Maternal Admixture and Population Structure in Mexican–Mestizos Based on mtDNA Haplogroups

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KEY WORDS mtDNA ancestry; maternal admixture; Mexican-Mestizos; mtSNPs; Y-SNPs

ABSTRACT The maternal ancestry (mtDNA) has important applications in different research fields, such as evolution, epidemiology, identification, and human population history. This is particularly interesting in Mestizos, which constitute the main population in Mexico (~93%) resulting from post-Columbian admixture between Spaniards, Amerindians, and African slaves, principally. Consequently, we conducted minisequencing analysis (SNaPshot) of 11 mitochondrial single-nucleotide polymorphisms in 742 Mestizos of 10 populations from different regions in Mexico. The predominant maternal ancestry was Native American (92.9%), including Haplogroups A, B, C, and D (47, 23.7, 15.9, and 6.2%, respectively). Conversely, European and African ancestries were less frequent (5.3 and 1.9%, respectively). The main characteristics of the maternal lineages observed in Mexican–Mestizos comprised the following: 1) contrasting geographic gradient of Haplogroups A and C; 2) increase of European lineages toward the Northwest; 3) low or absent, but homogeneous, African ancestry throughout the Mexican territory; 4) maternal lineages in Mestizos roughly represent the genetic makeup of the surrounding Amerindian groups, particularly toward the Southeast, but not in the North and West; 5) continuity over time of the geographic distribution of Amerindian lineages in Mayas; and 6) low but significant maternal population structure (F_{ST} = 2.8%; $P = 0.0000$). The average ancestry obtained from uniparental systems (mtDNA and Y-chromosome) in Mexican–Mestizos was correlated with previous ancestry estimates based on autosomal systems (genome-wide single-nucleotide polymorphisms and short tandem repeats). Finally, the comparison of paternal and maternal lineages provided additional information concerning the gender bias admixture, mating patterns, and population structure in Mestizos throughout the Mexican territory. Am J Phys Anthropol 151:526–537, 2013.

In Mexico, biological admixture arose during and after the Spanish Conquest in 1519, involving Native Americans, Spaniards, and African slaves. Although regulations were planned in Mexico to avoid admixture, hybrid populations increased intensively during the 17th and 18th Centuries (Moreno-Toscano, 2003). The resulting Mexican–Mestizo population currently constitutes ~93.3% of the total, and is defined as a person born in Mexico, with a Spanish-derived last name and Mexican ancestors traced back to the third generation (Gorodezky et al., 2001). During the admixture process, Pre-Hispanic demography appears to be a decisive factor that explains the heterogeneity found throughout the continent (Wang et al., 2008; Bryc et al., 2010), and particularly in Mexico (Rubi-Castellanos et al., 2009). The complexity of the admixture process during ~500 years supports the genetic analysis to be addressed, including different geographical regions and considerable numbers and types of DNA markers (Wang et al., 2008; Silva-Zolezzi et al., 2009).

Analysis of the genetic variation of mitochondrial DNA (mtDNA) has been an important tool for inferences on human evolution owing to its characteristics, such as high copy number per mitochondrion and cell, nonrecombining, high mutation rate, and maternal inheritance (Schurr et al., 1990). In addition, this uniparental

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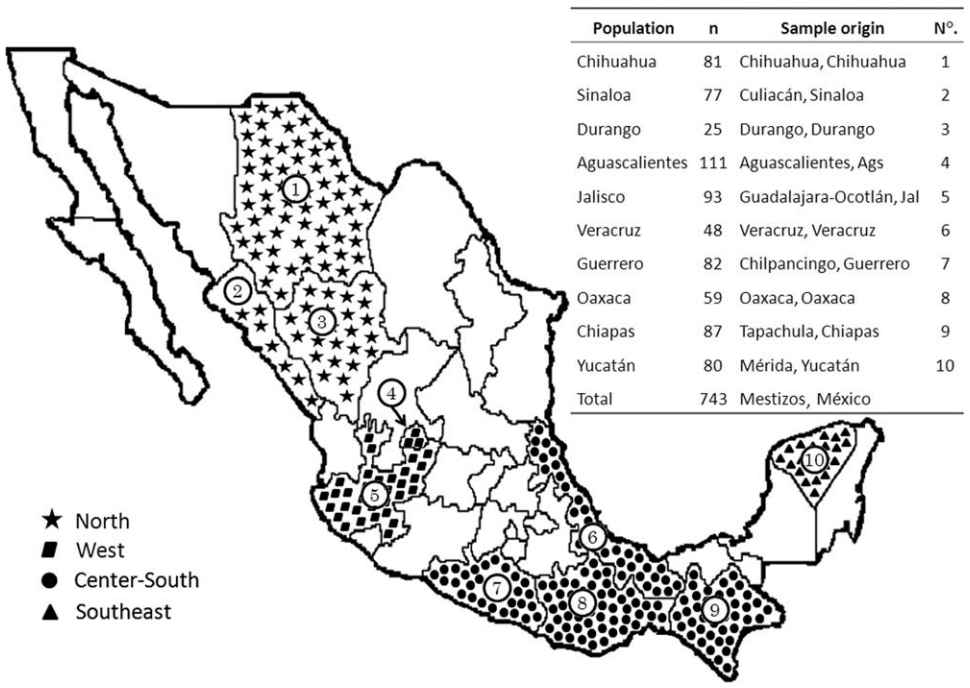

Population	n	Sample origin	N°.
Chihuahua	81	Chihuahua, Chihuahua	1
Sinaloa	77	Culiacán, Sinaloa	2
Durango	25	Durango, Durango	3
Aguascalientes	111	Aguascalientes, Ags	4
Jalisco	93	Guadalajara-Ocotlán, Jal	5
Veracruz	48	Veracruz, Veracruz	6
Guerrero	82	Chilpancingo, Guerrero	7
Oaxaca	59	Oaxaca, Oaxaca	8
Chiapas	87	Tapachula, Chiapas	9
Yucatán	80	Mérida, Yucatán	10
Total	743	Mestizos, México	

Fig. 1. Geographic location, origin, and sample size of the 10 Mexican–Mestizo populations analyzed in this study.

genetic system is more sensitive to genetic drift effects, becoming a better population structure marker (Shriver and Kittles, 2004). The mtDNA of Native Americans belongs to one of the five distinct founding lineages, designed initially as Haplogroups A, B, C, D, and X (Schurr et al., 1990; Brown et al., 1998). These haplogroups were further characterized as A2, B2, C1, and D1, respectively, including additional minor lineages denominated C4c, D2a, D3, D4h3a, X2a, and X2g (Tamm et al., 2007; Achilli et al., 2008; Perego et al., 2009). It should be noted that in the absence of control region sequence data, a combination of coding-region markers genotyping has been suggested to better define these haplogroups (Behar et al., 2007). These mitochondrial markers include both insertions–deletions (indels) and single-nucleotide polymorphisms (SNPs), which for practical purposes will be described in this manuscript as mitochondrial SNPs (mtSNPs).

Admixture analysis of Mexican–Mestizos from different regions of the country has been carried out with different genetic systems, such as genome-wide SNPs (Silva-Zolezzi et al., 2009), autosomal short tandem repeats (STRs) (Rubi-Castellanos et al., 2009), and *Y*-chromosome markers, including Y-STRs (Salazar-Flores et al., 2010) and Y-SNPs (Martínez-Cortés et al., 2012). Although these studies have described regional genetic differences, few analyses have described the maternal lineages variability (mtDNA) throughout the Mexican territory. In fact, the majority of these studies have described mtDNA haplogroup distribution from specific locations/states in Mexico, including Chihuahua state (Green et al., 2000); the city of Tlapa, Guerrero state (Bonilla et al., 2005); and Mexico City (Martinez-Marignac et al., 2007). The most recent study concerning mtDNA diversity in Mexican–Mestizos has claimed great diversity in Amerindian ancestry (Guardado-Estrada et al., 2009); however, the analyzed population sample mostly comprised individuals from Mexico City, central region, and the southern coastal region. Owing to the limited knowledge of matrilineal admixture and prevalence of mtDNA lineages in some regions of Mexico, such as western and southeastern regions, further evaluation is required.

In this study, we assessed the mtDNA variation in 10 populations from different regions of Mexico. The aims of this study were to provide information on the following: 1) distribution of maternal lineages and ancestry (Amerindian, European, Eurasian, and African); 2) genetic differentiation pattern or population structure; 3) genetic contribution of Amerindian groups to their nearby Mexican–Mestizo populations; and 4) comparison between assessments of admixture in Mexican–Mestizos based on uniparental and autosomal markers. Results revealed a low but significant population structure in Mestizos mainly defined by Native American lineages, which roughly correspond to the adjacent Amerindian groups in central and southeastern regions.

MATERIALS AND METHODS

Population samples

A total of 742 unrelated individuals of 10 Mexican–Mestizo populations from different geographic regions were analyzed (Fig. 1). Concerning the population clustering into regions, we must advise that readers could notice minor differences with other studies owing to the lack of uniform criteria to define geographic regions in Mexico. All individuals were self-classified as Mestizos because their parents and grandparents are Mexicans, speak Spanish, and do not belong to any specific ethnic group. Prior to the inclusion in our study, all volunteers signed an informed consent form according to the ethical

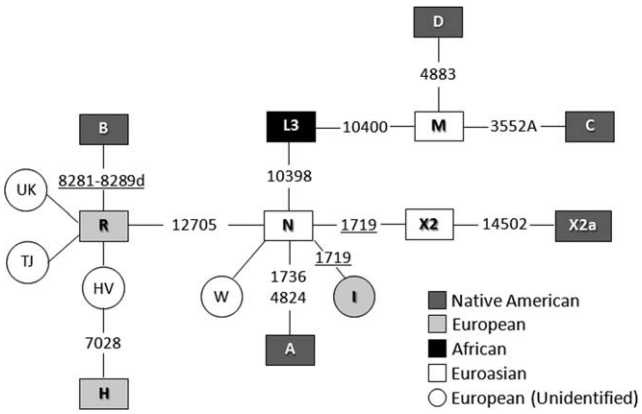

Fig. 2. Phylogenetic relationships between the 11 mtSNPs analyzed in this study to define some principal haplogroups and ancestries in 10 Mexican–Mestizo populations; recurrent mutations are underlined.

guidelines of the Helsinki Declaration and approved by the Ethical Research Committee at the CUCiénega (UdeG). DNA was extracted from blood samples by standard methods.

Multiplex polymerase chain reaction

Eleven mtSNPs were amplified in one reaction with primers and conditions that have been reported previously (Quintáns et al., 2004; Alvarez-Iglesias et al., 2007). This set of markers allowed defining mtDNA haplogroups with a specific Native American, European, Eurasian, and African origin (Fig. 2). Multiplex polymerase chain reactions (PCRs) were performed with the QIAGEN Multiplex PCR kit (QIAGEN, Hilden) using 10 ng of genomic DNA in a total volume of 10 μL. PCR products were observed by polyacrylamide gel electrophoresis followed by silver staining. After amplification, 0.2 μL of PCR product was purified with 1 μL of ExoSAP-IT to remove primers and unincorporated dNTPs (Amersham Biosciences). The mixture was incubated at 37°C for 15 min followed by 15 min at 80°C to inactive the enzyme.

Minisequencing (SNaPshot) and capillary electrophoresis

The minisequencing reaction was carried out in a total volume of 5 μL with 1 μL of purified PCR product, 2.5 μL of SNaPshot™ reaction mix (Applied Biosystems, Foster City, CA), and 0.7 μL of single-base extension (SBE) primer mix at optimal concentrations. The cycling conditions of the SBE reactions were as specified by Alvarez-Iglesias et al. (2007). Five microliters of SBE product was treated with 1 μL of enzyme SAP (Amersham Biosciences) for 60 min at 37°C, followed by 15 min at 80°C for enzyme inactivation. Later, 1 μL of SBE product was mixed with 9 μL of HiDi™ formamide and 0.3 μL of size standard Genescan LIZ-120 (Applied Biosystems), and it was submitted to capillary electrophoresis in the Genetic Analyzer ABI Prism 3130® (Applied Biosystems). SBE products were injected for 16 s at 1.2 kV and 5 μA at 60°C in a 36-cm length capillary filled with POP7®. GeneMapper® v3.2 (Applied Biosystems) software was employed for mtSNP allele designations. The mtSNP combinations found for each haplogroup are summarized in Supporting Information Table S1.

Hypervariable region-I sequencing

For individuals presenting two mtSNPs that define different haplogroups, we amplified the first hypervariable region (HVR1) using primers that have been described previously (Vigilant et al., 1989). PCR products were purified by agarose gel electrophoresis utilizing GFX PCR DNA and Gel Band Purification kits by following the manufacturers' protocol (Amersham Pharmacia Biotech, Uppsala, Sweden) and used as templates. DNA strands from the HVR1 (position 16,024–16,365) were sequenced with the chain termination method using fluorescence-labeled dideoxynucleotide (Big Dye v2.0, Applied Biosystems) according to the manufacturer' s instructions. The extension products were purified employing Centri-Sep Spin Columns (Princeton Separations, Adelphia, NJ) and separated in the ABI Prism 310 Genetic Analyzer (Applied Biosystems).

Data analyses. For each population, and globally, haplogroup frequencies were calculated by the gene counting method. Similarly, polymorphic variation of the sequences was estimated by haplotype diversity (*D*). Genetic differentiation among populations was evaluated by pairwise comparisons of the haplogroup distribution with Arlequin 3.11 software (Excoffier et al., 2007). Genetic distances (F_{ST}) were computed with Genetic Data Analysis (GDA) version 1.1 program (Lewis and Zaykin, 2001). Additionally, exact tests were carried out to compare the mtDNA haplogroup distribution here obtained by state and/or region with those previously reported from Mexican–Mestizo populations, and to compare Amerindians with Mestizos from the same geographical region. Exact tests were resolved by the multiple permutations method to reduce the effects of sample size differences (Evsiukov and Morozova, 2011). Similarly, the maternal ancestry was compared with its paternal counterpart based on Y-SNPs, which was previously obtained from most of the same population samples (Martínez-Cortés et al., 2012), and with assessments of autosomal ancestry in Mexican–Mestizos based on genome-wide SNPs (Silva-Zolezzi et al., 2009) and autosomal STRs (Rubi-Castellanos et al., 2009). Finally, genetic structure was evaluated by analysis of molecular variance (AMOVA), and considering geographic and genetic criteria to establish population clusters by means of the software spatial analysis molecular of variance (SAMOVA) (Dupanloup et al., 2002).

RESULTS

Distribution of mitochondrial haplogroups

The matrilineal diversity estimated in the 742 Mexican–Mestizos studied here was defined by nine haplogroups and paragroups (Table 1). In the whole population sample, Native American Haplogroups A, B, C, and D were prevalent (92.9%), with frequencies of 47, 23.7, 15.9, and 6.2%, respectively (Table 1). The genetic differentiation based on these Native American lineages among these 10 Mexican–Mestizo populations was significant ($P = 0.00086$), with a range of 87.1–97.9% for Jalisco and Veracruz, respectively (Fig. 3). However, some estimates deserve caution and must be considered

TABLE 1. Frequency (%), haplotype diversity (D), and ancestry of mtDNA haplogroups (Hg) observed in 10 Mexican–Mestizo populations from different geographic regions

	Region	North			West		Center-South				Southeast	
Haplogroup	Population[a] Ancestry[b]	Chi ($n = 81$)	Sin ($n = 77$)	Dur ($n = 25$)	Ags ($n = 110$)	Jal ($n = 93$)	Ver ($n = 48$)	Gue ($n = 82$)	Oax ($n = 59$)	Chia ($n = 87$)	Yuc ($n = 80$)	Global ($n = 742$)
A	NA	32.1	38.96	28	34.55	48.39	54.17	45.12	57.63	66.67	60	47.04
B	NA	24.69	23.38	40	25.45	18.28	22.92	28.05	25.42	20.69	20	23.72
C	NA	28.4	18.18	24	19.09	16.13	10.42	14.63	10.17	5.75	13.75	15.9
D	NA	3.7	7.79	4	11.82	4.3	10.42	7.32	3.39	3.45	3.75	6.2
R(xH,B)	Eu	3.7	3.9	4	1.82	3.23	2.08	–	3.39	–	–	2.02
H	Eu	3.7	3.9	–	3.64	4.3	–	1.22	–	1.15	–	2.16
I	Eu	–	–	–	–	1.08	–	1.22	–	–	–	0.27
N(xR,A,N1'5,X2)	EA[c]	1.23	3.9	–	0.91	1.08	–	–	–	–	–	0.81
L(xM,N)	Afr	2.47	–	–	2.73	3.23	–	2.44	–	2.3	2.5	1.89
	D	78.77	76.66	75.08	78.64	75.69	70.83	72.06	62.07	54.80	63.56	71.24

[a] Abbreviation of the Mexican–Mestizo populations: Chi, Chihuahua; Sin, Sinaloa; Dur, Durango; Ags, Aguascalientes; Jal, Jalisco; Gue, Guerrero; Oax, Oaxaca; Ver, Veracruz; Chia, Chiapas; Yuc, Yucatan

[b] Abbreviation of the ancestral origin of the mtDNA haplogroups: Eu, European; EA, Euroasian; NA, Native-American; Afr, African.

[c] Based on the historial records, the N-lineage was considered of European ancestry (**DISCUSSION**).

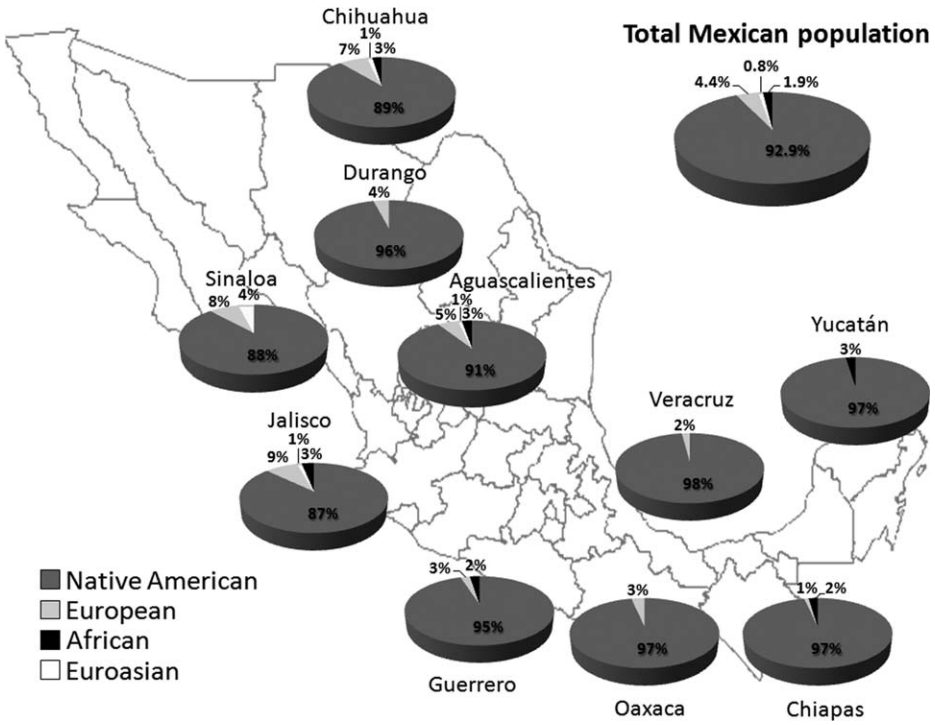

Fig. 3. Geographic distribution of the main maternal ancestries estimated in 10 Mexican–Mestizo populations.

as preliminary when they arise from small population samples, such as those obtained for Durango state ($n = 25$) (Fig. 1).

Concerning the Native American haplogroups, Lineages A and C displayed heterogeneous distribution throughout the Mexican territory ($P \leq 0.0015$), supporting an opposite geographic gradient: Haplogroup A increased toward the Southeast and C increased to the North and West (Table 1). Lineage A was the modal one in the majority of populations (range, 28–66.7%), increasing gradually from Northwest (28–48.4%) to Southeast (45.1–66.7%) and—slightly—to the Pacific-Ocean bordering states. Although the trend was a gradual reduction of Haplogroup C from the North (Chihuahua, 28.4%) to the South (Chiapas, 5.8%), in the state of Yucatán (Southeast) Lineage C increased (13.8%) in terms of populations of this region ($\leq$10.4%). Distribution of Haplogroup B was homogeneous throughout the country (18.3–28%; $P = 0.63497$), excluding the state of Durango where it became the modal lineage (40%). The Haplogroup D had the lowest total frequency (6.2%), without a clear geographical pattern. Finally, the Native American Haplogroup X2a was not observed in Mexican–Mestizos.

On the other hand, European maternal lineages constituted only 4.4% of the total Mexican population (Fig. 3), and were represented by Haplogroups R (xH,

TABLE 2. HVR-1 haplotypes of the Mexican–Mestizos with coding region mtSNPs that characterizes two distinct haplogroups (Hg)

Sample ID[a]	Hypervariable 1 (HVR1) haplotype	Possible Hg[b]	Concluded Hg[c]
Ags65	16086C, 16111T, 16223T, 16274A, 16290T, 16319A, 16362C	A,B	A2
Ags83	16111T, 16223T, 16290T, 16319A, 16342C, 16362C, 16390A	A,B	A2
Chi1	16111T, 16223T, 16274A, 16290T, 16319A, 16362C	A,B	A2
Chi21	16111T, 16223T, 16274A, 16290T, 16319A, 16362C	A,B	A2
Chi34	16111T, 16223T, 16274A, 16290T, 16319A, 16362C	A,B	A2
Chi79	16111T, 16223T, 16274A, 16290T, 16319A, 16362C	A,B	A2
Jal166	16111T, 16223T, 16274A, 16290T, 16319A, 16362C	A,B	A2
Ver12	*DNA not available for additional analysis*	A,B	A2[d]
Yuc48	16111T, 16223T, 16274A, 16290T, 16319A, 16362C	A,B	A2
Ver52	16111T, 16223T, 16274A, 16278T, 16290T, 16319A, 16362C	A,B	A2
Ags100	16182C, 16183C, 16189C, 16217C	A,B	B2
Ags104	16182C, 16183C, 16189C, 16217C	A,B	B2
Chia125	16092C, 16182C, 16183C, 16189C, 16217C	A,B	B2
Chia103	16111T 16183C 16189C 16223T 16290T 16291T 16304C 16319A 16362C	A,X2	A2
Chia104	16111T 16183C 16189C 16223T 16290T 16291T 16304C 16319A 16362C	A,X2	A2
Oax9	16111T, 16189C, 16223T, 16290T, 16319A, 16362C	A,X2	A2
Ver54	*DNA not available for additional analysis*	A,X2	A2[d]
Jal12	16183C, 16189C, 16193+C, 16217C, 16320T	B,X2	B2
Jal219	16092C, 16129A, 16148T, 16223T, 16354T, 16391A	I, X2	I5a[e]
Gue62	*DNA not available for additional analysis*	I, X2	I[d]

[a] The sample ID corresponds to their Mexican–Mestizo population origin. For abbreviations, refer to Table 1.
[b] Possible haplogroups defined through 11 coding-region mtSNPs (Supporting Information Table S1).
[c] Haplogroup concluded adding information from DNA sequence of HVR1.
[d] Probable haplogroup according to the Mexican samples with the same mtSNP haplotype.
[e] This haplogroup designation also included HVR2 sequence data: 73G, 199C, 204C, 250C, 263G, 309T, 315T, and 385G.

B), H, and I (2, 2.16, and 0.23%, respectively) (Table 1). The Eurasian paragroup N (xA, N1′5, X2, R) represented 0.81% (range, 0.9–3.9%) of the total Mexican–Mestizo population. Altogether, the European matrilineal ancestry was estimated at 5.26%, owing to the joining of European haplogroups and the Eurasian paragroup N, which is supported by historical records and previous genetic studies (**DISCUSSION**). Conversely, African lineage distribution was restricted to paragroup L (Table 1), which was low (1.89%) and homogeneous throughout Mexican–Mestizo population samples ($P = 0.84558$) (Fig. 3). The larger haplotype diversity was observed in the North and West ($D \geq 0.75$) rather than in the Center-South and Southeast ($D \leq 0.72$). Interestingly, this is related to the higher frequency of non-Native American haplogroups in populations from the North and West (Table 1).

Individuals with dubitable mitochondrial haplogroup

It was remarkable that 18 individuals (2.4%) simultaneously showed two diagnostic mtSNPs that characterize different maternal haplogroups. One set of these lineages carried markers of both Haplogroups A and B, whereas further lineages that presented mutations characteristic of Clades A and B were also carriers of G1719A—a homoplasic SNP that is diagnostic of several branches of the human mtDNA tree, including X2, R0b, H7a, L0f2a, L3d3, L3h1, and N1′5 (to which Haplogroup I belongs), among others (mtDNA tree build 14, phylotree.org, van Oven and Kayser, 2009). Once contamination was ruled out of these samples by use of negative controls and amplification of additional genetic systems, HVR1 sequence data were obtained from 17 samples, including one additional sample that showed the SNP 1719A (Table 2). The sequence data revealed that most of these individuals belong to Haplogroup A (A2 and A2d1a), and a minority were of the Lineage B (Table 2). The HVR1-haplotypes obtained for the majority of these subjects allowed us to confirm without doubt their mtDNA haplogroup, excepting sample Jal219 that required additional HVR2 DNA sequence data to display the European Haplogroup I5a (Table 2). This information was useful for inferring the haplogroup of samples without DNA available for further analysis. These results support that G1719A constitutes a hotspot for homoplasy (Reidla et al., 2003; Alvarez-Iglesias et al., 2007), similar to that observed for the 9-bp deletion at position 8,280 that characterizes Lineage B (Behar et al., 2007). In fact, the presence of the 9-pb deletion nested in a Haplogroup A background (A–B) in 10 individuals from seven Mexican–Mestizo populations (Table 2) is concordant with the previous observations in American populations (Torroni et al., 1993; Achilli et al., 2008), and has already been noted in Mexican subjects (Torroni et al., 1994; Green et al., 2000; Bonilla et al., 2005; Martinez-Marignac et al., 2007).

Matrilineal differentiation between Mestizos and surrounding Native groups

After the European contact, Native American populations suffered demographic decline owing to warfare and epidemic diseases (Schurr, 2004). However, the original size of Amerindian groups in Mexico was eventually reconstituted, given their high reproductive rate (Aguirre Beltrán, 1989). Moreover, indigenous migration toward large cities during the last decades could have changed the geographic distribution of the maternal genetic makeup over time. To detect these changes, we compared the regional distribution of Native American lineages (A–D) here described for Mestizos, with that of previously reported in nearby Amerindian groups (Table 3). Because of the scarce knowledge of mtDNA haplogroup distribution in Mexican Native groups, some

TABLE 3. Comparison of mtDNA Native American haplogroups between Mexican–Mestizos and nearby Amerindian groups (pooled) from the same geographical region

Region	Mestizo populations *P-value*	*n*	Nearby Amerindian groups (pooled)	*n*	Sample origin Reference(s)
North	Chihuahua[a]	72	Tarahumara	141	Peñaloza et al., 2007; Sandoval et al., 2009; Kemp et al., 2010
	$P = 0.00503$		Pima	97	Sandoval et al., 2009
West	Jalisco and Aguascalientes[a]	181	Purépecha	71	Peñaloza et al., 2007; Sandoval et al., 2009
			Huichol	77	Peñaloza et al., 2007; Kemp et al., 2010
	$P = 0.00069$		Cora	72	Kemp et al., 2010
Center	México City[b]	381	Nahua Xochimilco	78	Peñaloza et al., 2007; Sandoval et al., 2009
	$P = 0.02386$		Nahua Atocpan	109	Peñaloza et al., 2007; Kemp et al., 2010
			Otomí	68	Sandoval et al., 2009
	$P = 0.01160$[c]		*Aztecs (Ancient)*[c]	*23*	Kemp et al., 2005
Center-South	Veracruz[a]	47	Nahua Necoxtla	62	Peñaloza et al., 2007; Sandoval et al., 2009
	$P = 0.93374$		Nahua Ixhuatlancillo	57	Peñaloza et al., 2007; Sandoval et al., 2009
			Nahua Coyolillo	35	Peñaloza et al., 2007
			Otomí	35	Peñaloza et al., 2007
	Guerrero[a]	78	Nahua Zitlala	60	Peñaloza et al., 2007; Sandoval et al., 2009
	$P = 0.04266$		Nahua Chilacachapa	41	Peñaloza et al., 2007
	Oaxaca[a]	57	Mixtec (Alta)	31	Torroni et al., 1994; Peñaloza et al., 2007
	$P = 0.96310$		Mixtec (Baja)	25	Torroni et al., 1994; Peñaloza et al., 2007
			Triqui	107	Sandoval et al., 2009
			Mixtec	86	Sandoval et al., 2009; Kemp et al., 2010
			Mixe	68	Torroni et al., 1994; Kemp et al., 2010
			Zapotec	100	Torroni et al., 1994; Kemp et al., 2010
Southeast	Yucatán[a]	78	Tzeltal	35	Peñaloza et al., 2007
	$P = 0.65621$		Maya	52	Sandoval et al., 2009
	$P = 0.23823$[c]		*Maya from Xcaret (Ancient)*[c]	*24*	González-Oliver et al., 2001

[a] From this study subtracting non-Amerindian haplogroups.
[b] From Martinez-Marignac et al. 2007 subtracting non-Amerindian haplogroups.
[c] Ancient populations (italic) were not pooled with Native American groups for pairwise comparison purposes. They were compared independently against Mestizos and pooled Native Americans from the same state (homogeneity test; *P*-value).

regions are under-represented, which compels us to advise caution in interpreting these results. These comparisons revealed minor differences or nondifferentiation between Mestizos and Native Americans in the Center-South and Southeast of Mexico ($P \geq 0.656221$); the minor differences observed were for Guerrero ($P = 0.04266$) and Mexico City ($P = 0.02368$). Conversely, Mestizos from the North and West showed substantial differences with nearby Amerindian groups ($P \leq 0.00503$) (Table 3). Additionally, to explore continuity of the geographic distribution of Native American lineages over time, ancient DNA of Aztecs (Kemp et al., 2005) and Mayas (González-Oliver et al., 2001) was compared to Mestizos and Amerindian groups from the same region (Table 3). The ancient Maya sample did not present differences to both Mestizos of Yucatan and present-day Mayas ($P = 0.23833$), suggesting continuity of the maternal genetic makeup over time. In opposition, Aztecs showed moderate differences to Mestizos of Mexico City and Amerindian groups from the central region ($P = 0.01160$) (Table 3).

Comparison with previous mtDNA admixture estimates in Mestizos

The comparison of our maternal admixture estimates in Mexican–Mestizos with the previous studies did not show significant differences (Supporting Information Table S2). Interestingly, in the northern cities of Ojinaga and Ciudad Juárez located on the border between the United States of America and Mexico in the Chihuahua state, a similar European and African contribution was described previously (5.4 and 4.5%, respectively) (Green et al., 2000). However, we estimated higher European than African contribution in the close city of Chihuahua (8.6 and 2.5%, respectively) (Supporting Information Table S2). This increment of maternal African lineages could be explained by the recurrent human immigration that these border cities receive, which is in agreement with a previous census that noted an important displacement of individuals from different regions of Mexico and Central America.

Comparison between uniparental and autosomal ancestries

The maternal ancestry here estimated was compared with its paternal counterpart based on SNPs of the non-recombining region of the *Y*-chromosome (Y-SNPs), previously reported in the same Mexican–Mestizo populations (Martínez-Cortés et al., 2012). The maternal ancestry in Mexican–Mestizos was predominantly Native American, whereas the paternal complement was mainly European ($P < 0.00005$) (Fig. 4). Conversely, the African ancestry displayed a low frequency and homogeneous distribution throughout the Mexican territory for both uniparental systems (Fig. 4).

As we can hypothesize that the autosomal ancestry correlates with the average of uniparental ancestries (NRY and mtDNA), we tested this hypothesis in

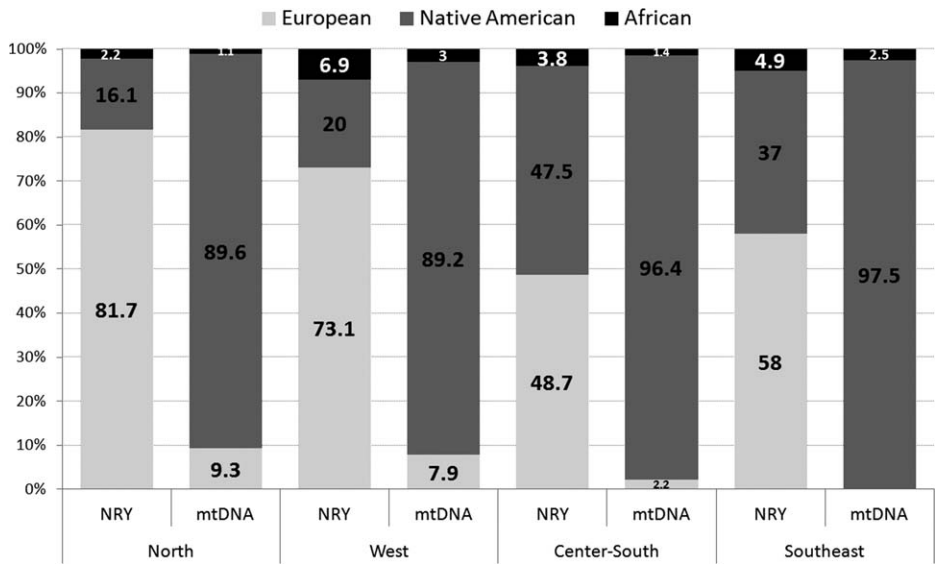

Fig. 4. Distribution of paternal (NRY) and maternal (mtDNA) ancestries in Mexican–Mestizos clustered by geographic regions.

Mexican–Mestizo populations with available data from the literature. The average ancestry obtained from maternal (this study) and paternal lineages (Martínez-Cortés et al., 2012) was correlated with the autosomal ancestry reported in Mexican populations based on genome-wide SNPs in Veracruz, Guerrero, and Yucatán (Silva-Zolezzi et al., 2009), and with those based on autosomal STRs from Chihuahua and Jalisco (Rubi-Castellanos et al., 2009) (Supporting Information Fig. S2). Although our results confirmed this hypothesis, it was noticeable that the correlation between ancestries that involved genome-wide SNPs was stronger ($r = 0.9773$, $P = 0.0100$) than the correlation implying autosomal STRs ($r = 0.9297$, $P = 0.0030$), especially for Native American and European components.

Mating patterns between maternal and paternal ancestry

To evaluate possible random mating deviations in Mexican–Mestizo populations, we analyzed human mating patterns in view of their paternal and maternal ancestries based on 478 males with haplogroup data for both mtSNPs (this study) and Y-SNPs (Martínez-Cortés et al., 2012). Ten different mating patterns were observed, with the European patrilineal and Native American matrilineal pattern (52.7%; range, 28–88.9%) standing out, and increasing toward the Northwest (Supporting Information Fig. S1). The second most frequent mating pattern involved Native American lineages for both uniparental systems (30.8%), which increase toward the Center and Southeast displaying a wide range of variation (0–60%). The third pattern involved the paternal African and maternal Native American ancestries (9.4%), displaying a range of 3.3–18.9%. This pattern in the Yucatán state was the unique departure from random mating expectations ($P = 0.00370$), whereas the remaining patterns of male/female contributions observed in Mexican–Mestizos were in agreement with this hypothesis ($P > 0.05252$). The fourth mating pattern engaged European components for both uniparental lineages (3.6%), and was not observed in the Southeast of Mexico. The remaining mating patterns were relatively rare in the total population sample (<1%) (Supporting Information Fig. S1).

Genetic relationships and genetic structure

The AMOVA test demonstrated a low but significant differentiation between populations ($F_{ST} = 2.85\%$; $P = 0.0000$). The geographic clustering of Mexican–Mestizo populations (SAMOVA) resulted in the following three groups ($F_{CT} = 4.62\%$; $P = 0.0000$): 1) Chihuahua, Durango, Sinaloa, Aguascalientes, Jalisco, and Guerrero; 2) Veracruz, Oaxaca, and Yucatán; and 3) Chiapas. The consistency of this outcome relied on the nonsignificant differentiation between populations within each group ($F_{SC} = 0.08\%$; $P = 0.42426$). In general, genetic distances and pairwise comparisons (data not shown) were consistent with the AMOVA results that were described previously. Additionally, we explored the correlation between the paternal and the maternal interpopulation differentiation in Mexican–Mestizos (F_{ST} values). Although these values were moderately correlated ($r = 0.4715$; $P = 0.012$), demonstrating that they are relatively symmetric, genetic differentiation was more evident via paternal (mtDNA $- F_{ST} = 2.85\%$ vs. NRY $- F_{ST} = 4.68\%$) (Martínez-Cortés et al., 2012).

DISCUSSION

To our knowledge, this study constitutes the largest effort to define the principal maternal lineages of Mexican–Mestizos, including nonpreviously analyzed populations and regions, such as the West and Southeast regions. In addition, in this article we estimated the maternal ancestry, genetic relationships, and population structure in Mestizos from different regions of Mexico. Regarding the impact of the knowledge generated, the maternal genetic variability in Mexican–Mestizos could serve as a reference in different fields. For instance, the population structure inferred here will address association study design involving mtDNA. Similarly, in

forensic genetics, the selection of mtDNA databases for interpretation of mtDNA profiles based on HVR1 and HVR2 must follow the guidelines of the population structure, such as that obtained here; this is important because of the scarcity of mtDNA databases from Mexican populations. Finally, in anthropology our results contribute to characterizing the complex biological identity of the Mexican–Mestizos, particularly from the maternal point of view.

It is noteworthy that we employed the old nomenclature to name Native American Haplogroups (A, B, C, and D) as we did not explore additional positions that allow a more refined classification of pan-Amerindian Haplogroups into A2, B2, C1, D1, D4h3a, and D4e1c (Tamm et al., 2007; Achilli et al., 2008; Perego et al., 2009; Sandoval et al., 2009; Kumar et al., 2011). Available evidence from control region sequences derived from Mexican populations (Guardado-Estrada et al., 2009, 2012; Sandoval et al., 2009) points to A2, C1, D1, and D4h3a as the sole members of Haplogroups A, C, and D who are present in these populations. Haplogroup B2 lineages do not carry any diagnostic control region mtSNP that would allow for distinguishing it from its parental Clades B4 and B4b, but so far B2 is the only Haplogroup B4 clade that has been found in Native Americans. Therefore, based on this background and comments describing analysis of coding-region mtSNP as the best strategy to define haplogroups (Behar et al., 2007), we can certainly assume that the great majority of Mexican–Mestizos characterized within Haplogroups A, B, C, and D will be of Native American origin.

Maternal ancestry in Mexican–Mestizos

Our results show that the mtDNA haplogroups of Mexican–Mestizos from different populations and regions were predominantly of Native American origin (~90%), represented by the four haplogroups originated in East Asia and widely distributed in the Americas (Ballinger et al., 1992; Torroni et al., 1993). In America, the Lineage A is described as decreasing from North to South, whereas the Haplogroup C diminishes in the opposite direction (Schurr, 2004). Interestingly, our results show a contrary pattern for these lineages throughout the Mexican territory, supporting a high genetic heterogeneity throughout the American continent (Wallace and Torroni, 2009). Although the distribution of Haplogroup B in Mexican–Mestizos is different from the clinal distribution for Mexican Native groups (Kemp et al., 2010), this is similar to the previous descriptions in Native American populations (Schurr, 2004). The lower frequency of Haplogroup D, especially in the Southeast (~3.5%), is consistent with continental studies, describing a low or even absent frequency of the Lineage D in Central America (Torroni et al., 1993, 1994; González-Oliver et al., 2001). Although the absence of Haplogroup X2a in our sample is in agreement with the previous studies of Mexican–Mestizo populations (Green et al., 2000; Guardado-Estrada et al., 2009), X2 has been detected at low frequencies in two northwestern Mexican Native populations (Peñaloza et al., 2007). In general, the presence of X2a is limited to the northernmost American populations (Brown et al., 1998; Malhi et al., 2001).

The European maternal ancestry in Mexican–Mestizos was defined by the joint frequency of European lineages and the Eurasian Lineage N (xR, A, I, X2). Although the limited panel of mtSNPs analyzed does not allow us to define the specific geographical origin for these N paragroup samples, they probably belong to Clade W, X1, X3, or X4 (Fig. 2). It should be noted that the assumption that N-derived lineages found in Mexico actually represent European ancestry is based on different sources: 1) historical records of the Mexican population (Aguirre Beltrán, 1989; 2) different genetic studies that consistently posit a negligible amount of Asian contributions to the Mexican population (Rubi-Castellanos et al., 2009; Silva-Zolezzi et al., 2009; Martínez-Cortés et al., 2012; and 3) the presence of such Eurasian haplogroups in Spain, the principal source of the European ancestry for the Mexican population (Gorodezky et al., 2001; Dahmany et al., 2006). Therefore, the European ancestry was 5.26%, with lower frequencies in Mexican–Mestizos from Center-South and Southeast regions than in the North and West ($P = 0.00401$), where 84.6% of the global European ancestry were concentrated (Table 1 and Fig. 3). The low Pre-Columbian population density that characterizes the North region, also known as Aridoamerica, probably helped to increase the European matrilineal ancestry in that area, which was poorly inhabited by nomadic people who came to be known as Chichimecas, meaning barbaric or uncivilized (Cordell and Fowler, 2005). Nevertheless, it is noteworthy that the European maternal origin here estimated (range, 0–11.7%) is smaller than the European paternal ancestry (range, 43.3–95%) calculated in the same 10 Mexican–Mestizo populations (Martínez-Cortés et al., 2012).

The total contribution of the matrilineal African ancestry in Mexican–Mestizos is minor (1.89%), and it can be explained by the records of slave trading in Mexico that involved—almost—exclusively African males (Aguirre Beltrán, 1989; Escalante-Gonzalbo et al., 2009). Interestingly, African maternal ancestry did not increase toward the coastal states of Guerrero, Oaxaca, or Veracruz, contrary to the previous descriptions for this component (Lisker, 1985; Gorodezky et al., 2001). However, our results are consistent with the studies asserting that African ancestry could be limited to specific coastal regions, but not for the majority of coasts in Mexico (Rangel-Villalobos et al., 2008; Rubi-Castellanos et al., 2009). Altogether, these data support a limited gene flow of the original African settlements or a "dilution effect" of African maternal lineages after European contact. In addition, the higher growth rates of Mexican Amerindian groups and prominent Pre-Hispanic population density of Mesoamerica probably helped to cause the African ancestry dilution effect (Aguirre Beltrán, 1989; Rubi-Castellanos et al., 2009). Finally, Mexican–Mestizos could have received African ancestry from Spaniards, considering the Islamic rule over the Iberian Peninsula during 8th Centuries (Rubi-Castellanos et al., 2009; Bryc et al., 2010).

Matrilineal genetic continuity over time

The null or minor differentiation between Mestizos and Amerindian groups in the Center-South and Southeast of Mexico indicates that Mexican–Mestizos roughly represent the matrilineal genetic pool of the nearby Native American groups (Table 3). Moreover, census data from the states of Veracruz, Oaxaca, and Mexico City, which report the highest number of immigrants (INEGI, 2005), are consistent with Native American gene flow toward Mestizo cosmopolitan cities.

Conversely, when Mestizos were pairwise compared individually with each nearby Native group (without pooling), differences were detected in all cases ($P < 0.001$; data not shown); this suggests that distribution of maternal Native American lineages from one specific Amerindian group does not explain that of the nearby Mestizos.

The genetic differentiation between Northwest Mestizo populations and nearby Amerindian groups suggests replacement of the maternal Native American lineages. Interestingly, this result can be related with the Pre-Hispanic demography because the North Region (including Aridoamerica) had a low population density, whereas the Central and Southeastern Region (representing Mesoamerica) was a densely populated area (González-José et al., 2007). In fact, the main settlement of the North region occurred after European contact and was promoted by the discovery of precious metals, mainly silver, as well as the emergence of agriculture, livestock breeding, and—eventually—cities (Moreno-Toscano, 2003; García-Martinez, 2009). This subsequent immigration would have changed the original distribution of mitochondrial lineages, adding haplotypes from different and probably distant regions, which is in agreement with the highest haplotype diversity observed in Northwest Mestizos (Table 1).

For Mayan populations, the inclusion of ancient DNA and contemporary Native groups (pooled by regions) allowed us to ascertain the matrilineal genetic continuity over time (González-Oliver et al., 2001), even including local Mestizos. The mtDNA homogeneity observed in Mayas is in agreement with the conclusions based on autosomal STRs that support theories of extensive trading throughout the Mayan-influenced areas (Ibarra-Rivera et al., 2008). The previous—and difficult—Spanish Conquest of the southeastern region probably helped to maintain the original maternal genetic makeup in this area (García-Martinez, 2009). This pattern of continuity was not observed in ancient Aztecs (Table 3), contrary to the report by Kemp et al. (2005). This difference between studies is most likely explained by the number of populations and the clustering method employed for comparison purposes. We tested the hypothesis of genetic continuity over time in smaller regions (states) and included a total of 15 Native American groups (Center, Center-South, and Southeast) (Table 3); conversely, Kemp et al. (2005) employed a lesser number of Native groups from a wider geographical area that showed non-differentiation with Mayas (current and Xcaret), Mixe-Mixtec, and Chibchan and Chocó (Central America), but displayed differences with Nahuas from Cuetzalan (Central Region, Mexico). In addition, this result could be related to the complex variability of Mexico City as a mega-metropolis with higher immigration rates (Martinez-Marignac et al., 2007).

Asymmetrical admixture or differential gene flow

The largest Native American maternal ancestry and predominant European paternal ancestry in Mexican–Mestizos evidenced strong sex-biased admixture (Fig. 4 and Supporting Information Fig. S1). This phenomenon also has been widely described in Latin America as asymmetrical admixture or differential gene flow (Torroni et al., 1994; Batista dos Santos et al., 1999; Carvajal-Carmona et al., 2000, 2003; Mesa et al., 2000; Campos-Sánchez et al., 2006; Wang et al., 2008; Rojas et al., 2010). However, mating patterns inferred from the paternal/maternal ancestries of Mexican–Mestizo males emphasize that European/Native American ancestry is more frequent in the North and West, whereas the Native American ancestry from both parents increases in the Center and Southeast. Interestingly, the increased frequency of unions between African males and Native American women (9.4%) contrasts with the number of African-European mates (0.6%), which is probably explained by sociocultural factors limiting some unions. Similarly, European women often had sons with European men (3.6%) and rarely with Native American men (0.8%). However, results suggest that these mating patterns are defined by the limited number of European women available for mating, because they are in agreement with random mating expectations. In general, these mating patterns are in agreement with historical records of the country during the first centuries after the Spanish conquest (Aguirre Beltrán, 1989; García-Martinez, 2009).

The heterogeneity caused by different admixture proportions is not exclusive to Mexican–Mestizo populations; it has also been described in Latin America with some peculiarities. Although Amerindian maternal ancestry is prevalent in Central America and the North region of South America (>80%), diverse South American populations show lower frequencies of this Native American ancestry (<50%), increasing the European and African ancestries in the same way (Carvalho-Silva et al., 2001; Sans et al., 2002; Carvajal-Carmona et al., 2003; Corach et al., 2010; Lao et al., 2010; Rojas et al., 2010). There are some interesting exceptions to the pattern in Latin America, such as the city of Melo (Uruguay) and Cuba, where the African maternal ancestry prevails with 45.3 and 52%, respectively (Sans et al., 2002; Mendizabal et al., 2008). It is of particular note that Mexican–Mestizos have the lowest frequency of African maternal lineages among the cited Latin-American populations. This observation is not surprising taking into account that historical records detail a range of 15,000–35,000 African slaves brought to Mexico (Aguirre Beltrán, 1989), a minor quantity in comparison with the 9.6–10.8 million Africans imported into the Americas (Lovejoy, 1989). In addition, the aforementioned dilution effect of African ancestry surely helped to reduce its present-day distribution in Mexican–Mestizo populations.

Comparison of ancestral components estimated with different genetic systems

The European and Native American ancestries based on genome-wide SNPs were correlated with the average ancestry obtained from uniparental systems (Supporting Information Fig. S2). Although autosomal and uniparental estimates of ancestry are obtained from different population samples, the positive correlation found in all cases (10 populations and 2 genetic systems) supports this conclusion. Therefore, based on NRY and mtDNA genetic data, we could approximate the autosomal ancestry of some Mexican–Mestizo populations where this information is not available. For instance, in the studied Mexican populations without estimates of autosomal ancestry we predicted values of European/Amerindian ancestry as follows: Durango, 49.5/50.5%; Sinaloa, 47.5/52%; Aguascalientes, 36.5/59%; Oaxaca, 23/73.5%; and Chiapas, 23.5/73%.

Patterns of mtDNA population structure

Although three population clusters were established among Mexican–Mestizos, the mtDNA population structure was defined by two groups geographically defined: 1) North-West, and 2) Center-South and Southeast. The two exceptions to this geographic pattern were Guerrero, which was clustered into the first group, and Chiapas, which remained isolated. In the case of Mestizos from Guerrero, they presented a haplotype distribution similar to Jalisco (West), including European and African haplotypes scarcely observed in the Center and Southwest (H, I, and L) that explain the reason why they were clustered in this group. With regard to Mestizos from Chiapas (city of Tapachula), they had the highest frequency of Haplogroup A, and low frequencies of the remaining Native American Lineages (B, C, and D) that explain their lowest haplotype diversity (Table 1). This differentiation pattern is explained by genetic drift effects suffered by the great number of Amerindian groups from Chiapas, which probably supplied an important quantity of matrilineal lineages to the local Mestizo population sample. Altogether, the variability in maternal admixture proportions estimated in Mexican–Mestizos throughout the country emphasizes the need to consider genetic structure effects in population studies. Finally, the general landscape of structure and relatedness obtained in this study, although valuable, should be improved by increasing the population sample sizes and number of Mexican–Mestizo populations analyzed.

CONCLUSIONS

In conclusion, the maternal ancestry of Mexican–Mestizos was largely Native American (92.86%) followed by European (5.26%) and African (1.89%). The mtDNA admixture and population structure inferred in Mexican–Mestizos was characterized by the following: 1) differences in Native-American lineage distribution (principally Haplogroups A and C); 2) increment of European haplogroups to the West and North; 3) low but homogeneous African maternal origin throughout the country; 4) Native American maternal lineages of Mexican–Mestizos roughly representing those of the surrounding Amerindian groups in the Center and Southeast, but not in the Northwest; 5) regional continuity of maternal lineages over time was consistent in the Southeast (Mayas), but modest in the Center (Aztecs); and 6) the average ancestry obtained from uniparental systems (NRY and mtDNA) was correlated with autosomal ancestry in Mexican–Mestizos. Finally, the landscape of asymmetric gene flow throughout the Mexican territory was improved.

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