9-2-2014

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Ana Itzel Juárez-Martín
Universidad Nacional Autónoma de México

Blanca Zoila González-Sobrino
Universidad Nacional Autónoma de México

Ángel Eduardo Camarena Olvera
Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas

Ramcés Falfán-Valencia
rias Ismael Cosío Villegas, rfalfanv@iner.gob.mx

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Ana Itzel Juárez-Martín[1], Blanca Zoila González-Sobrino[1], Ángel Eduardo Camarena Olvera[2], Ramcés Falfán-Valencia[2]

Keywords: HLA class II genes, Otomi, migratory history, genetic variability, DRB1, DQB1

[1] Instituto de Investigaciones Antropológicas/ Universidad Nacional Autónoma de México

[2] Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas

[1 2] All authors have contributed equally to the design, article preparation and final revision of the manuscript.

Correspondence to: Ramcés Falfán-Valencia, Laboratorio de HLA, Departamento de Inmunogenética, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas (INER). [Department of Immunogenetics, National Institute of Respiratory Diseases Ismael Cosío Villegas (INER)] Calzada de Tlalpan 4502, Colonia Sección XVI. México, D.F., 14080. Telephone: +52(55) 52 55 54 87, extension 5152. E-mail: rfalfanv@iner.gob.mx

Declarations. The present study was approved by the Science and Bioethics Committee of the National Institute of Respiratory Diseases Ismael Cosío Villegas (Approval Number B09-10). After having been informed of the purpose of the study, the volunteers signed a letter of consent.

Acknowledgments. The present study was conducted thanks to the support of the National Council of Science and Technology (CONACYT) through the National Scholarships Program (172908) and through legal funding from the PAPIIT-UNAM project IN400913.

Conflicts of Interest. The authors declare that there are no conflicts of interest. All authors have approved the final version of the article.

Abstract. From a historic and genetic point of view, the Otomi of the Mezquital Valley are a frontier people that have played an important role in the making of the population dynamics of the Mexican Central Plateau. Due to their antiquity in the area, the Otomi may be bearers of ancient genetic variability, shared mainly today with other groups belonging to the Otomanguean linguistic family and with the Nahua.

This study analyzes the HLA class II allele frequencies reported in Mexican indigenous populations, in order to provide an intra-regional level historical perspective of the genetic relationships between the Otomi of the Mezquital Valley and indigenous populations from other regions of Mexico. We examined genetic variation in *HLA-DRB1* and *-DQB1* loci in 66 non-related individuals belonging to seven indigenous communities from the Ixmiquilpan municipality in the Mezquital Valley, in the State of Hidalgo, Mexico.

The variability of the *HLA-DRB1* gene among the Otomi of the Mezquital Valley is mainly concentrated in five alleles: *-DRB1*08:02* (31.06%), *-DRB1*04:07* (25.77%), *-DRB1*14:06* (7.55%), *-DRB1*14:02* (6.06%) and *-DRB1*16:02* (4.55%), these alleles have been previously described in other indigenous populations. The most frequent alleles at the *HLA-DQB1* locus are *-DQB1*03:02* (34.09%), *-DQB1*04:02* (31.03%) and *-DQB1*03:01* (19.7%). Furthermore, *HLA-DQB1*02:02* allele is found in the Otomi group with a frequency of 2.27%, notably this allele has not been reported in Mexican indigenous populations.

In conclusion, the genetic constitution of the Otomi population is intermediate to the northern groups and the genetic variability shared by the peoples of the central regions of Mexico. Furthermore, *HLA-DRB1* and *-DQB1* allelic variability among the Otomi, provides an insight into the historical processes implied in the biological

admixture with European, Asian and African populations as well as in the admixture with the population of Mexico City associated to long-standing migratory processes.

Introduction. The Mezquital Valley comprises the western portion of the Mexican State of Hidalgo and it is inhabited mainly by Otomi or *Ñähñü* language speakers (Lastra 2006). Information provided by historical, archaeological and linguistic studies (Garrett 2012) has led to a widespread consensus that Otomi speaking groups are the oldest inhabitants of the region. Demographic data on the region (CLIN/ INALI 2008) reveals the there are approximately 95,057 Otomi speakers in Hidalgo and 23,261 (approximately 24.47%) reside in the municipality of Ixmiquilpan. Despite intense contact and continuous exchange with regions both north and south from the Mezquital Valley, the Otomi maintain many characteristic traits of Mesoamerican indigenous societies, including the privileged use of the local language, a traditional agrarian lifestyle based on collective ownership of land, and a social organization structured around cohesive domestic groups linked by extensive kinship ties (Baez 2012). Otomi communities in the Mezquital Valley are predominantly but not exclusively endogamous (Oliver et al. 2003, 2005; Franco 1992) and their social and cultural life is organized around a variety of traditional forms of non-market exchange (Medina and Quezada 1976), community-based collective labor and an elaborated rituality, which combines elements of Catholic and Pre-Columbian religions (Galinier 1990). Although the Otomi maintain a strong degree of ethnic identity they are not an isolated group; on the contrary, they have a long history of migration, first as semi-nomadic hunter-gatherers and after the Spanish conquest, as seasonal laborers in different regions both north and south from the Mezquital Valley (Ruiz de la Barrera 2000).

From a geographical point of view, the Otomi are located on a environmental frontier separating two of the main historical and climatic areas that constitute the Mexican territory: the great Mesoamerican region and the arid plains of northern Mexico, which begin at the Mezquital Valley (at 21st parallel north) (López-Aguilar

2005; Gerhard 1986) (Figure 1). Traditionally, the frontier between these two areas has been regarded as a clear-cut cultural boundary dividing agricultural peoples from hunter-gathering groups (Parsons 1998). Due to the lack of historical sources to reconstruct the history of contacts in this frontier (López-Aguilar 1991; Wright 2002) it is often assumed that the two regions developed in relative isolation from each other. Nevertheless, by studying the genetic constitution of the Otomi population it is possible to reconstruct a more complex and prolonged history of contacts.

The aim of this study is to report the allelic distribution of the HLA class II (-*DRB1* and -*DQB1*) genes among the Otomi population of the Mezquital Valley, as a frontier people who have played a unique role in the shaping of the genetic dynamics of the populations located along the corridor connecting the Northern Plains and the Central Plateau of Mexico. The great diversity observed among these populations is associated with a history of sustained contacts throughout this vast corridor, preserving a permanent dialogue with each other and with other regions during different periods and with different degrees of intensity. Furthermore, formation of the current genetic constitution of Mexican indigenous populations has been dynamic, as it has experienced changes in the distribution of Native-American, European and African alleles throughout time.

The extended collection of genes on the short arm of human chromosome 6 between regions 6p21.31 and 6p21.33 is called the Major Histocompatibility Complex (MHC) and is the most gene dense region of the human genome. The MHC class II region covers about 0.9 Mb and in the mosaic MHC sequence, 19 class II genes were counted including the classical genes HLA-DP, HLA-DQ and HLA-DR; the non-classical genes HLA-DM and HLA-DO; and 8 pseudogenes (Vandiedonck and Knight 2010).

One of the outstanding characteristics of the HLA system is its high degree of polymorphism and variability. According to the IMGT/HLA database (http://www.ebi.ac.uk/imgt/hla/stats.html) a total of 1,582 alleles have been identified for the *HLA-DRB1* locus and 712 for the *HLA-DQB1* locus. At a continental level, American indigenous populations exhibit reduced genetic diversity at the *HLA-DRB1* locus, while also exhibiting a number of unique alleles (Watkins et al. 1992; Cerna et al. 1993; Yunis et al. 1994; Trachtenberg et al. 1996; Erlich et al. 1997; Fernández-Viña et al. 1997; Parham et al. 1997; Blagitko et al. 1997; Hollenbach et al. 2001; Gorodesky et al. 2001; Layrisse et al. 2001; Loeza et al. 2002 Arnaiz-Villena et al. 2000, 2007; Zhang et al. 1993). Polymorphisms of the HLA system are relevant in epidemiological terms since the presence of certain alleles in populations might have occurred as an adaptive response to the presence of infectious agents or as the effect of genetic drift. Therefore, the extensive allelic polymorphisms and the linkage disequilibrium among different HLA loci are usually used as highly polymorphic genetic markers in anthropological studies (Shen et al. 2010).

An analysis based on genetic distances of *HLA-DRB1* and *-DQB1* genes was performed in order to provide an intra-regional level perspective of the historical relationship between the Otomi and other Mexican indigenous populations. To study the allelic distribution of *HLA-DRB1* both at regional and worldwide level, a Bayesian phylogeny and a Principal Components Analysis were also performed.

Materials and Methods

Sample. The sample was composed of 66 non-related individuals from seven communities: Peña II, El Tephé, Humedades, Cerritos, San Nicolás, Dios Padre and El Barrido, located in the municipality of Ixmiquilpan in the Mezquital Valley, Mexico

(Figure 2). The valley is located at longitude 98º56´42" and 99º51´18" and latitude 19º45´27" and 20º45´9", is part of the Neovolcanic Plateau physiographic province, situated in the western portion of the Sierra Madre Oriental mountain range (González-Quintero 1968).

According to data of the 13th National Population and Housing Census (2010), the Ixmiquilpan municipality is inhabited by 86,363 people. The age-average of the study participants was 49.7±10.6 years old. All the volunteers were residents of the Ixmiquilpan municipality with at least two recorded generations of Otomi descent and most of them spoke *Ñähñü* or Otomi language. After having been informed of the purpose of the study, all the volunteers signed a letter of consent. The present study has been approved by the Science and Bioethics Committee of the National Institute of Respiratory Diseases Ismael Cosío Villegas (Approval Number B09-10).

HLA Genotyping. The genomic DNA was obtained using the BD Tract DNA Isolation Kit (Maxim Biotech, San Francisco, CA, USA) from peripheral blood cells collected through venipuncture. The high-resolution genotyping of the HLA class II alleles was achieved through polymerase chain reaction with sequence-specific primers (PCR-SSP), which implemented the Fastype HLA-DNA SSP typing system (Bio-synthesis Inc.; Dallas TX, USA). This methodology has been accredited and certified by the American Society for Histocompatibility and Immunogenetics (ASHI).

Statistical analysis. Allele frequencies were determined by direct counting of molecular data. The statistical analysis was performed using Arlequin v.3.0 software (Excoffier et al. 2005). The software program was used to test for Hardy-Weinberg equilibrium, and to generate genetic distances (*F*st). Haplotype frequencies of two-loci

combinations were estimated by the maximum likelihood algorithm (Excoffier and Slatkin 1998). The linkage disequilibrium (LD) coefficient Δ (D) (Weir 1979) was calculated according to standard formula for joint probability for a set of events (Slatkin and Excoffier 1996); standardized Δ (D´) was calculated according to Lewontin (1964).

Comparisons between the fixation indexes *F*st and *Phi*st (Φ_{ST}) (Excoffier et al. 1992) were performed with sequences of 1022 bp obtained from the IMGT/HLA online database|EBI (http://www.ebi.ac.uk/imgt/hla/). The pair-wise allele distances were recorded as the number of nucleotide differences and used for the subsequent calculation of *Phi*st. Since *Phist* takes into account the nucleotide differences among alleles from a common ancestor, then comparisons between *Fst* and *Phist* can provide an insight into the ancestry and gene flow among populations throughout time. When *Fst* values are larger than corresponding *Phist* values, it can be inferred that isolation processes are more recent than allelic phylogenetic processes and gene flow. When *Phist* values are larger than *Fst* values, they indicate ancient isolation with less gene flow along history, if any. Phylogeny of *HLA-DRB1* alleles was reconstructed with the software MrBAYES 3.1.2 (Ronquist and Huelsenbeck 2003) applying the General Time-Reversible (GTR + G) model of nucleotide substitution. Software was executed for 10000000 generations using 4 Metropolis-Coupled Markov Chain Monte Carlo (MC^3) chains and the recommended random starting trees. A consensus tree was determined using the 50% majority-rule. Principal Components Analysis (PCA) was performed using the software *X*LSTAT (Version 2014.2.06) to explore the distribution of *HLA-DRB1* alleles worldwide.

Populations Comparisons. An analysis based on the allele frequencies of HLA class II genes (-*DRB1* and -*DQB1*) was conducted in twelve Mexican indigenous populations

and in one sample of admixed population from Mexico City, as previously reported (Table 1). The populations were organized according to their geographical distribution and grouped as follows: 1) the Sierra Madre Occidental mountain range is occupied by Mayo, Seri, and Tarahumara peoples, 2) the western part of the Sierra Madre Oriental mountain range is inhabited by Teenek or Huastec groups, 3) the Central Plateau consists of the Otomi, Nahua and Mazahua, 4) the Mazatec, Mixe and Mixtec peoples occupy the mountain areas in Oaxaca, 5) the Zapotec populations are mainly located in the Oaxacan central valleys, and 6) samples of Mayas of Yucatán and Guatemala were added representing Southeast region of the country. Although the latter are currently located in a different nation state, they maintain strong cultural, linguistic and historical links with the rest of populations settled in the Mexican territory. We have included Mexican indigenous and admixed populations, as well as Guatemalan Maya to perform the intra-regional level analysis. Geographic locations of studied populations are illustrated in Figure 1.

To assess the *HLA-DRB1* allele distribution at a worldwide level, PCA was performed considering a total of 70 populations. Extended references for the populations comprised in the analysis and the number of individuals in each sample (N) are detailed in Table 1. Numbers in square brackets correspond to the ID population number according to Table 1: the Mexican indigenous populations shown in Figure 1 [1-12]; Admixed groups from Mexico City [14, 15]; North America [39-42]; Central America/Caribbean [13, 16] South America [17-38]; Central-Asia [57-60]; Mediterranean European [61- 67]; Africa [68-70] and Siberia [43-56] populations.

Results

HLA class II alleles in the Otomi population. Table 2 shows the high-resolution *HLA-DRB1* and *-DQB1* allele frequencies reported in the Otomi population of the Mezquital Valley. The diversity of the *HLA-DRB1* gene among the Otomi is distributed in 17 alleles, however, most of the variability is restricted to two alleles: *-DRB1*08:02* (31.06%) and *-DRB1*04:07* (25.77%). The *-DRB1*14:06* allele represents 7.55% of the variation in the Otomi population, *-DRB1*14:02* (6.06%), *-DRB1*16:02* (4.55%) and *-DRB1*04:03, -DRB1*04:04* are at the same frequency (3.79%). The analysis of the *HLA-DQB1* locus among the Otomi population reveals ten alleles, although only three of these are present at frequencies higher than 10%: *-DQB1*03:02* (34.09%), *-DQB1*04:02* (31.06%), and *-DQB1*03:01* (19.7%). The Otomi of the Mezquital Valley also exhibits the *HLA-DQB1*02:02* allele at a frequency of 2.27%. It represents the only indigenous population in Mexico that exhibits this allele.

Haplotype analysis. The two-loci *HLA-DRB1-DQB1* haplotypes are listed in Table 3. According to the *HLA-DRB1* and *-DQB1* tests of linkage disequilibrium for all pairs of loci, the overall pairwise values LD: -260.35 and LE: -421.060 (X^2 =323.25; 144 df; P= 0.000) denote strong linkage disequilibrium. Furthermore, estimates of Hardy-Weinberg show a slight deviation from expected/observed heterozygosities (*locus* 1: exp het = 0.8313/ obs het= 0.8484; p-value= 0.024) (*locus* 2: exp het = 0.7469/ obs het= 0.7723; p-value= 0.136). Linkage disequilibrium and failure to comply with the Hardy-Weinberg law in a population with fewer heterozygotes than expected may be attributed to inbreeding at the community level (Oliver et al. 2003).

The Otomi population exhibits 18 haplotypes, of which, the five typical Native-American haplotypes are more frequent: *HLA-DRB1*08:02-DQB1*04:02* (31.06%), *HLA-DRB1*04:07-DQB1*03:02* (25.77%), *HLA-DRB1*14:06-DQB1*03:01* (7.55%),

*HLA-DRB1*14:02-DQB1*03:01* (6.06%), and *HLA-DRB1*16:02-DQB1*03:01* (4.55%). The Otomi share eight haplotypes with Mediterranean populations (mainly from Spain, Morocco, Tunis and Eastern-Europe Macedonians), including *HLA-DRB1*03:01-DQB1*02:01, HLA-DRB1*07:01-DQB1*02:02/*03:03, HLA-DRB1*01:02-DQB1*05:01* (exhibiting the same frequency of 3.03%), *HLA-DRB1*01:03-DQB1*05:01*, *HLA-DRB1*15:02-DQB1*06:01* (sharing the frequency of 2.27%), *HLA-DRB1*13:01-DQB1*03:01* and *HLA-DRB1*13:02-DQB1*06:04* (both with a frequency of 0.76%), showing clearly the admixture with non-Amerindian populations.

The Otomi also exhibit the same frequency of 3.79% for the haplotypes *HLA-DRB1*04:03-DQB1*03:02* and *HLA-DRB1*04:04-DQB1*03:02* that have been reported in several populations worldwide, including Mexican and South-American indigenous populations (Yukpa, Arsario, Kogi, Wayuu, Kaigang, Quechuas and Uros), but also among Asian Mongolians and Mediterranean populations (from Spain, Crete, Morocco and Tunis) (Vidal, et al. 2002; Sánchez-Velasco, et al. 2003; Arnaiz-Villena, et al. 1999; 2001; Gomez-Casado, et al. 2000 Hajjej et al. 2006; Machulla, et al. 2003).

The most probable origin of *HLA-DRB1-DQB1* haplotypes was inferred from data available from previous reports (Zuñiga et al. 2013; Barquera *et.al.* 2008; Yunis et al. 2003; Marsh et al. 1999). Haplotype analysis of *HLA-DRB1-DQB1* genes indicates that approximately 75.75% of the allelic variability among the Otomi is of Native American origin, while 22.73% derives from European-shared with other populations origin. The remaining 1.52% corresponds to haplotypes *HLA-DRB1*12:01-DQB1*03:01* and *HLA-DRB1*15:03-DQB1*06:03* of Asian and African origins, respectively.

Population analysis. Calculations of genetic distances *F*ST between Otomi and the rest of Mexican populations are shown in Tables 4 and 5. *HLA-DRB1* calculations of *F*ST in the Otomi reveal that the shortest genetic distances are with the Mazahua (0.017), Mixtec (0.021) and Zapotec (0.029) peoples. There is also a short *F*ST genetic distance between the aforementioned groups and the rest of the populations of the Mexican Central Plateau. According to the genetic distances *F*ST estimates for *HLA-DQB1*, after the Mixtec (Fst= 0.014) and the Zapotec (Fst= 0.015), the closest neighboring groups to the Otomi are the Seri (Fst= 0.018) and Tarahumara (Fst= 0.025). *HLA-DRB1* and -*DQB1* genetic distances among admixed population from Mexico City and the Otomi (Fst= 0.019 and Fst= 0.015, respectively) denote the intense gene flow existing between the Otomi and the inhabitants of the central regions of Mexico.

Figure 3 shows comparisons between fixation indices *F*st (for recent migrations) and *Phist* (for ancient migrations) calculated for *HLA-DRB1* and *–DQB1*. The *HLA-DRB1* gene provides evidence that the most intense gene flow occurs between Otomi, Mixtec, Zapotec and Mazahua groups, all belonging to the same Otomanguean linguistic trunk. Fst value of Mayo (Fst= 0.071) indicates that separation between these and the Otomi was shorter in previous times (*Phist*= 0.051). The Tarahumara is the northern population that has maintained the greatest level of gene flow with the Otomi (*Phist*= 0.05 *vs.* Fst= 0.04). Simultaneously, exchange between Otomi, Nahua and other groups in the Central Plateau has remained continuous since they settled in the region, although it has intensified in recent times (*Phist*= 0.04 *vs.* Fst= 0.03). Finally, according to *HLA-DQB1* there is an ancient genetic divergence between Otomi and Mixe populations (*Phist*= 0.17). Gene flow between Yucatan Maya and Otomi was more intense in the past (*Phist*= 0.099) than it is at present times (Fst= 0.21).

***HLA-DRB1* allele distribution among Mexican indigenous populations.** Figure 4 shows a Bayesian phylogeny based on the pair-wise allele distances *Phi*st in the *HLA-DRB1* alleles. Forty-seven alleles are present in Mexican indigenous populations. Population specific alleles account for 32% of the variability while the remaining 68% are shared alleles. Of the groups analyzed, those that exhibit increased allele diversity are the Guatemalan Maya and the Nahua. The *HLA-DRB1* alleles in these two groups represent approximately 70% of the total variability shown in Mexican indigenous populations. Of the populations analyzed, the genetic constitution of the Maya of Guatemala accounts for nearly 60% of the *HLA-DRB1* variation, while the Otomi carry about 40%. The populations carrying the largest number of European alleles are the Maya from Guatemala, the Nahua and the Otomi of the Mezquital Valley. If we consider the number of nucleotide changes in the sequences, *HLA-DRB1*01:01, *09:02, *10:01*, **07:01, *04:02* and **12:01* alleles are those that exhibit the largest number of accumulated differences (*Phist ca.*= 0.12), which suggests the previous divergence of these alleles. All the alleles aforementioned are of European, Asian or African origin.

Only the Native American alleles -*DRB1*08:02* and **04:07* are shared by all Mexican indigenous populations. Among the Native-American alleles, *HLA-DRB1*08:02* is the one with the largest nucleotide diversity, reaching its highest frequency in the northern region of Mexico (35% among the Tarahumara group). Allele -*DRB1*04:07* is found in frequencies greater than 10% among the Mayo, Seri, Teenek and Maya. The allele -*DRB1*16:02* may be of Mesoamerican origin since it is almost absent in northern populations of Mexico.

Principal Component Analysis. Figure 5 shows a Principal Component Analysis performed for *HLA-DRB1* frequencies, displaying the position of each population in two dimensions. The plot shows that the Otomi belongs to the same cluster as other Mexican indigenous groups and the remaining of American indigenous populations considered in this study. The PCA notably separates the Otomi from admixed populations of Mexico City, who are set between European, African and Central Asia populations, and the rest of Mexican indigenous populations (including the Otomi), which are closer to the Native-American cluster.

Historical background and discussion

According to colonial sources, the ancestors of the Otomi were among the earliest agricultural peoples of the Mexican Central Plateau, as Motolinía (16^{TH} century), Torquemada (16^{TH}-17^{TH} centuries), Clavijero (18^{TH} century), and Orozco y Berra (19^{TH} century) state in their studies of the region (Fournier 1996). Although the analysis of HLA class II genes does not allow us to assume that the genetic variability of contemporary Mezquital Valley groups corresponds to that of pre-Columbian Otomi populations, it is clear that the valley has been a site for complex genetic interaction as humans have occupied it for the last 11,000 years (Fournier 2012). Data obtained from the analysis of other genetic systems, such as mitochondrial DNA (mtDNA) supports the hypothesis on the antiquity of the Otomi of the Mezquital Valley. Sequences of the mtDNA control region [spanning nucleotide positions 16024-576] suggest that the Otomi were the first people to differentiate from the original genetic pool, which reinforces the claim that the Otomi were among the first settlers in the Mesoamerican territory (Gorostiza et al. 2012). As a result, it is most likely that the Otomi genetic constitution was formed gradually throughout time, by receiving inheritances from

different ethnolinguistic groups, as Wright (2005) has suggested based on the study of ancient Mixtec, Mexica (Aztec) and Otomi codex books.

The Otomi territory, which extended from the northern portion of the State of Mexico to the south of the State of Hidalgo, played the role of a buffering zone, dividing the Mexica and Purepecha territories and the lands of those groups generically known as Chichimeca, who occupied the northern portions of Mesoamerica (Parsons 1998). Therefore, the Otomi acted as intermediaries, brokering the relationships between hunter-gathering and agricultural-sedentary peoples. The boundaries shared by Otomi and Chichimeca groups were not fixed. Rather, this was an unstable frontier that was constantly expanding and contracting, but despite its instability, all groups shared a common linguistic background that allowed them to maintain continuous social, economic, cultural and genetic exchanges (Fournier and Vargas 2002). As the southern continuation of the Chihuahuan deserts, the Mezquital Valley has a strategic geographic location, as it is an unavoidable crossroad connecting northern and central Mexico, which has facilitated contacts among all the cultural regions of the country. At the same time, the Mezquital Valley serves as a hub, linking the country from the Gulf of Mexico to the Pacific coast (Reyes and Odena 2001). Residing in the frontier, the Otomi share genetic diversity with groups of the Central Plateau and of the populations beyond the northern border of Mesoamerica. We should bear in mind that the Otomi were capable of moving throughout this vast geographic corridor, a fact that would have facilitated gene flow with groups from the Northern Arid Plains and Mesoamerica. Gene flow between the Otomi and populations from northern and western Mexico (Tarahumara, Huichol, and Cora in the first case and Purepecha in the latter) becomes clear when we analyze the linguistic and historic traits of the Otomi and the data provided by other genetic systems such as mtDNA (Juárez-Martín 2010). The analysis of HLA class II

genes reinforces the previous assertion, as it provides evidence of the relative closeness between the Otomi and the Tarahumara and Seri populations from northern Mexico (Tables 4 and 5). The combined use of genetic data with linguistic, geographic and historical information also reveals that population movements did not take place solely on a north-south axis, but that there were also migrations and contacts that followed other geographic lines.

The Mazahua and the Otomi share a common linguistic origin but diverged from each other approximately 5.8 to 7.4 centuries ago (Lastra and Valiñas 2007). The Otomi have the shortest genetic distance with Mazahua (Fst= 0.018), demonstrating that gene flow between them has remained relatively stable throughout their history (*Phist*= 0.02) (Figure 3). Contacts between Nahua and Otomi populations have been intense and close at least since 11th Century BC, when both groups shared a vast common territory in central Mexico (Obregón 2001). However, after the rise of the Mexica (Aztec) Empire in the 15th century, náhuatl language spread at the expense of Otomi language, which was gradually displaced and fragmented into 13 different dialects (Lastra and Valiñas 2007). Nonetheless, this linguistic fragmentation did not interrupt contacts among Otomi groups and the gene flow was maintained. The genetic distance between Otomi and Nahua (Fst= 0.035) can be explained as a result of the intense gene flow that has taken place in the Central Plateau.

The development of lode mining in northern Hidalgo, Zacatecas and San Luis Potosí after the Spanish Conquest, changed the demographics of northern Mexico once more, as it created colonies of Nahua, Spanish, and African slaves, who settle in the Otomi region to work as miners during the 17th century (López-Aguilar 2005). These historical processes might explain the presence of some African alleles in the Otomi population as shown in the haplotypes *–DRB1*15:03-DQB1*06:03* and *–DRB1*13:02-*

*DQB1*06:04*. As mentioned before, haplotypes *–DRB1*15:02-DQB1*06:01* and *–DRB1*12:01-DQB1*03:01* can be traced back to the Asian occupation of the Otomi-Mazahua region, probably during colonial or even during recent times (Melville 1994).

HLA-DRB1 alleles reported (6/17) among the Otomi are of Native American origin, accounting for nearly 75% of the variability, while the rest of the alleles reflect a clear admixture with European, Asian and African populations. It is relevant that all Mexican indigenous populations carry non-Amerindian alleles (Figure 4), although such frequencies are low when contrasted with admixed populations of Mexico (Rubi-Castellanos et al. 2009; Wang et al., 2008; Vargas-Alarcón et al. 2010; Zúñiga et al. 2013). If we compare these values with the admixture calculations reported for *HLA-DRB1* in population of Mexico City, it results that 51.2% is of Native American origin, 40% has European origin and the remaining 5% comes from African and Asian regions (Zúñiga et al. 2013).

However, *HLA-DRB1* genetic distances among the Otomi and present-day inhabitants from Mexico City, reveal that the genetic exchange is ancient (*Phist*= 0.002) and it has kept intense in present days (Fst= 0.019) (Figure 3). The closeness between the Otomi and the admixed population of the Central Valleys is consistent with the data reported for other genetic markers such as STR-PCR polymorphisms (Barrot et al. 2005; González-Martín et al. 2008; Hernández-Gutiérrez et al. 2005). The Otomi became the largest indigenous group in Mexico City, reflecting a trend that can be traced back to colonial and Pre-Columbian times, but was intensified at the beginning of the 1960s. Towards 1990, over 30% of Mexico City´s population was composed by immigrants from the states of Hidalgo and Estado de México (Rubio 2000: 173).

Although *HLA-DRB1* and *-DQB1* genetic distances between Otomi and Mestizo are very short (Tables 4 and 5), they do not cluster in the PCA (Figure 5), which shows

that the Otomi follow the same genetic pattern of the rest of the Mexican indigenous populations in a different way from the admixed populations of Mexico City.

The interethnic dynamics among the populations that settled in the central regions of Mexico can be better understood by taking into account the role of the Otomi as a frontier people that hold a record of gene flow between populations in Northern and Central Mexico. Our intra-regional study permits integration of genetic data with linguistic, geographic and historical information, providing a detailed exploration that enhances the results achieved by analysis conducted at continental or worldwide levels.

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Table 1. Populations included in the statistical analysis

ID	POPULATION	N	LOCATION	REFERENCE
1	**OTOMI**	66	IXMIQUILPAN, HIDALGO/ MEXICO	Present study
2	**MAZAHUA**	65	ESTADO DE MÉXICO/ MEXICO	Arnaiz-Villena, et al. 2011
3	**NAHUA**	85	MORELOS/ MEXICO	Vargas-Alarcón, et al. 2007
4	**MAZATEC**	89	OAXACA/ MEXICO	Arnaiz-Villena, et al. 2000
5	**MIXE**	55	SIERRA DE OAXACA/ MEXICO	Hollenbach, et al. 2001
6	**ZAPOTEC**	90	VALLE DE OAXACA/ MEXICO	"
7	**MIXTEC**	103	MIXTECA ALTA, OAXACA/ MEXICO	"
8	**TEENEK**	55	SAN LUIS POTOSI/ MEXICO	Vargas-Alarcón, et al. 2006
9	**MAYO**	60	SINALOA/ MEXICO	Arnaiz-Villena, et al. 2007
10	**SERI**	31	SONORA/ MEXICO	Alaez, et al. 2002
11	**TARAHUMARA**	44	CHIHUAHUA/ MEXICO	García-Ortiz, et al. 2006
12	**MAYA (MXN)**	50	YUCATAN/ MEXICO	Vargas-Alarcón, et al.

				2011
13	**MAYA (GTM)**	132	QUETZALTENANGO/ GUATEMALA	Gómez-Casado, et al. 2003
14	**MEXICAN ADMIXED 1**	234	MEXICO CITY/ MEXICO	Zúñiga, et al. 2013
15	**MEXICAN ADMIXED 2**	99	MEXICO CITY/ MEXICO	Vargas-Alarcón, et al. 2010
16	**CUBANS**	78	CUBA	Alegre, et al. 2007
17	**CAYAPA**	100	ECUADOR	Trachtenberg, et al. 1994
18	**QUECHUA**	80	BOLIVIA	Martínez-Laso, et al. 2006
19	**UROS**	105	PERU	Arnaiz-Villena, et al. 2009
20	**YUKPA**	73	VENEZUELA	Layrisse, et al. 2001
21	**ARSARIO**	18	COLOMBIA	Monsalve, et al. 1998
22	**KOGI**	42	COLOMBIA	“
23	**ARHUACO**	107	COLOMBIA	“
24	**WAYUU**	88	COLOMBIA	“
25	**EAST TOBA**	134	ARGENTINA	“
26	**MATACO WICHI**	49	ARGENTINA	“

27	**TOBA PILAGA**	19	ARGENTINA	“
28	**CHIRIGUANOS**	56	ARGENTINA	Petlz-Erler, et al. 1997
29	**GUARANI**	32	BRAZIL	“
30	**KAINGANG 1**	225	BRAZIL	“
31	**XAVANTES**	74	BRAZIL	Monsalve, et al. 1998
32	**GUARANI-M´BYÁ**	93	BRAZIL	Tsuneto, et al. 2003
33	**GUARANI-KAIOWÁ**	160	BRAZIL	“
34	**GUARANI-ÑANDEVA**	87	BRAZIL	“
35	**ACHE**	87	BRAZIL	“
36	**KAINGANG 2**	235	BRAZIL	“
37	**AMAZONIAN**	41	BRAZIL	“
38	**QUECHUA**	44	BRAZIL	“
39	**ATHABASCAN**	62	CANADA	Monsalve, et al. 1998
40	**PENUTIAN**	26	CANADA	“
41	**TLINGIT**	53	ESKIMO-ALEUTIANO	“
42	**ZUNI**	50	ESKIMO-ALEUTIANO	“

43	**EVENK**	35	RUSSIA (SIBERIA)	Grahovac, et al. 1998
44	**KETS**	22	RUSSIA (SIBERIA)	“
45	**UDEGEY**	23	RUSSIA (SIBERIA)	“
46	**NIVKH**	32	RUSSIA (SIBERIA)	“
47	**CHUKCHI**	59	RUSSIA (SIBERIA)	“
48	**ESKIMOS**	80	RUSSIA (SIBERIA)	“
49	**KORYAK**	92	RUSSIA (SIBERIA)	“
50	**MANSI**	68	RUSSIA (SIBERIA)	Uinuk-ool, et al. 2002
51	**TODJA**	22	RUSSIA (SIBERIA)	“
52	**TOFALAR**	43	RUSSIA (SIBERIA)	“
53	**BURYAT**	25	RUSSIA (SIBERIA)	“
54	**NEGIDAL**	35	RUSSIA (SIBERIA)	“
55	**ULCHI**	123	RUSSIA (SIBERIA)	“
56	**OKHOTSK EVENKI**	25	RUSSIA (SIBERIA)	“
57	**TUVINIANS**	190	RUSSIA/ NORTH MONGOLIA	Martínez-Laso, et al. 2001
58	**KHALKHA**	100	MONGOLIA	Machulla, et al. 2003
59	**OOLD**	52	MONGOLIA	“

60	**TSAATAN**	72	MONGOLIA	“
61	**SPANIARDS (BCN)**	941	SPAIN	Vidal, et al. 2002
62	**SPANISH-BASQUES**	83	SPAIN (NORTH)	Sánchez-Velasco, et al. 2003
63	**CANTABRIANS**	83	SPAIN (NORTH)	“
64	**CABUERNIGOS**	95	SPAIN (NORTH)	“
65	**PASIEGOS**	88	SPAIN (NORTH)	“
66	**MACEDONIAN**	172	MACEDONIA	Arnaiz-Villena, et al. 2001
67	**CRETANS**	135	CRETE	Arnaiz-Villena, et al. 1999
68	**CAMERUNESES**	126	CAMEROON	Pimtanothai, et al. 2001
69	**MORROCCAN**	98	MOROCCO	Gomez-Casado, et al. 2000
70	**TUNISIAN**	104	TUNIS	Hajjej, et al. 2006

Table 2. Frequencies of the *HLA-DRB1* and *-DQB1* alleles in the Otomi population

Allele	Number observed	Frequency (%)
DRB1*		
01:02	4	3.03
01:03	3	2.27
03:01	4	3.03
04:03	5	3.79
04:04	5	3.79
04:07	34	25.77
04:10	1	0.76
07:01	4	3.03
08:02	41	31.06
12:01	1	0.76
13:01	1	0.76
13:02	1	0.76
14:02	8	6.06
14:06	10	7.55
15:02	3	2.27
15:03	1	0.76
16:02	6	4.55
DQB1*		
02:01	4	3.03

02:02	3	2.27
03:01	26	19.7
03:02	45	34.09
03:03	1	0.76
04:02	41	31.06
05:01	7	5.3
06:01	3	2.27
06:03	1	0.76
06:04	1	0.76

Table 3. Two-loci haplotype distribution of *HLA-DRB1-DQB1* in the Otomi population

HLA-DRB1	***HLA-DQB1***	# CASES	FREQUENCY (%)	Δ	Δ´	X^2
*DRB1*01:02*	*DQB1*05:01*	4	3.03	0.0287	1.000	73.6
*DRB1*01:03*	*DQB1*05:01*	3	2.27	0.0215	1.000	54.81
*DRB1*03:01*	*DQB1*02:01*	4	3.03	0.0294	1.000	132
*DRB1*04:03*	*DQB1*03:02*	5	3.79	0.0296	1.000	11.75
*DRB1*04:04*	*DQB1*03:02*	5	3.79	0.0296	1.000	11.75
*DRB1*04:07*	*DQB1*03:02*	34	25.77	0.1428	0.904	64.54
*DRB1*04:10*	*DQB1*03:02*	1	0.76	0.0049	1.000	1.88
*DRB1*07:01*	*DQB1*02:02*	3	2.27	0.0220	1.000	98.23
*DRB1*07:01*	*DQB1*03:03*	1	0.76	0.0073	1.000	98.23
*DRB1*08:02*	*DQB1*04:02*	41	31.06	0.2141	1.000	132
*DRB1*12:01*	*DQB1*03:01*	1	0.76	0.0061	1.000	4.31

*DRB1*13:01*	*DQB1*03:01*	1	0.76	0.0061	1.000	4.31
*DRB1*13:02*	*DQB1*06:04*	1	0.76	0.0075	1.000	132
*DRB1*14:02*	*DQB1*03:01*	8	6.06	0.0340	0.691	17.43
*DRB1*14:06*	*DQB1*03:01*	10	7.55	0.0538	0.876	35.58
*DRB1*15:02*	*DQB1*06:01*	3	2.27	0.0222	1.000	132
*DRB1*15:03*	*DQB1*06:03*	1	0.76	0.0075	1.000	132
*DRB1*16:02*	*DQB1*03:01*	6	4.55	0.0368	1.000	26.9

Δ Linkage disequilibrium coefficient (D)

Δ´ Standardized linkage disequilibrium (D´)

All values are significant. Level of significance $p \leq 0.05$

Table 4. *HLA-DRB1 F*st genetic distances among Mexican populations of the study

POPULATION	NAHUA	MAZAHUA	TEENEK	SERI	TARAHUMARA	MAYO	MAZATEC	MIXE	MIXTEC	ZAPOTEC	MAYA (GTM)	MEXADM
MAZAHUA	0.048											
TEENEK	0.039	0.086										
SERI	0.098	0.103	0.080									
TARAHUMARA	0.064	0.065	0.125	0.094								
MAYO	0.081	0.112	0.032	0.055	0.163							
MAZATECO	0.018	0.051	0.027	0.08	0.094	0.061						

				8								
MIXE	0.035	0.059	0.087	0.12	0.096	0.134	0.034					
				0								
MIXTECO	0.047	0.045	0.035	0.06	0.082	0.064	0.015	0.019				
				1								
ZAPOTECO	0.024	0.041	0.067	0.11	0.071	0.112	0.023	0.018	0.031			
				5								
MAYA	0.046	0.061	0.028	0.02	0.093	0.015	0.034	0.081	0.029	0.069		
(GTM)				6								
MEX ADM	0.016	0.028	0.057	0.09	0.056	0.095	0.024	0.043	0.034	0.018	0.049	
				8								
OTOMÍ	0.035	0.017	0.058	0.04	0.041	0.071	0.034	0.042	0.021	0.029	0.035	0.01
				4								9

All values are significant. Level of significance $p \leq 0.01$

Table 5. *HLA-DQB1 F*st genetic distances among Mexican populations of the study

POPULATION	NAHUA	TEENEK	SERI	TARAHUMARA	MAYO	MAZATEC	MIXE	MIXTEC	ZAPOTEC	MAYA (MXN)	MAYA (GTM)	MEXADM
TEENEK	0.103											
SERI	0.089	0.082										
TARAHUMARA	0.049	0.174	0.072									
MAYO	0.101	0.008	0.056	0.16								
MAZATEC	0.038	0.027	0.05	0.0.82	0.032							
MIXE	0.035	0.138	0.084	0.02	0.14	0.05						
MIXTEC	0.021	0.079	0.037	0.027	0.072	0.02	0.019					
ZAPOTEC	0.017	0.074	0.038	0.03	0.07	0.016	0.017	0.001*				

MAYA (MXN)	0.045	0.082	0.036	0.055	0.059	0.044	0.075	0.029	0.031			
MAYA (GTM)	0.058	0.056	0.038	0.086	0.037	0.039	0.098	0.04	0.042	0.167		
MEX ADM	0.015	0.120	0.065	0.022	0.108	0.049	0.031	0.015	0.016	0.213	0.030	
OTOMI	0.042	0.106	0.018	0.025	0.085	0.046	0.043	0.014	0.015	0.021	0.038	0.015

* Not significant value. Level of significance $p \leq 0.01$

Figure 1

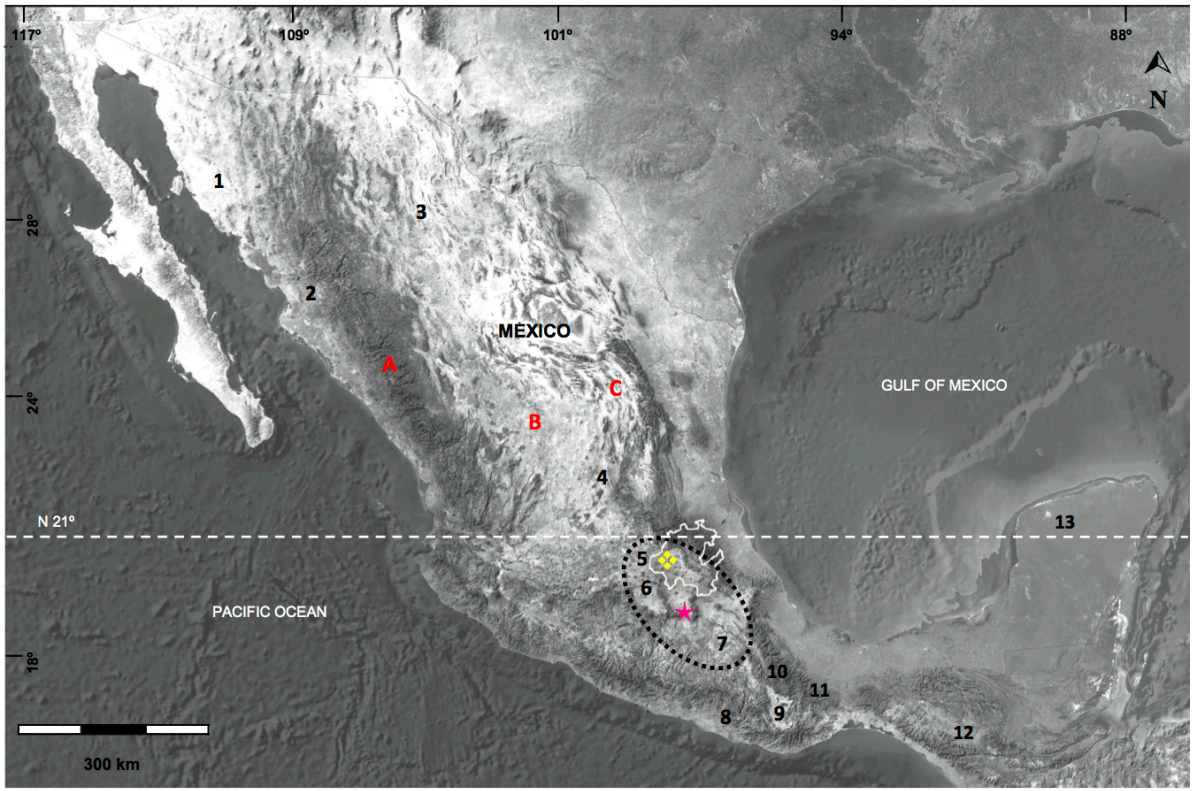


Figure 1. Physiographic map of Mexico. Geographical location of the Mexican populations analyzed in this work. Orographic features are indicated with letters and studied populations with numbers.

SYMBOLS

A Sierra Madre Occidental Mountain range ··· Mexican Central Plateau

B Northern Arid Plains --- Climatic Frontier

C Sierra Madre Oriental Mountain range — Limits State of Hidalgo

(Guatemala)

❖ Mezquital Valley

★ Mexico City

1 Seri
2 Mayo
3 Tarahumara
4 Teenek
5 Otomi
6 Mazahua
7 Nahua
8 Mixtec
9 Zapotec
10 Mazatec
11 Mixe
12 Maya
13 Maya (Mexico)

Figure 2

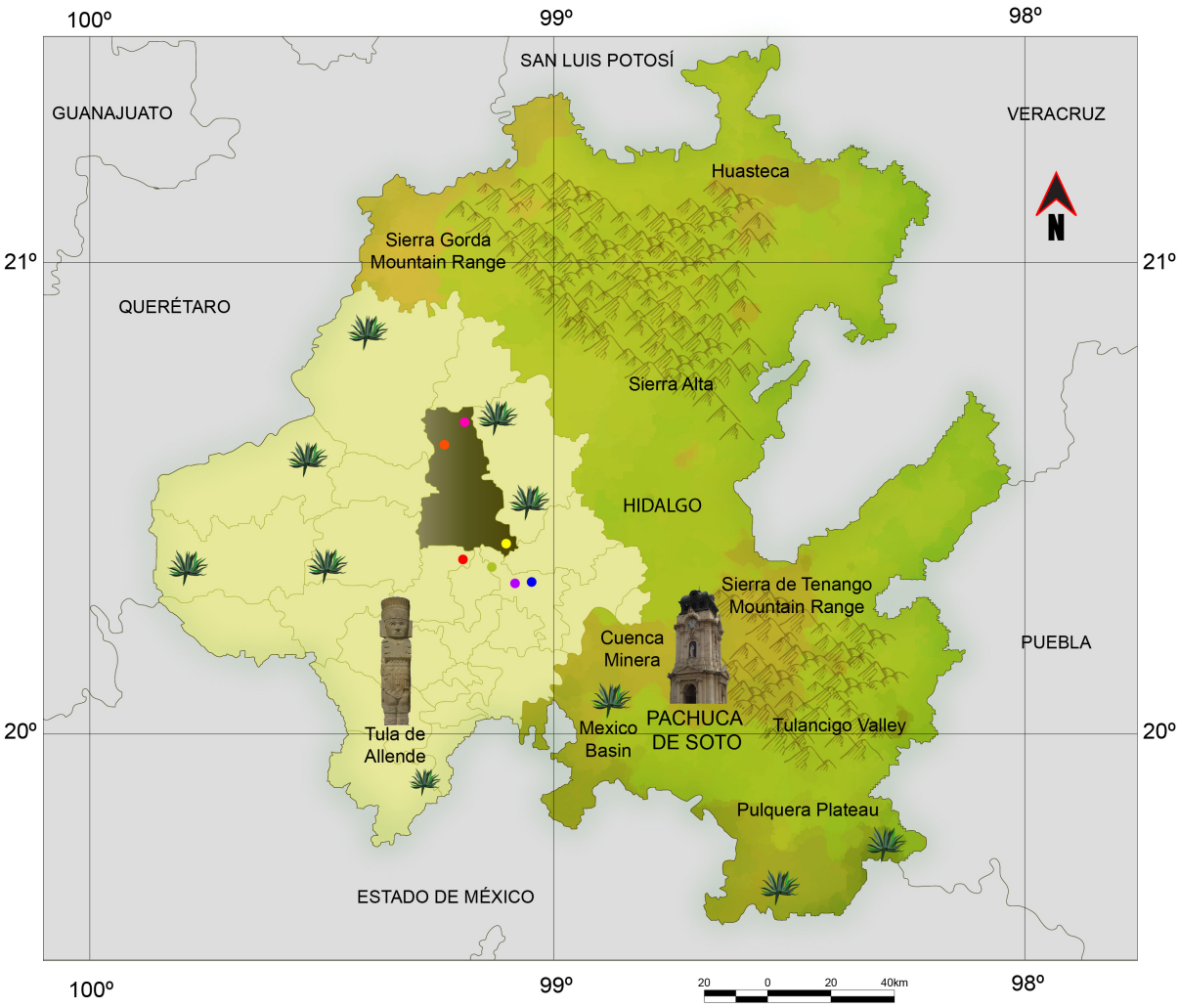


Figure 2. Communities of Ixmiquilpan municipality where samples were collected.

SYMBOLS

The Mezquital Valley

Municipality of Ixmiquilpan

- San Nicolás
- Cerritos
- Dios Padre
- Las Humedades
- El Tephé
- Peña II
- El Barrido

Figure 3

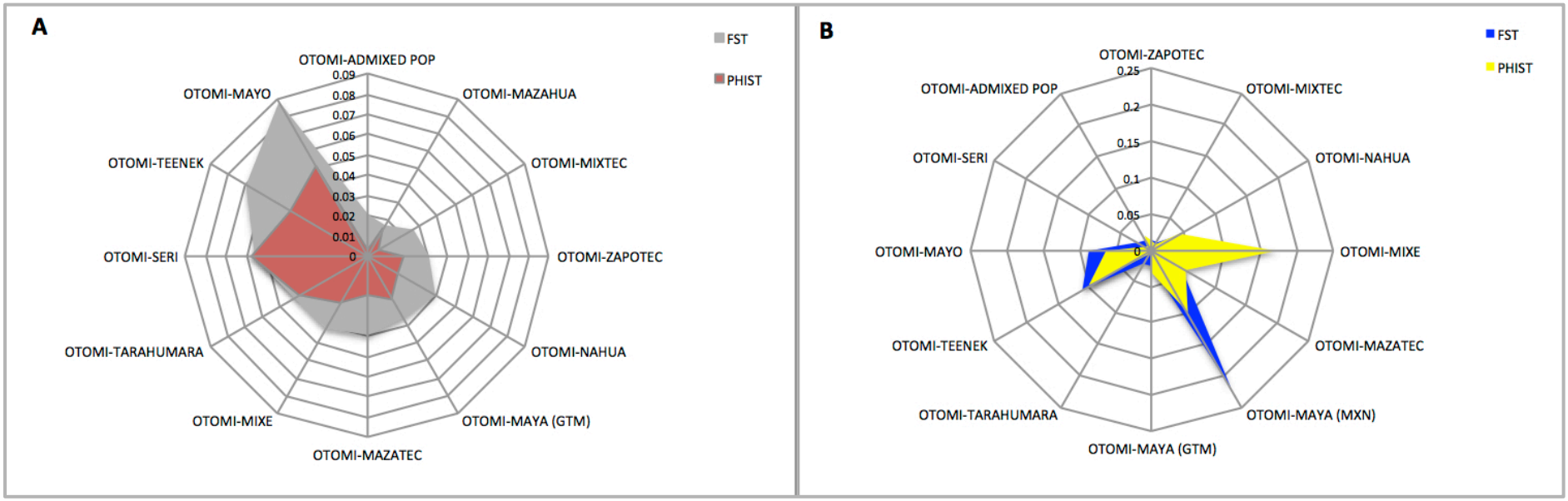
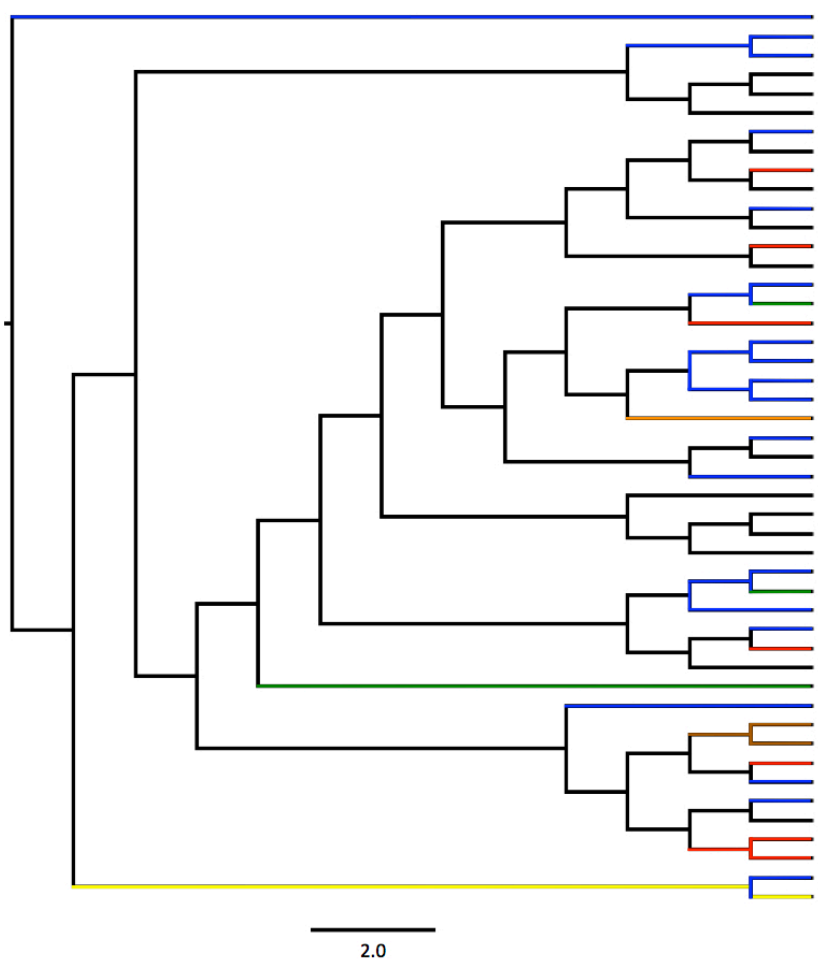


Figure 3. Comparisons between fixation indexes *F*st and *Phi*st in *HLA-DRB1* [A] and *HLA–DQB1* [B]. *F*st values represent recent migrations, while *Phist* (for molecular sequence data) show the oldest migrations by taking into account the mutational 'distances' between alleles.

Figure 4



	SERI	MAYO	TARAHUMARA	TEENEK	OTOMI	MAZAHUA	NAHUA	MIXTEC	ZAPOTEC	MAZATEC	MIXE	MAYA (GTM)
*01:01		0.8				13				0.7		0.7
*01:02					3.03							1.1
*01:03					2.27							
*04:19										1.4		
*04:32							2.1					
*04:25							0.7					
*03:01		1.7	1.13		3.03		0.7					2.3
*03:06							0.7					
*14:02	16.1	0.8	31.81	5.6	6.06		9.5	4.9	4.5	3.5	3.6	4.1
*14:46		0.8		0.9								
*14:01			2.27				4.7	4.9	1.1			1.1
*14:10							0.7					
*14:06		11.8	1.13	13	7.55	12	10.2	9.7	6.3	8.4	7.3	
*14:13				0.9								
*08:01		0.8										0.4
*08:04						3	1.4					0.4
*08:02	33.9	9.3	35.22	5.6	31.06	31	11.5	21.4	22.2	13.3	28.2	18.9
*11:01			1.13				0.7			0.7	1.8	1.5
*11:04							0.7					0.7
*13:01		2.5	1.13		0.76		2.7					1.1
*13:02			1.13		0.76		0.7	0.5	2.8			1.5
*12:01					0.76	2						0.4
*11:02			1.13									
*13:10		0.8										
*13:03												0.4
*13:04												0.4
*13:47		3.3										
*14:27		0.8										
*14:29		0.8					0.7					
*15:01			2.27			1	0.7					2.3
*15:03					0.76					0.7		
*15:02					2.27			0.5		4.2		
*16:01							2.6					
*16:02				8.3	4.55	3	11.5	18.4	18.8	18.3	30.9	2.6
*16:04				0.9								
*10:01							11	0.5	3.4	0.7		0.7
*04:02							0.7					0.4
*04:03		5.8	3.4	4.6	3.79	14	2.6	3.9	5.7		3.6	2.7
*04:04	1.6	7.5		9.3	3.79	1	4.1	1	15.3	9.8	5.5	3
*04:07	48.4	50.1	11.36	39.8	25.77	16	18.4	29.1	11.4	28.1	18.2	38.6
*04:08				0.9		3			1.7			0.7
*04:05						1		0.5	2.3			0.4
*04:09										1.4		
*04:10		0.8			0.76			1.5	0.6		0.9	0.7
*04:11			2.27	10.2				1.5	1.7	7.7		2.6
*07:01		0.8			3.03		0.7		1.7	0.7		4.5
*09:012			4.54									0.7

Figure 4. Bayesian phylogeny of *HLA-DRB1* alleles in Mexican indigenous populations. Representative allele frequencies in populations appear on the right side. Populations were geographically ordered following a north-south pattern. Each allele is colored according to its probable origin.

Allele color code

Red: Native American; Blue: Caucasian-shared with other populations; Green: African; Orange: Asian; Yellow: Asian/Siberian; Brown: Allele reported in several populations worldwide; Black: Uncommon allele

Figure 5

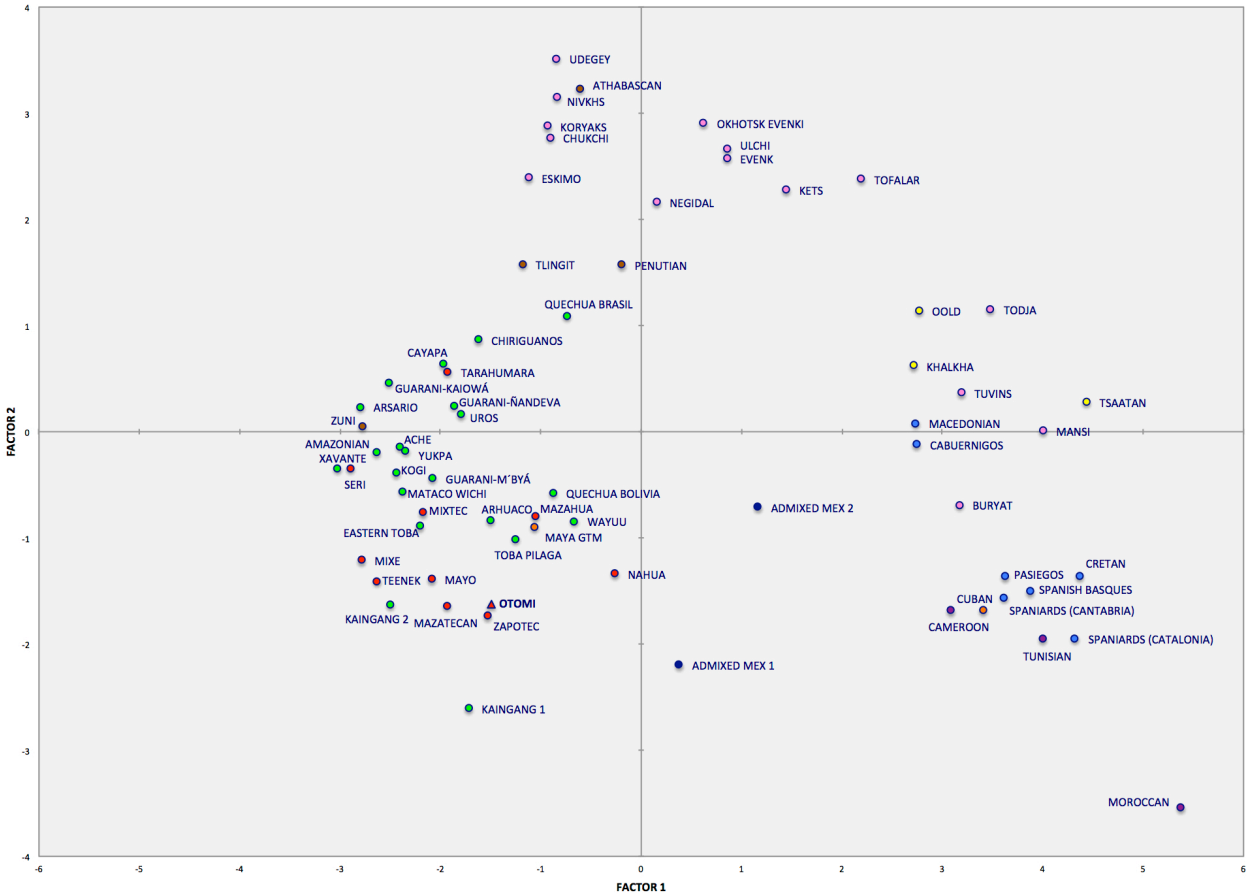

UDEGEY
ATHABASCAN
NIVKHS
KORYAKS
CHUKCHI
OKHOTSK EVENKI
ULCHI
EVENK
ESKIMO
KETS
TOFALAR
NEGIDAL
TLINGIT
PENUTIAN
QUECHUA BRASIL
OOLD
TODJA
CHIRIGUANOS
CAYAPA
KHALKHA
TARAHUMARA
GUARANI-KAIOWÁ
TUVINS
TSAATAN
ARSARIO
GUARANI-ÑANDEVA
UROS
ZUNI
MACEDONIAN
MANSI
AMAZONIAN
ACHE
CABUERNIGOS
XAVANTE
YUKPA
KOGI
SERI
GUARANI-M´BYÁ
MATACO WICHI
QUECHUA BOLIVIA
ADMIXED MEX 2
BURYAT
MIXTEC
ARHUACO
MAZAHUA
WAYUU
EASTERN TOBA
MAYA GTM
TOBA PILAGA
MIXE
NAHUA
CRETAN
PASIEGOS
TEENEK
MAYO
SPANISH BASQUES
CUBAN
OTOMI
SPANIARDS (CANTABRIA)
KAINGANG 2
MAZATECAN
ZAPOTEC
CAMEROON
SPANIARDS (CATALONIA)
TUNISIAN
ADMIXED MEX 1
KAINGANG 1
MOROCCAN
FACTOR 2
FACTOR 1

Figure 5. Principal Component Analysis (PCA) showing a global view of the relationship between the Otomi and worldwide populations based on *HLA-DRB1* allele frequencies.

Populations color code

Red: Mexican indigenous [a]; Dark Blue: Mexican admixed; Green: Native American; Orange: Central America and Caribe; Brown: Eskimo-Aleutian; Pink: Asian/ Siberian;

Yellow: Central Asia; Light Blue: Mediterranean Europeans; Purple: African. [a] Otomi population is highlighted with a red triangle