

Original Synthesis Report

Pacific Islanders and Amerindian relatedness according to HLA autosomal genes

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Abstract - Americas peopling has recently been explained based only on genetic data. While different First America inhabitants' ethnic groups, Amerindians, Na-Dene speakers, Aleuts and Eskimo there exist, there is no either genetic, cultural or anthropological homogeneity within these groups. In the present work, we have particularly addressed the relatedness of First America Inhabitants with Pacific Islanders by using autosomal genetic markers: the HLA alleles. HLA is the most polymorphic human genetic system accounting for 9,438 alleles; this is most useful for comparing populations relatedness. Ethnic groups of Pacific Islanders and First America Inhabitants have been used. A *genealogic* study and also a *frequency comparison* study by using HLA alleles and haplotypes have been carried out. Our conclusions are: 1- Aleuts seem to be a genetic and linguistic separate group which may be related to northern European Lapps, both of them originated in southern Siberia Baikal Lake area. 2- First America Inhabitants, including all analyzed Amerindians, Na-Dene speakers and Eskimo have had genetic flow with Pacific Islanders: the latter share autosomal HLA alleles and haplotypes with First America Inhabitants. This could have been bidirectional. 3- Particularly, Easter Islanders show a probable cultural and genetic exchange with Titikaka Lake Aymaras. This civilisation also shares significant traits with European Iberian megalithic builders. 4- Mesoamericans may be grouped together because of they bear more ancient Olmec culture traits and present paper HLA results. 5- Genetics is not able by itself to uncover in space and time Americas peopling and First America Inhabitants relatedness with Pacific Islanders.

Keywords: Alberite Dolmen, Aleuts, America peopling, Amerindians, Australia, Easter Island, HLA, Lapps, Melanesia, Micronesia, Pacific, Polynesia, Tiwanaku

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Introduction

The First Amerindian Natives are postulated to have come from Asia through the Bering land bridge between 30,000–12,000 years before the present (BP). These conclusions have been based on cultural, morphological and genetic similarities between American and Asian populations. Both Siberia (Crawford, 1998) and Mongolia (Kolman et al., 1996; Merriwether et al., 1996) have been put forward as the most likely places of origin in Asia.

Greenberg first postulated the triple migration theory for explaining the peopling of the Americas (Greenberg et al., 1986): Amerindians (most North and South American Indians; 12,000 years BP), Na-Dene (Athabaskans, Navajo, Apache; 8,000 years BP) and Eskimo-Aleuts (6,000 years BP). Research carried out before the widespread use of Y Chromosome (Y Chr) and other nuclear DNA markers including mtDNA (Wallace & Torroni, 1992) for the study of populations (Cavalli-Sforza et al., 1994; Parham & Ohta, 1996) supported the three-wave model. However, other mtDNA studies have not (Horai et al., 1993; Torroni et al., 1993); other authors postulate only one wave coming from Mongolia / North China as giving rise to the First Native American ancestors (Kolman et al., 1996; Merriwether et al., 1996). The study of Y Chromosome DNA markers seemed to suggest the existence of a single major paternal haplotype in both North and South American Native populations (Karafet et al., 1997; Santos et al., 1996). However, other studies on Y Chromosome show that more than one paternal founder haplotypes arrived in America during different migrations (Karafet et al., 1999), probably from Siberia (Santos et al., 1999).

Alu-insertion investigations have also been carried out to ascertain the origin of First Americans (Novick et al., 1998). The results are not concordant with the multiple-wave migration hypothesis; a surprisingly short genetic distance between Chinese and Native Americans was found and explained by a recent gene flow from Asia (Novick et al., 1998).

More recently, new mtDNA analysis has suggested that all mtDNA lineages must have been isolated in Asia before entering the New World by at least 7-15 thousand years. They even suggest that this place must have been Beringia (Mulligan et al., 2008). Also, a dispersal of Amerindians coming from Asia has been put forward through Coastal Pacific line (Goebel et al., 2008) based on all available archaeological, anthropological and mtDNA and genetic data.

All these calculations are done by using paternal (Y Chr) or maternal (mtDNA) lineages may be biased when populations displacements are concerned, as in the putative Amerindians displacement from Asia to the Americas. In addition, other authors (Uinuk-Ool et al., 2002) using nuclear histocompatibility (HLA) markers do not regard as important and possible to establish the number and timing of migration waves. The important issue is whether immigrants (Amerindians) were already differentiated (in Asia) into such ethnic groups whose descendants are still to be found in Asia. If they were differentiated then the question of how and when they crossed the Bering Land Bridge is a secondary one (Uinuk-Ool et al., 2002).

A Trans-Pacific route of American peopling from Asia or Polynesia has been suggested because HTLV-1 virus strains shared identical sequences in Japan and in the northern coast of South America (Leon-S et al., 1996) and some HLA alleles may have been introduced by the same Trans-Pacific route (Arnaiz-Villena et al., 2009; Cerna et al., 1993). In the same way, “quasi-specific” Amerindian HLA alleles, like A*02:12 or B*39:05 (Arnaiz-Villena et al., 2005, Arnaiz-Villena, unpublished), have been found in several unrelated individuals of Easter Island, which suggests an early contact between Easter Islanders Polynesians and Amerindians (Lie et al., 2007). Recent genetic studies have identified Polynesian mtDNA haplogroups in remains (skulls) of Botocudo Amerindians from Brazil (Amerindian group extinct by the end of 19th century) (Goncalves et al., 2013). Other signs may indicate a communication between these

groups of populations, like the presence of South American sweet potato in earlier Pacific sites (Lawler, 2010) or the finding of chicken remains of Polynesian type in El Arenal (Chile) dated by radiocarbon back to 1300-1400 AD (Storey et al., 2007; Storey et al., 2008). Furthermore, skeletal remains of pre-Columbian individuals with Polynesian ancestry and several Mapuche artefacts which are similar to Polynesian ones at Mocha island (Chile) have been recently reported (Lawler, 2010; Matisoo-Smith & Ramirez, 2010). All these facts provide evidences for this Trans-Pacific route that could have occurred in both ways at different times.

Finally, both genetic (Bruges-Armas et al., 1999) and archaeological (Holden, 1999) evidence suggests that a two-way Trans-Atlantic traffic occurred before Columbus discovered America; archaeologists in New Mexico have recently found tools used 20,000 years ago in Spanish Solutrean culture (Holden, 1999; Stanford & Bradley, 2012).

In the present work, we have studied the North, Meso and South American Amerindians' HLA allele frequencies and have compared them with those of other North American First Inhabitants and Asians, particularly with Central and South East Asia, and Pacific populations. HLA genes have been analyzed for the following Amerindian ethnic groups: Mayans (Gomez-Casado et al., 2003), Mixe, Mixtecos, Zapotecos (Petzl-Erler et al., 1997), Lakota Sioux (Leffell et al., 2004), Mazatecos (Arnaiz-Villena et al., 2000), Lamas (Moscoso et al., 2006), Quechuas (Martinez-Laso et al., 2006), Aymaras (Arnaiz-Villena et al., 2005), Uros (Arnaiz-Villena et al., 2009), Tarahumaras (Garcia-Ortiz et al., 2006), Mapuches (Rey et al., 2013), Toba Pilaga, Mataco Wichí, and Eastern Toba (Cerna et al., 1993).

Our aims are: 1) To determine the HLA class II (DRB1 and DQB1) quasi-specific Amerindian allelic lineages (hereafter "alleles" for simplicity) and specific class II HLA haplotypes by using DNA sequencing; in other words, the most frequent HLA alleles and haplotypes in Amerindians which do not exist or exist in very low frequency in other populations, i.e.: genealogy comparisons and 2) To compare the Amerindians HLA allele frequencies with those of other First American Natives (Na-Dene, Eskimo and Aleuts) and also those of Asian and Pacific populations with computer programs in order to study the HLA relatedness with peoples most likely to be candidates for First American Peoples ancestors; this would clarify the still unclear peopling of the Americas and the origins of Amerindians, i.e.: groups of genes frequencies comparisons by using class II HLA alleles and haplotypes frequencies.

Table 1. Worldwide populations included in Present Paper Analysis. A Total of 8014 Chromosomes were Analysed.

Population	N	Reference	Population	N	Reference
Aborigines	152	(Lester et al., 1995)	Mayans	132	(Gomez-Casado et al., 2003)
Ainu	50	(Bannai et al., 1996)	Mazatecans	89	(Arnaiz-Villena et al., 2000)
Aleuts	85	(Rey et al., 2010)	Mixe	55	(Petzl-Erler et al., 1997)
Athabaskans	62	(Monsalve et al., 1998)	Mixtecans	103	(Petzl-Erler et al., 1997)
Aymaras	102	(Arnaiz-Villena et al., 2005)	Negidal	35	(Uinuk-Ool et al., 2002)
Buryat	25	(Uinuk-Ool et al., 2002)	Nganasan	24	(Uinuk-Ool et al., 2004)
Chukchi	59	(Grahovac et al., 1998)	Nivkhs	32	(Grahovac et al., 1998)
Chuvashians	82	(Arnaiz-Villena et al., 2003)	Papua New Guinean	65	(Gao et al., 1992)
Easter Island	48	(Lie et al., 2007)	Quechuas	80	(Martinez-Laso et al., 2006)
Eastern Toba	135	(Cerna et al., 1993)	Samoa	29	(Mack et al., 2000)
Eskimo	35	(Grahovac et al., 1998)	Taiwan	48	(Zimdahl et al., 1999)
Evenks	35	(Grahovac et al., 1998)	Tarahumara	44	(Garcia-Ortiz et al., 2006)
Han Chinese	264	(Trachtenberg et al., 2007)	Tlingit	53	(Imanishi et al., 1992a)
Hottentot	91	(Imanishi et al., 1992a)	Toba Pilaga	19	(Cerna et al., 1993)
Kets	22	(Grahovac et al., 1998)	Tofalar	43	(Uinuk-Ool et al., 2002)
Koryaks	92	(Grahovac et al., 1998)	Todja	22	(Uinuk-Ool et al., 2002)
Khalk Mongolians	202	(Munkhbat et al., 1997)	Tuvinians	197	(Martinez-Laso et al., 2001)
Khoton Mongolians	85	(Munkhbat et al., 1997)	Kinh Vietnam	103	(Vu-Trieu et al., 1997)
Lakota Sioux	302	(Leffell et al., 2004)	Udegeys	23	(Grahovac et al., 1998)
Lamas	83	(Moscoso et al., 2006)	Ulchi	73	(Uinuk-Ool et al., 2002)
Malaysia	74	(Mack et al., 2000)	Uros	105	(Arnaiz-Villena et al., 2009)
Mansi	68	(Uinuk-Ool et al., 2002)	Yupik	252	(Leffell et al., 2002)
Mapuches	104	(Rey et al., 2013)	Zapotecans	75	(Petzl-Erler et al., 1997)
Mataco-Wichi	49	(Cerna et al., 1993)			

N, number of individuals. Population names are ordered in alphabetical order

Material and Methods

Population sample

Eight thousand and fourteen chromosomes from samples of forty seven different populations in several geographical locations (Central and South-East Asia, Australia, Pacific Islands and North, Meso and South America and others) were compared in this study. These samples belong to different ethnic groups, as classified by Greenberg et al in Ref 4: Amerindians, Na-Dene, Eskimo, Orientals, Australian aborigines, Polynesians and Melanesians (and controls). Populations are detailed in Table 1.

HLA genotyping

High resolution HLA class II (DRB1 and DQB1) was performed by PCR-SSOP-Luminex technique (Itoh et al., 2005). This methodology consists of: a) PCR using specific primer pairs of provided by the manufacturers (Luminex Corporation, Austin, TX, USA). All of these primers are 5'-biotined and they are specific to determine sequences of exons 2 and 3 (or only exon 2 for HLA class II) of HLA genes; b) hybridization: products of PCR biotin-labelled were denaturalized at 97 °C and then were able to hybridize to complementary DNA probes associated to microbeads; and c) assignation of the HLA alleles: the complex resulting of the hybridization was introduced in Luminex platform, this system identify the fluorescent intensity of fluorophores on each oligobead that has hybridized with the biotin-labelled PCR product. Software of Luminex assigns the HLA alleles for each sample of DNA (Itoh et al., 2005). HLA-DRB1 and -DQB1 allele DNA automated sequencing (ABI PRISM 3700/ ABI PRISM 3730. Applied Biosystems; California) was only done when DNA typing yielded ambiguous results (Arnaiz-Villena et al., 1992).

Aymara (Bolivia), Uros (Peru) and Lamas (Peru) populations were specifically HLA retyped for present work analyses, because of relevance for conclusions.

Statistical analysis

Statistical analysis was performed with Arlequin v2.0 software kindly provided by Excoffier and Slatkin (Schneider et al., 2000). In summary, this program calculated HLA-DRB1 and -DQB1 allele frequencies, Hardy-Weinberg equilibrium and the linkage disequilibrium between n alleles at n different loci. Their level of significance (p) for 2×2 comparisons was determined as previously described (Imanishi et al., 1992b; Imanishi et al., 1992c). In addition, the most frequent complete haplotypes were deduced from: 1) the 2 HLA loci haplotype frequencies (Imanishi et al., 1992b; Imanishi et al., 1992c); 2) the previously described haplotypes in other populations (Imanishi et al., 1992b; Imanishi et al., 1992c); and 3) haplotypes if they appeared in two or more individuals and the alternative haplotype was well defined (Imanishi et al., 1992b; Imanishi et al., 1992c). Phylogenetic trees (dendrograms) were constructed with the HLA-DRB1 allelic and HLA class II haplotypic frequencies using the Neighbour-Joining (NJ) method (Saitou & Nei, 1987) with the genetic distances between populations (DA) (Nei, 1972), using DISPAN software comprising the programs GNKDST and TREEVIEW (Nei, 1973; Nei et al., 1983). Correspondence analysis in three dimensions and its bidimensional representation was carried out using the VISTA v5.05 computer program (Young & Bann, 1996, <http://forrest.psych.unc.edu>). Correspondence analysis consists of a geometric technique that may be used for displaying a global view of the relationships among populations according to HLA (or other) allele or haplotype frequencies. This methodology is based on the genetic distances (DA) variance among populations (similar to the classical principal components methodology) and of a statistical visualization of the differences.

Table 2. Shared HLA-DRB1-DQB1 Haplotype Frequencies (%) in Different Pacific, Siberian and Amerindian Populations.

Haplotype HLA-DRB1-DQB1	Samoa	Papua	Easter Island	Taiwan	Ainu	Buryat	Evenks	Ulchi	Negidal	Eskimo	Aleuts	Yupik	Athabaskans
DRB1*04:01-DQB1*03:01 (A)	—	—	—	—	—	8.0	5.7	4.1	9.6	26.2	9.7	22.8	—
DRB1*08:01-DQB1*04:02 (B)	—	—	—	—	—	2.0	—	—	1.4	—	12.5	—	—
<i>DRB1*14:01-DQB1*05:03 (C)</i>	—	7.6	—	—	20.0	—	—	4.8	1.4	4.4	—	6.7	16.9
<i>DRB1*14:02-DQB1*03:01 (D)</i>	1.7	—	—	—	—	—	—	4.1	5.7	20.0	—	22.0	34.7
<i>DRB1*08:02-DQB1*04:02 (E)</i>	—	—	7.3	—	10.0	—	—	—	4.3	11.3	2.8	13.3	4.8
DRB1*04:07-DQB1*03:02 (F)	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>DRB1*04:03-DQB1*03:02 (G)</i>	17.2	—	6.3	9.5	3.0	8.0	2.9	—	5.7	—	—	3.0	9.5

Haplotype HLA-DRB1-DQB1	Sioux	Tarahumara	Mixe	Mixtecs	Mazatecs	Zapotecs	Mayans	Lamas	Uros	Aymara	Quechuas	Mapuches	Toba Pilaga	Eastern Toba	Mataco Wichi
DRB1*04:01-DQB1*03:01 (A)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
DRB1*08:01-DQB1*04:02 (B)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>DRB1*14:01-DQB1*05:03 (C)</i>	—	—	—	4.9	—	1.2	—	—	—	—	—	1.0	—	—	—
<i>DRB1*14:02-DQB1*03:01 (D)</i>	—	27.3	2.9	4.4	—	4.1	1.1	8.4	11.6	10.7	6.5	14.9	7.9	10.4	22.4
<i>DRB1*08:02-DQB1*04:02 (E)</i>	—	35.2	28.0	21.6	—	21.5	15.4	2.4	23.6	22.1	27.9	9.6	10.5	18.9	1.0
DRB1*04:07-DQB1*03:02 (F)	9.3	11.4	17.8	28.9	16.6	9.9	34.8	10.1	2.3	5.4	6.8	2.9	5.3	5.9	—
<i>DRB1*04:03-DQB1*03:02 (G)</i>	2.2	3.4	3.9	3.9	—	5.2	1.4	7.8	11.7	—	2.9	14.9	—	—	—

These Pacific Islands populations (Samoa, Papua and Easter Island) were chosen because of data availability for comparisons and for covering all Pacific Islands distances. Letters in brackets in first column name haplotypes shown in Figure 1.

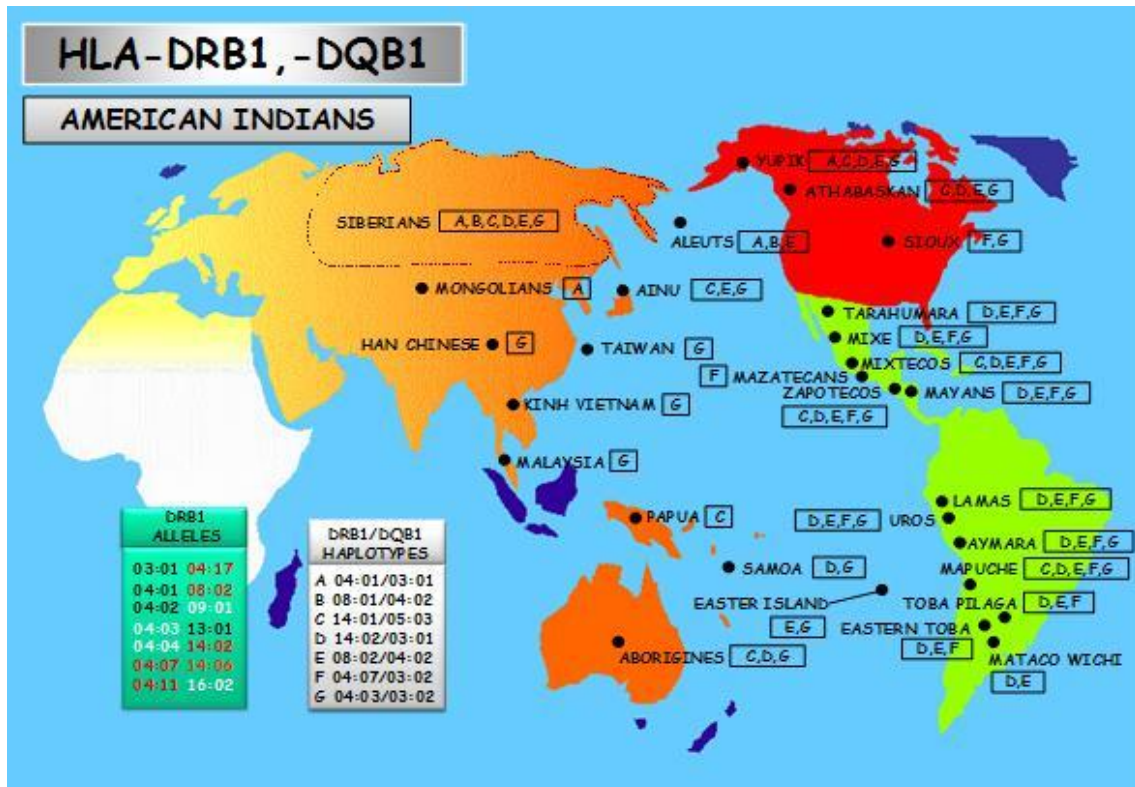


Figure 1. Map showing HLA-DRB1 alleles and HLA-DRB1-DQB1 haplotypes in Siberian, Pacific and Amerindian populations.

Haplotypes are defined by letters from A to G (see Table 2). Alleles are colored according to whether they are present in Amerindians but also in other World populations (black), they are shared by Amerindian and Pacific populations in high frequency (white) or they are mainly present in Amerindians (red). Data from populations were from references stated in Table 1. Amerindian populations living place is coloured in green. Reddish to yellow colours mean a gradient of HLA relatedness according to geography.

Amerindians are shown as apart in all computer analyses.

Results and Discussion

The expected and observed gene frequency values for HLA-A, -B, -DRB1 and –DQB1 loci do not differ significantly in studied populations and results are in Hardy-Weinberg equilibrium (results not shown).

We have previously observed that Amerindian HLA frequencies profile (Arnaiz-Villena et al., 2010a, 2010b) does not correlate with either their linguistic branch or their geographical present placements. Clear cut conclusions about Pacific/Amerindian relationships could not be reached, but only some indications (Arnaiz-Villena et al., 2010). However, from Table 2, it may be drawn that:

- 1) Aleuts have a different HLA profile (as already found, Moscoso et al., 2008; Rey et al., 2010, see C and D haplotypes)
- 2) Haplotype F is only found in Amerindians, having Mesoamerican Amerindian its highest frequency. This separates either in space and/or time Amerindians (particularly those from Mesoamerica) from Athabaskans, Asians and Pacific Islanders.
- 3) Haplotype G relates Pacific Islanders with Amerindians, particularly South American Amerindians, suggesting a more ancient and/or intense gene exchange between Pacific Islanders and Amerindians. This is also supported because Athabaskans, Yupik (Alaska Eskimos) and Siberians also bear this Pacific Islanders HLA “high” frequency haplotype. Note again that Aleuts lack this haplotype stressing their HLA genetic uniqueness (Moscoso et al., 2008). Gene flow between Amerindian and Pacific Islanders seems to have existed: two Pacific Islanders populations from Samoa and Easter Island share high frequencies (particularly Samoans) with South American Amerindians Mapuches and Uros, together with Taiwan populations. The latter have been postulated by some authors as the first Pacific Prehistoric settlers (Sykes et al., 1995).

In conclusion, Mesoamerican Amerindians (related to Maya and Olmec cultures) seem less related with Pacific Islanders than some South Americans Amerindians (Table 2).

Figure 1 shows in a map how haplotype G relates all Siberians, Eskimo, Athabaskans, Amerindians and Pacific Islanders. Again it is striking its highest frequency in Pacific Islanders and South American Amerindians. If this fact is due to a founder effect then a part of America peopling should have come through the South. Other possibilities are open (see below). It is also remarkable that Aleuts do not have this common haplotype (G). This stresses again its unique origin (in space and/or time) from Baikal Lake Area, in Siberia (Moscoso et al., 2008).

Also this figure shows that DRB1*04:03, 04:04, 09:01 and 16:02 are DRB1 alleles extensively shared with Pacific Islanders; DRB1*03:01, 04:01, 04:02 and 13:01 are present in Amerindians but also in other World populations (Gonzalez-Galarza et al., 2011). HLA DRB1*04:07, 04:11, 04:17, 08:02, 14:02 and 14:06 are mainly present in Amerindians.

Aymaras and Easter Islanders

There is little discussion that population of Pacific Islands started in southern China / Taiwan about 5000 years before present (BP). Settlers would first arrived to Melanesia (islands surrounding North and East Australia) and mixed with local Lapita culture people (their origin is not fully explained) (Kayser et al., 2000). Later they would have migrated eastwards to Tonga and Samoa and finally arrived in Easter Island at about 1000 AD (Hunt & Lipo, 2006; Martinson-Wallin & Crockford S.J., 2002). However some prehistoric cultural traits are pointed out to be shared with South American Amerindians: bottle gourd (Green, 2000) and sweet potato (Wallin et al., 2005; Yen, 1974) were cultivated in Easter Island before Europeans officially arrived to Easter Island at the end of 18th Century (Yen, 1974). Fishing (Martinez, 1979), and linguistic and other cultural traits (Jones, 2010; Klar, 2010) strengthen the hypothesis that Easter Island / South Amerindians cultural flow had been established. Titikaka Lake giant stone statues from Tiwanaku culture (like, Monolito Ponce, Monolito Fraile and Monolito Bennet) (Wikipedia, 2013b) are very similar to the giant Easter and other Pacific Islands statues. This led to Thor Heyerdahl to postulate that Amerindians had populated Pacific Islands including Easter Island (Heyerdahl, 1952). Efforts have been made to find out traces of a possible gene flow between America and Pacific Islands with a remarkable success (Goncalves et al., 2013).

Tiwanaku culture at Titikaka Lake Area had constructed interesting artefacts like giant statues similar to those found also in Pacific Islands; also, stone holes in temples, which amplify sound, like megaphones (the so-called “ritual ears”) are also found both in megalithic-dolmenic Spain (Alberite Dolmen, build up 5000 years ago) and Tiwanaku archaeological (Arnaiz-Villena et al., 2013). Tiwanaku culture was developed by Aymara Amerindians themselves (Arnaiz-Villena et al., 2005) or by both Aymara and other Amerindians previously settled in the area, i.e.: Uros, living on reed-made floating islands at Titikaka Lake, who probably came from Amazon Basin in prehistoric times (Arnaiz-Villena et al., 2009).

Haplotype HLA-A*02-B*39-DRB1*09:01-DQB1*03:03 was found in Aymaras (Arnaiz-Villena et al., 2005) and later shown that its class I A*02:01-B*39:09 part was shared with Easter Islanders (Thorsby, 2012); this haplotype is postulated to be derived from Titikaka Lake living people, Aymara (Arnaiz-Villena et al., 2005).

However, Tiwanaku and Easter Island cultures similarities are more significant than genetic coincidences, also more cultural traits are common (Green, 2000; Lawler, 2010; Storey et al., 2007; Storey et al., 2008; Wallin et al., 2005). Ancient gene flow between both Aymara people and Easter Islanders may have been diluted with time and in this particular case may be as useful as cultural shared traits, at present. The same is true for discovering the general relationship between Amerindians and other Pacific Islanders.

Mesoamerican (Mayans, Mixe, Zapotecs, Mixtecs) vs. South American Amerindians

DA genetic distances (Table 3) show a general view: Mesoamericans seem to be less related to Pacific Islanders than South American Amerindians by using two dimension HLA gene frequencies analyses (Table 3).

However, if genealogy analysis of haplotype G (Table 2, Figure 1, HLA-DRB1*04:03-DQB1-03:02) is used Samoans (17.2 %), Taiwanese (9.5 %), Mapuches (14.9 %), Uros (11.7 %) and Lamas (7.8 %) cluster together in a high frequency group. Mesoamericans cluster together because of a lower frequency with an apparent separation of Pacific Islanders: Zapotecs (5.2 %), Mixe (3.9 %), Mixtecs (3.9 %) except perhaps with Easter Islanders (6.3 %). This HLA haplotype is also found in the

Mediterranean area and other parts of the World in low frequency. Isolation and founder effect may have given Samoa people this highest recorded haplotype G frequency.

Table 3. Genetic Distances (DA) Between Pacific Populations and Other Populations ($\times 100$) Obtained by Using HLA-DRB1-DQB1 Haplotypic Frequencies.

Samoa		Papua		Easter Island		Taiwan	
Population	DA (%)	Population	DA (%)	Population	DA (%)	Population	DA (%)
Taiwan	1.55	Ulchi	4.30	Taiwan	3.84	Samoa	1.55
Easter Island	5.88	Evenks	8.10	Samoa	5.88	Evenks	3.80
Buryats	6.72	Taiwan	8.55	Evenks	6.86	Easter Island	3.84
Evenks	6.84	Ainu	8.99	Buryats	8.73	Buryats	5.14
Lamas	8.60	Lakota Sioux	9.57	Lakota Sioux	8.83	Lakota Sioux	5.93
Lakota Sioux	9.13	Easter Island	10.65	Negidal	9.59	Papua	8.55
Negidal	10.62	Mazatecans	12.22	Toba Pilaga	10.05	Lamas	11.06
Mapuches	11.15	Buryats	12.96	Lamas	10.32	Ulchi	11.27
Ulchi	13.36	Samoa	13.43	Papua	10.65	Negidal	11.98
Papua	13.43	Negidal	15.23	Zapotecans	10.90	Mazatecans	13.12
Mataco Wichi	15.01	Mataco Wichi	15.87	Ainu	11.02	Mapuches	16.47
Uros	17.19	Toba Pilaga	16.04	Mapuches	11.95	Mataco Wichi	16.74
Toba Pilaga	17.67	Aleuts	16.75	Quechuas	11.96	Ainu	16.79
Mazatecans	17.76	Lamas	18.83	Uros	12.04	Toba Pilaga	16.90
Ainu	19.10	Eastern Toba	22.62	Ulchi	13.30	Aleuts	17.61
Zapotecans	19.26	Zapotecans	23.71	Eastern Toba	13.43	Zapotecans	20.46
Aleuts	22.01	Aymara	24.43	Aymara	14.23	Uros	21.65
Quechuas	22.28	Mapuches	24.86	Aleuts	14.98	Eastern Toba	23.42
Eastern Toba	23.30	Quechuas	28.13	Mazatecans	15.11	Quechuas	23.62
Aymara	24.94	Uros	31.49	Mataco Wichi	15.95	Aymara	25.21
Athabaskans	26.96	Athabaskans	32.62	Mixe	16.75	Mixe	28.42
Mixe	27.59	Mixe	33.82	Mayans	22.5	Mayans	30.93
Mayans	31.79	Mayans	33.89	Mixtecans	26.48	Athabaskans	34.98
Mixtecans	34.82	Eskimo	34.88	Athabaskans	32.10	Mixtecans	36.60
Yupik	35.60	Mixtecans	35.98	Yupik	33.05	Yupik	40.68
Eskimo	38.58	Yupik	38.32	Eskimo	33.54	Eskimo	41.28
Tarahumara	42.63	Tarahumara	54.20	Tarahumara	35.06	Tarahumara	48.99

Mesoamericans tend to cluster together according to HLA allele frequencies in Correspondence and NJ tree analyses Figure 2 (Arnaiz-Villena et al., 2000; Gomez-Casado et al., 2003), and Mayans and Mixe speak clearly Mayan languages and the other Mesoamerican ethnic groups are not very firmly attached to languages different to Mayans (Arnaiz-Villena et al., 2000; Gomez-Casado et al., 2003). Because of this, it is likely that based on these genetic and linguistic evidences, Olmec culture may have given rise to all other Mesoamerican ethnic group cultures. This is supported both on archaeological and genetic data (Arnaiz-Villena et al., 2000; Gomez-Casado et al., 2003). Particularly, Zapotecans constructed Monte Alban, which is one of the most ancient pyramid based complex (Monte Alban, Oaxaca, by 500 years BC) (Arnaiz-Villena et al., 2000) and Mayans imposed their type of languages in a widespread Mesoamerica area (Gomez-Casado et al., 2003). Both Monte Alban and Yucatan first pyramids were constructed by Zapotecans and Mayans respectively by around 500 years BC (Wikipedia, 2013a). Thus, it would seem that Mesoamericans have less relationship with Pacific Islanders.

America peopling: Amerindians, Na Dene, Eskimo, Aleuts and Pacific Islanders relationship according to HLA autosomal genetic markers

- Genetics

1. Frequencies

It is clear that from HLA genetic frequencies and cultural data some conclusions may be drawn about Amerindians and Pacific Islanders relationship. Conclusions are:

- 1) A three waves model for America peopling is not supported (Arnaiz-Villena et al., 2000; Arnaiz-Villena et al., 2010).
- 2) Mesoamericans seem to cluster together according to HLA genes and cultures (Olmecs seem to have given rise to Mayans, Zapotecans, Mixtecs, Mazatecs and Mixe cultures) (Arnaiz-Villena et al., 2000; Arnaiz-Villena et al., 2010; Gomez-Casado et al., 2003).
- 3) All North, Meso and South American Amerindians cluster together and are separated from other World populations. Amerindians follow little geographical gradient in this HLA frequency analyses, except for

Mesoamerican groups (Arnaiz-Villena et al., 2000; Arnaiz-Villena et al., 2010; Gomez-Casado et al., 2003; Rey et al., 2013).

- 4) Aleuts cluster separately from other groups and may be more related to Baikal Area first inhabitants and immigrated to North Europe, together with Lapps (Moscoso et al., 2008).

2. Genealogy

- 1) Amerindians and Pacific Islanders extensively share high frequency and rare (in other parts of World) HLA haplotypes (Table 2: DRB1*04:01-DQB1*03:01, DRB1*08:01-DQB1*04:02, DRB1*14:01-DQB1*05:03, DRB1*14:02-DQB1*03:01, DRB1*08:02-DQB1*04:02, DRB1*04:07-DQB1*03:02, DRB1*04:03-DQB1*03:02; Figure 1: DRB1*04:03, DRB1*04:04, DRB1*09:01, DRB1*16:02).
- 2) Cultural and genetic data show an HLA relationship of Easter Islanders and Aymaras (haplotypes A*02:01-B*39:09 and DRB1*08:02-DQB1*04:02, Table 2) (Arnaiz-Villena et al., 2005; Thorsby, 2012).
- 3) Genealogy data in Athabaskans do not discard a two way Amerindian migration through or around Beringia at different times (Arnaiz-Villena et al., 2010).
- 4) Genealogy data show that Aleuts should be separated in terms of HLA genetics from Eskimo, Athabaskans and Amerindians (Table 2). Also, Aleut language is different from Eskimo and other ancient American languages (Moscoso et al., 2008).

In summary, Pacific peopling seems more complex than thought and genetics by itself may not be a unique tool to uncover it. For instance, it is not easily understandable that Taiwanese and South Chinese populated Pacific Islands for the first time 5000 years BC and in addition found some local aboriginal culture elements like those of Lapita culture (Kayser et al., 2000). This remarks that Pacific Islands were already probably inhabited.

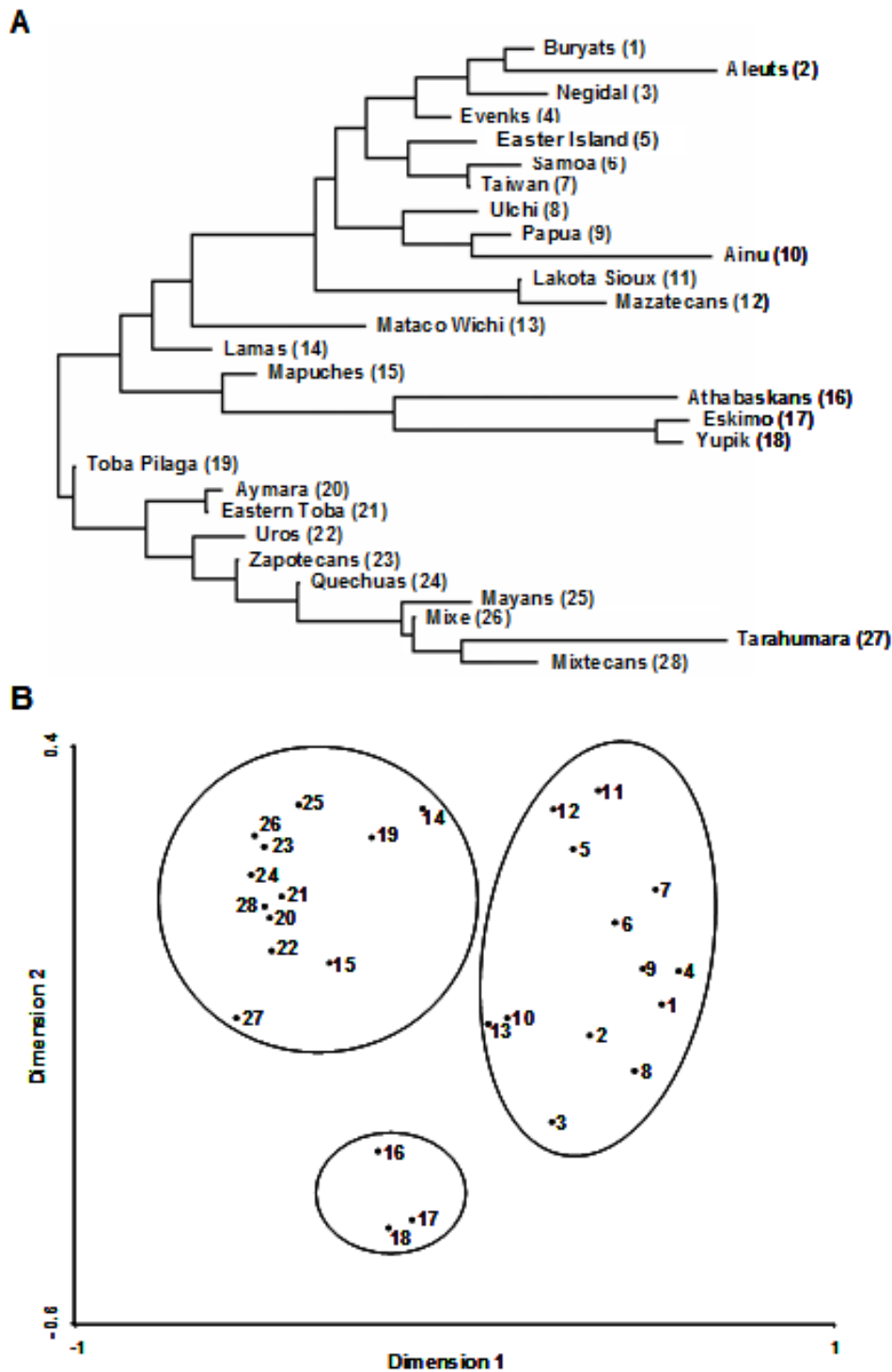


Figure 2. A: Neighbour-Joining (NJ) dendrogram showing relatedness between Amerindians, Pacific and Siberian populations.

Genetic distances between populations (DA) were calculated by using HLA-DRB1-DQB1 haplotypes (high resolution). B: Correspondence analysis showing a global view of the relationship between Amerindian, Pacific and Siberian populations according to HLA-DRB1-DQB1 haplotype frequencies in three dimensions (bidimensional representation). Populations: Buryats (1), Aleuts (2), Negidal (3), Evenks (4), Easter Island (5), Samoa (6), Taiwan (7), Ulchi (8), Papua (9), Ainu (10), Lakota Sioux (11), Mazatecans (12), Mataco Wichi (13), Lamas (14), Mapuches (15), Athabaskans (16), Eskimo (17), Yupik (18), Toba Pilaga (19), Aymara (20), Eastern Toba (21), Uros (22), Zapotecans (23), Quechuas (24), Mayans (25), Mixe (26), Tarahumara (27) and Mixtecans (28). Data from populations were from references stated in Table 1.

- A multidisciplinary approach

On the other hand, genetics study of present day populations may not be accurate enough to explain both Pacific (Sykes et al., 1995) and America peopling (Arnaiz-Villena et al., 2010). A solely genetical interpretation of ancient and past peopling may be biased for one or several of the following facts:

- 1) An ancient founder effect may have disappeared because of a continuous new population admixture effect.
- 2) Europeans induced a remarkable bottleneck effect in America after 1492: about 85 % of American First Inhabitants died because of European borne-diseases during 16th Century (Dobbins, 1993). Amerindians lacked appropriate HLA molecules for starting immune response against new European pathogens. This suggests that Amerindians had a different HLA profile to Europeans before 1492 and that after 16th Century this profile could have farther changed. This putative initial different Amerindian HLA profile may have been more similar to the European one than this which is observed at present, because prehistoric isolation may have not been absolute.
- 3) Stress (like epidemics) induces appearance of new HLA alleles in spermatozoa as demonstrated by single spermatozoa PCR (Huang et al., 1995). A set of new alleles may have appeared after 1492 in Amerindians suffering epidemics of new European borne pathogens.

Genetic HLA input in America (and reversal output) from Pacific (Arnaiz-Villena et al., 2010) and Atlantic Oceans (Solutreans from Iberia) (Stanford & Bradley, 2012) may explain that Amerindians shared cultural traits with both Pacific (Green, 2000; Lawler, 2010; Storey et al., 2007; Storey et al., 2008; Wallin et al., 2005) and Iberian Solutreans (Stanford & Bradley, 2012) and Iberian megalithic builders (Arnaiz-Villena et al., 2013). Caucasoid Kennewick Man skull found in Columbia River mouth (Morell, 1998), prehistoric Caucasoid skulls found in Brazil (Neves & Pucciareli, 1991) and the fact the most ancient American archaeological sites are in South America (Monte Verde, Pedra Furada) (Dillehay, 1997) points out that American peopling has been more complex than thought (Greenberg et al., 1986). Also it appears to have been varied and complex, and also stratified in space and time. At present, it is difficult to describe it in

detail. But all genetic, linguistic, anthropological and cultural findings have to be taking into account.

Finally, there are many questions yet to be answered about America peopling but many more about Pacific Islands peopling. It is clear that a multidisciplinary approach to these problems is necessary, in addition genetics. Results only based on present day genetics may have more than one interpretation of past history and conclusions only drawn from it are all arguable.

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