



Forensic Population Genetics – Letter to the Editor

Analysis of 10 X-STRs in three population groups from Ecuador

Dear Editor,

X-chromosomal short tandem repeat (X-STR) loci provide a useful tool for forensic purposes [1]. Due to a sex-based mode of inheritance, the use of this genetic system has become extremely valuable in paternity testing, especially in deficiency cases involving female offspring [2].

Ecuador is a multi-ethnic country in which the admixed population of Mestizos represents 72% of the population while Native Americans account for 7% with Kichwa being the largest [3]. A second population deserving interest is the Waorani, the last population of Native American hunter-gatherers in the Eastern region of the nation. The population is composed of ~3000 individuals [4] and has been previously characterized as genetically unique [5] attributed to prolonged isolation, high inbreeding and low population size. Previous studies focused on autosomal and uniparentally inherited markers [6–11] have shown evidence of the high diversity existing in the Ecuadorian population as a result of its complex history and multi-ethnic richness. However, current information on the variability of X-chromosome in Ecuador is limited [5]. This study aimed to explore the genetic structure and dynamics of Ecuadorian Waorani, Kichwa and Mestizo populations from the perspective of the X chromosome based on 10 X-STRs [12].

A population sample of 139 maternally unrelated individuals born and living in Ecuador were selected: 32 Waoranis (17 males and 15 females) and 65 Kichwas (27 males and 38 females) from the Amazonian provinces; and 42 Mestizos (28 males and 14 females) from different regions of the country. All donors gave their informed consent prior to their inclusion in the study.

DNA was extracted from saliva swab samples and blood stains on FTA cards (Whatman Inc., Clifton, NJ) using the Chelex extraction procedure [13]. X-STR amplification (DXS8378, DXS9898, DXS7133, GATA31E08, GATA172D05, DXS7423, DXS6809, DXS7132, DXS9902 and DXS6789) was performed according to Gusmao et al. [12]. Alleles were separated and detected using an ABI® Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Allele frequencies were calculated using the direct counting method. Gene diversities, Hardy–Weinberg equilibrium (females), linkage disequilibrium (males) and pairwise exact test of population differentiation were tested using Arlequin software v3.0 [14]. Pairwise population comparisons between the Ecuadorian populations were performed at a single locus level (exact test of population differentiation and *Fst* genetic distance analysis) and for the 10 X-STRs (*Fst* genetic distance analysis). Additionally, pairwise *Fst* genetic distances were calculated between the populations from Ecuador and other available populations with the same 10 X-STRs [15–20].

Unweighted pair group method with arithmetic mean was used to build a phylogenetic tree based on *Fst* distances using the option

neighbor and drawtree in the PHYLIP software package [21] and visualized with the TREEVIEW software [22]. Statistical analysis included the following forensic efficiency parameters: power of discrimination in females (PD_F) and males (PD_M), mean exclusion chance in trios involving daughters (MEC_T) and father/daughter duos (MEC_D) [23].

Allele frequencies obtained for the ten X-STR loci studied in the three Ecuadorian population groups are shown in Table S1. Male and female samples were pooled to calculate allelic frequencies after the exact test of differentiation revealed no significant differences. Genetic diversity values for loci were above 46%, and the population of Mestizos showed a higher average of genetic diversity (67% for all 10 X-STR markers) than Kichwa (61%) and Waorani (59%). Therefore, the admixed population showed evidence of a greater diversity than the two Native American, as was expected, given the fact that the first group includes a more geographically and culturally diverse subset of individuals.

Population genetic profiles are presented in Table S2. All male haplotypes were unique, with the exception of one haplotype that was shared by three Waorani individuals. There was no evidence of departure from Hardy–Weinberg equilibrium in female samples, after Bonferroni correction for multiple tests ($p \leq 0.005$).

Significant association was not determined for linkage disequilibrium between loci in Kichwas and Mestizos after Bonferroni correction ($p \leq 0.0011$). This result suggests the absence of significant associations due to genetic substructure. In contrast, DXS6809–DXS7133 ($p = 0.0010$) and DXS7423–DXS8378 ($p = 0$) showed significant results in Waorani population. These findings could be a consequence of demographic factors (e.g. population structure, endogamy or founder effects) [24] or sampling effects [25].

Forensic efficiency statistical parameters are presented in Table S4. The most polymorphic loci were DXS6789, DXS6809 and DXS7132 in Waorani, Kichwa and Mestizo groups, respectively. DXS8378 showed to be the least discriminative marker in all populations. The overall values of PD_M and PD_F were higher in Mestizo ($PD_M = 99.998551\%$ and $PD_F = 99.9999991\%$) than in Kichwa ($PD_M = 99.995006\%$ and $PD_F = 99.999991\%$) and Waorani ($PD_M = 99.986216\%$ and $PD_F = 99.99995\%$) populations. The same pattern was observed for combined MEC_T and MEC_D , as they were higher in Mestizos ($MEC_T = 99.9945\%$ and $MEC_D = 99.9728\%$) than in Kichwas ($MEC_T = 99.9797\%$ and $MEC_D = 99.6348\%$) and Waorani ($MEC_T = 99.9442\%$ and $MEC_D = 99.1804\%$). The results obtained support the potential of this decaplex system for forensic identification and paternity testing, especially in the Mestizo population.

When considering both methodologies used for population comparisons between Ecuadorian groups at single locus level, significant values were obtained for 6 markers after Bonferroni correction ($p \leq 0.005$) (DXS6789, DXS6809, DXS7132, DXS7133, GATA172D05 and GATA31E08) (Table S5). Significant pairwise *Fst* genetic distances were determined ($p \leq 0.005$) between the three

populations for the complete STR panel (Table S6). Therefore, statistical analysis regarding *Fst* between Kichwas and Mestizos were not in accordance with the expected admixture given their coexistence and subsequent gene flow.

Pairwise genetic distance analysis (Table S6) carried out with European, American and African sample sets revealed significant genetic distances between the Ecuadorian groups and all populations ($p \leq 0.005$). The Waorani exhibited the highest distance from the tested populations, whereas the Mestizo population was the closest (Fig. S1). These results are consistent with the history of these Ecuadorian groups. In this regard, the high Native American component present in the Waorani and Kichwa, absent or minor in the rest of the populations, could explain the observed distance. Although the Native American ancestry component is still predominant, the relative proximity of the Mestizo population to other Latin American and European groups can be attributed to the European contribution in its gene pool.

In conclusion, X-chromosome markers revealed significant differences between the three ethnic groups studied from Ecuador, suggesting that specific reference databases or correction factors are required for forensic testing calculations when individuals from these groups are involved.

This paper follows the guidelines for publication of population data requested by the journal [26] and the International Society for Forensic Genetics (ISFG), as well as abiding to ISFG recommendations on the analysis of DNA polymorphisms [27].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fsigen.2012.08.004>.

References

[1] R. Szibor, M. Krawczak, S. Hering, J. Edlmann, E. Kuhlisch, D. Krause, Use of X-linked markers for forensic purposes, *Int. J. Legal Med.* 117 (2003) 67–74.
 [2] R. Szibor, I. Plate, J. Edlmann, S. Hering, E. Kuhlisch, M. Michael, D. Krause, Chromosome X haplotyping in deficiency paternity testing principles and case report, *Int. Congr.* 1239 (2003) 815–820.
 [3] INEC, Instituto Nacional de Estadística y Censos, Ecuador, 2010.
 [4] CODENPE, Council of Development of the Nationalities and Peoples of the Ecuador, 2008. www.codenpe.gov.ec.
 [5] S. Cardoso, M. Alfonso-Sánchez, L. Valverde, D. Sánchez, M. Zarrabeitia, A. Odrizola, B. Martínez-Jarreta, M.M. de Pancorbo, Genetic uniqueness of the Waorani tribe from the Ecuadorian Amazon, *Heredity* (2012), PMID:22234246.
 [6] S. Cardoso, M.A. Alfonso-Sánchez, F. González-Andrade, L. Valverde, A. Odrizola, A.M. Pérez-Miranda, J.A. Peña, B. Martínez-Jarreta, M.M. de Pancorbo, Mitochondrial DNA in Huaorani (Ecuadorian amerindians): a new variant in haplogroup A2, *Forensic Sci. Int. Genet. Suppl.* 1 (2008) 269–270.
 [7] M. Geppert, M. Baeta, C. Núñez, B. Martínez-Jarreta, S. Zweynert, O.W. Cruz, F. Gonzalez-Andrade, J. Gonzalez-Solorzano, M. Nagy, L. Roewer, Hierarchical Y-SNP assay to study the hidden diversity and phylogenetic relationship of native populations in South America, *Forensic Sci. Int. Genet.* 5 (2011) 100–104.
 [8] F. Gonzalez-Andrade, L. Roewer, S. Willuweit, D. Sanchez, B. Martinez-Jarreta, Y-STR variation among ethnic groups from Ecuador: Mestizos, Kichwas, Afro-Ecuadorians and Waoranis, *Forensic Sci. Int. Genet.* 3 (2009) e83–e91.
 [9] F. Gonzalez-Andrade, D. Sanchez, Martinez-Jarreta B, Genetic profile of the Ecuadorian Mestizo population (Ecuador-South America) by using the Power Plex 16 System Kit, *Forensic Sci. Int.* 135 (2003) 64–66.
 [10] M. Baeta, C. Núñez, F. González-Andrade, C. Sosa, Y. Casalod, M. Bolea, S. Zweynert, O.W. Vacas Cruz, J. González-Solorzano, M. Geppert, L. Roewer, Martínez-Jarreta B, Mitochondrial analysis revealed high homogeneity in the Waorani population: the last nomadic group of hunter-gatherers from Ecuador, *Forensic Sci. Int. Genet. Suppl.* 2 (2009) 313–314.
 [11] M. Baeta, C. Núñez, C. Sosa, M. Bolea, Y. Casalod, F. Gonzalez-Andrade, L. Roewer, B. Martínez-Jarreta, Mitochondrial diversity in Amerindian Kichwa and Mestizo populations from Ecuador, *Int. J. Legal Med.* (2011), PMID:22189782.

[12] L. Gusmao, C. Alves, P. Sánchez-Diz, M.T. Zarrabeitia, M.A. Abovich, I. Aragón, B. Arce, G. Arrieta, E. Arroyo, I. Atmetlla, C. Baeza, M.C. Bobillo, L. Cainé, R. Campos, L. Caraballo, E. Carvalho, M. Carvalho, R.M.B. Cicarelli, D. Comas, D. Corach, M. Espinoza, M.R. Espinheira, F. Rendo, O. García, I. Gomes, A. González, A. Hernández, M. Hidalgo, P. Lozano, M. Malaghini, D. Manzanares, B. Martínez, J.A. Martins, K. Maxzud, I. Miguel, N. Modesti, M. Montesino, R. Ortiz, J.J. Pestano, M.F. Pinheiro, L. Prieto, E. Raimondi, J.A. Riancho, M.B. Rodríguez, I. Salgado, N. Salgueiro, J.J. Sánchez, S. Silva, U. Toscanini, C. Vidales, C.V. Silva, M.C. Villalobos, C. Vullo, I. Yurrebaso, A.I. Zubillaga, A. Carracedo, Amorim A, Results of the GEP-ISFG collaborative study on an X-STR Decaplex, *Forensic Sci. Int. Genet. Suppl.* 1 (2008) 677–679.
 [13] P.S. Walsh, D.A. Metzger, Higuchi R, Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material, *Biotechniques* 10 (1991) 506–513.
 [14] L.G.L. Excoffier, S. Schneider, Arlequin ver. 3.0: an integrated software package for population genetics data analysis, *Evol. Bioinformatics* 1 (2005) 47–50.
 [15] L. Gusmao, P. Sánchez-Diz, C. Alves, I. Gomes, M.T. Zarrabeitia, M. Abovich, I. Atmetlla, C. Bovillo, L. Bravo, J. Builes, L. Cainé, R. Calvo, E. Carvalho, M. Carvalho, R. Cicarelli, L. Catelli, D. Corach, M. Espinoza, O. García, M. Malaghini, J. Martins, F. Pinheiro, M.J. Porto, E. Raimondi, J.A. Riancho, A. Rodríguez, A. Rodríguez, B. Rodríguez Cardozo, V. Schneider, S. Silva, C. Tavares, U. Toscanini, C. Vullo, M. Whittle, I. Yurrebaso, A. Carracedo, Amorim A, A GEP-ISFG collaborative study on the optimization of a X-STR decaplex: data on 15 Iberian and Latin American populations, *Int. J. Legal Med.* 123 (2009) 227–234.
 [16] M.T. Zarrabeitia, F. Pinheiro, M.M. de Pancorbo, L. Caine, S. Cardoso, L. Gusmao, Riancho JA, Analysis of 10 X-linked tetranucleotide markers in mixed and isolated populations, *Forensic Sci. Int. Genet.* 3 (2009) 63–66.
 [17] U. Toscanini, L. Gusmao, G. Berardi, Raimondi E, Genetic data of 10 X-STR in two Native American populations of Argentina, *Forensic Sci. Int. Genet. Suppl.* 2 (2009) 405–406.
 [18] B. Garcia, M. Crespillo, M. Paredes, Valverde JL, Population data for 10 X-chromosome STRs from north-east of Spain, *Forensic Sci. Int. Genet.* 6 (2012) e13–e15.
 [19] M. Illescas, A. Pérez, J. Aznar, L. Valverde, S. Cardoso, J. Algorta, M. de Pancorbo, Population genetic data for 10 X-STR loci in autochthonous Basques from Navarre (Spain), *Forensic Sci. Int. Genet.* 6 (2012) e146–e148.
 [20] I. Gomes, V. Pereira, V. Gomes, M.J. Prata, N. Pinto, A. Carracedo, A. Amorim, L. Gusmao, The Karimojong from Uganda: genetic characterization using an X-STR decaplex system, *Forensic Sci. Int. Genet.* 3 (2009) e127–e128.
 [21] J. Felsenstein, PHYLIP: Phylogeny Inference Package (Version 3.2), *Cladistics* 5 (1989) 164–166.
 [22] R. Page, Treeview: an application to display phylogenetic trees on personal computers, *CABIOS* 12 (1996) 357–358.
 [23] D. Desmarais, Y. Zhong, R. Chakraborty, C. Perreault, Busque L, Development of a highly polymorphic STR marker for identity testing purposes at the human androgen receptor gene (HUMARA), *J. Forensic Sci.* 43 (1998) 1046–1049.
 [24] F.P. Leite, S.E. Santos, E.M. Rodriguez, S.M. Callegari-Jacques, D.A. Demarchi, L.T. Tsuneto, M.L. Petzl-Erler, F.M. Salzano, M.H. Hutz, Linkage disequilibrium patterns and genetic structure of Amerindian and non-Amerindian Brazilian populations revealed by long-range X-STR markers, *Am. J. Phys. Anthropol.* 139 (2009) 404–412.
 [25] I. Gomes, C. Alves, K. Maxzud, R. Pereira, M.J. Prata, P. Sanchez-Diz, A. Carracedo, A. Amorim, Gusmao L, Analysis of 10 X-STRs in three African populations, *Forensic Sci. Int. Genet.* 1 (2007) 208–211.
 [26] A. Carracedo, J.M. Butler, L. Gusmao, W. Parson, L. Roewer, P.M. Schneider, Publication of population data for forensic purposes, *Forensic Sci. Int. Genet.* 4 (2010) 145–147.
 [27] W. Bär, B. Brinkmann, B. Budowle, A. Carracedo, P. Gill, P. Lincoln, W. Mayr, B. Olaisen, DNA recommendations. Further report of the DNA Commission of the ISFG regarding the use of short tandem repeat systems, *Forensic Sci. Int.* 87 (3) (1997) 179–184.

Miriam Baeta^{a,*}
 Carolina Núñez^a
 José María Aznar^b
 Cecilia Sosa^a
 Yolanda Casalod^a
 Miguel Bolea^a
 Fabricio González-Andrade^c
 Marian M. de Pancorbo^b
 Begoña Martínez-Jarreta^a

^aLaboratory of Forensic Genetics, Faculty of Medicine, University of Zaragoza, 50009 Zaragoza, Spain
^bBIOMICS Research Group, University of the Basque Country UPV/EHU, Vitoria-Gasteiz, Spain
^cScience and Technology Department, Ministry of Public Health, Quito, Ecuador

*Corresponding author. Tel.: +34 976762110
 E-mail address: mibaeta@hotmail.com (M. Baeta)