizations throughout the world. Mesoamerican architecture was also unique and distinguished by preferences for stepped pyramids, stucco floors, and ballcourts. Finally, and perhaps most importantly for development in the Americas, was the cultivation of specialized foods including maize, beans, and squash, together with cacao (chocolate) and fermented beverages made from maguey. Many of these foods became the staples of a world-wide diet we continue to thrive on today (Carmack et al. 1996; Kirchhoff 1943).

Mesoamerica once boasted a population of over 50 million people living throughout an area roughly contiguous with the modern nations of México, Guatemala, Honduras,

Belize, and El Salvador. The principal factors that contributed to the origins of Mesoamerica's civilizations are debated by scholars working throughout the region today but most believe that the inequalities between rulers and ruled, a condition of all early civilizations, developed with the consolidation of social power by chiefs who coordinated agricultural labor and supervised the storage and redistribution of crop surpluses that ensured group survival against drought and other natural calamities. Once foods had been domesticated as staples, they would have been available to any population interested in shifting from foraging to agriculture and sedentary life. Agriculture can support large populations but it demands ever more intensive forms of cultivation. Mesoamerican people met the challenge by developing a wide variety of agricultural techniques, from terracing mountain sides to digging canals or even creating artificial wetlands. Mesoamericans domesticated dogs and turkeys, but wild animals like deer were naturally drawn to gardens where they could be easily captured and tethered. The cultivation of fruit trees attracted a wide variety of tropical birds whose colorful plumage was coveted for displaying wealth and prestige (Palerm 1972).

The successful development of agricultural intensification allowed Mesoamerican civilizations to build surpluses that not only insured themselves against catastrophes like drought but also led to social specialization and ultimately to the creation of specialized classes of merchants, warriors, artisans, and aristocracies of kings. Mesoamerican peoples were sufficiently impressed with their accomplishments as agricultural engineers that they even commemorated foods, like squash for example, in artistic masterpieces of precious greenstone. As "big men" were called upon to sponsor tribal feasts, they enlarged their own quarters and expanded the open yards surrounding their dwellings to accommodate more of their clients. Eventually prestige came to be marked by house size, and chiefs used their dwellings to display their elevated status by literally raising their homes on artificial platforms and restricting surrounding space to specialized ritual activities. From these humble beginnings came the magnificent palaces, temples, and plazas that continue to awe us today. Click on Image for more detail (López-Austin and López- Luján 2001; Palerm 1972).

3.2 Archeological record

Mesoamerican chronology divides the history of pre-Columbian Mesoamerica into a number of named successive eras or periods, from the earliest evidence of human habitation through to the early Colonial period, which followed the Spanish colonization of the Americas. These are known, with slight variation depending on region, as the Paleo-Indian, the Archaic, the Preclassic (or Formative), the Classic, and the Postclassic periods. The last three periods, representing the core of Mesoamerican cultural fluorescence, are further divided into two or three sub-phases. Most of the time following the arrival of the Spanish in the 16th century is grouped into the Colonial period (Weaver Muriel 1993).

The differentiation of early periods (i.e., up through the end of the Late Preclassic) generally reflects different configurations of socio-cultural organization that are characterized by increasing socio-political complexity, the adoption of new and different subsistence strategies, and changes in economic organization (including increased interregional interaction). The Classic period through the Postclassic are differentiated by the cyclical crystallization and fragmentation of the various political entities throughout Mesoamerica. Each period showed a particular architecture, nowadays is capture in the cultural records preserved in diverse Mesoamerican archeological sites (see Table 1).

Table 1: Chronological Summary of Mesoamerican Cultures

Period	Timespan	Important cultures and cities
Paleo-Indian	10,000-3,500 BC	Honduras, Guatemala; Belize (obsidian and pyrite points) Iztapan
Archaic	3,500-1800 BC	Agricultural settlements, Teotihuancan
Preclassic (Formative)	BC 2,000-259 AD	Unknown culture in La Blanca and Ujuxte, Monte Alto Culture
Early Preclassic	BC 2,000-1,000	Olmec area: San Lorenzo Tenochtitlan; Central Mexico: Chalcatzingo; Valley of Oaxaca: San José Mogote; The Maya Area: Nakbe, Cerros
Middle Preclassic	BC 1,000-400	Olemc area: La Venta, Tres Zapotes; Maya area: El Mirador, Izapa, Lamanai, Xunantunich, Nja Tunich, Takalik Abaj, Kamimaljuyú, Uaxactun; Valley of Oaxaca: Monte Alban
Late Preclassic	BC 400-200 AD	Maya area: Uaxactun. Tikal, Edzná, Cival, San Bartolo, Altar de Sacrificios, Piedras Negras, Ceibal, Rio Azul; Central Mexico: Teotihuacan; Gulf Coast: Epi-Olmec culture; Western Mexico: Shaft Tomb Tradition
Classic	200-900 AD	Classic Maya Centers, Teotihuacan, Zapotec
Early Classic	200-600 AD	Maya area: Calakmul, Chunchucmil, Copán, Naranjo, Palenque, Quiriguá, Tikal, Uaxactun, Yaxha; Central Mexico: Teotihuacan apogee; Zapotec apogee; Western Mexico: Teuchitlan tradition
Later Classic	600-900 AD	Maya area: Uxmal, Toniná, Cobá, Waka, Pusilhá, Xultún, Dos Pilas, Cancuen, Aguateca; Central Mexico: Xochicalco, Cacaxtla; Gulf Coast: El Tajín and Classic Verazruz culture; Wertern Mexico: Teuchtitlan tradition
Terminal Classic	800-900/1000 AD	Maya area: Puuc sites-Uxmal, Labna, Sayil, Kabah
Postclassic	900-1519 AD	Aztec, Tarascans, Mixtec, Totonac, Pipil, Itzá, Ko'woj, Kiche', Poqomam, Mam
Early Postclassic	900-1200 AD	Cholula, Tula, Mitla, El Tajín, Tulum, Topoxte, Kaminanljuyú, Joya de Cerén
Later Postclassic	1200-1519 AD	Tenochtitlan, Cempoala, Tzintzuntzan, Mayapán, Tího, Utatlán. Iximche, Mixco Viejo, Zaculeu
Post Conquest	Until 1697 AD	Central Peten: Tayasal Zacpeten

In blue: Principal Eras.

3.3 Linguistic classification

The similarities noted between many Mesoamerican languages and the millennia in which these native speakers were engaged in contact produced that the languages began to change and show similarities with one another. This has resulted in Mesoamerica evolving into a linguistic area of diffusion, a *sprachbund*, where most languages, even though they have different origins share some important linguistic traits (Campbell et al. 1986). These authors employed a rigid linguistic analysis which demonstrated that the similarities between a numbers of languages were indeed considerable, with the conclusion that their origins were very likely caused by diffusion rather than inheritance the standard criteria for defining a *sprachbund* (Campbell et al. 1986). Five traits in particular were shown to be widely attested among the languages, with boundaries coinciding with that of the Mesoamerican region and having a probable origin through diffusion. These authors compared the five traits with the traits defining other linguistic areas considered to be well established like the East Asian-*sprachbund* and Balkan linguistic union, concluding by comparison that the proposed Mesoamerican Linguistic Area could indeed be considered a well founded area, argued that some of the discarded traits might also be taken into consideration as strengthening the proposal but, they were not sufficient by themselves to act as foundation and other well documented traits of a more ethno-linguistic characters might not be considerable as truly linguistic traits but rather cultural (Campbell 1997; Campbell et al. 1986).

Mesoamerican languages are the indigenous languages to the Mesoamerican cultural area; this area is characterized by extensive linguistic diversity containing several hundred different languages and seven major language families. This *sprachbund* is defined by an array of syntactic, lexical and phonological traits as well as a number of ethnolinguistic traits found in the languages of Mesoamerica, which belong to a number of linguistic families, such as Uto-Aztecan, Mayan, Totonacan, Oto-Manguean and Mixe-Zoquean languages as well as some language isolates and unclassified languages known to the region (see Fig. 16).

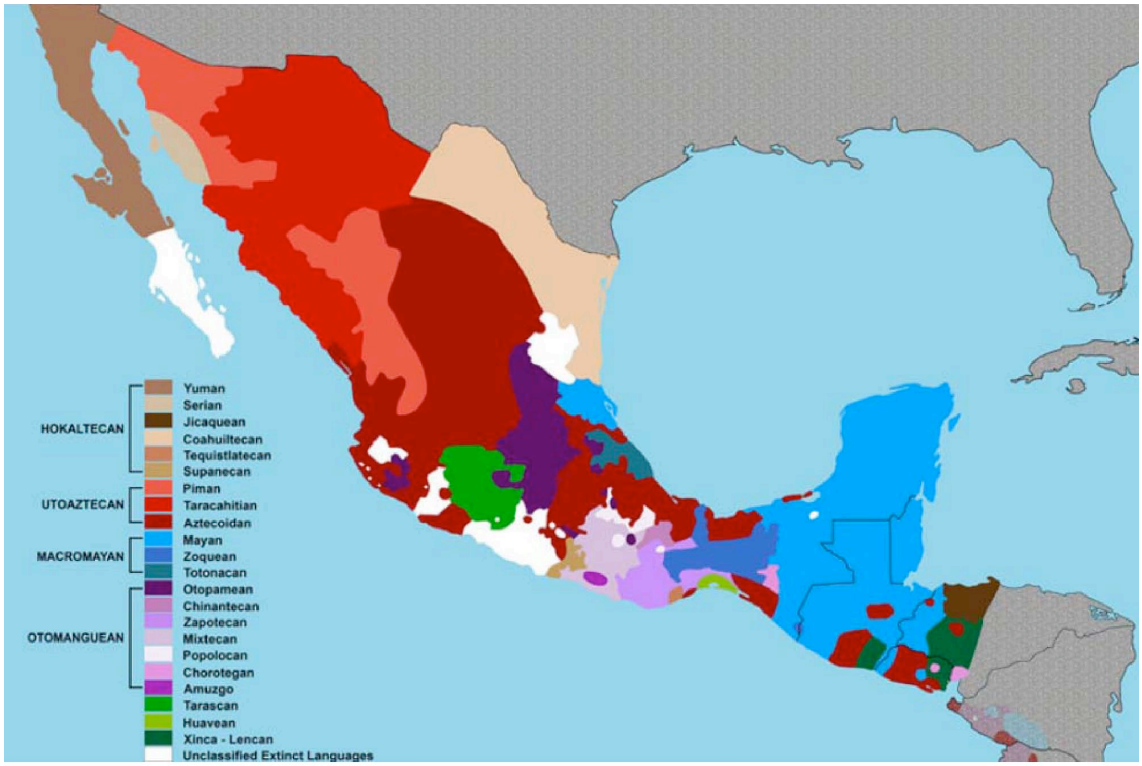

Figure 16: Linguistic Map based on the previous linguistic maps compiled in The Handbook of Middle American Indians (McQuown 1968).

Mesoamerican languages were also among the first to evolve independent traditions of writing. The oldest texts date to approximately 1,000 B.C. while most texts in the indigenous scripts (such as Maya) date to ~600-900 A.D. Following the arrival of the Spanish in the 16th century, and continuing up until the 19th century, most Mesoamerican languages were written in Latin script. Many Mesoamerican languages today are either endangered or already extinct, but others, including the Mayan languages, Nahuatl, Mixtec and Zapotec, have several hundred thousand speakers and remain viable (Suárez 1983).

4 Native Mexican Populations

Ancient Mexico is classified into three major regions based on the history of native populations and cultural evidence; these regions are called cultural regions and have been dived in: Arid America (Northeast Mexico and Baja California peninsula); Oasis America (Northwest Mexico) and Mesoamerica (Central-South Mexico, Guatemala, Belize, Honduras, El Salvador, Nicaragua and Costa Rica) (López-Austin and López-Luján 2001). Actually Mexico is defined as a "pluricultural" nation in recognition of the diverse ethnic groups that constitute it, and in which the indigenous peoples are the original founders. According to the National Commission for the Development of the Indigenous Peoples there are ~12 million indigenous people in Mexico, of many different ethnic groups, which constitute about 11-13% of the population in the country, the number of Mexican indigenous is estimated to be somewhere between 14% and 30% of the overall Mexican population (estimated approximately in 111 million people) (http://www.cdi.gob.mx/index.php?id_seccion=3). The Mexican census does not report racial-ethnicity but only the political-ethnicity of indigenous communities who hold political autonomy and preserve their indigenous languages, traditions, beliefs, and cultures.

Law of Linguistic Rights of the Indigenous Languages recognizes 62 indigenous languages as "national languages" which have the same validity as Spanish in all territories in which they are spoken. According to the National Institute of Statistics, Geography and Data Processing (INEGI 2007), approximately 6.7% of the population speaks an indigenous language, that is, approximately half of those identified as indigenous. The National Commission for the Development of the Indigenous Peoples identifies 62 indigenous language groups in Mexico although certain languages have multiple dialects each of which is unique and may be mutually unintelligible. The majority of the indigenous population is concentrated in the central and southern states: Yucatán, 59%; Oaxaca, 48%; Quintana Roo, 39%; Chiapas, 28%; Campeche, 27%; Hidalgo, 24%; Puebla, 19%; Guerrero, 17%; San Luis Potosí, 15% and Veracruz, 15% (Lizcano Fernández 2005).

4.1 Cultural traits

The pre-Columbian civilizations of what now is known as Mexico are usually divided in two regions: Mesoamerica, in reference to the cultural area in which several complex civilizations developed before the arrival of the Spanish in the sixteenth century, and Aridoamerica (or the North) in reference to the arid region north of the Tropic of Cancer in which few civilizations developed and was mostly inhabited by nomadic or semi-nomadic groups (López-Austin and López- Luján 2001). Mesoamerica was densely populated by diverse indigenous ethnic groups which, although sharing common cultural characteristics, spoke different languages and developed unique civilizations (Aguilar-Moreno 2004). One of the most influential civilizations that developed in Mesoamerica was the Olmec civilization, sometimes referred to as the "Mother Culture of Mesoamerica". The later civilization in Teotihuacán reached its peak around 600 AD, when the city became the sixth largest city in the world, which's cultural and theological systems influenced the Toltec and Aztec civilizations in later centuries. Evidence has been found on the existence of multiracial communities or neighborhoods in Teotihuacan (and other large urban areas like Tenochtitlan). The Maya civilization, though also influenced by other Mesoamerican civilizations, developed a vast cultural region in south-east Mexico and northern Central America, while the Zapotec and Mixtec culture dominated the valley of Oaxaca, and the Purépecha in western Mexico (Hamnett 1999).

By the time of the arrival of the Spanish in Mesoamerica, many of the diverse ethnic civilizations (with the notable exception of the Tlaxcaltecs and the Tarascan Kingdom of Michoacán) were loosely joined under the Aztec empire, the last Nahua civilization to flourish in Central Mexico. The capital of the empire, Tenochtitlan, became one of the largest urban centers in the world, with an estimated population of 350,000 inhabitants (Hamnett 1999). During the conquest of the Aztec Empire, the conquistadores, only a handful compared to the millions of indigenous peoples, used the ethnic diversity of the country and exploited the discontentment of the subjugated groups, making important alliances with rivals of the Aztecs (Aguilar-Moreno 2004). While the alliances were decisive to their victory, the indigenous peoples were soon subjugated by an equally impressive empire. Wars and forced labor were accompanied by the spread of European diseases previously unknown in the New World. Pandemics

wrought havoc, killing between 90% and 95% of the pre-contact population according to some estimates (Hamnett 1999). At first, the colonial system imposed a system of castes, in which the indigenous peoples were marginalized. Nevertheless, a cultural symbiosis took place: the indigenous peoples adopted and syncretized Roman Catholicism, and a new ethnic group was born: the Mestizo, of mixed European and indigenous ancestry (López Guerra and Flores Chávez 2004).

4.2 Linguistic Diversity

The vast Amerindian linguistic family is present in most of the continent, covering part of North America and all Central and South America, Mexico being a target of special interest due to its particular geographic position in the colonization of the continent, between North and Central America, and because it harbors one of the greatest ethnic and linguistic diversity. Nowadays, there are more than 68 living native indigenous populations and 298 languages have been described, of which 291 are living and 7 are extinct (Campbell 1977). The indigenous languages of Mexico are classified into three linguistic stocks (Hokan, Otomaguean and Uto-Aztecan), beside six linguistics families that are not related to other languages, and, therefore, not grouped into the three linguistic stocks (Algonquian, Huevan, Mayan, Mixe-zoquean, Tarascan and Totonacan). There are currently 120 ethnic groups in Mexico that are grouped into 12 large linguistic families (Manrique Castañeda 1987). These peculiarities, among others, make the region one of the most anthropologically diverse and interesting of the continent.

4.3 Genetic Diversity

In terms of the currently available genetic data there is a lack of an exhaustive evaluation of the genetic landscape of diversity in Mexico. Some genetic studies based on uniparental markers (Bonilla et al. 2005; Cerda-Flores et al. 2002a; Cerda-Flores et al. 2002b; Cerda-Flores et al. 2002c; Green et al. 2000) showed a relative genetic homogeneity of Mexican Indians. Other studies are based on mixed urban samples (Mestizos) and showed that these groups are basically the result of admixture between

Native Americans, Europeans and, to a lesser extent, West Africans, but give a poor idea about the genetic structure and diversity of indigenous populations (Silva-Zolezzi et al. 2009). Moreover, studies focused on ancient mitochondrial DNA (mtDNA) have shown the presence of A, B and C mtDNA lineages in pre-Columbians. Focusing on highly informative genetic markers, such as those on the mtDNA and the Y-chromosome, in native populations will give insights, not only on the genetic diversity of Mexicans, but also on the understanding of the colonization of the Americas (Bonilla et al. 2005).

OBJECTIVES

As humans colonized the world, populations diverged and thus differentiated genetically. Hence the dispersal of modern humans can be inferred from extant diversity observed in different parts of our genome (e.g., autosomes, mtDNA or the Y chromosome). Indeed, the history of human expansions into the New World is genetically supported by patterns of allele frequency variation (Cavalli-Sforza et al. 1994). The spread, time, and effective population size of the first settlers into the Americas are complex topics that still need to be elucidated. The extant cultural, linguistic, archeological, and autochthonous genetic variability harbored by present indigenous groups are valuable sources to find new answers to past questions.

Within this framework, Mesoamerica deserves special attention: it is one of the main cultural areas in the Americas where more genetic, cultural and archeological record exists. In addition, its geographic position is a key factor, because acting as a natural geographic corridor between North and Central-South America; it has been a direct witness not only of the initial and subsequent migration waves but also of the many human civilizations that flourished later on.

Following a molecular anthropological approach, this thesis evaluates the genetic diversity of a representative sample of the extant Native American gene pool within Mexico, and by constructing continental datasets it also intends to contribute to the history reconstruction of Mesoamerica and the peopling of the Americas. For that purpose, the study of uniparental markers (i.e. mitochondrial DNA and Y-chromosome) constitutes the main part of the work, but also autosomal STR variation, linguistic diversity, and ethnographic data were complementary investigated.

The particular aims of the present thesis are as follows:

⇒ To characterize mtDNA and Y-chromosome variation in Native Mexican populations by means of DNA sequencing, SNP typing and STR amplification, followed by haplogroup determination and statistical analyses.

⇒ To gain a better understanding of current genetic structure of Native Mexican groups in order to search for the presence of founding diversity pre-dating the *Mestizaje* process.

⇒ To use the genetic data generated herein and collect comparable publicly available data from the literature in order to construct continent-wide datasets and trace both maternal and paternal lineages throughout the Americas with the ultimate goal of contributing to the understanding of the tempo and mode of the settlement of the New World and the reconstruction of the historical events during the evolution of Native American populations.

RESULTS

Chapter 1: Nutritional status of the poor, marginalized adults of the Triqui ethnic group in Oaxaca, Mexico.

Rosa María Ramos Rodríguez and **Karla Sandoval Mendoza**

Revista Panamericana de Salud Pública. 2007. 22(4):260–267

Ramos RM, Sandoval K. Estado nutricional en la marginación y la pobreza de adultos triquis del estado de Oaxaca, México. 2007; 22(4): 260-67.

Chapter 2: Linguistic and maternal genetic diversity are not correlated in Native Mexicans

Karla Sandoval, Leonor Buentello-Malo, Rosenda Peñaloza-Espinosa, Heriberto Avelino, Antonio Salas, Francesc Calafell and David Comas.

Human Genetics. 2009 Oct;126(4):521-31.

Sandoval K, Buentello-Malo L, Peñaloza-Espinosa R, Avelino H, Salas A, Calafell F, et al. Linguistic and maternal genetic diversity are not correlated in Native Mexicans. 2009; 126(4): 521-31.

Chapter 3: Paternal lineages in Native Mexicans show a sex-biased admixture and lack of genetic structure in Mesoamerica

Karla Sandoval, Leonor Buentello-Malo, Rosenda Peñaloza-Espinosa, Heriberto Avelino, Andres Ruíz-Linares, Francesc Calafell and David Comas

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Paternal lineages in Native Mexicans show a sex-biased admixture and lack of genetic structure in Mesoamerica

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Running headline: Y-chromosome diversity of Native Mexican Populations

Abstract

The genetic characterization of Native Mexicans is of great interest to understand the population dynamics of past and present Mexican populations, often of admixed origin, as well as to aid in the reconstruction of the history of Mesoamerica and the peopling of the Americas. Here, we describe the Y-chromosome genetic diversity and haplogroup composition of 197 Native Mexican individuals from eleven populations, and intersected our results with publicly available data from 911 individuals from 36 additional Native populations from throughout the Americas. We found extensive heterogeneity among Native Mexican populations but lack of continental structure, and lack of correlation between linguistic classification and genetic diversity within Mexico. Populations falling outside Mesoamerica (i.e., Pima and Tarahumara), the northernmost sampled populations, show the greatest genetic differentiation. Most of these observations are in agreement with the results found for the mtDNA on the same population samples. However, contrary to what was observed in the female lineages, we found a remarkable frequency of European (and to a lesser extent African) Y-chromosome haplotypes, suggesting a strong female Native American and male European (and African) sex-biased contribution to the Native Mexican genomes. We also found differences in the Y-chromosome admixture proportions among populations, revealing different demographic histories within Mexico. Our results point towards a complex genetic makeup of Native Mexicans whose population history has been differently recorded in different regions of the genome.

Introduction

During the last two decades the peopling of the Americas has been subject to intensive research by geneticists, refining previous knowledge reported by archeologists, linguists, historians and anthropologists. Despite the controversy of the colonization of the New World by humans, genetic studies have agreed on two major issues: a single migration originated in East Asia crossed the Bering land before entering to the new continent; and this process produced a strong population bottleneck, such that modern Native Americans show significant reductions in their genetic variation, as a consequence of substantial genetic drift (Kitchen et al. 2008). However some questions are still waiting to be solved, like the timing of migration (leaving Asia and entering into the Americas); the size of founding populations; the nature of the migration from Asia (was it continuous, slow or fast) and, the migration route(s) taken within the Americas.

Despite the abundant genetic data on different Native American populations across the continent, there are specific areas for which such genetic information continues to be scanty, such as the cultural area known as Mesoamerica. In terms of human population history, only a few molecular studies have specifically addressed this region. Most of these studies have been focused on the analysis of Mestizo samples using autosomal markers (Cerda-Flores et al. 2002a; 1989; 1987; 2002b; Felix-Lopez et al. 2006; Rangel-Villalobos et al. 1999) and Y-chromosome markers (Gorostiza et al. 2007; Gutierrez-Alarcon et al. 2007; Hernandez-Gutierrez et al. 2005; Licea-Cadena et al. 2006; Luna-Vazquez et al. 2008; Luna-Vazquez et al. 2005; Luna-Vazquez et al. 2003; Rangel-Villalobos et al. 2001a; Rangel-Villalobos et al. 2001b; Rangel-Villalobos et al. 2008; Rangel-Villalobos et al. 2000; Rubi-Castellanos et al. 2009a; Rubi-Castellanos et al. 2009b; Salazar-Flores et al. 2009; Torres-Rodriguez et al. 2006).

These studies in Mestizos in Mesoamerica have shown that the Native American contribution to extant populations is relevant, especially in the maternal side (González-Oliver et al. 2001; Green et al. 2000; Peñaloza-Espinosa et al. 2007; Sandoval et al. 2009; Torroni et al. 1994). In order to describe the Native component in Mesoamerica, a few studies have recently been published on Native Mexican groups, mainly in maternal lineages (Barrot et al. 2005; Felix-Lopez et al. 2006; Paez-Riberos et al. 2006; Rangel-Villalobos et al. 2000). However, the genetic characterization of paternal lineages on Native Mexican populations is very scanty and it is needed to give more insights into both inter and intra variability among Mesoamerican groups, providing another piece to complete the Peopling of the Americas' puzzle.

Overall, studies based on Y-chromosome diversity have shown a consistent pattern of scarce haplotypic diversity in Native American populations (Ruiz-Linares et al. 1999), also these findings which suggested almost no or very restricted variability were contradicted by posterior studies which documented more variation, although it is generally less marked than those found in the mtDNA or autosomal regions (Salzano 2002). Concerning Y-chromosome variation, haplogroup Q is present in about 76% of Native Americans (Zegura et al. 2004b), and jointly with haplogroup C, these are the common paternal lineages in the Americas and rare elsewhere, with the exception of northern Asia, where they reach frequencies of 28% and 18%, respectively (Mulligan et al. 2004). Since the identification of the Q-M242 SNP (Seielstad et al.2003), the efforts to find newly Native American haplogroups and date them have continuously increased. Based on STR mutation rates, the age of M242 haplotypes in the Americas was calculated at ~18,000–15,000 ybp (Bortolini et al. 2003; Seielstad et al. 1999). Most of the remaining Y-chromosome haplotypes belong to one of several different haplogroups, and comprise only 5% of Native American Y-chromosomes, having

limited distributions throughout the continent. C-M130 haplotypes have only been detected among the Na-Dene-speaking Tanana, Navajo, and Chipewyan, and the Amerindian Cheyenne (Bergen et al. 1999; Bortolini et al. 2002; Bortolini et al. 2003; Karafet et al. 1999). This have been dated at ~30,000–25,000 ybp (Karafet et al. 1999; Underhill et al. 2000), generally consistent with its broad distribution in East and Southeast Asia, where it appears to have originated, and its haplotypic diversity in eastern Siberian and Asian populations (Lell et al. 2002). Age estimates for the entire C lineage and the Native American-specific C-P39 are 27,500±10,100 and 2,550±1910 ybp, respectively (Hammer and Zegura 2002; Karafet et al. 2002).

In this study, we present Y-chromosome STR and SNP data for 197 unrelated individuals from eleven native populations from Mexico belonging to two independent cultural and geographical areas: Tarahumara and Pima in northern Mexico; and Triqui, Mixtec, Otomí, Nahua Xochimilco, Nahua Zitlala, Nahua Atocpan, Nahua Ocotitlán, Purépecha, and Maya, in Mesoamerica. The aim of the present study is to analyze the diversity of the Y chromosome in these native samples in order to determine the genetic structure of the paternal lineages and its correlation to geographical, cultural, and linguistic factors. In addition, we report the male counterpart of the maternal lineages previously investigated by means of mtDNA analysis on the same Native Mexican populations (Sandoval et al. 2009) in order to establish the possible admixture sex bias in Mesoamerican samples.

Materials and Methods

Populations studied

A total of 197 Native Mexican male individuals were analyzed: 22 Triqui, 18 Tarahumara, 2 Mixtec, 7 Otomí, 22 Nahuas from Xochimilco, 22 Nahuas from Zitlala,

9 Nahuas from San Pedro Atocpan, 17 Nahuas from Santo Domingo Ocotitlán, 8 Purépecha, 19 Maya, 51 Pima. An additional sample of 11 Quechuas from Peru were also analyzed and used for inter population comparisons. DNAs from Pima, Maya and Quechua were provided from the laboratory of Judith R. Kidd and Kenneth K. Kidd (Yale University, New Haven, Conn., USA) where lymphoblastoid cell lines are maintained. All samples were collected with the appropriate informed consent and all individuals were native speakers with local ancestors; their geographic location is shown in Figure 1.

Y chromosome genotyping

DNA concentration of all samples was normalized to 1ng/µl using the Quantifiler™ Human DNA Quantification Kit (Applied Biosystems). Three SNPs (M45, which defines the major haplogroup P; M242, which defines haplogroup Q; and M207, which defines haplogroup R) were genotyped in all individuals in 5µl reactions containing 1µl of DNA template, 0.25µl of 20x SNP Genotyping Assay, 1.25µl of sterile-filtered water and 2.5µl of TaqMan® Universal PCR Master Mix amplified with a standard conditions each individual by TaqMan® probes (Applied Biosystems). For those individuals whose lineage was not assigned within the major haplogroup P, marker M130 (which defines haplogroup C) was genotyped using the same PCR conditions described above. After the PCR amplification, allelic discrimination was done with SDS Software TM v2.3 (Applied Biosystems) in order to determine the haplogroups defined by the SNPs previously described.

A total of 17 Y-STRs loci (DYS19, DYS385a/b, DYS389I, DYS389II, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, GATA-H4) were amplified with the AmpFlSTR® Yfiler™ PCR Amplification Kit (Applied Biosystems). PCR products

were discriminated in an ABI 3130xl genetic analyzer and the allele sizes were determined with GeneMapper v3.2.software. The haplogroup assignation defined by the SNPs was confirmed using the profile based on STRs, with the haplogroup predictor with the Batch Processing Program (http://www.hprg.com/hapest5/page5.html).

Statistical Analysis

STR-haplotype data was used to estimate intra population genetic diversity parameters such as: gene diversity, mean pairwise differences and theta values using Arlequin program v3.11 (Schneider et al. 2005). To compare the Mexican results with other Native American populations, haplotype information of seven Y-STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393) from 36 Native American populations were collected from the literature (Supplementary Table S1). Individuals belonging to non-Native American haplogroups (Bortolini et al. 2003) were excluded for all estimations except for the Correspondence Analysis (see below). For some analyses, population samples were grouped into major subcontinental areas: North (586 individuals of 23 populations from Alaska to Mexico), Central (79 individuals of four populations from Guatemala to Panama) and South America (246 individuals of nine populations from Colombia southwards) (see Table S1).

Population genetic structure was tested in Mexican and Native American populations through the analysis of molecular variance (AMOVA) (Excoffier et al. 1992) using the Arlequin program v3.11 (Excoffier et al. 2005). Genetic relationships among Mexican and continental populations were analyzed by two approaches. The first approach was based on genetic distances (F_{ST} and R_{ST}) calculated with Arlequin program v3.11 (Excoffier et al. 2005) and represented in a Multidimensional Scaling plot (MDS). The second approach was based on haplogroup absolute frequencies in

order to construct a Correspondence Analysis (CoA) plot. Both analyses were performed with STATISTICA 7 package (http://www.statsoft.com).

Median joining networks were constructed using Network 4.5.1.0 package (Bandelt et al. 1999) for the Native American haplogroup Q (Bortolini et al. 2003). The time to the most recent common ancestor (TMRCA) has been estimated for the Q lineage. Y-STRs weights were determined according with Hurles et al. (2002). The mutation rate considered was one transition per 571 years; these have been calibrated on the evolutionary rate of each locus reported in Y-chromosome haplotype reference database (http://www.yhrd.org/).

Results

Paternal lineage composition in Native Mexicans

Few SNPs are needed to define the major haplogroups found in the Americas since only two major haplogroups are associated with native American populations: haplogroup Q, and at lower frequency, haplogroup C (Zegura et al. 2004a). After genotyping four Y-chromosome SNPs (see Table 1), the 197 Native Mexican individuals were classified into major haplogroups (Table 1), and subsequently confirmed and refined using the 17-STR profile with the Batch Processing Program (http://www.hprg.com/hapest5/page5.html). The most frequent haplogroup in Native Mexican populations was haplogroup Q (80.92%) which agrees with what was previously reported by Zegura et al (2004a) being the major lineage among Native Americans. The other native haplogroup C was not found in native Mexicans. Other haplogroups with European and African origin were found in our sample set, such as R, which is the most common haplogroup in European populations (Karafet et al. 1998), and was the second more frequent haplogroup in Mexican samples (12.26%), followed

at lower frequencies by haplogroups J, E, I, G, and T (see Table 1). The presence of these no-native lineages in Native Mexicans might be the result of recent admixture. In almost all Mexican populations the proportion of the admixture was similar except for the Nahuas Xochimilco, where significant differences were observed when compared to Triqui (exact test of differentiation, p=0.003) and Pima (p=0.0007) samples. These two populations showed a high percentage of Q native haplogroup (100%) and (96.08%).

A total of 106 different haplotypes (Supplementary Table S2) were observed in 165 Native Mexican individuals who belong to Q haplogroup. Table 2 summarizes the results for the 17 STRs analyzed on native Mexican samples and the eight native samples from Quechuas used for comparison. Interestingly, Pimas and Tarahumara, the northernmost samples, exhibit the lowest diversity values (average genetic diversity and pairwise differences).

An analysis of molecular variance (AMOVA) based on F_{st} and R_{st} genetic distances was performed in order to define the population structure of Mexican populations according to sample size, geographic and linguistic criteria (Table 3). In order to avoid biases due to admixture of different non-native haplogroups, AMOVA estimations were performed only with native haplotypes, i.e. those belonging to haplogroup Q. To determine whether the four Nahua groups had to be considered as a single group, an AMOVA was performed among the Nahua samples. Certain heterogeneity ($p<0.05$) among Nahuas was found (3.19%, for F_{st} values; and 10.18%, for R_{st} values). To corroborate this result, an exact test of population differentiation was performed ($p<0.05$). Since both results showed a clear differentiation within the Nahua populations, for most of the analyses they were considered as four separated groups, except for those analyses in which sample size was relevant. Particularly, Xochimilco and San Pedro Nahuas were treated as a single group, because they share the same

linguistic family, are close geographically, and no statistical differences were observed when an AMOVA was performed between these two populations (4.51%, p=0.1 for F_{st} values; and 3.58%, p=0.2 for R_{st} values).

The AMOVA showed that, when all Mexican populations were considered as a single group (11 populations), 14.09% (F_{ST} values) and 16.30% (R_{ST} values) ($p<0.05$) of the genetic variance was found between populations, showing a relevant genetic heterogeneity among native Mexican populations. When two groups (northern samples versus Mesoamerican samples) were compared, the AMOVA showed significant differences between the two groups 8.94% (F_{ST} values) and 11.03% (R_{ST} values) ($p<0.05$). Four major linguistic families described in Mexico were represented in our population samples: Oto-Manguean (Mixtec, Triqui and Otomí), Uto-Aztecan (the four Nahua populations, Tarahumara and Pima), Tarascan (Purépecha), and Mayan (Maya) (Mithun 1999). Non-significant proportions of the variance (-3.34% for the F_{ST} p=0.9 and -2.17% for the R_{ST} p=0.7) were explained by the fact of belonging to these linguistic groups, which suggest a lack of correlation between Y-chromosome STR haplotype diversity and linguistic classification. When only populations with at least 10 individuals were considered (Mixtecs, Purépechas and Otomíes were discarded due to their low sample size, and Nahuas from Xochimilco and San Pedro Nahuas were consider together as a single group), 12.86% (F_{ST}) and 16.80% (R_{ST}) ($p<0.05$) of the genetic variance was found between all populations, showing again relevant genetic heterogeneity among native Mexican populations. When cultural groups (northern versus Mesoamericans) were considered, significant values were found (7.11%, $p<0.05$ and 11.38%, $p<0.05$ for F_{ST} and R_{ST}, respectively). When the linguistic classification (three families represented) was considered, again non-significant values were found (-3.63%, p=0.7 and -3.09%, p=0.8 for F_{ST} and R_{ST}, respectively).

For the Correspondence Analysis (CoA) performed with Mexican populations, only samples with more than ten individuals were considered (Mixtecs, Otomíes and Purépechas were excluded). The CoA plot accounts for 74.82% of the total variation, where 48.47% is explained by the first dimension. Santo Domingo Nahuas, Zitlala Nahuas, Pimas and Triquis were grouped together, although no particular clustering of populations can be clearly detected (Supplementary Figure S1). In the MDS calculated with Mexican populations based on F_{ST} and R_{ST} genetic distances, Tarahumara and Pima are located apart from the rest in the first or second dimensions, whereas the rest of the populations do not show a clear clustering (Figure S2).

Mexican populations within the American genetic landscape

To compare the genetic diversity found in Mexico with the rest of Native American populations at a continental scale, a Correspondence Analysis (CoA) based on absolute frequencies of 12 haplogroups predicted by Y-chromosome 7 STRs was performed with all samples in the American dataset (see Table S1). No particular clustering of Mexican samples is shown in the two-dimension plot (Figure S3A), which accounts for 43.76% of the total variation (23.59% is explained by the first dimension and 20.17% by the second dimension). It is noteworthy that the four Central American samples were grouped together. When the CoA was performed only with the two Native American haplogroups (Q and C) (74.42% of the total variation: 44.14% and 30.28% in the first and second dimensions, respectively), the same pattern was observed: no clustering of Mexican samples and clustering of the four Central American samples (Figure S3B).

A different pattern is exhibited when a MDS analyses are performed at a continental scale including the genetic distances based on 7 STRs from the 36

populations. In the MDS based on F_{ST} (stress value = 0.194) and R_{ST} (stress value = 0.112) no clear clusters are observed, with the exception of the two Yanomami groups, and some northern populations: Dogrib and Tanana, both belonging to same linguistic family (Na-Dene-Atapaskan), Aleuts (Alaska Peninsula), Minnesota Chippewa (Northeast USA), and Sisseton Wahpeton-Sioux (Northeast USA).

In order to formally test if there is a clustering among Native American samples, an AMOVA considering the American database was performed (Table 3). When samples were considered as a single group, a high degree of genetic heterogeneity between populations 16.96% (F_{ST}) and 20.50% (R_{ST}) was found ($p<0.05$). In order to ascertain whether this heterogeneity was caused by geographical factors, three continental groups (North, Central and South) were considered. The AMOVA showed that a non-significant 1.15% (F_{ST}, $p>0.05$) and 1.51% (R_{ST}, $p>0.1$) of the genetic variance was due to differences between these three sub-continental groups, whereas the genetic variance within the three groups remained highly significant (16.26% for F_{ST}, and 19.55% for R_{ST}; $p<0.05$), suggesting an important genetic heterogeneity within the sub-continental areas and a lack of continental structure. When Mexican samples were considered as a separate category from North, Central and South groups, a non-significant 0.67% (F_{ST}, $p>0.01$) of the genetic variance was attributed to differences among groups, similar non-significant results were obtained when the R_{ST} values were considered (0.88 %, $p>0.02$), whereas the genetic heterogeneity within groups remained highly significant (16.69% (F_{ST}) and 20.11%, (R_{ST}); $p<0.05$). These results suggest that Mexican populations do not constitute a separate category with respect to the North, Central and South American populations and emphasize the lack of continental structure.

In order to test the correlation between paternal lineages and linguistic groups, an additional AMOVA was performed classifying the 36 Native American populations into 19 different linguistic families (see Table S1). It is noteworthy that significant differences between linguistic groups were found: 6.83% (F_{ST}) and 11.60 % (R_{ST}) ($p<0.05$), suggesting a genetic differentiation of linguistic groups in the Americas.

Analysis of the haplogroup Q

In order to establish the genetic relationships between the Y-STRs haplotypes observed in our sample set, a median joining network was constructed for haplogroup Q, in which only native Mexican haplotypes with 17 STRs were included. A star-like pattern without population clustering is observed, with the exception of one cluster mainly formed by Pima individuals (Figure 2). This cluster comprises 10 different haplotypes, from which nine are found in Pimas (29 individuals) and one in Tarahumaras (4 individuals). It is noteworthy that these two populations are the northernmost samples in the analysis and they do not belong to what is strictly defined as Mesoamerica. This cluster is characterized by one haplotype shared between 12 Pimas (13, 13, 17, 12, 16, 28, 23, 10, 14, 13, 14, 11, 12, 19, 17, 15, 22, 11, same STR order as the one described in *Y chromosome genotyping* section). The estimated age for the haplogroup Q in native Mexicans is 6,436 ($\pm$913) ybp.

Discussion

Due to the presence of a substantial proportion of non-native American male lineages in our samples, the Y-chromosome haplogroup composition of native Mexican populations shows a remarkable contrast with previous results from the mtDNA counterpart for the same populations, in which almost only native female lineages

(haplogroups A2, B2, C1, D1, and D4h3) were found (Sandoval et al. 2009). We have found a remarkable frequency of European (and to a lesser extent African, represented by haplogroups E1b1b and E1a1b) Y-chromosome lineages in native Mexicans (almost 20%), whereas the non-native mtDNA lineages in native Mexicans is negligible (less than 0.5%, Sandoval et al. 2009), suggesting a sexual bias in the introgression of non-native American lineages in native populations, fact consistent with previous studies performed in different Amerindian populations (Carvajal-Carmona et al. 2003; Dipierri et al. 1998; Gonzalez-Andrade et al. 2007; Mendizabal et al. 2008; Sans et al. 2002).

Although the general observation is consistent with a model of sex-biased admixture characterized by a predominance of European males and Native American females in the ancestral genetic pool of native Mexicans, it is noteworthy the variation in the Y-chromosome admixture proportions among native Mexican populations. This can be observed in the significant differences in haplogroup composition between Xochimilco Nahuas and Triqui or Pima. While the former showed the highest proportion of European haplotypes (32%), the other two populations exhibit the highest proportion of native American haplotypes (100% and 96%, respectively), explaining the resulting significant values of the exact test of differentiation. This is consistent with the demographic history of these populations since Xochimilco Nahuas represent an urban sample within Mexico City, which have been largely exposed to possible admixture events, whereas Triqui and Pima are two indigenous groups well known to be characterized by their geographic and cultural isolation. In addition, the finding of African Y-chromosome haplotypes in our samples (haplogroups E1b1b and E1a1b), although not as frequent as haplotypes of European origin, is not negligible, and indicates that the involuntary migration of Africans through slave trade appears to have left a trace in Native Mexican populations proximal to coastal routes (e.g. Purépecha).

Interestingly, Pimas and Tarahumara, the northernmost samples, exhibit the lowest diversity values (average genetic diversity and pairwise differences), which is in total agreement with our previous observations based on mtDNA diversity of the same set of samples, where we found a clear differentiation of these two populations from the rest of Native Mexicans (Sandoval et al. 2009). In addition, they present significant differences in Y-chromosome lineages to the Mesoamerican samples as shown in the AMOVAs, and some of their lineages cluster differentially in the network of the haplogroup Q. Pima and Tarahumara are the only sampled populations outside the cultural geographic region of Mesoamerica, whose differentiation could be indicative of a remarkable transition of genetic patterns between North America and Mesoamerica, where probably due to geographic and weather conditions a major part of the continental diversity is likely to have accumulated over millennia.

We found extensive heterogeneity among Native Mexican populations but a lack of genetic structure at the continental scale, which may be the result of extensive genetic drift occurring in most Native American populations. Likewise, we did not find a correlation between linguistic classification and Y-chromosome diversity, which is also consistent with previous observations of mtDNA diversity in Native Mexicans (Sandoval et al. 2009). However, certain correlation between linguistic and genetic diversity is found for the paternal lineages at a continental scale, despite the lack of correlation with geography. This fact is remarkable since most of the genetic and linguistic correlations found in human populations have been attributed to their correlation with geography; i.e. genes and languages are correlated as a result of geography (Comas et al. 2008). In the analysis of male lineages in Native Americans, there is no correlation between geography and genetic differentiation, but certain correlation between male lineages and linguistic families is observed, suggesting a

genetic link between linguistically close populations regardless their geographical location.

Finally, we conducted a phylogenetic analysis on the native haplotypes in order to perform some historical inferences in Mexican populations. Haplogroup Q (defined by M242 mutation) is one of the two main branches of the major Y-chromosome lineage P, which originated approximately 26,600 to 41,400 ybp (Karafet et al. 2008). The bearers of M242 mutation migrated eastward across Siberia until they reached the north-eastern point of Asia trough the Bering ice corridor about 15,000 to 20,000 years ago. Haplogroup Q represents a recent paternal founder for the Native American populations into the peopling of the Americas (Bortolini et al. 2003; Seielstad et al. 2003). This haplogroup is distributed widely in North Eurasia and found at high frequencies in some Siberian groups and at low frequencies in Europe, East Asia and the Middle East (Karafet et al. 2002). The diversity of haplogroup Q in native Mexicans is around 6,436 ($\pm$913) ybp, that is, well within the aforementioned ranges, pointing to a recent origin of these haplotypes. It is noteworthy the common and recent origin of a cluster in Pima and Tarahumara, which could be related to a common expansion in the area located northern of the Mesoamerica border.

Overall, our results offer a detailed picture of the male-specific genetic composition of a dense panel of Native Mexican populations and show the importance of complementing different approaches for comprehensively reconstructing the demographic history of human populations, especially from the Americas where the genetic make up of native populations can be additionally complex due to recent admixture. Further studies at larger, genome-wide, scales are necessary in order to extend such characterization to other regions of the Native Mexican genome.

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Table 1 Y-chromosome haplogroups found in eleven native Mexican samples

	SNPs genotyped					Haplogroup frequencies (%)						
Population	n	M242[a]	M207[b]	M45[c]	M130[d]	Q	R	J	E	I	G	T
Triqui	22	22	-	-	-	100	0	0	0	0	0	0
Tarahumara	18	13	4	1	-	72.2	22.2	0	0	0	0	5.6
Mixtec	2	2	-	-	-	100	0	0	0	0	0	0
Otomíe	7	4	2	1	-	57.1	28.6	0	0	14.3	0	0
Xochimilco_Nahua	22	14	7	1	-	63.6	31.8	0	0	0	5	0
Zitlala_Nahua	22	19	3	-	-	86.4	13.6	0	0	0	0	0
San Pedro_Nahua	9	7	1	1	-	77.8	11.1	11.1	0	0	0	0
Santo Domingo_Nahua	17	15	2	-	-	88.2	11.8	0	0	0	0	0
Purépecha	8	6	-	2	-	75	0	12.5	12.5	0	0	0
Maya	19	14	3	2	-	73.7	15.8	5.3	5.3	0	0	0
Pima	51	49	-	2	-	96.1	0	0	0	0	3.9	0
Total	197	165	22	10	-	80.9	12.3	2.6	1.6	1.3	0.8	0.5

[a]Derived allele: haplogroup Q; [b]Derived allele: haplogroup R; [c]Ancestral allele: individuals not belonging to major haplogroup P; [d]Derived allele: haplogroup C.

Table 2 Diversity parameters for the 17 Y-STRs for haplogroup Q.

Population	N	Hp	K	Haplotype diversity	Average GD	Mean PW	Theta*
Triqui	22	15	16	0.95+/-0.02	0.47+/-0.25	8.12+/-3.92	20.43 (14.54)
Tarahumara	13	10	14	0.92+/-0.06	0.43+/-0.24	7.38+/-3.69	10.56 (11.28)
Mixtec	2	1	0	0.00+/-0.00	0.00+/-0.00	0.00+/-0.00	UC
Otomíe	4	4	12	1.00+/-0.17	0.50+/-0.35	8.50+/-4.98	UC
Xochimilco Nahua	14	10	14	0.95+/-0.03	0.48+/-0.26	8.16+/-4.03	20.08 (19.26)
Zitlala Nahua	19	17	17	0.98+/-0.02	0.55+/-0.29	9.40+/-4.52	82.59 (153)
San Pedro Nahua	7	7	15	1.00+/-0.07	0.50+/-0.30	8.57+/-4.51	UC
Santo Domingo Nahua	15	8	12	0.91+/-0.04	0.45+/-0.24	7.65+/-3.78	9.28 (5.52)
Purépecha	6	6	16	1.00+/-0.09	0.55+/-0.34	9.46+/-5.07	UC
Maya	14	13	17	0.98+/-0.03	0.55+/-0.30	9.38+/-4.58	88.08 (260)
Pima	49	16	13	0.89+/-0.02	0.32+/-0.17	5.60+/-2.73	7.08 (2.25)
Quechua	8	7	15	0.96+/-0.07	0.44+/-0.26	7.50+/-3.92	25.27 (59.97)

N: number of individuals; Hp: number of different haplotypes; K: number of polymorphic loci; Average GD: average gene diversity; Mean PW: mean pairwise differences.

*Theta, in parenthesis standard deviation

UC: Unable to compute

Table 3 Analysis of molecular variance (AMOVA) of Native Americans.

	Groups	Among groups	Among populations within groups	Within populations
Mexicans[a]				
	11 native samples		14.09*/16.30*	85.91*/83.70*
	4 linguistic families	-3.34ns/-2.17ns	16.11*/17.64*	87.22*/84.53*
	northern versus Mesoamerican	8.35*/8.43*	8.94*/11.03*	82.71*/80.55*
Mexicans[b]				
	7 native samples		12.86*/16.80*	87.14*/83.20*
	3 linguistic families	-3.63ns/-3.09ns	14.83*/18.53*	88.81*/84.56*
	northern versus Mesoamerican	8.96*/8.22*	7.11*/11.38*	83.94*/80.40*
America database[c]				
	One single group		16.96*/20.50*	83.04*/79.50*
	North, Central and South America	1.15ns/1.51ns	16.26*/19.55*	82.59*/78.94*
	America database vs 11 Mexicans groups	0.67ns/0.88ns	16.69*/20.11*	82.65*/79.01*
	19 linguistic families	6.83*/11.60*	10.46*/9.42*	82.72*/78.98*

* $P < 0.05$; ns: non-significant
Left numbers based on F_{ST} distances and right numbers based on R_{ST} distances

[a] Eleven native Mexican populations

[b] Seven native Mexican populations with more than 10 individuals

[c] Data from 36 native American populations based on haplotype information from 7 Y-STRs (see Supplementary Table S1)

Figure Legends

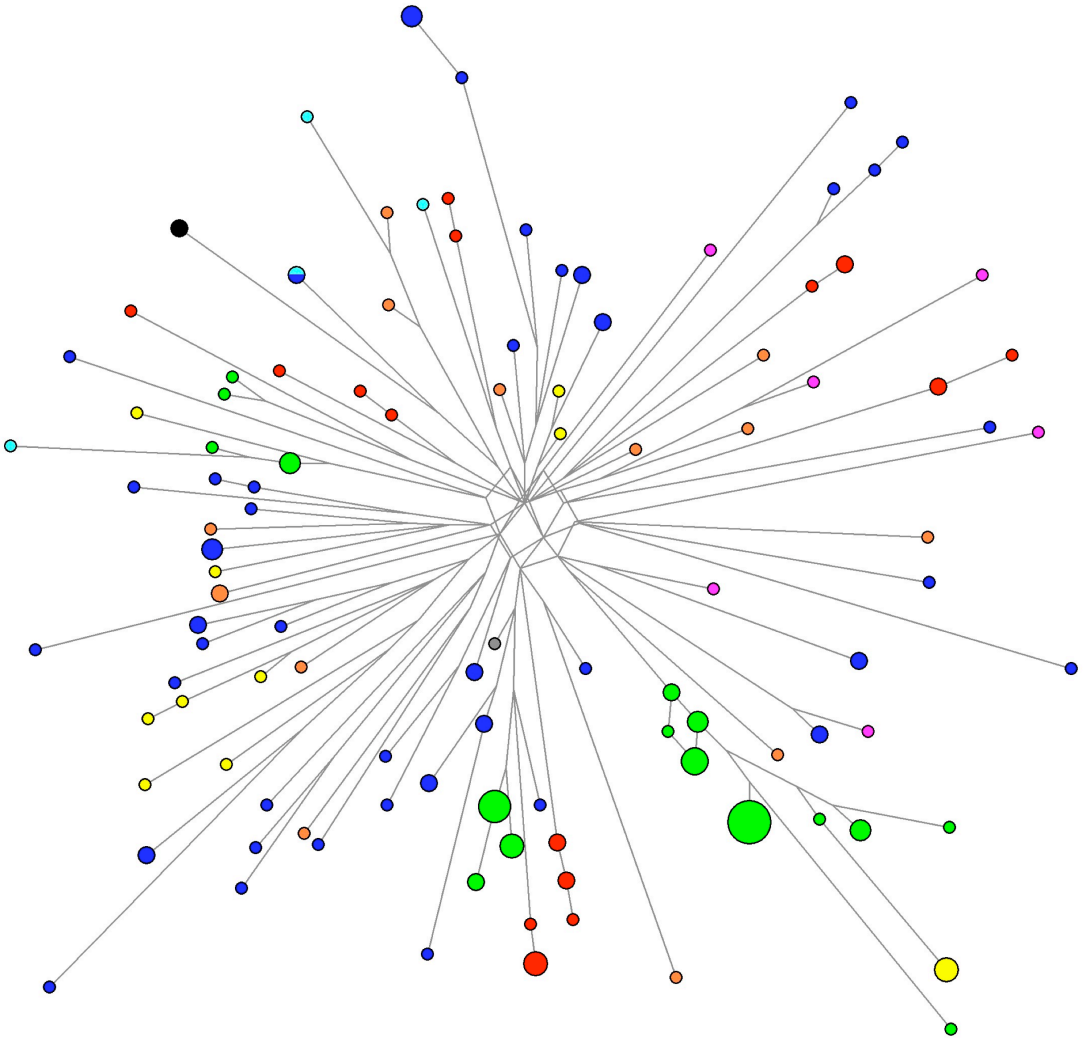
Figure 1. Geographical location of native Mexican populations sampled within the continental framework. Names of the populations as shown in Supplementary Table 1.

Figure 2. Network of haplogroup Q in native Mexican samples for 17 Y-STRs. Color code: Triquis (in red), Tarahumaras (yellow), Mixtecs (black), Otomíes (aqua), Four Nahuas (blue), Purépechas (pink), Mayas (orange), and Pimas (in green).

Figure 1.


Tanana (Ta)
Dogrib (Do)
Aleut (Au)
Navajo (Na)
Sioux (SI)
Chippewa (MC)
Stillwell Cherokee (SC)
Apache (Ap)
Cherokee (OC)
Apache (SA)
Choctaw (CO)
Huasteco (Hu)
Seri (Se)
Creek (CR)
Otomí (Os)
Tarahumara (R)
Otomí (Ov)
Pima (PM)
Otomí (O)
Nahua (SP)
Maya (MY)
Kuna (KU)
Nahua (NX)
Bari (BV)
Nahua (SD)
Purépecha (P)
Yanomami (YSV)
Nahua (NZ)
Mixtec (M)
Triqui (T)
Ngobe (NG)
Yanomami (YB)
Emera (Em)
Wounan (Wo)
Kichwa (K)
Beni (BB)
Waorani (WA)
Kaingang-Guarani (KG)
Quechua (Q)
Toba (To)

Figure 2.



Supplementary Material

Supplementary Table S1, References and general information of the 36 Native American populations conformed the America Data Base used it for comparison to the Mexican sequences analyzed.

Supplementary Table S2 Haplotypes based on 17 Y-chromosome STRs of the AmpFlSTR® Yfiler™ kit for Mexican populations reported in this study: Triqui, Tarahumara, Purépecha, Otomí, Mixtec, Nahua from Xochimilco, Nahua from Zitlala, Nahua from Ixhuatlancillo, Nahua from Necoxtla, Maya, Pima, and Quechua populations.

Supplementary Figure S1

Two-dimension Correspondence Analysis (CoA) plot of Native Mexican samples with more than 10 individuals, based on absolute frequencies of seven haplogroups found. In blue populations than belong to Uto-Aztecan linguistic family, in red Otomanguean, in black Mayan, and in grey and underlined the haplogroups.

Supplementary Figure S2

A) Two-dimensional MDS plot of Native Mexicans samples based on F_{ST} genetic distances. Populations in blue Uto-Aztecan, in green Otomanguena, and in black Mayan linguistic families.
B) Two-dimensional MDS plot of Native Mexican samples based on R_{ST} genetic distances. Populations in blue Uto-Aztecan, in green Otomanguena, and in black Mayan linguistic families.

Supplementary Figure S3

A) Two-dimension Correspondence Analysis (CoA) plot of Native American populations based on absolute frequencies of twelve haplogroups found. North populations in blue, Central in green, South in black, Mexicans in red and haplogroups in grey and underlined.

B) Two-dimension Correspondence Analysis (CoA) plot of Native American populations based on absolute frequencies of the two (C and Q) native haplogroups found. North populations in blue, Central in green, South in black, Mexicans in red and Haplogroups in grey and underlined.

Supplementary Table S1. Data from 36 Native American populations based on haplotype information from 7 Y-STRs: DYS19, DYS389I, DYS389II DYS390, DYS391, DYS392 and DYS393 (only individuals with native-American haplogroups are considered).

Sub-continental region	Population	Code	Linguistic Family[a]	n	Reference
North-America	Aleut	Au	Eskimo-Aleut	53	Zlojutro M et al. (2009)
	Choctaw	CO	Muskogean	10	Bolnick AD et al. (2006)
	Creek	CR	Muskogean	9	Bolnick AD et al. (2006)
	Minnesota Chippewa	MC	Algonkian	9	Bolnick AD et al. (2006)
	Oklahoma Red Cross-Cherokee	OC	Iroquoian	16	Bolnick AD et al. (2006)
	Stillwell Cherokee	SC	Iroquoian	13	Bolnick AD et al. (2006)
	Sisseton Wahpeton-Sioux	SI	Siouan	20	Bolnick AD et al. (2006)
	Apache	Ap	Na-Dene, Athapaskan	89	Zegura S et al. (2004)
	Navajo	Na	Na-Dene, Athapaskan	71	Zegura S et al. (2004)
	Dogrib	Do	Na-Dene, Athapaskan	9	Malhi RS et al. (2008)
	Tanana	Ta	Na-Dene, Athapaskan	10	Zegura S et al. (2004)
	Seri	Se	Serian	12	Malhi RS et al. (2008)
	San Carlos Apache	SA	Na-Dene, Athapaskan	21	Malhi R et al (2008)
	Huasteco	Hu	Mayan	38	Barrot C et al. (2007)
	Otomíes del Valle	Ov	Otomanguean	25	Barrot C et al. (2007)
	Otomíes de la Sierra	Os	Otomanguean	26	Barrot C et al. (2007)
	Triqui	T	Otomanguean	22	This study
	Tarahumara	R	YutoAztecan	13	This study
	Nahua Xochimilco and San Pedro	NX_SP	YutoAztecan	21	This study
	Nahua Zitlala	NZ	YutoAztecan	21	This study
	Nahua Santo Domingo	SD	YutoAztecan	15	This study
	Maya	MY	Mayan	14	This study
	Pima	PM	YutoAztecan	49	This study
Central-America	Ngobe	NG	Chibchan	30	Ascune MA et al. (2008)
	Kuna	KU	Chibchan	18	Ascune MA et al. (2008)
	Emera	Em	Choco	17	Ascune MA et al. (2008)
	Wounan	Wo	Choco	14	Ascune MA et al. (2008)
South-America					
	Yanomami	YSV	Yanomam	10	Roewer L 1993[b]
	Kichwa	K	Quechuan	79	González AF et al (2007)
	Waorani	WA	Waorani	35	González AF et al (2009)
	Kaingang and Guarani	KG	Tupian	27	Leite F et al (2008)
	Bari	BV	Chibchan	16	Pardo T & Borjas L[b]
	Beni	BB	Arawakan	34	Arroyo E et al.[b]
	Yanomami	YB	Yanomam	10	Kayser M et al.[b]
	Toba	To	Guaicuran	27	Toscanini U et al (2008)
	Quechua	Q	Quechuan	8	This study

[a] Linguistic classification based on Mithun and Marianne (1999)
[b] Haplotype data obtained from the data base http://www.yhrd.org/

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Supplementary Table S2. 17-STR haplotypes found in haplogroup Q in native Mexicans and Quechua.

Haplotype[a]	Triqui n=22	Tarahumara n=13	Mixtec n=2	Otomí n=4	Xoc_N[b] n=14	Zit_N[c] n=19	SP_N[d] n=7	SD_N[e] n=15	Purépecha n=6	Maya n=14	Pima n=49	Quechua n=8	Total n=173
13-15-15-13-29-24-11-14-14-14-11-11-20-18-18-22-11	1	0	0	0	0	0	0	0	0	0	0	0	1
13-14-15-13-29-24-11-14-14-14-11-11-19-18-18-22-11	2	0	0	0	0	0	0	0	0	0	0	0	2
13-15-16-13-30-23-10-14-13-14-11-12-19-15-16-22-13	4	0	0	0	0	0	0	0	0	0	0	0	4
13-15-16-13-30-23-10-14-13-14-11-12-19-15-17-22-13	1	0	0	0	0	0	0	0	0	0	0	0	1
13-14-17-14-31-25-10-16-13-14-11-11-21-15-19-22-11	1	0	0	0	0	0	0	0	0	0	0	0	1
13-14-15-13-31-23-10-14-13-14-11-10-21-15-16-20-11	1	0	0	0	0	0	0	0	0	0	0	0	1
13-14-15-13-30-23-10-16-13-14-11-11-19-15-16-22-13	1	0	0	0	0	0	0	0	0	0	0	0	1
13-14-15-13-30-23-10-14-13-14-11-10-20-15-16-20-11	1	0	0	0	0	0	0	0	0	0	0	0	1
13-14-15-13-31-23-10-14-13-14-11-10-20-15-16-20-11	2	0	0	0	0	0	0	0	0	0	0	0	2
14-16-16-14-31-24-10-15-13-14-12-13-20-14-15-22-12	2	0	0	0	0	0	0	0	0	0	0	0	2
15-15-16-13-29-26-10-14-12-14-11-11-18-15-16-22-11	2	0	0	0	0	0	0	0	0	0	0	0	2
13-14-17-14-30-25-10-16-13-14-11-11-21-15-19-22-11	1	0	0	0	0	0	0	0	0	0	0	0	1
15-15-16-13-29-27-10-14-12-14-11-11-18-15-16-22-11	1	0	0	0	0	0	0	0	0	0	0	0	1
13-15-15-15-32-24-10-14-13-14-11-11-19-16-15-22-12	1	0	0	0	0	0	0	0	0	0	0	0	1
13-15-16-15-32-24-10-14-13-14-11-11-19-16-15-22-12	1	0	0	0	0	0	0	0	0	0	0	0	1
15-14-15-13-30-23-10-15-13-14-11-11-21-16-15-22-12	0	4	0	0	0	0	0	0	0	0	0	0	4
13-14-14-12-28-23-10-14-13-14-13-13-19-18-15-22-11	0	1	0	0	0	0	0	0	0	0	0	0	1
15-14-14-12-29-23-10-14-13-14-11-12-19-15-15-22-12	0	1	0	0	0	0	0	0	0	0	0	0	1
13-15-17-14-31-24-11-15-13-14-11-13-20-17-16-22-12	0	1	0	0	0	0	0	0	0	0	0	0	1
15-14-15-13-30-22-10-14-13-14-11-11-19-17-16-22-11	0	1	0	0	0	0	0	0	0	0	0	0	1
13-14-16-13-30-24-10-14-13-14-11-12-19-16-16-22-12	0	1	0	0	0	0	0	0	0	0	0	0	1
15-14-15-13-30-23-10-14-13-14-11-11-21-16-15-22-12	0	1	0	0	0	0	0	0	0	0	0	0	1
13-15-18-14-31-25-10-15-13-14-11-13-20-17-18-22-12	0	1	0	0	0	0	0	0	0	0	0	0	1
15-14-14-13-30-23-10-14-13-14-11-11-20-15-16-22-12	0	1	0	0	0	0	0	0	0	0	0	0	1
13-14-17-13-30-24-10-14-13-14-11-13-19-16-15-22-12	0	1	0	0	0	0	0	0	0	0	0	0	1

13-14-18-13-18-24-10-16-14-14-12-11-21-15-17-22-13	0	0	2	0	0	0	0	0	0	0	0	0	2
13-15-17-14-30-24-10-15-13-14-11-11-20-15-14-22-12	0	0	0	1	0	0	0	0	0	0	0	0	1
13-13-14-12-28-23-10-13-14-14-11-11-20-15-18-22-12	0	0	0	1	0	0	0	0	0	0	0	0	1
14-15-17-14-31-24-10-15-13-14-11-12-19-17-18-22-12	0	0	0	1	0	0	0	0	0	0	0	0	1
13-14-19-12-28-24-10-16-14-14-11-12-20-16-15-22-12	0	0	0	1	1	0	0	0	0	0	0	0	2
13-15-17-14-31-24-10-15-13-14-11-11-20-15-14-22-11	0	0	0	0	1	0	0	0	0	0	0	0	1
14-14-17-12-30-24-10-16-13-14-11-11-20-16-15-22-12	0	0	0	0	2	0	0	0	0	0	0	0	2
13-15-17-12-28-24-9-13-13-14-11-12-20-15-19-22-12	0	0	0	0	2	0	0	0	0	0	0	0	2
13-14-16-13-29-25-10-14-13-14-11-11-19-15-16-22-12	0	0	0	0	2	0	0	0	0	0	0	0	2
16-14-16-12-26-24-10-14-13-14-11-11-19-16-15-22-12	0	0	0	0	2	0	0	0	0	0	0	0	2
13-14-18-13-30-25-11-16-12-14-11-12-21-18-17-22-11	0	0	0	0	1	0	0	0	0	0	0	0	1
13-14-18-13-30-25-11-16-12-14-11-12-20-18-17-22-11	0	0	0	0	1	0	0	0	0	0	0	0	1
13-14-17-13-30-24-10-14-13-14-11-12-21-16-17-22-11	0	0	0	0	1	0	0	0	0	0	0	0	1
14-14-20-13-29-24-10-13-13-14-11-11-20-15-18-22-12	0	0	0	0	1	0	0	0	0	0	0	0	1
13-14-17-13-29-24-10-16-13-14-11-11-20-15-16-22-11	0	0	0	0	0	2	0	0	0	0	0	0	2
13-15-18-12-28-25-9-13-13-14-11-13-20-15-18-23-12	0	0	0	0	0	1	0	0	0	0	0	0	1
13-14-21-13-32-25-10-14-13-14-11-11-20-16-19-22-12	0	0	0	0	0	1	0	0	0	0	0	0	1
14-14-17-13-32-24-10-14-13-14-11-12-20-16-18-22-12	0	0	0	0	0	1	0	0	0	0	0	0	1
14-15-17-12-28-23-9-13-13-14-11-12-20-15-18-22-11	0	0	0	0	0	2	0	0	0	0	0	0	2
13-14-16-13-30-23-11-14-13-14-11-11-19-17-16-25-13	0	0	0	0	0	1	0	0	0	0	0	0	1
14-14-17-13-31-24-10-16-13-14-11-13-19-16-17-22-12	0	0	0	0	0	1	0	0	0	0	0	0	1
13-13-15-13-31-24-11-14-14-15-11-10-19-15-16-23-11	0	0	0	0	0	1	0	0	0	0	0	0	1
13-13-13-14-31-23-10-14-14-14-10-11-19-15-18-22-11	0	0	0	0	0	1	0	0	0	0	0	0	1
13-15-17-12-27-24-10-15-13-14-11-13-20-14-17-23-12	0	0	0	0	0	1	0	0	0	0	0	0	1
13-15-15-12-30-24-10-14-13-14-11-10-19-18-18-24-11	0	0	0	0	0	1	0	0	0	0	0	0	1
13-14-17-13-30-24-10-15-13-14-11-13-19-15-18-22-12	0	0	0	0	0	1	0	0	0	0	0	0	1
13-14-19-13-29-25-10-13-13-14-11-12-21-15-18-23-12	0	0	0	0	0	1	0	0	0	0	0	0	1
13-15-16-12-29-24-10-15-13-16-11-10-19-16-19-24-11	0	0	0	0	0	1	0	0	0	0	0	0	1
13-16-21-12-28-24-10-13-13-14-11-12-20-15-19-22-12	0	0	0	0	0	1	0	0	0	0	0	0	1

16-14-18-12-29-25-10-13-13-14-11-11-21-18-18-22-12	0	0	0	0	0	1	0	0	0	0	0	0	1
13-14-17-13-29-24-10-16-13-14-11-11-20-15-16-22-11	0	0	0	0	0	2	0	0	0	0	0	0	2
13-14-18-13-30-24-11-16-12-14-10-12-19-18-17-22-11	0	0	0	0	0	0	1	0	0	0	0	0	1
13-15-18-13-29-24-10-16-13-14-11-11-19-17-17-22-11	0	0	0	0	0	0	1	0	0	0	0	0	1
13-14-19-12-28-23-9-13-13-14-11-13-20-15-17-23-12	0	0	0	0	0	0	1	0	0	0	0	0	1
13-16-16-13-30-25-11-15-13-14-11-12-19-15-15-22-12	0	0	0	0	0	0	1	0	0	0	0	0	1
13-14-18-13-30-24-10-16-13-14-11-11-20-15-18-22-12	0	0	0	0	0	0	1	0	0	0	0	0	1
13-14-16-12-29-24-11-14-13-14-11-13-19-15-18-24-12	0	0	0	0	0	0	1	0	0	0	0	0	1
13-14-17-12-29-24-11-14-13-14-11-13-19-15-18-24-12	0	0	0	0	0	0	1	0	0	0	0	0	1
13-14-16-13-30-24-11-14-13-14-11-13-19-16-16-22-11	0	0	0	0	0	0	0	2	0	0	0	0	2
13-14-17-13-31-24-11-16-13-14-11-11-20-14-16-22-11	0	0	0	0	0	0	0	1	0	0	0	0	1
13-14-15-14-31-22-10-16-13-14-11-11-21-13-15-22-12	0	0	0	0	0	0	0	3	0	0	0	0	3
13-14-15-13-30-22-10-16-13-14-11-11-21-14-15-22-12	0	0	0	0	0	0	0	1	0	0	0	0	1
13-14-17-14-32-24-11-14-13-14-11-11-20-15-19-22-11	0	0	0	0	0	0	0	1	0	0	0	0	1
13-14-18-13-29-23-10-16-13-14-11-12-20-15-17-22-11	0	0	0	0	0	0	0	2	0	0	0	0	2
13-12-17-13-29-24-10-14-13-14-11-13-19-16-16-22-12	0	0	0	0	0	0	0	2	0	0	0	0	2
13-15-16-13-29-24-11-15-13-14-11-10-19-15-17-22-12	0	0	0	0	0	0	0	3	0	0	0	0	3
14-14-17-12-28-24-10-13-13-15-11-11-20-15-17-22-12	0	0	0	0	0	0	0	0	1	0	0	0	1
13-15-18-12-28-24-9-13-13-15-11-13-20-15-20-22-12	0	0	0	0	0	0	0	0	1	0	0	0	1
13-15-19-12-28-24-9-13-13-14-11-12-20-15-18-22-12	0	0	0	0	0	0	0	0	1	0	0	0	1
14-13-18-13-30-24-9-14-13-14-11-11-20-15-16-22-11	0	0	0	0	0	0	0	0	1	0	0	0	1
13-16-17-13-31-24-10-14-14-14-11-12-19-18-15-22-12	0	0	0	0	0	0	0	0	1	0	0	0	1
13-13-16-12-28-22-9-14-13-14-13-11-19-16-14-22-11	0	0	0	0	0	0	0	0	1	0	0	0	1
13-14-14-12-30-23-9-13-13-14-12-12-20-15-17-23-11	0	0	0	0	0	0	0	0	0	1	0	0	1
14-16-17-13-31-24-11-15-13-14-11-12-19-15-15-22-13	0	0	0	0	0	0	0	0	0	1	0	0	1
13-13-15-12-29-25-10-14-13-14-11-11-19-16-15-22-11	0	0	0	0	0	0	0	0	0	1	0	0	1
13-13-17-14-32-24-11-14-13-14-12-12-20-15-14-22-12	0	0	0	0	0	0	0	0	0	2	0	0	2
13-14-16-13-30-25-10-14-13-14-11-13-19-18-17-23-12	0	0	0	0	0	0	0	0	0	1	0	0	1
13-14-18-13-29-23-10-14-13-15-11-13-19-15-17-22-12	0	0	0	0	0	0	0	0	0	1	0	0	1

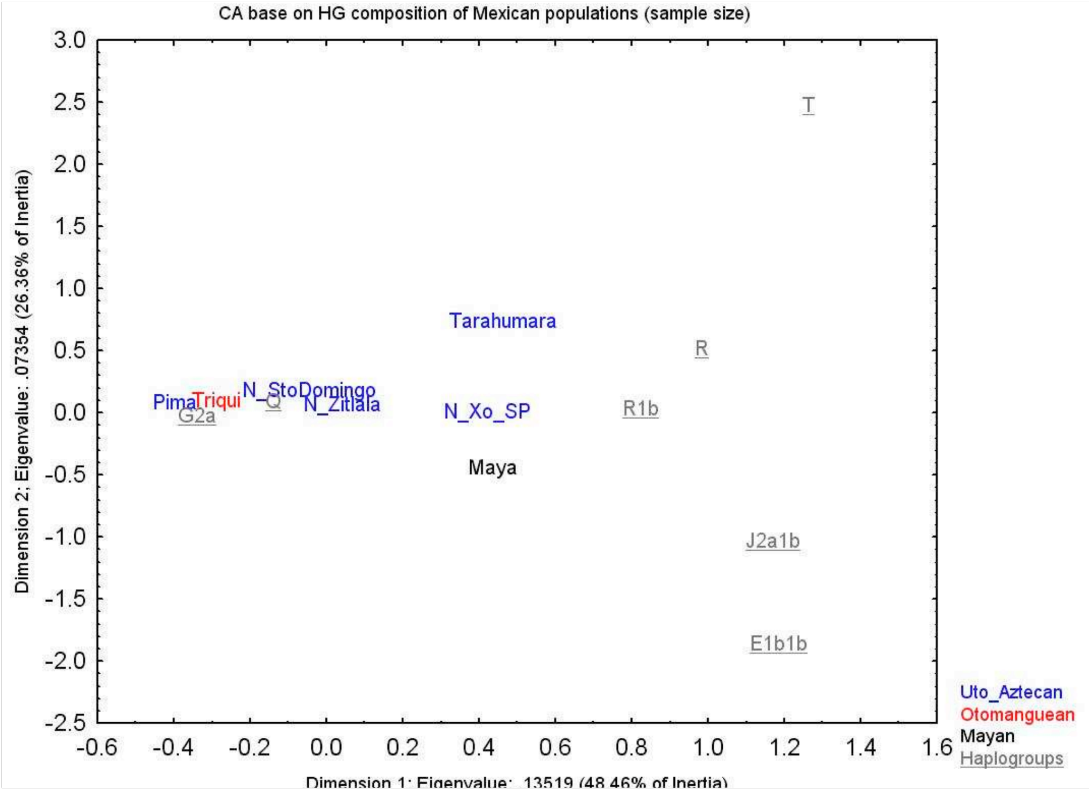
13-14-18-12-29-23-10-13-14-14-11-12-20-15-18-22-12	0	0	0	0	0	0	0	0	0	1	0	0	1
13-14-14-12-29-23-10-13-14-14-11-12-20-15-18-22-12	0	0	0	0	0	0	0	0	0	1	0	0	1
13-14-17-13-28-24-10-15-13-14-11-12-19-15-16-22-12	0	0	0	0	0	0	0	0	0	1	0	0	1
15-15-17-14-31-24-10-14-13-14-11-12-22-18-18-22-11	0	0	0	0	0	0	0	0	0	1	0	0	1
13-16-17-14-31-24-10-14-13-14-11-12-19-16-16-22-11	0	0	0	0	0	0	0	0	0	1	0	0	1
14-14-16-13-30-24-10-14-13-14-11-11-21-17-16-22-12	0	0	0	0	0	0	0	0	0	1	0	0	1
13-15-18-13-31-23-10-14-12-14-11-13-19-17-16-22-11	0	0	0	0	0	0	0	0	0	1	0	0	1
13-13-17-12-28-23-10-14-13-14-13-12-19-17-15-22-11	0	0	0	0	0	0	0	0	0	0	3	0	3
13-14-16-12-28-23-10-14-13-14-13-12-19-16-15-22-11	0	0	0	0	0	0	0	0	0	0	1	0	1
13-14-16-12-28-23-10-14-13-14-11-12-19-15-15-22-11	0	0	0	0	0	0	0	0	0	0	2	0	2
13-15-17-14-31-24-10-15-13-14-11-11-20-18-15-22-12	0	0	0	0	0	0	0	0	0	0	1	0	1
13-14-17-14-33-23-10-16-13-14-11-11-19-15-16-22-11	0	0	0	0	0	0	0	0	0	0	2	0	2
13-14-16-12-28-23-10-14-13-14-11-12-19-16-15-22-11	0	0	0	0	0	0	0	0	0	0	3	0	3
13-14-16-14-30-24-10-16-13-14-12-13-19-15-15-22-12	0	0	0	0	0	0	0	0	0	0	1	0	1
13-13-17-12-28-23-10-14-13-14-11-12-19-17-15-22-11	0	0	0	0	0	0	0	0	0	0	13	0	13
13-14-17-14-32-24-10-16-13-14-11-11-19-15-16-22-11	0	0	0	0	0	0	0	0	0	0	7	0	7
13-14-15-12-28-23-10-14-13-14-11-12-19-16-15-22-11	0	0	0	0	0	0	0	0	0	0	5	0	5
13-13-17-12-26-23-10-14-13-14-11-11-19-17-15-22-11	0	0	0	0	0	0	0	0	0	0	1	0	1
13-14-15-12-28-23-10-14-13-14-11-12-19-15-15-22-11	0	0	0	0	0	0	0	0	0	0	1	0	1
13-14-16-14-30-24-10-16-13-13-12-13-19-15-16-22-12	0	0	0	0	0	0	0	0	0	0	1	0	1
13-14-18-15-32-24-10-16-13-14-11-11-19-15-16-22-11	0	0	0	0	0	0	0	0	0	0	4	0	4
13-15-17-14-31-24-10-15-13-14-11-12-20-18-16-22-12	0	0	0	0	0	0	0	0	0	0	3	0	3
13-13-17-13-28-23-10-14-13-14-13-12-19-17-15-22-11	0	0	0	0	0	0	0	0	0	0	1	0	1
Native Mexicans	22	13	2	4	14	19	7	15	6	14	49	0	165
13-15-19-14-31-23-10-15-14-14-11-13-20-15-16-22-12	0	0	0	0	0	0	0	0	0	0	0	1	1
13-15-18-14-30-23-10-16-13-14-11-14-20-15-17-22-12	0	0	0	0	0	0	0	0	0	0	0	1	1
14-17-17-13-29-24-10-15-13-14-11-14-20-15-15-22-13	0	0	0	0	0	0	0	0	0	0	0	1	1
13-14-18-13-31-23-10-14-13-14-11-12-20-15-17-22-12	0	0	0	0	0	0	0	0	0	0	0	2	2
13-15-16-13-29-23-10-15-13-14-12-12-19-16-16-22-11	0	0	0	0	0	0	0	0	0	0	0	1	1

13-11-17-13-29-24-10-14-13-14-11-13-20-15-18-23-12	0	0	0	0	0	0	0	0	0	0	0	1	1
13-15-17-13-31-24-10-14-13-14-11-14-20-15-16-22-13	0	0	0	0	0	0	0	0	0	0	0	1	1
Quechua individuals	0	0	0	0	0	0	0	0	0	0	0	8	8
Total all native populations	22	13	2	4	14	19	7	15	6	14	49	8	173

[a] Constructed using the marker order: DYS19, DYS385a, DYS385b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, GATA-H4

Nahuas from [b] Xochimilco, [c] Zitlala, [d] San Pedro, and [e] Santo Domingo

Figure S1.


CA base on HG composition of Mexican populations (sample size)
Dimension 2; Eigenvalue: .07354 (26.36% of Inertia)
Dimension 1; Eigenvalue: .13519 (48.46% of Inertia)
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2.0
1.5
1.0
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-0.5
-1.0
-1.5
-2.0
-2.5
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
T
Tarahumara
R
Pima
Triqui
N_StoDomingo
Q
N_Zitlala
G2a
N_Xo_SP
R1b
Maya
J2a1b
E1b1b
Uto_Aztecan
Otomanguean
Mayan
Haplogroups

Figure S2.

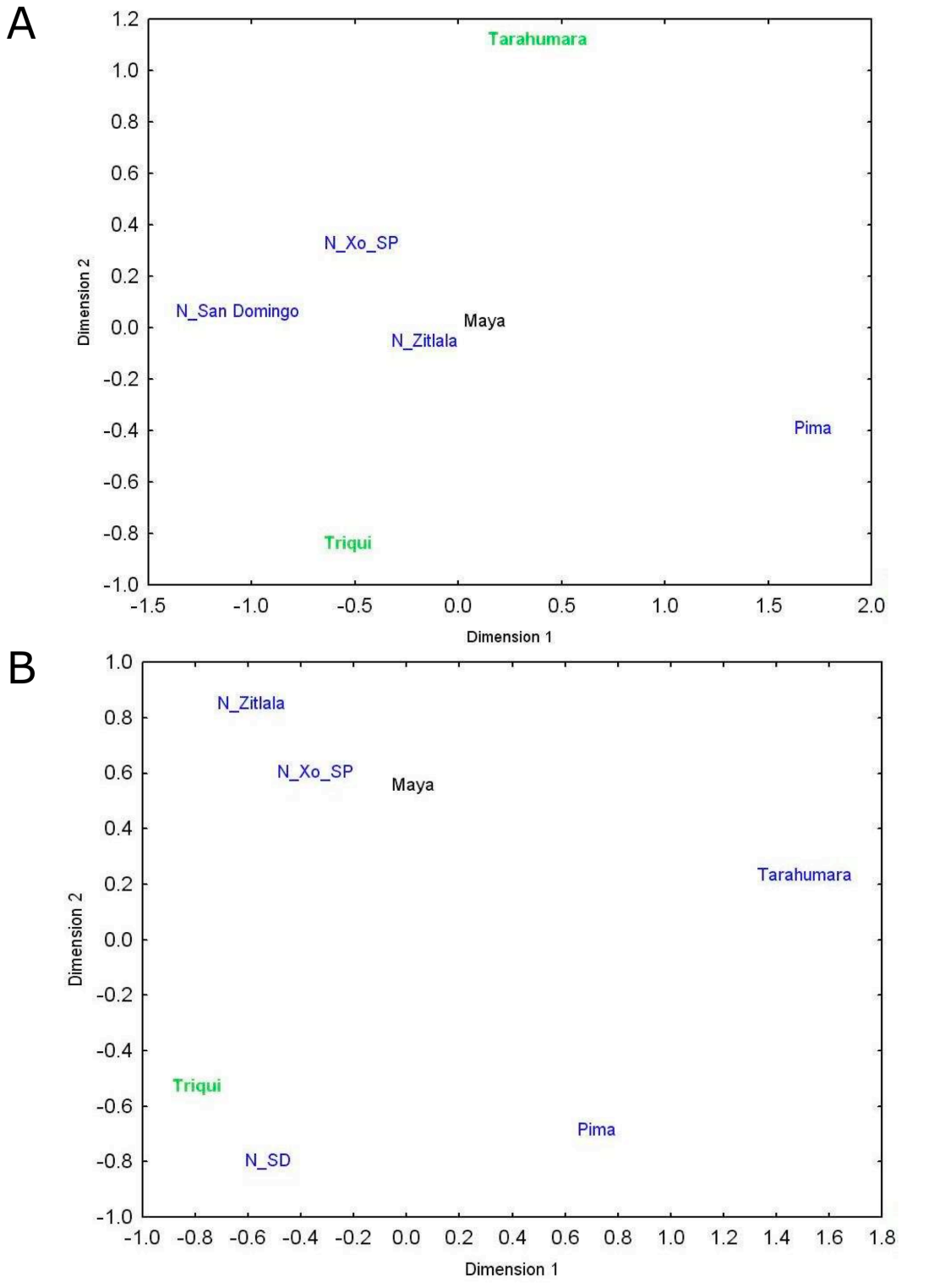
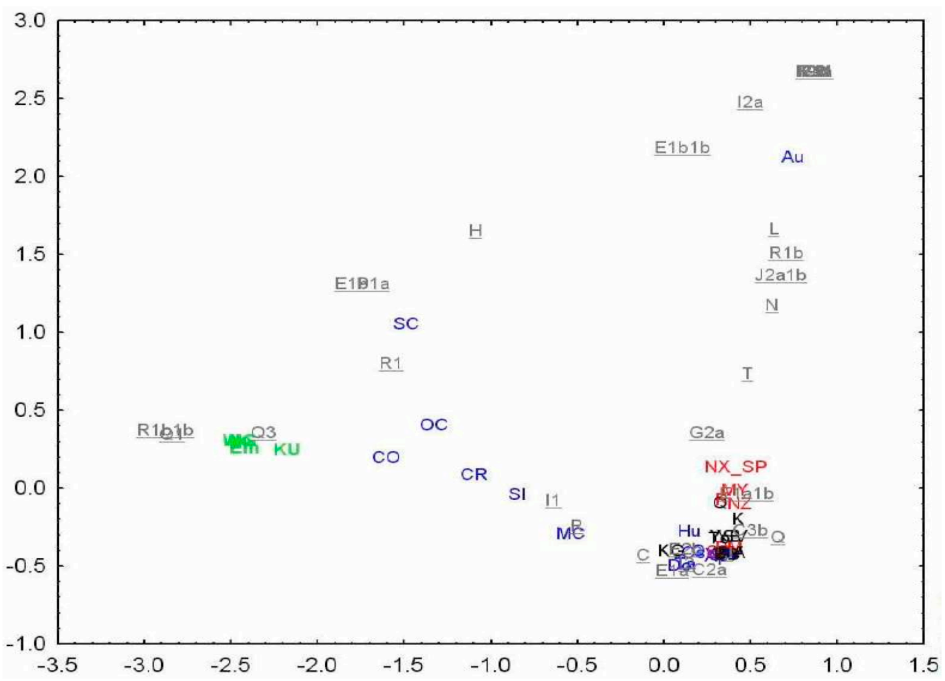

A
Tarahumara
N_Xo_SP
N_San Domingo
Maya
N_Zitlala
Pima
Triqui
Dimension 2
Dimension 1
B
N_Zitlala
N_Xo_SP
Maya
Tarahumara
Triqui
Pima
N_SD
Dimension 2
Dimension 1

Figure S3.

A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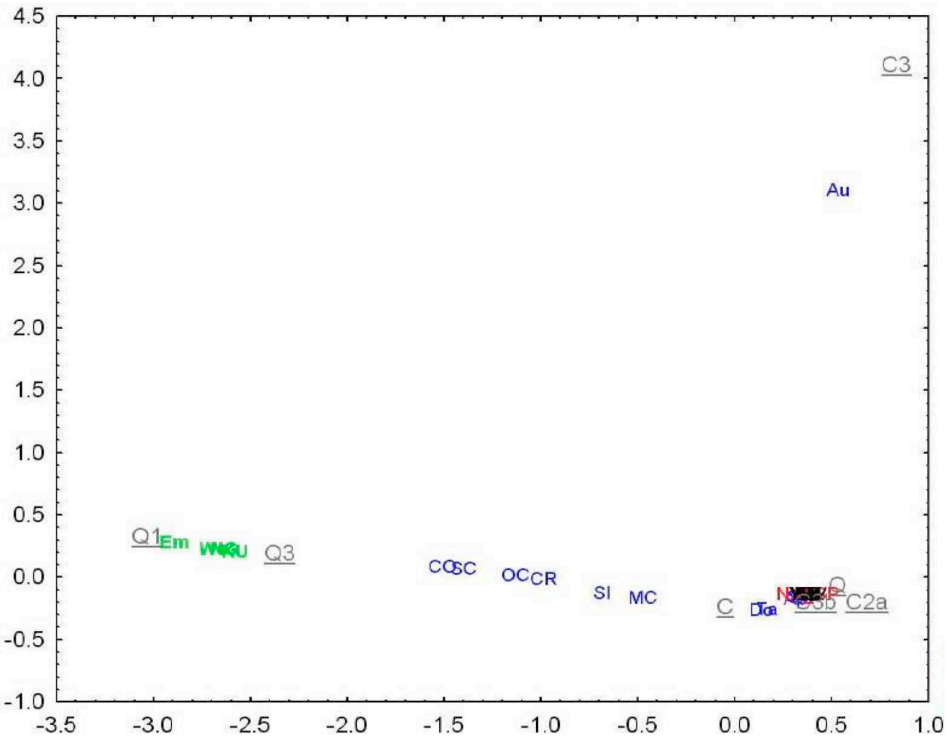
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C3
Au
Q1
Em
Q3
CO
SC
OC
CR
SI
MC
C
C2a

Chapter 4: Origin and Genetic Differentiation of Three Mexican Native Groups (Purépechas, Triquis and Mayas): Contribution of CODIS-STRs to the History of Human Populations of Mesoamerica

Martínez-Cortés G, Nuño-Arana I, Rubi-Castellanos R, Vilchis-Dorantes G, Luna-Vázquez A, Coral-Vázquez RM, Canto-Cetina T, Salazar-Flores J, Romero-Rentería O, Muñoz-Valle JF, **Sandoval-Mendoza K**, Gamero-Lucas JJ and Rangel-Villalobos H

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Origin and Genetic Differentiation of Three Native Mexican Groups (Purépechas, Triquis, and Mayas): Contribution of CODIS-STRs to the History of Human Populations of Mesoamerica

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Running headline: Origin and genetic differentiation of three Native groups from Mexico

Keywords: STRs; CODIS; Mexico; Native groups, Mesoamerica.

Abstract

Background: CODIS-STRs in Native Mexican groups have been rarely analyzed for both human identification and anthropological purposes. *Aim*: To analyze the genetic relationships and population structure among three Native Mexican groups from Mesoamerica. *Subjects and methods:* 531 unrelated Native individuals from Mexico were PCR-typed for 15 and 9 autosomal STRs (Identifiler™ and Profiler™ kits, respectively), including five population samples: Purépechas (Mountain, Valley, and Lake), Triquis, and Yucatec Mayas. Previously published STR data were included to the analyses. *Results*: Allele frequencies and statistical parameters of forensic importance were estimated by population. The majority of Native groups were not differentiated pairwise, excepting Triquis and Purépechas, which was attributable to their relative geographic and cultural isolation. Although Mayas, Triquis and Purépechas-Mountain presented the highest number of private alleles, suggesting recurrent gene flow, the elevated differentiation of Triquis indicates a different origin of this gene flow. Interestingly, Huastecos and Mayas were not differentiated, which is in agreement with the archeological hypothesis that Huastecos represent an ancestral Maya group. Interpopulation variability was larger in Natives than in Mestizos, both significant. *Conclusion*: Although results suggest European admixture has increased the similarity between Native Mexican groups, the differentiation and inconsistent clustering by language or geography stresses the importance of serial founder effect and/or genetic drift to depict their present genetic relationships.

Introduction

Patterns of the current population structure provide an important source of data for inferences regarding recent demographic history. Genetic variation among human populations has shown that groups living on the same continent are relatively homogeneous (Bamshad et al., 2004). However, Native American populations exhibit considerable interpopulation variability indicating differences between populations from North and South America (Bortoloni et al., 2003; Mao et al., 2007; Wang et al., 2007). The pre-Columbian civilizations of most of Mexico and Central America, together comprising Mesoamerica, participated in the same system of beliefs and rites; they shared a certain lifestyle –sedentary–, as well as social and political organization. A relative cultural homogeneity based on archaeological and anthropological data has been described (Duverger, 2007). However, also observed is a linguistic and genetic heterogeneity in Mesoamerica, shaped by both demographic and biological factors (Wang et al., 2008). In Mexico, the present indigenous population numbers 10.2 million, representing 9.6% of the total Mexican population. There is a spread of 156,557 native settlements in 803 localities, in which >30% of the population speak an indigenous language. Using language as a criterion selection, it is possible to estimate that in Mexico there are >68 native groups >85 languages and variant dialects described until now (Cisneros, 2004; National Institute of Statistics, Geography, and Informatics-Mexico [INEGI], 2005); nearly 80% of this population is concentrated in eight Mexican States as follows: Chiapas; Oaxaca; Guerrero; Hidalgo; Yucatán; Campeche; Veracruz, and San Luis Potosí.

Among the Native Mexican groups analyzed in this work, Purépechas –also known as Tarascos– constituted one of the most important Mesoamerican cultures at the time of

Spanish contact, coming to control a vast area of western Mexico (70,000 km^2) including the State of Michoacán and part of the states of Guanajuato, Guerrero, Jalisco, Colima, Querétaro, and Mexico. In point of fact, the Purépechas were one of the few groups that resisted the Aztec expansion prior to the Spanish Conquest (Michelet, 2001). They derived from admixture of different Chichimecas groups, a term referring to nomad hunters from Aridoamerica. According to the Relation and Chronicles of Michoacán, the Chichimecas migrated with Aztecs and other Native groups from the mythic site, *Chicomoztoc*; they separated to the East and arrived at Michoacán, where they admixed with local Nahuas already settled in Michoacán territory, giving rise to the Native group known as pre-Tarascos (Kirchhof, 1956). Other sources claim they formed a social organization structured in small groups that arrived first at Zacápu and Naranxán in the state of Michoacán ca. 4,000 ybp; they eventually migrated and congregated at Pátzcuaro and contiguous Lakes (Jiménez-Moreno, 1948; Schöndube, 1996; Michelet, 1996, 2001). The second Native group analyzed in this study comprised the Triquis, who presumably originated in the Central Valley of Oaxaca State –probably Monte Alban–, and were eventually banished by the Zapotecans. Subsequently, they arrived at their current location in the western Oaxaca mountain region at nearly 2,000 ybp. At the beginning of the XV century, the Triquis were subjugated by the Aztecs and were forced to paid tribute (Lewin-Fisher and Sandoval-Cruz, 2007). At the time of the Spanish contact, the Triquis already constituted a cultural and linguistic island in the High Mixteca region of Oaxaca. At present, the Triquis comprise two principal regions with cultural and linguistic differences: San Juan Copala (Low), and Chicahuaxtla (High); access to their territory is difficult due to its location at the confluence of the Sierra Madre Oriental with the Sierra Madre Occidental, comprising an extension of 500 km^2 (Huerta-Ríos, 1995; Lewin-Fisher and

Sandoval-Cruz, 2007). Finally, we analyzed the Yucatec Mayas, who constituted one of the most important Mesoamerican cultures because of their ancient cultural and scientific legacy. The Maya civilization inhabited a large area of southeastern Mexico and Central America, with a history of ca. 3,000 ybp. During this time, hundreds of dialects were spoken in these regions, generating nearly 44 different contemporary Mayan languages. Records and archeological data indicate that pre-Columbian Mayas of the Yucatán Peninsula achieved two large migrations during the Late Classic and Early Post-Classic ages, including one from the Central Uplands of Mexico across the coastal plain of the Gulf of Mexico, and another, yet more ancient, from the Petén area in the Maya Uplands at the South of Yucatan Peninsula (Nalda, 2005; Schmidt, 2007). The identity of these culture remains in force to the present day with the concurrence of at least three factors: the everyday use of the Mayan language; the permanence of religious rituals and customs, and a social organization of autonomous communities. Their social and political conditions were markedly inferior during the three centuries following the Spanish Conquest (Ruz, 2006).

To unravel the differentiation processes that generated the population's genetic heterogeneity, microsatellites –or Short tandem repeats (STRs)– constitute ideal polymorphic markers, whose relatively high mutation rate allows assessment of the biological diversity and elucidation of the history of human populations (Bosch et al., 2000; Zhivotovsky et al., 2003; Sahoo and Kashyap, 2005; Liu et al., 2006). In this context, we highlight the autosomal STRs included in the Combined DNA Index System (CODIS), which are widely used for human identification purposes. The correct interpretation of CODIS-STR-generated DNA profiles in forensic casework requires knowledge of the allele distribution and some statistical parameters in the population in which the system will be

applied; thus, worldwide-population STR datasets have been generated for this purpose. In Mexico, despite the large number of Native groups, only a few molecular studies have been conducted with autosomal STR loci in these populations (Rangel-Villalobos et al., 2000; Sánchez et al., 2005; Barrot et al., 2005; Ibarra-Rivera et al., 2008; González-Martín et al., 2008).

In this work, we obtained CODIS-STR population data in order to estimate statistical parameters of forensic importance of five population samples from the following three Native Mexican groups: Purépechas; Triquis, and Mayas. In addition, we analyzed the genetic relationships and population structure (AMOVA) in these native groups (clustered by geographic and linguistic criteria), including previously reported ancestral populations (African and European), Mestizos, and Natives from Mexico. Anthropological discussion addressed both pre-Columbian records and the possible present-day effects of gene flow among these Native populations.

Methods

Population Sample

A total of 531 unrelated individuals from five indigenous communities were studied. Prior to the inclusion in our study, all volunteers signed an informed consent letter, according to the ethical guidelines of the Helsinki Declaration; they were classified into three Native Mexican groups: (i) 333 Purépechas from three areas of the western state of Michoacán, including the localities of Zipiajo (n = 168), Angahuan (n = 103), and Puácuaro (n = 62) from the Mountain, Valley, and Lacustrine Regions, respectively; these three population samples were analyzed individually; (ii) 108 Triquis from the District of San Juan Copala in the Mixteca region of the eastern state of Oaxaca, and (iii) 90 Mayas from different

localities around Mérida, the largest city of the Yucatán peninsula, in Mexico's southeastern region. DNA was extracted from fresh blood samples by the salting-out method (Miller et al., 1988) and from buccal swabs by Chelex® 100 method (Walsh et al., 1991). For interpopulational analyses, we included previously published Native Mexican and Mestizo populations (Table I); their geographic location throughout the Mexican Republic is presented in Figure 1. In addition, considering the origin of European conquerors and African slaves who came to Mexico since the European contact with the New World (Rubi-Castellanos et al., 2009), population samples from Spain and Central-Western Africa were included for this purpose. The European population sample consisted of a gene pool obtained from different autochthonous communities of Spain; similarly, the African sample included a gene pool of Fangs and Equatorial Guinea populations located in Western Africa (Table I). For Native groups, their linguistic classification is indicated in Figure 2 (Gordon, 2005; National Institute of Indigenous Languages-Mexico [INALI], 2008).

PCR amplification and genotyping

We used the Profiler Plus™ kit in Mayas and Triquis, and Identifiler™ kit in Purépechas, both kits for human identification from Applied Biosystems (Foster City, CA, USA), which are designed for co-amplification of the following autosomal STR loci: D8S1179; D21S11; D7S820; vWA; D18S51; D3S1358; D13S317; D5S818, and FGA (Profiler Plus™ PCR kit). Additionally, CSF1PO, D19S433, TPOX, TH01, D16S539, and D2S1338 were analyzed in Purépechas (Identifiler™ PCR kit). The amplified products were separated by capillary electrophoresis using the ABI Prism™ 310 Genetic Analyzer following

manufacturer recommendations. The allelic ladder provided with the kit and GeneMapper ID software version 3.2 were utilized for genotyping.

Data analyses

Allele distribution and statistical parameters of forensic importance were computed with the PowerStats program (Tereba, 1999). For each population sample, Hardy-Weinberg expectations and two-loci equilibrium were verified by exact tests with a 95% confidence interval (95% CI) with the Genetic Data Analysis (GDA) program version 1.1 (Lewis and Zaykin, 2001). Bonferroni correction was applied to evaluate these *p*-values according to the loci-number of Profiler and Identifiler kits ($p < 0.0055$ and $p < 0.0033$, respectively). Gene flow among Native groups was assessed as the number of migrants per generation (Nm) according to the equation of Wright (Wright, 1951). In addition, we estimated the following parameters of genetic diversity in each Native group: (i) mean allele number, (ii) average expected heterozigosity, and (iii) number of alleles exclusively observed in one population or "private alleles". To evaluate private alleles we applied the computer program ADZE (Allelic Diversity AnalyZEr) that uses a rarefaction approach to trim unequal samples to the same standardized sample size (g), and to assess the sample size-corrected private allelic richness to any set of populations (Szpiech et al. 2008). For interpopulational analysis, we included STR data from previously published populations described in Table I. For consistent comparison, data of only 9 STR loci included in the Profiler™ kit analyzed in all these populations were employed for this purpose. Genetic differentiation was evaluated by normalized F_{ST} distances and pairwise F_{ST} *p*-values, computed with the Arlequin 3.1 software (Excoffier et al., 2005). Bonferroni correction was implemented to evaluate multiple F_{ST} *p*-values by population. F_{ST} distance was selected

because represent genetic differentiation patterns by drift, corresponding with both genetic and archeological records of human populations (Pérez-Lezaún et al. 1997). Genetic distances were displayed on a multidimensional scaling (MDS) plot to explore the genetic relationships among populations with the SPSS for Windows program version 10.0. Analysis of molecular variance (AMOVA) was carried out placing Mestizos and Natives populations in different clusters based on geography and linguistic classification, as properly described in the text. Additionally, we utilized spatial analysis of molecular variance (SAMOVA), which is similar to the traditional AMOVA, but allow clustering populations in groups geographically are genetically homogeneous (Dupanloup et al., 2002). To establish whether decrease of homozygosity (or increase of heterozygosity) reflects European admixture in Native groups, we reviewed correlation of the decrease of homozygosity with the genetic distance between each group and the southwestern Spanish population. For each Native group, this European admixture marker (a decrease in homozygosity) was correlated with its geographic distance and altitude to the nearest Mexican-Mestizo population. Thus, the final purpose was to verify whether geographic distance and altitude influence European admixture in these Native groups. In order to investigate whether isolation-by-distance (IBD) could explain genetic differentiation among Native populations, we revised the correlation between genetic and geographic distances among these groups (Ramachandran et al., 2005). The statistical significance of these correlations was evaluated by the Mantel test. Distances in km between populations were computed employing geographic coordinates with the Great Circle Calculator program (http://www.gb3pi.org.uk/great.html). Concurrently, we examined possible landscapes of genetic and geographic differentiation processes by means of AIDA program software (Bertorelle and Barbujani, 1995).

Results

Statistical Parameters of Forensic Importance and Genetic Diversity

Allele distribution and statistical parameters of forensic importance of the Native Mexican groups Purépechas (West), Triquis (South), and Mayas (South-East) were estimated and provided as electronic supplementary material (ESM) (Tables S1-S5). In general, for all five Native population samples, genotype distribution by locus and two loci combination were in agreement with Hardy-Weinberg and linkage equilibrium, respectively. Only two loci displayed significant *p*-values for HWE test after applying the Bonferroni correction: D3S1358 in Purépechas-Lake, and D7S580 in Triquis; these *p*-values were close to the Bonferroni limit and represented unique events by population. Therefore, they do not support significant immigration or endogamy processes in these Native groups; thus, we did not consider they deserve further discussion. The combined Power of discrimination (PD) and Power of exclusion (PE) for both STR systems were ≥0.9999 and ≥0.99752, respectively.

The genetic diversity parameters of these groups are presented in Table II. Purépechas-Mountain had the largest number of private alleles with six, followed by the Purépecha-Valley and Mayas, with three private alleles each Native group. For the mean allele number, again Purépechas-Mountain had the maximum value, followed by Mayas and Choles. Finally, the average of expected heterozigosity pointed out Otomi-Sierra, Choles and Purépechas-Mountain, respectively, as the Native groups with larger genetic diversity, whereas the smallest value was observed in Triquis. Conversely, the ADZE program revealed that the mean number of private alleles in standardized sample sizes (g) was higher in Mayas, Triquis, Purépechas-Mountain, respectively (Figure 3).

Genetic Differentiation among Populations

The MDS plots based on pairwise normalized Fst values (Figure 4) shows the genetic relationships among populations. The stress values for both MDS plots (Figure 4A and 4B) were 0.0010 and 0.1143 respectively, indicating that the data represent an appropriate configuration in their spatial distribution. As could be expected, the majority of Mexican Mestizos displayed smaller genetic distances with the European population than the Native groups (data not shown), as graphically represented in the MDS plot (Figure 4A). Additional discussion concerning genetic differentiation among Mexican Mestizos will be omitted, considering that this has been conducted in a recent report (Rubi-Castellanos et al., 2009). Regarding Native groups, Triquis and Purépechas from Valley and Lake presented significant differences with all the Mestizo populations included herein ($p < 0.0056$; data not shown), in agreement with their position in the MDS plot (Figure 4A). This significant differentiation with Mestizos suggests low European admixture in these three Native populations, contrasting with a previous observation of elevated European admixture in Purépechas in view of their high heterozygosity and similar STR allele frequencies to western Mestizos ($p >0.05$) (Rangel-Villalobos et al., 2000); the low number of markers and the small size ($n = 25$) and geographical origin of the Purépecha population sample previously studied appear to be relevant in explaining this difference.

Conversely, the Tepehuas, Otomíes-Sierra, Otomíes-Valley, Mayas, and Choles were genetically closer to Mestizos from Central and southeastern regions, including the Valley of Mexico, Hidalgo, Puebla, Veracruz, and Yucatán (Figure 4A). This result, in conjunction with the non-significant differentiation of these Native groups with Mestizos ($p < 0.0056$; data not shown), suggests the presence of certain European admixture level in these Native

populations, as previously reported for the Chol population sample (González-Martín et al., 2008). However, the position of the populations in the MDS plot formally does not represent the European admixture level in these Native groups, which would require a deeper analysis with suitable loci as ancestry informative markers (AIMs); thus, these comments concerning admixture must be taken with caution. Concurrently, pairwise comparisons showed non-significant differentiation among Tepehuas, Otomíes-Sierra, Otomíes-Valley, Mayas, and Choles (Table III).

The correlation was not significant between homozygosity in Native groups and the increase of genetic distance to the Spanish population of reference (r^2= 0.587; p = 0.0550), indicating that homozygosity was not a suitable European admixture marker for our data (plot not shown). This conclusion was confirmed when correlation test was repeated without Triquis, the most differentiated Native group, diminishing the estimated correlation (r^2 = 0.072; p = 0.3320).

Genetic structure (AMOVA)

Analysis molecular of variance (AMOVA) tests consistently demonstrated that the majority of genetic variability for the 9 STR system in Mexican populations is at the intra-individual level (F_{IT} = 98.8–99.3%), which was moderated and not significant, attributable to the polymorphism of the 9 STRs. Although the coefficient that describes the intrapopulation inbreeding (F_{IS}) was either positive or negative, in all population groups it was low and not significant (Table IV). Conversely, inter-population variability in Native groups was larger than in Mestizos (F_{ST} = 1.25 vs. 0.26%), both of these significant. In Purépechas the inter-population variability was significant and similar to all 10 Mexican Native groups (F_{ST} = 1.21; p= 0.0000), whereas in Maya groups was lower but also significant (F_{ST} = 0.45; p=

0.0088). In both Purépecha and Maya groups the into-individual variability was not significant (Table IV). The following AMOVA test clustering Mestizos vs. Native groups indicated low internal consistency –or high heterogeneity– into these clusters, because the genetic differentiation among populations into groups was larger than the differentiation among groups (F_{SC} 0.61 vs. F_{CT} 0.38%), both of these significant (Table IV).

Finally, a set of AMOVA and SAMOVA tests were carried out for grouping Native groups according to linguistic and geographic criteria, respectively (Table IV). Results revealed that on increasing linguistic criteria for clustering Native groups (stock and family, particularly), differentiation among groups also increased slightly (F_{CT} = 0.2–0.62%), decreasing differentiation among populations into groups (F_{SC} = 1.10–0.74%). The best SAMOVA results for grouping populations considering both genetic and geographic criteria separated consistently to the Triquis, Purépechas and the Native groups from Hidalgo, whereas the Huastecos were either separated or into the Maya-Choles group (Table IV). However, the variability at both intra and inter population level was significant.

Landscapes of Genetic and Geographic Differentiation Patterns

Although the geographic distance (km) and genetic differentiation (F_{ST}) among Native Mexican groups was not correlated (r^2 = –0.0167; p = 0.4300), the autocorrelation plot allowed shaping (arbitrarily) of three population clusters based on the following genetic and geographic criteria: 1) Purépechas, Otomíes, Huastecos, and Tepehuas (< 500 km, and F_{ST} < 0.02); , 2) Mayas and Choles (> 800 km, and F_{ST} < 0.02), and 3) Triquis (F_{ST} > 0.02) (Figure 5). In the correlation test by cluster, only the geographically more remote native groups (Mayas and Choles) presented a significant correlation (r^2 = –0.5095; p = 0.0040). Concurrently, analysis with AIDA software displayed an apparent random pattern and few

significant values (4/9) that do not support the IBD differentiation model in Native groups from Mexico. Interestingly, the most significant value in the AIDA autocorrelogram plot appears to represent the geographical distance of the Triquis; subsequent analysis without this dataset clearly generates a random differentiation pattern (autocorrelogram plot not shown).

Discussion

Allele Distribution and Statistical Parameters of Forensic Importance

The STR allele frequencies and statistical parameters are useful for anthropological and human identification purposes (Evett and Weir, 1998). Particularly, genetic data of these widely employed STR systems are scarce in Native Mexican groups; as observed, these populations have a distinctive distribution regarding the admixed Mexican Mestizos, supporting the establishment of local STR databases for confident DNA profile interpretation in forensic casework.

AMOVA and Genetic Differentiation among Populations

Although our STR data do not allow the performance of a formal admixture analysis, the non-significant differentiation observed among Tepehuas, Otomíes-Sierra, Otomíes-Valley, Mayas, and Choles (Table III), and between these Native groups with Mestizos suggest either a shared Native American component in all these populations or a relative presence of European component in the cited Native groups (Figure 4A). The last option would imply that the European component could be acting as a homogenizing factor that has increased similarity among Native American populations. A similar observation has been reported in three of the seven indigenous groups studied with the Polymarker system (PM)

including Mixteca Alta, Mixteca Baja, and Nahuas of Xochimilco (Buentello-Malo et al., 2003). This is in agreement with the AMOVA results indicating the lowest variability is apportioned "among groups", when Mexican Mestizos and Native groups were clustered and compared (F_{CT}= 0.38%; Table IV); consequently, admixture occurring after European contact with New World populations came to diminish Native population genetic differentiation, previously generated by processes such as serial founder effect and random genetic drift as described for human populations (Ramachandran et al., 2005; Zhang and Dolan, 2008). Unfortunately, we could not use homozygosity as European admixture marker in these Native American populations. Probably the homozygosity usefulness diminished by a similar –although probably low– admixture level in the mentioned Mesoamerican Native groups. Finally, to estimate correctly the presence of European and/or African admixture in these groups, a deeper analysis with further loci would be needed (i.e., with Ancestry informative markers [AIMS]).

The larger genetic differentiation among populations in groups than among groups (Table IV), is consistent with the proposal of heterogeneity as a major characteristic of Mexican populations (Bonilla et al., 2005; Wang et al., 2008), although in contrast to a previous report claiming genetic homogeneity for seven Native Mexican groups based on five PM-system loci (Buentello-Malo et al., 2003); unfortunately, the authors did not apply a significance test to evaluate F_{ST}. The greater resolution power of the 9 STRs to disclose population genetic structure –with respect to the PM system– could explain the contrasting conclusions of these studies in Mexican populations.

The poor quality of both linguistic and geographic (SAMOVA test) criteria for clustering Native groups was particularly noteworthy because in all cases, differentiation among populations into groups was significant (p = 0.0000). Taken together, these results

emphasize the importance of the differentiation processes that acted upon Native American populations (Wang et al., 2007). Results of AIDA software and correlation tests indicated that, at the geographical level of these Native groups it is not possible to invoke a simple population pattern of genetic differentiation. However, it should be considered that genetic similarity at short distance (e.g., IBD) is likely to produce decreasing autocorrelation indices (Bertorelle and Barbujani, 1995). Therefore, the data available did not allow ruling out the possibility that significant patterns of geographic structure, rather than random variation, could exist in Mesoamerica when more Native American populations are studied. In addition, more complex evolutionary landscapes could fit better to explain the genetic differentiation presently observed among Native groups from Mesoamerica, such as Isolation by Migration (IM) models (Hey, 2005; Kitchen et al., 2008).

With respect to the genetic relationships among Native groups, we omitted discussing Otomíes from the Valley and Sierra, Tepehuas, and Huastecos (central region) because this has been previously addressed (González-Martín et al., 2004, 2008). Particularly, caution must be taken with respect to the lack of differentiation of the Tepehuas from the majority of Native groups (Table III), because this population sample had many STR data lost and was relatively small (n= 47); consequently, discussion about the Tepehuas genetic relationships will be avoided.

Therefore, we present a particular discussion of the results concerning the population samples studied herein:

Purépechas

The pairwise normalized F_{ST} genetic distances (Table III), graphically represented in the MDS plot (Figure 4B), depicted the Triquis and Purépechas from the Lake and Valley as

the most differentiated Native groups, respectively; these were probably influenced by cultural and geographic isolation, and the small effective population size of these groups, promoting differentiation processes as random genetic drift. In agreement with this differentiation, the Purépecha language has been described as an isolated dialect that is not related with any other linguistic family from Mexico (INALI, 2008) (Figure 2). In addition, some authors have suggested that the Purépechas received one or several migrations from Peru that landed on the Pacific Coast in the Mexican state of Michoacán; because they possess a distinctive archeology, anthropology, culture, and language (Ruiz, 1891; Peñaloza et al., 2001). However, this assertion is difficult to confirm, bearing in mind that the Purépechas rarely touched or lived on the coast; in addition, historical, archeological and anthropological records are not sufficient for supporting this theory (Michelet, 2001; Márquez-Joaquín, 2007). Conversely, Purépechas from the Mountain represented the population with the third most quantity of private alleles (Figure 3), behind Mayas and Triquis, respectively, without a significant increment in genetic diversity (Table II). Although for STRs we could not apply a neutrality test to evaluate the excess of private alleles with respect to the mutation-drift equilibrium expectation, it has been demonstrated that the excess of private alleles is a consequence of population amalgamation (Chakraborty et al., 1988), and particularly this effect has been observed in Native American populations by means of mitochondrial DNA (mtDNA), implying recurrent and high levels of gene flow (Fuselli, 2003). Concurrently, AMOVA test demonstrated significant differences among Purépecha populations, suggesting genetic heterogeneity (Table IV). In addition, preliminary studies of Native American paternal lineages defined by the mutation M3 (Páez-Riberos et al., 2006), allowed the proposal that the even distribution of Purépecha Y-STR haplotypes throughout the network joining tree including different Native Mexican

groups is a consequence of the pre-Columbian multiethnic origin of this Native group rather than of European admixture (via Mestizos). Similarly, mtDNA-haplogroups have revealed that Purépechas present an intermediate position between two clusters in a principal components plot (Peñaloza-Espinoza et al., 2007). In brief, these results are in agreement with the hypothesis that Purépechas is an ancient and cystic group in their own territory that, once it was shaped by admixture between Chichimecas and local Nahuas previously living in western Mesoamerica, most of the Purépecha group –in general– remained in the same place and had low admixture with other Mesoamerican groups (Jiménez-Moreno, 1948; Schöndube, 1996; Michelet, 1996, 2001). However, important differences in pre-Hispanic gene flow could exist between geographical regions, as observed in the Purépechas-Mountain population sample with respect to those of the Valley and Lake (Table II; Figure 3). Finally, to explain the present genetic background of this group, the recent Purépecha gene flow should not be disregarded, considering that census data (2000–2005) recorded a total of 1,498 Purépecha speakers living in Michoacán state migrated mainly to the states of Jalisco, Baja California, and Mexico, and to the U.S.A. (INEGI, 2007).

Mayas

In agreement with their same linguistic affiliation within the Maya-Totonaco group, Mayanse stock, and Maya family, the small and non-significant F_{ST} values indicate that Yucatec Mayas were not genetically differentiated from Huastecos and Choles (Figure 2; Table III). However, Huastecos showed significant differences from Choles, inducing the significant differentiation among the three Maya groups (Table IV), probably attributable to the higher genetic differentiation of Huastecos, and to recent gene flow that Choles have received from other ethnic groups (probably Highlands central groups), and/or from

Mexican Mestizos (Alejos-García and Martínez-Sánchez, 2007). This non-differentiation between Huastecos and Yucatec Mayas is important because is in agreement with the hypothesis that Huastecos could represent an ancestral Maya group that separated and remained in the Huasteca zone during migrations occurring 3,000 ybp (Ekholm, 1944). Concurrently, the non-differentiation between the Maya groups (from Yucatán and Choles from Campeche) from Otomíes could be indicative of gene flow among these central and southeastern Native groups, as a consequence of multiple human movements and arrangements throughout Mesoamerica since the fall of Teotihuacan up to the Early Post-Classic age (1,200–700 ybp), especially in the central highlands and Maya region (Nalda, 2005). This controversial theory of Toltec migration to Yucatán is supported by historical, archeological, pictography, social, and political organization, as well as the religion and militarism present in peninsular Mayas (Morley, 1946). In this context, based on 9 STR data, we can infer the cited Toltec migration to Yucatán could have increased the gene diversity in Mayas, in agreement with the largest number of private alleles estimated for Mayas (Figure 3), and with the elevated migration rate estimated in this work for these Native groups from central and southeastern regions of Mexico (Nm = 38.8). Similarly, Y-linked markers have displayed an elevated migration rate throughout these regions (Nm = 24.76), that could explain the relative homogeneity observed among Native groups from Mesoamerica (Rangel-Villalobos et al., 2008). Additionally, the influence of gene flow on Native groups from southeastern Mexico is supported by archeological references concerning pre-Columbian Mayas, who carried out several migration stages especially during the Late Classic and Early Post-Classic age (Nalda, 2005). In fact, multiple dates have catalogued this age as a "dynamic era" of Maya history (Soustelle, 1993; Nalda, 2005; Schmidt, 2007; Ibarra-Rivera et al., 2008).

Triquis

Triquis had the lowest average of genetic diversity (h = 0.6953), and the most distant MDS-plot position, presenting significant F_{ST} values with the majority of Native Mexican groups, suggesting that additional and/or more profound genetic differentiation processes have occurred in this population (i.e., inbreeding, founder effect, etc.). However, Triquis had the largest number of private alleles, only after that of the Mayas, suggesting they also could have received gene flow from Native groups, but different to that received by Mayas and Purépechas. Demographic data indicate that the total Triqui population throughout the Mexican territory is relatively small (~25,000 inhabitants), and recently a certain fraction has migrated to the States of Morelos, Veracruz, and Sonora, and to Mexico City, in addition to the U.S.A (Lewin-Fisher and Sandoval-Cruz, 2007). Particularly, the Triqui territory of the Lower Region (that belongs to the San Juan Copala, the origin of the population sample) is a small town with limited communication with Mexican Mestizos or nearby native groups (i.e. Mixtecos), aided by their rugged geographic location in an steep, difficult-to-access mountainous region. In addition, they have a cultural commitment to maintain their language and traditions, and limited confidence in persons from the outside (Huerta-Ríos, 1995; Lewin-Fisher and Sandoval-Cruz, 2007). Therefore, both geographic and cultural aspects have operated simultaneously, probably since pre-Columbian times, to shape the current differentiation of this Native group.

Conclusion

The CODIS-STR data here obtained validate the use of these markers for human identification purposes in these Native Mexican groups. A significant differentiation of Triquis and Purépechas from Valley and Lake was demonstrated, attributable to their

relative geographic and cultural isolation. Although a relative homogeneity was detected among Mesoamerican groups, particularly those inferred with higher European admixture, the large interpopulational variability rendered it impossible to shape consistent population clusters, stressing the importance of serial founder effect and genetic drift to depict their genetic relationships. Also, geographic and/or linguistic elements constituted a limited tool for explaining their current genetic relationships, presumably due to the complex historic and demographic events of the human populations from Mesoamerica, both prior to and after the Spanish Contact.

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Table I. Description of the Mexican and Worldwide populations used for interpopulation analysis.

Population	Abbr.	Sample	Geographical	Reference
Native American		**size**	**Origin**	
Chol	Chol	106	Campeche State	Sánchez et al., 2005
Tepehua	Tep	47	Hidalgo State	González-Martín et al., 2008
Otomi Sierra	OtoS	83	Hidalgo State	Barrot et al., 2005
Otomi Valley	OtoV	82	Hidalgo State	Barrot et al., 2005
Huastecos	Hua	133	Hidalgo State	Barrot et al., 2005
Maya	May	90	Yucatan State	This study
Triqui	Tri	108	Oaxaca State	This study
Purépechas	Pur	333	Michoacán State (Mich)	This study
Zipiajo	Pur M	168	Zipiajo, Mich (Mountain)	This study
Angahuan	Pur V	103	Angahuan, Mich (Valley)	This study
Puacuaro	Pur L	62	Puacuaro, Mich (Lake)	This study
Mestizos				
Chihuahua	Chi	162	North Central	Martínez-González et al., 2005
Nuevo León	NL	143	North East	Cerda-Flores et al., 2002
Jalisco	Jal	309	West	Rubi-Castellanos et al., 2008
Veracruz	Ver	170	Central	Licea-Cadena et al., 2006
Valley of Mexico	Mex	242	Central	Luna-Vázquez et al., 2005
Hidalgo	Hid	106	Central	Gorostiza et al., 2007
Puebla	Pue	313	Central	Rubi-Castellanos et al., 2008
Yucatán	Yuc	262	South-East	Rubi-Castellanos et al., 2008
Worldwide				
European	Eur	138	Southern Spain	Gamero-Lucas et al., 2000
African	Afr	132	North Africa	Gamero-Lucas et al., 2000

Table II. Parameters of Genetic Diversity based on nine CODIS-STRs estimated in ten Native groups from Mexico

Mexican Native group	Number of private alleles	Mean allele number	Average of expected heterozigosity
Chol	2	8.889	0.76410
Purépecha-M	6	9.556	0.76345
Purépecha-V	3	7.778	0.73520
Purépecha-L	2	7.444	0.74122
Tepehua	0	7.111	0.74830
Otomi-S	1	8.333	0.76631
Otomi-V	0	7.889	0.75463
Huasteco	1	8.333	0.74048
Triquis	2	8.000	0.69526
Mayas	3	9.111	0.75660

Table III. Pairwise F_{ST} distances (below diagonal), and F_{ST} *p*-values* (above diagonal) among 10 Native groups from Mexico

	PurM	PurV	PurL	Tep	OtoS	OtoV	Hua	Chol	May	Tri
PurM	*****	*0.0000*	*0.0000*	0.27051	0.00098	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*
PurV	0.01519	*****	0.97168	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*
PurL	0.01416	-0.00284	*****	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*
Tep	0.00095	0.00992	0.01106	*****	0.97656	0.82812	0.03711	0.98047	0.99023	*0.0000*
OtoS	0.00371	0.01189	0.01007	-0.00395	*****	0.0459	0.00391	0.01172	0.01172	*0.0000*
OtoV	0.01169	0.01612	0.01768	-0.00238	0.0025	*****	*0.0000*	0.02637	*0.0000*	*0.0000*
Hua	0.00975	0.01167	0.01288	0.00337	0.00417	0.01043	*****	0.00098	0.0127	*0.0000*
Chol	0.00885	0.0138	0.01346	-0.00343	0.00318	0.00313	0.00575	*****	0.05566	*0.0000*
Tri	0.02506	0.03521	0.03916	0.01157	0.02328	0.03014	0.02386	0.02371	*****	*0.0000*
May	0.00415	0.0088	0.00742	-0.00431	0.00335	0.00721	0.00309	0.0021	0.02422	*****

* Bonferroni correction indicated significance at $p < 0.0056$

Table IV. AMOVA and SAMOVA tests in Mexican populations based on 9 CODIS-STRs

MEXICAN POPULATIONS	**Nº Pop**	**Nº Groups**	**Into populations F_{IT} (%)**	**Inter populations F_{ST} (%)**	
Mestizos	8	1	99.27; *p*= 0.04203	F_{ST} = 0.26%; *p*= 0.0000	
Amerindians	10	1	98.83; *p*= 0.02542	F_{ST} = 1.25%; *p*= 0.0000	
MESTIZO/AMERINDIANS			**Into populations F_{IT} (%)**	**Among groups F_{CT} (%)**	**Populations into Groups F_{SC} (%)**
Mestizos *vs*. Amerindians	18	2	98.72; *p*= 0.0000	0.38; *p*= 0.0000	0.61; *p*= 0.0000
AMERINDIANS GROUPED					
Linguistic Group classification [a]	10	3	98.77; *p*= 0.0332	0.20; *p*= 0.0449	1.10; *p*= 0.0000
Linguistic Trunk classification [a]	10	5	98.71; *p*= 0.0273	0.62; *p*= 0.0000	0.74; p= 0.0000
Linguistic Family classification [a]	10	6	98.76; *p*= 0.0263	0.56; *p*= 0.0048	0.75; p= 0.0000
Geographic location [b]	10	5	98.74; *p*= 0.0293	0.63; *p*= 0.0000	0.71; *p*= 0.0000
Geographic location [c]	10	4	98.69; *p*= 0.0273	0.68; *p*= 0.0000	0.70; *p*= 0.0000

a. See linguistic classification criteria in Figure 2
b. May, Chol *vs.* Hua *vs.* PurM, PurV, PurL *vs.* Tri *vs.* Tep, OtoS, OtoV.
c. May, Chol, Hua *vs.* PurM, PurV, PurL *vs.* Tri *vs.* Tep, OtoS, OtoV.

FIGURE LEGENDS

Figure 1. Geographical locations of Mexican populations studied herein, and those used for comparison purposes. The previously published populations are indicated by black stars (Mestizos) and black points (Native American groups). Black triangles indicate populations reported in this study.

Figure 2. Linguistic classification of Native Mexican populations used for interpopulational analyses (Gordon, 2005; INALI, 2008). Underlined groups are reported on in this study.

Figure 3. Mean number of private alleles with standardized sample sizes (g) in Native Mexican populations obtained with the program ADZE.

Figure 4. Multidimensional scaling (MDS) plot based on normalized F_{ST} distances between (A) Mestizos, Native American, and Ancestral populations (European and African); (B) only Native American groups. See Table I for description of abbreviations.

Figure 5. Overlapped plots representing correlation between geographical and genetic distances (black lines) and the AIDA autocorrelogram (grey lines). Correlation plot displays the following three groups: Purépechas, Otomíes, Huastecos, and Tepehuas (black circles); Triquis (black squares), and Mayas and Choles (black triangles). In the autocorrelogram, filled diamonds indicate significant *p*-values ($p < 0.05$).

Figure 1.


Mestizos
Ethnic groups
Reported in this study
Chihuahua
Nuevo León
Otomíes
Tepehuas
Huastecos
Yucatán
Mayas
Choles
Jalisco
Hidalgo
Purépechas
Puacuaro
Zipiajo
Angahuan
Mexico city
Veracruz
Puebla
Triquis

Figure 2.

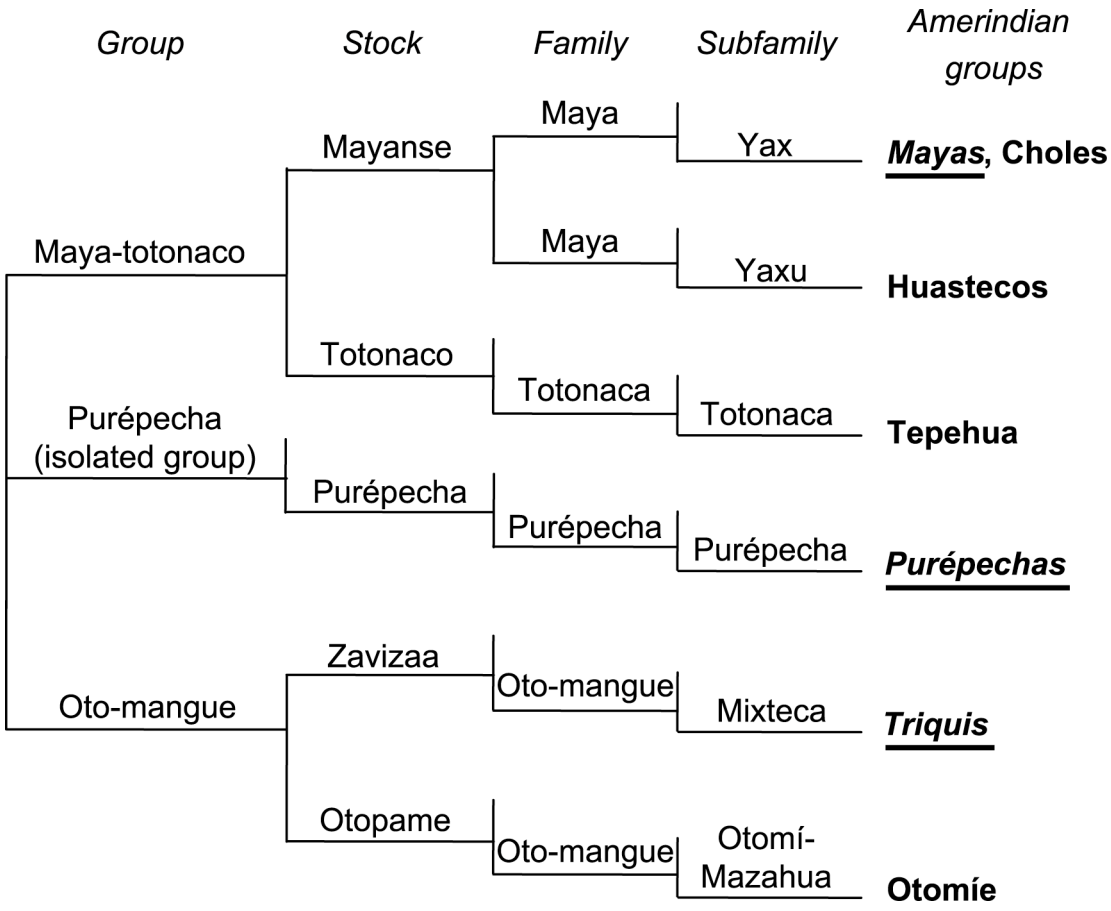

Group
Stock
Family
Subfamily
Amerindian groups
Maya-totonaco
Purépecha (isolated group)
Oto-mangue
Mayanse
Totonaco
Purépecha
Zavizaa
Otopame
Maya
Maya
Totonaca
Purépecha
Oto-mangue
Oto-mangue
Yax
Yaxu
Totonaca
Purépecha
Mixteca
Otomí-Mazahua
Mayas, Choles
Huastecos
Tepehua
Purépechas
Triquis
Otomíe

Figure 3.

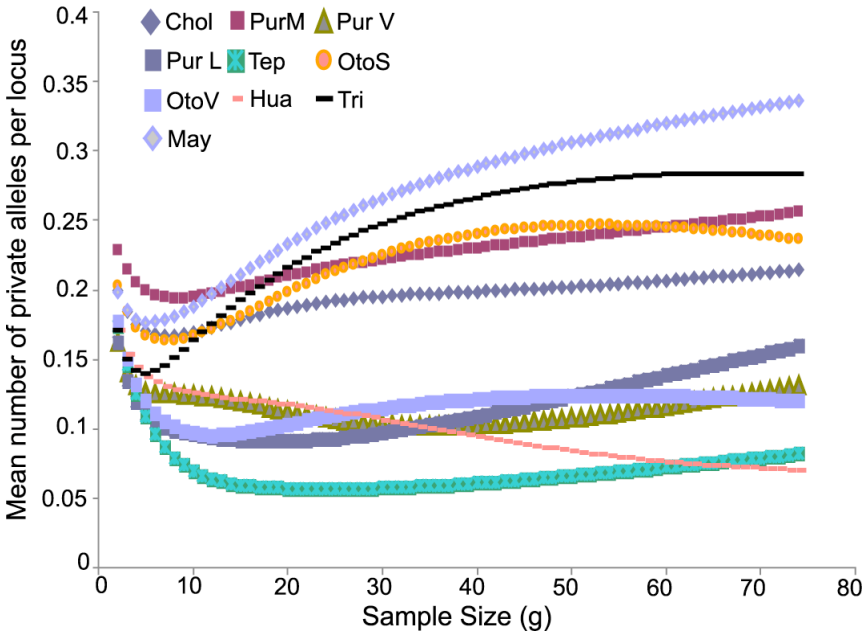

Chol
PurM
Pur V
Pur L
Tep
OtoS
OtoV
Hua
Tri
May
Mean number of private alleles per locus
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
40
50
60
70
80
Sample Size (g)

Figure 4.

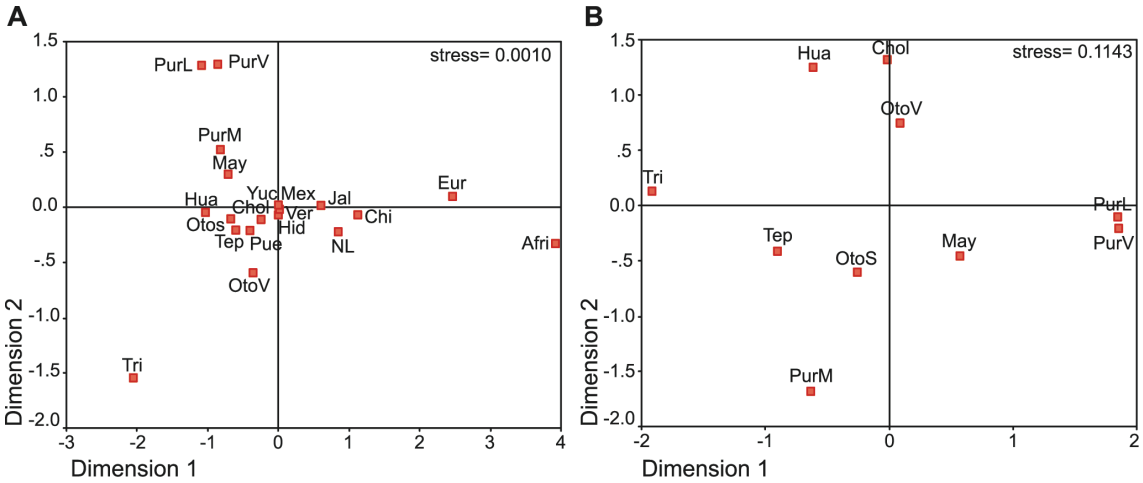
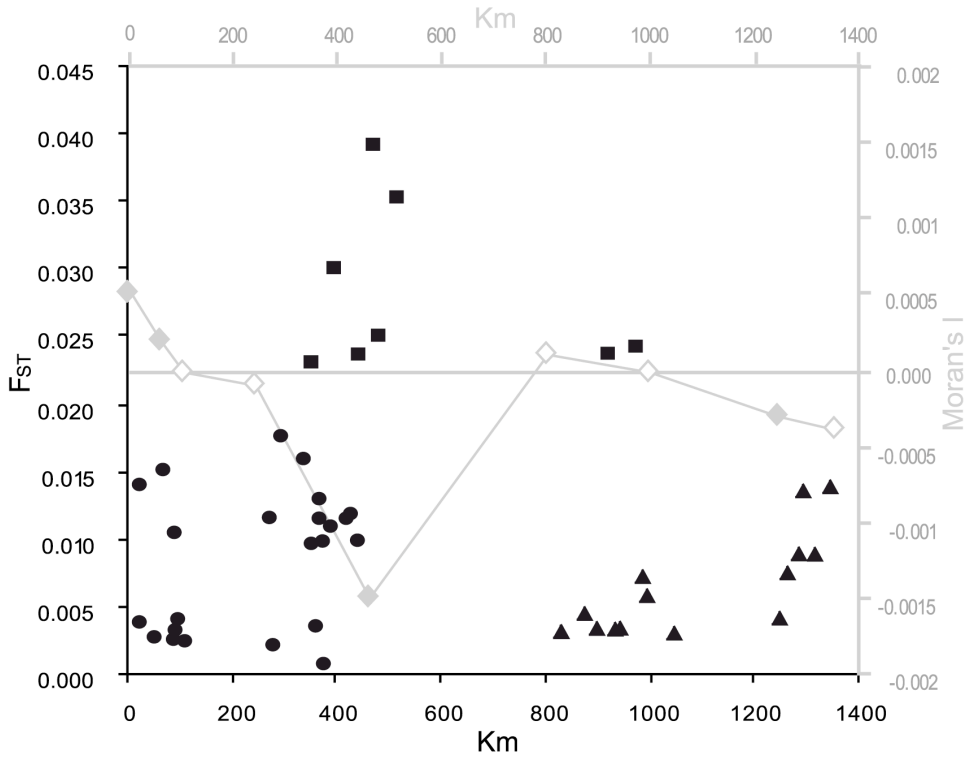

A
stress= 0.0010
PurL
PurV
PurM
May
Hua
Yuc
Mex
Jal
Eur
Chol
Ver
Chi
Otos
Hid
Tep
Pue
NL
Afri
OtoV
Tri
Dimension 2
Dimension 1
B
stress= 0.1143
Hua
Chol
OtoV
Tri
PurL
Tep
May
OtoS
PurV
PurM
Dimension 2
Dimension 1

Figure 5.


Km
0
200
400
600
800
1000
1200
1400
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
FST
0.002
0.0015
0.001
0.0005
0.000
-0.0005
-0.001
-0.0015
-0.002
Moran's I
Km

Supplementary Tables

S1. Allele frequency distribution for 15 STR loci (AmpfSTR® Identifiler™), and statistical parameters of forensic importance in Purépechas of Zipiajo (Mountain).

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
6						0.2589						0.0029			
6.3						0.0059									
7						0.5595								0.1160	
8			0.1084			0.0357	0.0208	0.0029				0.4613			
9	0.0029		0.0542			0.0238	0.3452	0.0299				0.0386	0.0029	0.0952	
9.3						0.1160									
10	0.1131		0.1777	0.2365			0.1517	0.3233		0.0059		0.0089	0.0239	0.0238	
11	0.0357		0.3162	0.2814			0.1428	0.2634				0.2291	0.0119	0.5476	
11.2										0.0059					
12	0.0952		0.3192	0.4161	0.0089		0.1875	0.3083		0.0297		0.2410	0.0688	0.1577	
12.2										0.0029					
13	0.4613		0.0090	0.0568	0.0029		0.1101	0.0628		0.2440		0.0178	0.1137	0.0535	
13.2										0.1250					
14	0.2113		0.0090	0.0029	0.0238		0.0416			0.2381	0.0238		0.1586	0.0059	
14.2										0.1636			0.0029		
15	0.0773		0.0030		0.5714			0.0089		0.0476	0.1398		0.2006		
15.2			0.0030							0.0714					
16	0.0029			0.0029	0.2410				0.0238	0.0416	0.4107		0.0898		
16.2										0.0238					
17				0.0029	0.0922				0.0565		0.1964		0.1047		
18					0.0297				0.0506		0.1815		0.1197		0.0180
19					0.0297				0.4017		0.0476		0.0149		0.0572
20									0.1458				0.0509		0.0722
21									0.0238				0.0119		0.1024
22									0.0416				0.0179		0.1024
23									0.1428						0.1475
24		0.0029							0.0267				0.0029		0.1144
24.2		0.0089													
25									0.0773						0.2289
26									0.0059				0.0029		0.0662
27															0.0873
28		0.0654													0.0030
29		0.2142							0.0029						
30		0.1726													
30.2		0.0089													
31		0.1041													
31.2		0.1220													
32		0.0178													
32.2		0.1547													

33.2		0.1160													
34		0.0059													
34.2		0.0059													
MAF	0.0162	0.0175	0.0169	0.0156	0.0151	0.0146	0.0169	0.0171	0.0165	0.0179	0.0178	0.0153	0.0181	0.0158	0.0173
PD	0.8826	0.9581	0.8911	0.8403	0.7966	0.7966	0.9227	0.8606	0.9277	0.9464	0.8724	0.8495	0.9687	0.8421	0.9679
PE	0.4413	0.6281	0.5149	0.3507	0.2923	0.2327	0.5408	0.5597	0.4797	0.6739	0.6623	0.3144	0.6837	0.3872	0.5681
TPI	1.7143	2.7097	2.0244	1.4153	1.2537	1.1053	2.1538	2.2568	1.8667	3.1111	3.0000	1.3125	3.2115	1.5273	2.3056
PIC	0.6788	0.8397	0.7114	0.6307	0.5580	0.5508	0.7595	0.6757	0.7608	0.8098	0.6998	0.6202	0.8678	0.6170	0.8579
H	0.7083	0.8155	0.7530	0.6467	0.6012	0.5476	0.7679	0.7784	0.7321	0.8393	0.8333	0.6190	0.8443	0.6726	0.7831
HWE*	0.6069	0.1310	0.0602	0.0488	0.7339	0.5225	0.1202	0.5839	0.1169	0.3724	0.0758	0.4887	0.0480	0.2751	0.1622

MAF: minimum allele frequency; PD: power of discrimination; PE: power of exclusion; TPI = typical paternity index; PIC: polymorphism information content; H: heterozigosity expected; HWE: Hardy-Weinberg equilibrium test (p-value). * Bonferroni correction to evaluate HWE test (p< 0.0033)

S2. Allele frequency distribution for 15 STR loci (AmpfSTR® Identifiler™), and statistical parameters of forensic importance in Purépechas from Angahuan (Valley).

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
5						0.0049									
5.3						0.0049									
6						0.3398									
7						0.3495								0.0728	
8			0.0588				0.0147					0.5097			
9	0.0049		0.0049	0.0050		0.0243	0.3431	0.0340				0.0049		0.0146	
9.3						0.2718									
10	0.0534		0.2108	0.1634		0.0049	0.2255	0.2573		0.0098				0.0194	
11	0.0291		0.3775	0.3911	0.0049		0.1863	0.2670				0.1990	0.0147	0.5971	
12	0.0340		0.3284	0.3317			0.0931	0.3883		0.0098		0.2767	0.0833	0.2961	
13	0.4612		0.0196	0.1040	0.0049		0.0882	0.0534		0.2647			0.0784		
13.2										0.1569					
14	0.2864			0.0050			0.0490			0.2647	0.0686	0.0097	0.2255		
14.2										0.0490					
15	0.1311				0.4757					0.1029	0.0539		0.1078		
15.2										0.0294					
16					0.3932				0.0049	0.0833	0.4167		0.1912		
16.2										0.0294					
17					0.0825				0.0728		0.3137		0.1912		
18					0.0388				0.0097		0.1275		0.0441		0.0248
19									0.4029		0.0196		0.0098		0.1535
20									0.2039				0.0049		
21									0.0049				0.0196		0.2178
22									0.0728				0.0294		0.1436
22.2		0.0049													
23									0.1796						0.0297
24									0.0388						0.1436
24.2		0.0922													
25									0.0097						0.1337
26															0.1436
27															0.0099
28		0.0388													
29		0.1505													
30		0.1553													
31		0.1019													
31.2		0.1845													
32		0.0049													
32.2		0.1845													
33.2		0.0583													
34.2		0.0243													
MAF	0.0254	0.0304	0.0259	0.0258	0.0246	0.0257	0.0286	0.0260	0.0275	0.0286	0.0269	0.0256	0.0284	0.0238	0.0294
PD	0.8455	0.9557	0.8499	0.8566	0.7705	0.8378	0.9045	0.8453	0.8977	0.9381	0.8416	0.7620	0.9479	0.7179	0.9527
PE	0.3974	0.8014	0.4222	0.3881	0.3298	0.4268	0.6623	0.4574	0.5919	0.6623	0.5182	0.4119	0.6434	0.2595	0.6978

TPI	1.5606	5.1500	1.6452	1.5303	1.3553	1.6613	3.0000	1.7758	2.4524	3.0000	2.0400	1.6094	2.8333	1.1704	3.3667
PIC	0.6352	0.8449	0.6461	0.6455	0.5343	0.6241	0.7460	0.6545	0.7189	0.7892	0.6561	0.5574	0.8301	0.4821	0.8289
H	0.6796	0.9029	0.6961	0.6733	0.6311	0.6990	0.8333	0.7184	0.7961	0.8333	0.7549	0.6893	0.8235	0.5728	0.8515
HWE *	0.5272	0.3438	0.0877	0.4143	0.8747	0.8478	0.8218	0.1703	0.5975	0.9131	0.2729	0.0183	0.0230	0.5562	0.8851

MAF: minimum allele frequency; PD: power of discrimination; PE: power of exclusion; TPI = typical paternity index; PIC: polymorphism information content; H: heterozigosity expected; HWE: Hardy-Weinberg equilibrium test (p-value). * Bonferroni correction to evaluate HWE test (p< 0.0033)

S3. Allele frequency distribution for 15 STR loci (AmpfSTR® Identifiler™), and statistical parameters of forensic importance in Purépechas from Puacuaro (Lake).

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
6						0.3548									
7						0.3306								0.121	
8			0.0738									0.4655			
9	0.0081		0.0082			0.0161	0.379	0.0323							
9.2						0.0081									
9.3						0.2903									
10	0.0403		0.1393	0.1475			0.25	0.2016		0.0088				0.0323	
11	0.0484		0.4016	0.3852			0.1532	0.2581				0.2328		0.5726	
12	0.0565		0.377	0.3607			0.0565	0.4274		0.0175		0.3017	0.0702	0.2661	
12.2										0.0088					
13	0.3952			0.0984			0.129	0.0726		0.2368			0.0614	0.0081	
13.2										0.1667					
14	0.2581			0.0082	0.0565		0.0323	0.0081		0.2018	0.0776		0.2544		
14.2										0.0263					
15	0.1855				0.4758					0.1579	0.0862		0.0614		
15.2										0.0175			0.0088		
16	0.0081				0.379				0.0242	0.114	0.3966		0.2018		0.0082
16.2										0.0439					
17					0.0806				0.0484		0.2845		0.2193		
18					0.0081				0.0242		0.1121		0.0614		0.0082
19									0.4194		0.0431		0.0351		0.1311
20									0.2258				0.0088		
21									0.0081				0.0088		0.1885
22									0.0484						0.1475
23									0.1774				0.0088		0.041
24									0.0242						0.1393
24.2		0.0726													
25															0.1557
26															0.1557
27															0.0246
28		0.0081													
29		0.1532													
30		0.1694													
30.2		0.0081													
31		0.0806													
31.2		0.1613													
32.2		0.2661													
33.2		0.0484													
34.2		0.0323													
MAF	0.0413	0.0452	0.041	0.0442	0.0452	0.0422	0.0446	0.0405	0.0436	0.0483	0.049	0.043	0.0483	0.0387	0.0478
PD	0.8954	0.9454	0.8272	0.8315	0.6738	0.8096	0.8871	0.8663	0.8897	0.9406	0.8401	0.7907	0.9394	0.7508	0.9465
PE	0.4184	0.6416	0.3632	0.5455	0.6416	0.4693	0.6111	0.3710	0.5521	0.6121	0.6847	0.3624	0.6121	0.2683	0.7323
TPI	1.6316	2.8182	1.4524	2.1786	2.8182	1.8235	2.5833	1.4762	2.2143	2.5909	3.2222	1.4500	2.5909	1.1923	3.8125

PIC	0.6957	0.8159	0.6104	0.6334	0.5479	0.6128	0.7121	0.6554	0.6996	0.8130	0.6947	0.5653	0.8076	0.5266	0.8378
H	0.6935	0.8226	0.6557	0.7705	0.8226	0.7258	0.8065	0.6613	0.7742	0.8070	0.8448	0.6552	0.8070	0.5806	0.8689
HWE*	0.7216	0.7078	0.6751	0.8954	0.0029	0.2366	0.7843	0.5635	0.6443	0.3687	0.2684	0.8256	0.2848	0.2654	0.5605

MAF: minimum allele frequency; PD: power of discrimination; PE: power of exclusion; TPI = typical paternity index; PIC: polymorphism information content; H: heterozigosity expected; HWE: Hardy-Weinberg equilibrium test (p-value). * Bonferroni correction to evaluate HWE test ($p < 0.0033$)

S4. Allele frequency distribution for 9 STR loci (AmpF*l*STR® Profiler Plus™), and statistical parameters of forensic importance in the Triquis.

Allele	D851179	D21511	D75820	D351358	D135317	VWA	D18S51	D55818	FGA
7								0.01389	
8	0.00463		0.08333						
9					0.35648			0.06019	
10	0.25463		0.28704		0.08333			0.04167	
11	0.05093		0.36111		0.11574			0.67593	
12	0.08333		0.19907	0.00926	0.18056		0.09722	0.20833	
13	0.43519		0.06944		0.15741		0.13426		
13.2							0.00463		
14	0.09722			0.06019	0.10185	0.00926	0.10185		
15	0.06481			0.65741	0.00463	0.03241	0.15741		
16	0.00926			0.20833		0.63889	0.06019		
17				0.06019		0.22685	0.24537		
18				0.00460		0.06481	0.10648		
19						0.01852	0.00463		0.09722
19.2									0.00463
20						0.00926			0.00463
21							0.02315		0.02315
22							0.00463		0.07407
23							0.01389		0.09722
24							0.02315		0.41204
25							0.00926		0.18056
26							0.01389		0.09259
28									0.00463
29		0.18056							0.00926
30		0.29630							
31		0.18056							
31.2		0.12963							
32		0.04167							
32.2		0.10648							
33.2		0.04167							
34.2		0.02315							
FAM	0.02544	0.02861	0.02302	0.02289	0.02631	0.02252	0.02733	0.02118	0.02528
PD	0.87439	0.92995	0.88580	0.70799	0.91735	0.74811	0.96313	0.69239	0.91598
PE	0.50976	0.77278	0.28208	0.27135	0.59218	0.24090	0.68032	0.15010	0.49406
TPI	2.00000	4.50000	1.22727	1.20000	2.45454	1.12500	3.17647	0.91525	1.92857
PIC	0.68569	0.79083	0.69145	0.47252	0.75666	0.48727	0.84607	0.44894	0.73843
H	0.75000	0.88889	0.59259	0.58333	0.79629	0.55556	0.84259	0.45370	0.74074
HWE*	0.65042	0.15033	0.00423	0.08302	0.47083	0.84357	0.86308	0.29691	0.38377

MAF: minimum allele frequency; PD: power of discrimination; PE: power of exclusion; TPI = typical paternity index; PIC: polymorphism information content; H: heterozigosity expected; HWE: Hardy-Weinberg equilibrium test (p-value).
* Bonferroni correction to evaluate HWE test (p< 0.0055).

S5. Allele frequency distribution for 9 STR loci (AmpF*l*STR® Profiler Plus™), and statistical parameters of forensic importance in the Mayas.

Allele	D8S1179	D21S11	D7S820	D3S1358	D13S317	VWA	D18S51	D5S818	FGA
7			0.00556					0.07778	
8			0.02222		0.02222			0.00556	
9	0.00556		0.03889		0.29444			0.03333	
10	0.03889		0.20000		0.18889		0.01111	0.07778	
11	0.04444		0.34444		0.15000		0.01111	0.57778	
12	0.11667		0.35556		0.21111		0.07222	0.17778	
13	0.39444		0.03333		0.06667	0.00556	0.09444	0.05000	
13.2							0.00556		
14	0.27778			0.05556	0.06667	0.10556	0.18889		
15	0.10000			0.56111		0.05000	0.15000		
16	0.00556			0.26667		0.39444	0.13889		
17	0.01667			0.07778		0.28889	0.14444		
18				0.02778		0.12778	0.11667		0.01111
19				0.01111		0.02222	0.04444		0.05556
20						0.00556	0.01667		0.05000
21							0.00556		0.11111
21.2									0.00556
22									0.05556
23									0.10000
24									0.17778
25									0.24444
26		0.00556							0.15556
26.2									0.00556
27		0.00556							0.02222
28		0.03333							0.00556
29		0.23333							
29.2		0.01667							
30		0.23333							
30.2		0.05000							
31		0.10000							
31.2		0.08889							
32.2		0.14444							
33		0.00556							
33.2		0.08333							
FAM	0.03108	0.03220	0.02966	0.02661	0.03284	0.02966	0.03319	0.02844	0.03319
PD	0.88960	0.94840	0.86099	0.79410	0.91510	0.87460	0.95830	0.81430	0.95310
PE	0.57860	0.66229	0.46347	0.22940	0.70580	0.46350	0.72800	0.36290	0.72800
TPI	2.36840	3.00000	1.80000	1.09760	3.46150	1.80000	3.75000	1.45160	3.75000
PIC	0.70260	0.82285	0.66039	0.55140	0.77300	0.68930	0.85770	0.58610	0.83650
H	0.78890	0.83330	0.72220	0.54440	0.85560	0.72220	0.86670	0.65560	0.86670
HWE	0.44304	0.17097	0.12665	0.47690	0.06516	0.19504	0.05293	0.25293	0.26113

MAF: minimum allele frequency; PD: power of discrimination; PE: power of exclusion; TPI = typical paternity index; PIC: polymorphism information content; H: heterozigosity expected; HWE: Hardy-Weinberg equilibrium test (p-value).
* Bonferroni correction to evaluate HWE test (p< 0.0055).

DISCUSSION

In the present thesis I have comprehensively studied several Native Mexican populations from a molecular anthropological point of view: from carrying out field work, collecting ethnographic data, to analyzing genetic markers such as mtDNA, Y-chromosome and autosomal STRs. The results encompass three different but closely related studies that share the main goal of carrying out an interdisciplinary research, within the Molecular Anthropology theoretical framework, to better understand Mexican indigenous and Native American genetic diversity. The main findings will be shortly discussed below.

Inside an indigenous community: Ethnography and field work

Mexico is a country characterized by its high cultural and linguistic diversity as a result of the presence of numerous native or indigenous groups. Nowadays Mexico is mainly inhabited by two distinct population groups: the so-called *Mestizo* and the Native Indigenous Mexican groups defined by having specific languages, cultural traits, ethnicity, oral history, and customs as part of their cultural complexity. Despite the fact that the proportion of the Mestizo population represents nearly 95% of the Mexican population (INEGI 2007), the Native component is present in the daily life of each of the 32 states conforming the Mexican territory, which covers 1,972,550 km^2 (the 15^{th} largest country in the world and the 5^{th} largest country in the Americas).

Motivated by such high cultural and ethnic diversity, one of my main purposes was to understand indigenous diversity from a closer point of view: field work. Initially, I carried out different seasons of field work during the years 2000 to 2002, particularly with the Triqui indigenous group in southern Mexico (San Juan Copala, Oaxaca). The understanding of how an indigenous community works is a valuable tool, not only because of their cultural, folkloric, and demographic information, but also because it offers a window to the understanding of their beliefs, cosmovision, and how they understand their own origin and past history. Some of the ethnographic techniques consisted in; 1) extensive bibliographic research, 2) direct and indirect observation, 3) personal and community interviews, 4) surveys, 5) informative talks (about my work within the group), 6) anthropometric measures, and finally 7) population sampling.

The anthropometric results that we obtained were associated with the current nutritional status of the Triqui population, which gave us interesting insights into their growth process in relation to their socioeconomic status (see Results section: chapter 1).

Thanks to the special and particular interest that the Triqui group had on knowing more about their own history and *"the record that was in their blood"* (literal expression cited by them), my anthropological interest went beyond the ethnographic work, leading me to the fascinating field of population genetics.

On the genetic diversity of Mexican Populations

The case of the Triqui population may be an example that shares sociological similarities with other indigenous communities throughout the country, but Mexican indigenous populations are also characterized by large geographical and demographic differences. Some populations, such as the Triqui indigenous group, are extremely isolated. Others groups may show extensive admixture in some cases, especially those located in urban areas, while some others exhibit remarkably different demographic histories so that they are more or less likely to have contributed to the genetic pool of the admixed population of present day Mexico. Thus, the relationship between the past and present history is reflected in the actual genetic composition. Molecular genetic markers are extremely useful to investigate not only the genetic composition across the indigenous and Mestizo populations, but also to trace maternal and paternal lineages back in time, allowing us to answer pivotal questions about human history.

On the mtDNA Diversity

In chapter 2 of the Results section, I present the results from a genetic study performed with 11 native populations from throughout Mexico. We showed how the sampled populations are mostly composed by Native American haplotypes. The main findings are the followings.

1) The majority of the mtDNAs of autochthonous Mexicans individuals can be allocated to one of the four most common Native American haplogroups (A2, B2, C1 and D1) (Achilli et al. 2008); 2) The absence of X2 in our samples supports the idea that

Mesoamerica played an important role during the colonization of the continent, restricting this haplogroup to the northernmost lands and shaping the diversity of the other founder haplogroups on their way down to Central and South America; 3) These results show a common origin of Native American populations, including our Mexican groups, with an extensive isolation and genetic drift, which might have produced an extreme heterogeneity in their haplogroup patterns; 4) A correlation between linguistic classification and their mtDNA gene pool was not found; 5) The northernmost groups Tarahumara and Pima show a certain degree of genetic differentiation from the rest, these populations are outside of the Mesoamerican area; and finally 6) Despite the mtDNA represents just a small fraction of the human genome, it has demonstrated once more to be a useful marker to trace back demographic events in human population history.

On the Y-chromosome Diversity

In chapter 3 (Results section) I present the male counterpart of the female-based analyses of mtDNA in the same panel of population samples. The main findings are the followings.

1) A substantial presence of non-Native American male lineages in our sample set was observed. The Y-chromosome haplogroup composition of Native Mexican populations shows a remarkable contrast with our previous findings from the mtDNA results for the same populations, in which almost only Native female lineages were found (Sandoval et al. 2009); 2) We found an excess of European (and to a lesser extent African) Y-chromosome haplotypes and a higher proportion of mitochondrial haplotypes of Native American origin, suggesting a strong female Native American and male European and African sex bias contribution, consistent with previous studies performed in different Amerindian populations. 3) These results are congruent with the demographic history of the studied populations, in which Nahuas Xochimilco showed the highest proportion of European haplotypes. This population represents an urban sample within Mexico City, which is likely to have been largely exposed to multiple admixture events, whereas Triqui and Pima are two indigenous groups well known to be characterized by their geographic and cultural isolation; 4) The finding of African Y-chromosome haplotypes in our samples, although not as frequent as haplotypes of other origin, is not neglectable

since it indicates that the involuntary migration of Africans through slave trade appears to have left a clear trace in Native Mexican populations proximal to coastal routes (e.g. Purepecha); 5) Pimas and Tarahumara, the northernmost samples, exhibit the lowest diversity values, which is in total agreement with our previous observations based on mtDNA diversity of the same set of samples (Sandoval et al. 2009), these groups are the only sampled populations outside the cultural geographic region of Mesoamerica, 6) A extensive heterogeneity, lack of genetic structure at a continental scale and a lack of correlation between linguistic classification and Y-chromosome diversity were observed, and 7) Being our estimated age of Q haplogroups around 6,436 (±913) ybp, we can confirm that all the Q haplogroups found in our sample set belong to the main Native American Q-M3 lineage.

Similar results, in which no concordance based on maternally inherited genetic systems and those based on paternally inherited genetic systems, were observed in a previous study that we performed in extant Mestizo Cuban populations (see Appendix 1). In that study we documented clear evidence for significant differences in the geographic patterning of mtDNA diversity versus Y-chromosome diversity, differences that may be related to different male and female demographic histories (Bamshad et al. 1998; Hammer et al. 1998; Seielstad et al. 1998).

On STR variation

In chapter 4 of the Results section, I present the results of CODIS-STRs system, which is normally used with forensic purposes. These markers have been scarcely analyzed in Native Mexican groups, both for human identification or anthropological purposes. The main objective was to analyze the genetic relationships and population structure among three Native Mexican groups from Mesoamerica; Purepechas from Valley and Lake, Triquis and Mayas. Our results showed that the majority of Native groups were not differentiated, except for Triquis and Purepechas (Valley and Lake), attributable to their relative geographic and cultural isolation. Conversely, Purepechas-Mountain presented an elevated number of rare alleles, suggesting recurrent gene flow into this group. It was interesting that Huastecos and Yucatec Mayas were not differentiated, which is in agreement with the archeological hypothesis that Huastecos represent an ancestral Maya group. We suggest that European admixture has increased the similarity among Native

Mexican groups, and again like aforementioned studies (Chapters 2 and 3) a geographic and linguistic clustering was not found within the Native groups, which stresses the importance of serial founder effect and/or genetic drift to depict their present genetic relationships.

With this kind of studies is possible to establish an interrelation between the past and present of the genetic and demographic histories of each native population. In fact we can make some inferences about the population density, the original genetic inputs that clearly shape the current genetic populations not only of indigenous groups but also the Mestizos. Some insights obtained from this works, have been show that the major concentration of the native Mexican groups were from the central to south of the country, according with the historical records (pictography, codices and oral), where the main pre-Columbian empires were established mainly from the central-south part of Mexico, delineating the Mesoamerica cultural area.

Concluding Remarks

Although the mtDNA and the Y-chromosome represent only a small part of the whole human genome, they are still highly informative and both have the capacity to be reliable tools for molecular anthropology studies.

Our results both support previous general observations and offer a more detailed view of the genetic composition of Native Mexican populations, particularly with respect to their uniparental lineage histories.

This work stresses the importance of conducting complementary studies in order to contrast the information offered by analyzing mtDNA (allowing to trace maternal lineages), and that offered by genotyping markers on its natural counterpart, the Y-chromosome (which allows to trace back paternal lineages).

The present thesis has the main goal of offering an interdisciplinary view, and to contribute not only to the comprehension of Mexican diversity but also to the historical reconstruction of important cultural areas like Mesoamerica, in order to provide insights into the Peopling of the Americas.

In this respect, one of the main findings obtained from the mtDNA analysis is the absence of X2 haplogroup in Mexican native populations, which can define a geographical frontier in its distribution, confirming that Mesoamerica played an important role during the colonization. The presence of the other four native haplogroups in the Mexican native groups, allows us to make some inferences about the possible migratory routes of the first settles of the continent.

Further analysis of Y-lineages and mtDNA-lineages present in the Americas will require more microsatellites as well as additional slowly evolving biallelic markers for the former and complete mtDNA sequences of Native American, Siberian and East Asia populations. This will be extremely useful to capture the entire genetic variability spectrum in order to trace back the actual DNA tracks bearing particular founding diversity. All this will lead scientists and the general community to have a better understanding about the Native American origins and contribute to the reconstruction of the human history of the American continent.

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APPENDIX

Appendix 1: Genetic origin, admixture, and asymmetry in maternal and paternal human lineages in Cuba

Isabel Mendizabal, **Karla Sandoval**, Gemma Berniell-Lee, Francesc Calafell1, Antonio Salas, Antonio Martínez-Fuentes, and David Comas

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