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# Archaeogenetics: DNA and the population prehistory of Europe

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## Chapter 21

# Morphological and Molecular Evolution in Prehistoric Skeletal Populations

Neskuts Izagirre, Patricia Artiach & Concepcion de la Rua

*For a long time, morphometric traits of the skeletal remains had been the only data available to discuss the biological relationships among past populations. In this work we analyze, by means of the RFLPs, the mtDNA recovered from the skeletal remains excavated in three prehistoric sites in the Basque Country (SJAPL, Longar and Pico Ramos). The frequency of the mtDNA haplogroups show a similar distribution in the samples of SJAPL and Pico Ramos, which present the lowest distance values and clustering together in the trees. Longar, shows higher distances with each of them, although lower than with modern samples. On the other hand, the analysis of the frequency of the cranial non-metric traits shows that SJAPL (Araba) and Longar (Nafarroa) are not statistically different populations, clustering together in the UPGMA tree. We have estimated the relative advantages of both data (genetic and morphologic) and we have also discussed the discrepant results obtained in this work, as well as their use in determining the biological relationships between different human groups.*

For a long time the morphometric characteristics of the skeleton provided the only method available for determining the biological relationships between human past groups. However, these characteristics are polygenic in nature and their expression is controlled by an unknown combination of environmental and genetic factors (Cavalli-Sforza & Bodmer 1971). This means that it is hard to interpret the results and difficult to establish whether the similarities observed between populations are due to genetic relationships between them or to environmental factors of various kinds.

The development of molecular biology techniques (i.e. PCR: Saiki *et al.* 1985; Mullis & Faloona 1987) has enabled us now to obtain genetic data on extinct populations. In this way we can characterize prehistoric human groups from both a morphological and a genetic perspective.

In this study we analyzed 62 non-metric cranial traits, and the variability of mtDNA by means of RFLP analyses, performed on human remains recov-

ered from three prehistoric sites in the Basque Country: SJAPL, Longar and Pico Ramos. Our intention was to compare the information obtained with morphological and molecular markers.

### Materials and methods

The remains analyzed came from the following sites:

\* SJAPL, located in the upper Ebro valley in the province of Araba. The remains belong to the late Neolithic period (5070±150 to 5020±140 years BP, according to <sup>14</sup>C dating of two bone samples: Etxebarria & Vegas 1988). The minimum number of individuals (MNI) recovered is 289, and genetic analyses were performed on 63 individuals.

\* The Pico Ramos cave on the Atlantic coast of the Basque Country (Bizkaia province). The remains belong to the Chalcolithic period based on the stratigraphic sequence (4110±110 to 4790±110 years BP)

(Zapata 1995). At least 104 individuals were recovered, and 24 individuals were analyzed using RFLPs.

\* The Longar burial chamber located in the province of Nafarroa, dated at 4445±70 to 4580±90 years BP, corresponding to the Late Neolithic-ancient Chalcolithic (Armendariz & Irigarai 1995). The MNI for this site is 69, and RFLP analyses were performed on 27 individuals.

#### *Analysis of non-metric cranial traits*

Tests were performed to check for the presence/absence of a total of 62 non-metric cranial traits, as described in Hauser & De Stefano (1989), Castro & Quevedo (1983-84) and Klug & Wittwer-Backofen (1983). Of all these characteristics, only those showing significant heredability (Sjøvold 1984) were used to calculate the MMD (Mean Measure Divergence) distance matrix (Sjøvold 1973).

The results for the non-metric cranial traits obtained here were compared with data on other present-day, historical and prehistoric populations. The prehistoric populations used were Turks, Syrians, Lebanese (Klug & Wittwer-Backofen 1983), Ukrainians (Cesnys & Konduktorova 1982; in Hauser & De Stefano 1989) and Siberians (Kozintsev 1972; in Hauser & De Stefano 1989); medieval populations from Hito in Cantabria (Galera 1989) and Garai in the Basque Country (unpublished data) were used, along with the population from Aldaieta (Basque country, sixth century) (unpublished data) and present-day population samples of Spaniards (Gil 1985), Romans (Vecchi 1968), Sardinians (Cosseddu *et al.* 1979), Dutch (Perizonius 1979), Bedouins (Henke & Disi 1981; in Klug & Wittwer-Backofen 1983) and Basques (unpublished data).

#### *Analysis of mtDNA*

MtDNA variability was studied by means of RFLP analysis (Torrioni *et al.* 1996). The DNA for this was extracted from intact teeth using the phenol-chloroform method. These teeth were decontaminated with acid to eliminate any contaminant DNA from their surfaces. The use of intact teeth reduces the risk of contamination of the dental pulp by modern DNA and prevents the destruction of the endogenous DNA by acids.

To analyze the restriction polymorphisms a set of primers was designed to amplify fragments less than 120 pbs long, located all over the mitochondrial genome (Izagirre & de la Rua 1999).

The analyses included two types of control: a blank control on the extraction to check for contamination during the extraction process at the laboratory, and a negative control of amplification to check for contamination during the amplification stage.

The series used to compare genetic data were Tuscans, Finns, Swedes, and Basques from the present day, as published by Torrioni *et al.* (1996).

#### **Results**

##### *Non-metric cranial traits*

The frequency of each non-metric trait was checked, and comparisons between populations were made using a  $\chi^2$  test.

The prehistoric sample from Pico Ramos was not included in the  $\chi^2$  tests because the remains were highly fragmented, which limited the number of observations for each characteristic. A comparison of all the Basque populations taken as a whole (prehistoric, medieval and present day) showed statistically significant differences. ( $\chi^2 = 702.777$ , g.f. = 207,

**Table 21.1.** Comparison via the  $\chi^2$  test between the Basque populations analyzed in this study and a set of Eurasian populations.

	SJAPL	Longar	Pais Vasco	Aldaieta	Garai	Hito	Spaniards	Romans
SJAPL		49,364	119.825**	103.039**	194.87**	79.845**	717.738**	328.068**
Longar			67.284*	37,908	90.548**	16,627	205.584**	42.990**
Pais Vasco				63,153	153.094**	49.59**	689.548**	159.538**
Aldaieta					144.213**	13,919	229.966**	103.158**
	Sardinians	Dutch	Turks	Syrians	Lebanese	Bedouins	Ukrainians	Siberians
SJAPL	244.456**	796.336**	170.581**	215.229**	253.253**	270.170**	412.487**	296.620**
Longar	24,458	209.883**	69.139**	98.997**	89.888**	140.861***	96.679**	159.538**
Pais Vasco	119.28**	413.867**	92.245**	102.042**	146.777**	337.476**	245.438**	179.199**
Aldaieta	88.223**	393.903**	83.647**	126.909**	102.835**	138.888**	125.146**	180.386**

\*  $p < 0.05$

\*\*  $p < 0.01$

$p < 0.0001$ ), from which we can deduce that there is heterogeneity among the Basque populations analyzed for this set of characteristics.

The  $\chi^2$  test comparison of the populations analyzed in this study together with the other populations indicated in the bibliography (Table 21.1) shows statistically significant differences between the prehistoric population of SJAPL and all the others, except the prehistoric population from Longar. Similar results were found on comparing the prehistoric sample from Longar with the remaining populations, except that there were no significant differences with the populations from Hito (Cantabria), Sardinia and Aldaieta (Basque Country).

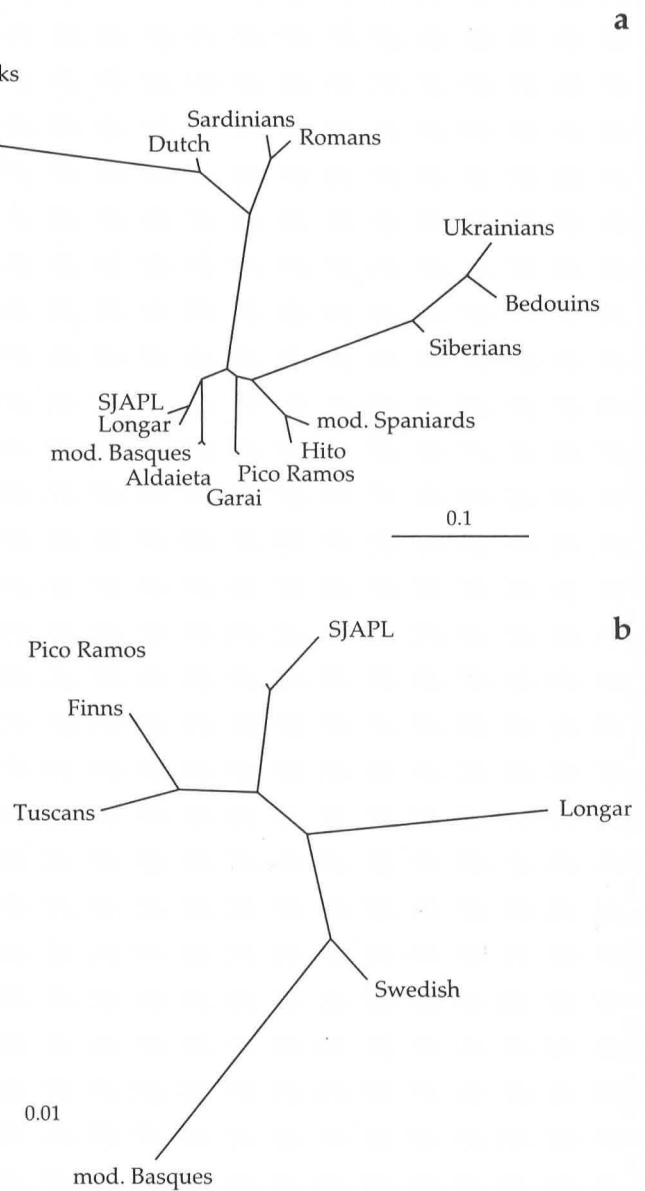
We then calculated the MMD biological distance matrix (Sjøvold 1973), using the data on the 14 characteristics showing significant heredability. Figure 21.1a shows the neighbour-joining tree constructed on the basis of this MMD matrix, where all the Basque populations can be seen to form a group to which the two populations from the Iberian Peninsula (medieval from Hito and present-day Spaniards) are linked. The Western Asia populations are split into two different groups and the European are together in another group.

*Analysis of mtDNA haplogroup diversity*

Genetic analysis was performed only on those samples which presented negative amplification and extraction controls. To double check the results obtained with dental samples, we carried out a parallel analysis on 92 bone samples (left femur) recovered from the SJAPL site. Although it was not possible to associate the bone samples with any particular dental remains, we analyzed 4577 *Nla*III restriction site on both types of sample (bone and teeth). The same digestion pattern was found in the bone samples as in the teeth, i.e. presence of cleavage for 4577 *Nla*III. A more detailed discussion of the mtDNA analysis method can be found in Izagirre & de la Rua (1999).

Table 21.2 shows the haplogroup distribution found in the remains recovered from the three prehistoric Basque sites. The distribution of haplogroups H, K and U is similar at all three sites, with haplogroup H having the highest frequency in all areas. It is also noteworthy that haplogroups I, W and V were not found at any of the prehistoric sites.

Two peculiarities were observed at the Longar site: the absence of haplogroup J and the presence of



**Figure 21.1.** Neighbour-joining trees: a) Based on the MMD matrix for the non-metric skull characteristics; and b) constructed on the basis of the distance matrix from Cavalli-Sforza & Edwards' (1967) cord measurement and mtDNA data.

individuals which we were unable to classify in any of the haplogroups described.

Diversity analysis shows similar diversity values in ancient and modern populations (Table 21.3). This, and the fact that three different sites are involved, suggests that few if any individuals from the same family were sampled.

The neighbour-joining tree constructed on the basis of Cavalli-Sforza & Edwards' (1967) cord dis-



**Table 21.2.** *MtDNA haplogroup frequencies at the three prehistoric sites analyzed in this study and their corresponding standard deviations (se).*

Haplogroup	Prehistoric sites		
	SJAPL Frequency $\pm$ se	Longar Frequency $\pm$ se	Pico Ramos Frequency $\pm$ se
H	0.377 $\pm$ 0.060	0.408 $\pm$ 0.094	0.374 $\pm$ 0.091
J	0.164 $\pm$ 0.047	–	0.167 $\pm$ 0.070
K	0.230 $\pm$ 0.054	0.222 $\pm$ 0.080	0.167 $\pm$ 0.070
U	0.180 $\pm$ 0.049	0.148 $\pm$ 0.068	0.125 $\pm$ 0.062
T, X	0.049 $\pm$ 0.027	0.148 $\pm$ 0.070	0.167 $\pm$ 0.070
W	–	–	–
I	–	–	–
V	–	–	–
Others	–	0.074 $\pm$ 0.050	–

SJAPL (Araba); Longar (Nafarroa) y Pico Ramos (Bizkaia).

**Table 21.3.** *MtDNA diversity based on mtDNA haplogroup frequencies in modern European populations and on three prehistoric Basque samples.*

Populations	$h^a$
MODERN POPULATIONS	
Tuscans (Italy)	0.769
Finns (Finland)	0.785
Basques (Gipuzkoa)	0.713
Swedes (Sweden)	0.762
PREHISTORIC BASQUE SAMPLES	
SJAPL <sup>b</sup> (Araba)	0.756
Pico Ramos (Bizkaia)	0.793
Longar (Nafarroa)	0.762

<sup>a</sup> genetic diversity (Nei 1987)

<sup>b</sup> SJAPL: San Juan Ante Portam Latinam

tance shows that the prehistoric populations from SJAPL and Pico Ramos have the least distance. The present-day Basque sample shows the greatest distance as regards all the populations included in our analysis. The prehistoric sample from Longar is least distant from the Swedish population (0.036), owing to the absence or low frequency of haplogroup J in both populations. However, Longar shows lesser distances from the other two prehistoric Basque populations than from the remaining populations analyzed.

## Discussion

In this study we observe that in the neighbour-joining trees constructed on the basis of the non-metric trait data (Fig. 21.1a), all the Basque populations

(both prehistoric and modern) are grouped together, regardless of their temporal and spatial origin. Although the expression of non-metric cranial traits is due to an unknown combination of genetic and environmental factors, these results seem to support the hypothesis that the influence of the genetic component in this case is highly important.

Discrepancies appear in the results of analyses of the relationships between the prehistoric populations from the Basque Country depending on whether non-metric cranial traits or genetic data are considered. The least distance based on non-metric traits is between the prehistoric populations of SJAPL and Longar, but on the basis of genetic distance it is

between SJAPL and Pico Ramos. This discrepancy is hard to evaluate, because for the Pico Ramos prehistoric population it was only possible to collect non-metric cranial trait data from a very small sample, because of the fragmented condition of the bones recovered. We believe that these data are not significant, so Pico Ramos is not considered in the comparisons between populations by means of  $\chi^2$  testing (Table 21.1). However the differentiation of Longar on the basis of genetic data is explained by the absence of individuals from haplogroup J and the presence of individuals classified under the haplogroup 'others'. Furthermore, from an archaeological viewpoint, the burial structure of Longar is peculiar, and unlike the Chalcolithic structures typically found in the Basque Country (the dolmens in the Rioja Alavesa area). Burial structures similar to that at Longar have been found in the south of the Iberian Peninsula and in the Paris Basin. However the personal effects found at Longar are similar to those described for nearby sites (Armendariz & Irigarai 1995).

In this study we have arrived at results as regards relationships between populations when using morphological data which are different from those obtained with genetic data. The differences are difficult to resolve with the data available at present. Among other factors, we could point to the scant mtDNA data available on extinct populations and the fact that since mtDNA is maternally inherited it reflects only the history of women. Differences in migration patterns between men and women, which have begun to show up in various studies of present-day populations (Cavalli-Sforza & Minch 1997; Seielstad *et al.* 1998;

Simoni *et al.* 1999) are also a factor which must be taken into account in interpreting genetic data on the prehistoric populations analyzed here.

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### References

- Armendariz, J. & S. Irigarai, 1995. *La Arquitectura de la Muerte: el Hipogeo de Longar (Viana, Navarra), un Sepulcro Colectivo de 2.500 A.C.* Estella: Centro de Estudios Tierra-Estella, Lizarraldeko Ikastetxea.
- Castro, M. & S. Quevedo, 1983-84. Proposiciones metodológicas para el estudio de los rasgos no-métricos en el cráneo humano. *Boletín del Museo Nacional de Historia Natural de Chile* 40, 173-210.
- Cavalli-Sforza, L.L. & W.F. Bodmer, 1971. *The Genetics of Human Populations*. San Francisco (CA): W.H. Freeman.
- Cavalli-Sforza, L.L. & A.W.F. Edwards, 1967. Phylogenetic analysis: models and estimation procedures. *Evolution* 32, 550-70. (Also *American Journal of Human Genetics* 19, 233-57.)
- Cavalli-Sforza, L.L. & E. Minch, 1997. Paleolithic and Neolithic lineages in the European mitochondrial gene pool. *American Journal of Human Genetics* 61, 247-51.
- Cesnys, G. & T.S. Konduktorova, 1982. Non-metric features of the skull in people of the Chernyakhovskaya culture. *Voprosy Antropologii* 70, 62-76.
- Cosseddu, G.G., G. Floris & G. Vona, 1979. Sex and side differences in the minor non-metrical cranial variants. *Journal of Human Evolution* 8, 685-92.
- Etxebarria, F. & J.I. Vegas, 1988. ¿Agresividad social o guerra durante el Neo-eneolítico en la cuenca media del Valle del Ebro? A propósito de San Juan ante Portam Latinam. *Munibe* 6, 105-12.
- Galera, V., 1989. La Población Medieval Cantabria de Santa Maria de Hito. Aspectos Paleobiodemográficos, Morfológicos, Paleopatológicos, Paleoepidemiológicos y de Etnogénesis. Unpublished Ph.D. thesis, Universidad de Alcalá, 82-6.
- Gil, C., 1985. Análisis de la simetría de los rasgos epigenéticos craneales bilaterales en una población esquelética española. *Actas IV Congreso Español de Antropología Biológica* (Barcelona), 473-82.
- Hauser, G. & G.F. De Stefano, 1989. *Epigenetic Variants of the Human Skull*. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung.
- Henke, W. & A. Disi, 1981. The craniology of recent Jordanian Bedouins. *Journal of Mediterranean Anthropology and Archaeology* 1, 217-48.
- Izagirre, N. & C. de la Rúa, 1999. An mtDNA analysis in ancient Basque populations: implications for haplogroup V as a marker for a major Paleolithic expansion from southwestern Europe. *American Journal of Human Genetics* 65, 199-207.
- Klug, S. & U. Wittwer-Backofen, 1983. Diskreta im populationsvergleich. *Homo* 34, 153-68.
- Kozintsev, A.G., 1972. Non-metrical traits on crania of 1 thousand BC from Minusinsk hollow. *Arch. Anat. Histol. Embryol.* (Leningrad) 62, 53-9.
- Mullis, K.B. & F.A. Faloona, 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalysed chain reaction. *Methods in Enzymology* 155, 335-50.
- Nei, M., 1987. *Molecular Evolutionary Genetics*. New York (NY): Columbia University Press.
- Perizonius, W.R.K., 1979. Non-metric cranial traits: symmetry and side difference. *Proceedings van de Koninklijke Nederlandse Akademie van Wetenschappen*, 82, 91-112.
- Saiki, R.K., S. Scharf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Erlich & N. Arnheim, 1985. Enzymatic amplification of  $\beta$ -globulin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230, 1850-54.
- Seielstad, M.T., E. Minch & L.L. Cavalli-Sforza, 1998. Genetic evidence for a higher female migration rate in humans. *Nature Genetics* 20, 278-80.
- Simoni, L., F. Calafell, J. Bertranpetit & G. Barbujani, 1999. Geographical patterns of mtDNA diversity in Europe. Euroconference: 'Human Diversity in Europe and Beyond: Retrospect and Prospect'. Cambridge, 1999.
- Sjøvold, T., 1973. The occurrence of minor non-metrical variants in the skeleton and their quantitative treatment for population comparisons. *Homo* 24, 204-33.
- Sjøvold, T., 1984. A report on the heritability of some cranial measurements and non-metric traits, in *Multivariate Statistical Methods in Physical Anthropology*, eds. GN. Van Vark & W.W. Howels. Boston (MA): D. Reidel, 289-321.
- Torroni, A., K. Huoponen, P. Francalacci, M. Petrozzi, L. Morelli, R. Scozzari, D. Obinu, M-L. Savontaus & D.C. Wallace, 1996. Classification of European mtDNAs from an analysis of three European populations. *Genetics* 144, 1835-50.
- Vecchi, F., 1968. Sesso e variazione di caratteri discontinui del cranio. *Rivista di Antropologia* 55, 283-90.
- Zapata, L., 1995. La excavación del depósito sepulcral calcolítico de la cueva Pico Ramos (Muskiz, Bizkaia). La industria ósea y los elementos de adorno. *Munibe* 47, 35-90.