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The Western Pygmies from the Central African Republic: new results on autosomal loci

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In a recent paper we tested two different hypotheses concerning the origin of African Pygmies, termed "recent divergence and differential admixture" (RDDA) and "pre-Bantu divergence" (PBD) (Destro-Bisol *et al.*,, 2004). The RDDA hypothesis sustains that the genetic differentiation observed between Eastern Pygmies (settled in Zaire) and Western Pygmies (Cameroon, Central African Republic and Congo) is due to the combined effect of differential admixture and genetic drift (see also Cavalli-Sforza, 1986). This theory suggests that the occupation of rainforest and deforestation by Bantu farmers (around 2-3 kiloyears ago) progressively reduced the Pygmies' habitat, eventually leading to a biological separation of Pygmy groups settled in the eastern and western sides of the tropical rainforest. The Eastern Pygmies remained relatively isolated from neighbouring populations, whereas Western Pygmies admixed with Bantus. The PBD hypothesis, on the other hand, states that the separation between Eastern and Western Pygmies occurred well before 2-3 Kya, independently from the Bantu expansion. It takes into consideration some archeological studies in the tropical rainforest which indicate the presence of a hunter-gatherer community in the tropical forest of Ituri of the Northeastern Congo Basin around 18 Kya (Mercader, 2002). These sites might be the remains of the nucleus from which present-day Eastern Pygmies evolved after the separation from the Western Pygmy branch. Therefore, the 18 Kya date could be tentatively regarded as the *ante quam* date for the divergence between the two Pygmy groups. It is also worth noting that the Last Glacial Maximum dates back to around this time, and that there is a broad consensus sustaining that during this time the area occupied by rainforest was considerably reduced compared to today (Cornellissen, 2002). This can be considered as a possible ecological cause of separation between the two groups of Pygmies, whose present distribution resembles the probable forest *refugia* of the Last Glacial Maximum (Sayer *et al.*, 1992).

Destro-Bisol *et al.* (2004) tested the RDDA and PBD hypotheses considering the variation of mitochondrial DNA in Western Pygmies (Mbenzele and Biaka from the Central African Republic) and Eastern Pygmies (Mbuti from the Popular Republic of Congo). Some Bantu-speaking farmer populations were also considered. We observed that distribution, sequence variation, and age of haplogroups, along with genetic distances, estimates of divergence times among populations, and simulations based on the coalescent approach, met the expectations of the PBD hypothesis, whereas they do not support the RDDA hypothesis. Given that mtDNA may be regarded as a single locus in evolutionary terms, the conclusions of the study mentioned above need further checks through the use of other genetic systems. In order to do this, we here present a synthesis of new results and published data regarding autosomal variation

(ten microsatellites and an Alu insertion) in the Mbenzele, Western Pygmies from the Central African Republic.

According to the RDDA hypothesis, the Western Pygmies may be regarded as a hybrid population between the Mbuti-like ancestors and Bantus. If this were to be the case, it follows that the gene frequency Western Pygmies should fall into the range of those observed in the two parental populations. Gene frequencies of numerous polymorphisms at protein level in Western Pygmies fit those expected for a hybrid population between Eastern Pygmies and Bantu farmers (Cavalli-Sforza *et al.,* 1969; Wjisman, 1986). However, polymorphisms of autosomal loci have are intrinsically limited in that an identical electrophoretic mobility or immuno-reactivity does not necessarily indicate identity by descent but only identity by state. We tested the compliance of gene frequencies of Eastern and Western Pygmies and Bantus by analyzing the association bertween the CD4 microsatellite and an Alu linked polymorphism. This test is a valid alternative to the use of protein loci since we combined a locus (ALU) which is stable from a phylogenetic point of view with another more variable and subjected to a high mutation rate (CD4 microsatellite). Our results do not support the RDDA hypothesis (figure 1). In fact, the frequency of association between the ALU deletion and the most representative microsatellite alleles (composed of 6 and 11 repeat units) in the Mbenzele, fall outside of the range of values for the Eastern Pygmies and the Bantus. It is also possible that this lack of agreement between CD4/Alu variation and that expected for a dihybrid model could be due to the effect of genetic drift on gene frequencies. However, it is worth noting that the conclusion is the same when considering another Western Pygmy population, the Bakola from Cameroon (see figure 1; Destro-Bisol *et al.,* unpublished data).

Goldstein *et al.* (1995) in their microsatellite loci studies postulated that under a stepwise mutation model the time periods since population splits are proportional to the squared difference in average allele lengths ($\delta\mu^2$) between populations. We applied this method to data published for African Pygmies (Destro-Bisol *et al.*, 2000; Pérez Lezaun *et al.*, 1997). The date of population splits were calculated using autosomal microsatellite data following the method of Goldstein *et al* (1995). We used μ = 3.73 x 10-4 and μ = 2.80 x 10-4 and generation times of 20 and 25 years to estimate a range of values (Chakraborty *et al.*, 1997). We obtained a separation time of 29115-48382 years for Mbenzele and Mbuti and 21513-35223 for Biaka and Mbuti. Given that we could use only six autosomal loci and some populations have been

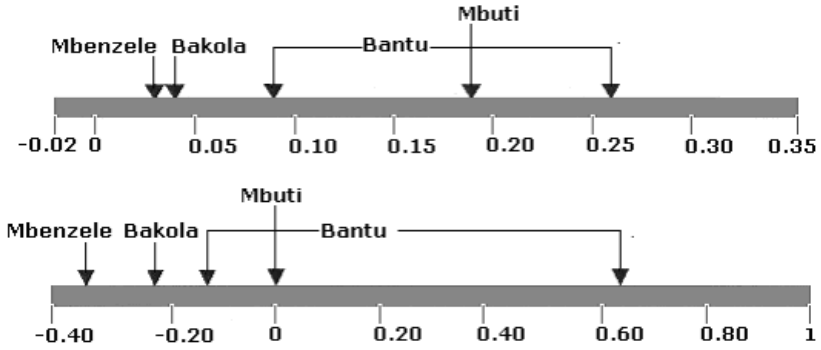

Fig. 1 - Association between the CD4 alleles"6" (top) and "11" (bottom) and the ALU deletion on the short arm of chromosome 12, as measured by the "d" parameter (Bengtsson & Thomas, 1981), in Western Pygmies (Mbenzele and Bakola) and Eastern Pygmies (Mbuti) and in Bantu populations. The data for comparison are from Tishkoff* et al*. (1996). Genotyping was carried out as described in Destro-Bisol* et al*. 1999). CD4 Alu haplotype frequencies were inferred by maximum likelihood using the Arlequin software (Schneider* et al*. 1997).

analyzed for only a low number of chromosomes (Biaka and Mbuti; Pérez Lezaun etal. 1997), the values are inevitably approximate. Furthermore, the method used assumes that gene flow is negligible and that genetic drift contributes less than mutation to population divergence. However, despite these caveats, we believe that our results merit discussion. Due to its high density of vegetation, the large area which separates the Ituri Forest and the territories of Western Pygmies (~1500 km measured as air distance) is very difficult to cross without the help of an adequate technology, such as the iron metallurgy practised by Bantu peoples but unknown to African Pygmies (de Maret & Nsuka, 1977). Therefore, the level of gene flow between the two Pygmy groups should have been relatively low. Concerning the problem of genetic drift, it is worth noting that no significant decrease in heterozygosity and number of alleles is detected in Pygmies relative to Bantus or other worldwide populations (these results; Calafell *et al.,* 1998; Destro-Bisol *et al.,* 2000b). This suggests that no dramatic bottleneck shaped the genetic structure of autosomal loci in African Pygmy populations.

In conclusion, the pattern of association between the CD4 microsatellite and an Alu linked polymorphism does not support a model in which the Western Pygmies are a hybrid between Eastern Pygmies and Bantus, as implied by the RDDA hypothesis. Furthermore, the population split estimates based on autosomal data provide results which are congruent with the PBD hypothesis, since they suggest that the separation between Western and Eastern Pygmies occurred much earlier then the Bantu expansion, but fail to support the RDDA hypothesis which posits the population split as a consequence of the Bantu expansion.

Info on the web

CD4/Alu data of Mbenzele and Bakola are available at www.isita-org.com, as supplementary material to this article.

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