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Further Comments on the Characterization of Founder Amerindian Mitochondrial Haplotypes

To the Editor:

We have detected the presence of 8–10 founder mitochondrial haplotypes in extant Amerindian populations (Bailliet et al. 1994; Bianchi et al. 1995), and we identified four of these haplotypes by use of the acronyms "A_1," "A_2," "D_1," and "D_2." Easton et al. (1996) confirmed most of our findings and used the nomenclature system proposed by us.

In the October 1996 issue of the *Journal,* Forster et al. (1996) verify that the number of ancestral Amerindian mitochondrial haplotypes is greater than the four variants reported by Torroni et al. (1992); and they too use the symbols A_1, A_2, D_1, and D_2 for identification. However, in their report, these authors state that "the decision taken by Bailliet et al. (1994) and Bianchi et al. (1995) to identify founding haplotypes according to 16517 *Hae*III status is therefore phylogenetically questionable, and we do not recommend the use of their haplogroups or their nomenclature" (Forster et al. 1996, p. 938). Moreover, in the legend to their figure 1, Forster

et al. (1996, p. 938) say that "the nomenclature used here is not related to that of Bailliet et al. (1994)." In light of these two comments, we think that it is worthwhile to assess the soundness of Forster et al.'s arguments.

The A_1/A_2 and D_1/D_2 haplotypes used by Forster et al. (1996) are differentiated by a base substitution in mtDNA positions 16111 and 16271, respectively. On the other hand, our subgroups A_1/A_2 and D_1/D_2 depend on a base substitution at position 16519. Thus, in both reports haplotype grouping depends on a single transition. Forster et al. (1996) indicate that the T→C transition used by us is recurrent and, according to them, unsuited for phylogenetic use. Yet, the same authors, despite finding that transitions at positions 16325 and 16362 are also recurrent, use them for haplotyping and phylogenetic reconstruction (Forster et al. 1996). Moreover, Torroni et al. (1993), in table V of their report, show that 3 of the 11 cases of Amerindians/Asians having the founder haplotype B exhibit also the T→G transition at position 16111, which is a clear demonstration of recurrence for this site. This is not surprising. Recently, Howell et al. (1996) made a direct appraisal of the mutation rate in human mtDNA and found an average of one nucleotide substitution every 25 generations. Accordingly, if this finding is confirmed, most mitochondrial mutations would be expected to be recurrent.

Transitions at positions 16111 and 16271 produce the loss of a *Cac*81 and a *Bfa*I site, respectively. By analyzing the data in the literature and by restricting a series of A_1/A_2 and D_1/D_2 haplotypes by use of the aforementioned enzymes, we have determined that 92%–95% of these haploforms can be identified as indicated in table 1. The data in table 1 maintain the nomenclature system proposed by us (Bailliet et al. 1994) and used by Forster et al. (1996) but give a better definition of the haplotypes.

Table 1

Characterization of A_1/A_2 and D_1/D_2 Mitochondrial Haplotypes

	STATUS FOR RESTRICTION ENZYME (POSITION)[a]		
HAPLOTYPE	*Hae*III (663)	*Cac*81 (16111)	*Hae*III (16517)
A_1	+	+	+
A_2	+	−	−
	*Alu*I (5176)	*Bfa*I (16271)	*Hae*III (16517)
D_1	−	+	+
D_2	−	−	−

[a] A plus sign (+) denotes presence, and a minus sign (−) denotes absence.

Approximately 4% of the haplotypes reported by Torroni et al. (1992) in Amerindians did not show any of the markers characterizing the four founder haplotypes proposed by Forster et al. Therefore, Torroni et al. (1992) grouped them under the name of "others" and assumed that they occurred because of Caucasian admixture. We had provided evidence showing that many of these "other" haplotypes were in fact founder Amerindian haplotypes, and we had used the letter "E" to identify this haplogroup (Bailliet et al. 1994). Easton et al. (1996) confirmed our assumption, changed the letter "E" to "X," and reported X_6 and X_7 as two forms of founder haplotypes corresponding to the haplogroup that we formerly had designated as "E." Forster et al. (1996) also named as "X" one additional founder haplotype within what we formerly had called haplogroup "E." However, since the X haplotype of Forster does not correspond to X_6 or X_7, we propose to name it "X_8," and we recommend using the letter "X" instead of the letter "E," to avoid confusion with the haplogroup "E" reported by Torroni et al. (1994) in Tibetans. It is worth mentioning here that we have found the X_8 haplotype in 6 of 41 Sioux individuals studied. Thus far, the following founder Amerindian haplotypes have been proposed: A_1, A_2, D_1, and D_2 (Bailliet et al. 1994; Easton et al. 1996; Forster et al. 1996); B_1 and B_2 (Easton et al. 1996); C_1 and C_2 (Bailliet et al. 1994; Easton et al. 1996); X_{6-8} (corresponding to what we formerly had called haplogroup "E") (Bailliet et al. 1994; Easton et al. 1996; Forster et al. 1996); and A/B, A/C, and A/D (Bailliet et al. 1994; Bianchi et al. 1995).

Finally, there are still important gaps in our knowledge of the biology of mtDNA. By using pedigree analysis, Howell et al. (1996) found that mitochondrial mutation seems to be 200-fold higher than previously had been assumed (Howell et al. 1996). Haplotype changes in a given maternal lineage may occur rather frequently and in a few generations (Gill et al. 1994; Chen et al. 1995; Howell et al. 1996). mtDNA recombination is possible, although the frequency of this event is not yet known (Howell et al. 1996). There is now evidence showing that the occurrence of some mitochondrial deletions may be under the control of nuclear genes (Zeviani et al. 1989; Suomalainen et al. 1995). Moreover, it has been proposed that some mitochondrial mutations may have epigenetic effects changing the mutational rate at other mitochondrial positions (Howell 1996) and perhaps giving rise to mutational "frozen" sites. We believe that most of the disagreement among different groups of researchers working on mtDNA is due to the eagerness to use mtDNA beyond the limitations of the method. If the aforementioned findings are confirmed by other groups, the chronologies of human evolution that are based on mtDNA will need revision in the future. This opinion is shared by Howell et al. (1996) and was also expressed by us in a letter to the editor (Bianchi and Rothhammer 1995) in reply to Torroni and Wallace (1995), who, in another letter to the editor (Bailliet et al. 1994), had criticized our paper, proposing the existence of more than four founder Amerindian mitochondrial haplotypes.

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Reply to Bianchi and Bailliet

To the Editor:

In their original study in 1994, Bianchi and Bailliet (Bailliet et al. 1994) suggested that np 16519, analyzed by *Hae*III digestion, is a phylogenetically useful site for tracing ancient migration patterns, and they used the presence/absence of this site to classify Native American mtDNA variants. In the letter above, they maintain their stance, although studies on Native Americans (Torroni et al. 1993*a*, 1993*b*), Africans (Chen et al. 1995), and Europeans (Torroni et al. 1996) have demonstrated that np 16519 is hypervariable. Bianchi and Bailliet now argue that, since all sites are subject to recurrent mutations, np 16519 is as informative as any. This argument avoids the fact that np 16519 mutates faster, on average, than other sites, such as np 16111 and 16271, which we used for phylogenetic analysis. To obtain an impression of how much faster np 16519 mutates, we counted the number of recurrent transitions of np 16519, np 16111, and np 16271 in most parsimonious trees of African mtDNA variants: the most parsimonious trees for the Chen et al. (1995) restriction-site data (140 Mandenka, Wolof, Pular, other Senegalese, Mbuti, and Biaka) require 8–10 mutations for np 16519, whereas no other site is estimated to have undergone >3 mutations. If one retained in this tree only the 99 individuals sampled from the Mandenka, Mbuti, and Biaka, then still 7 or 8 mutations at np 16519 are observed. Control-region sequences for these 99 individuals are available in the data sets of Graven et al. (1995) (Mandenka) and Vigilant (1990) (Mbuti and Biaka). Neither these 99 nor the complete set of 156 published control-region sequences from the three populations show any transitions at np 16111 or np 16271.

Bianchi and Bailliet claim that their groups A1/A2 and D1/D2 based on np 16519 are congruent with ours. This may appear to apply to published Eskimo data, which lack the *Hae*III np 16517 site (Merriwether et al. 1996) and are predominantly A2 (Forster et al. 1996); however, the *Hae*III site is also absent in the Kuna (Tor-

roni et al. 1993*a*), who instead belong to A1 (identifiable by a 6-bp deletion at np 106; see Batista et al. 1995). Nor does group D1 (i.e., group II) in the data of Horai et al. (1993) correlate with np 16519. It thus appears that the correlation illustrated by Bianchi and Bailliet in their table 1 does not conform with previously published data sets.

Next, we would like to elaborate their narrative of group X nomenclature. Bailliet et al. (1994) were the first to notice that published sequence data indicated the presence of five founding Amerind mtDNA types, rather than four as had previously been believed (and is still occasionally reported, e.g., see Easton et al. 1996, p. 214), and the existence of this additional sequence type (which they termed ''V'') was confirmed by Bandelt et al. (1995), Forster et al. (1996), and Scozzari et al. (1997). Forster et al. (1996) renamed it ''X,'' since the same mtDNA motif is found in the European group X (Torroni et al. 1996); however, Bailliet et al. (1994) also reanalyzed RFLP types, and they made the unfortunate aphylogenetic decision to group all RFLP types that lack the diagnostic A, B, C, or D RFLP sites into a new group ''E.'' Merriwether et al. (1996) and Easton et al. (1996) followed this decision but renamed ''E'' as ''X'' and split it into further subgroups, such as ''X6'' and ''X7''; however, one should bear in mind that a diagnostic RFLP marker can occasionally be lost because of reversion, which can often be detected by phylogenetic analysis; for example, see the discussion by Torroni and Wallace (1995) versus that by Bianchi and Rothhammer (1995). For instance, among the Yanomami there is a group D subset that is characterized by three control-region mutations, one of which is a transversion (e.g., see Yanomama 44 in the work of Torroni et al. 1993*a*). One such Yanomama (Yan 31) in the data of Easton et al. (1996) has suffered a reversion of its D-specific restriction marker, and, according to their definition, now belongs to group ''X6.'' Therefore, it appears that groups ''X6'' and ''X7'' of Easton et al. (1996) both represent mixed bags of reverted C and D types and do not correspond to group X of Forster et al. (1996).

We also disagree that the mtDNA chronologies of human evolution need major revisions because of the 200-fold- and 9-fold-faster mutation rates proposed for coding and control regions, respectively, by Howell et al. (1996). The 200-fold-higher rate is partly due to the inclusion, among the four pedigrees used to calculate the mutation rate, of two pedigrees (QLD1 and NWC1) that were already known by 1991 (Howell et al. 1991*a*, 1991*b*) to harbor recent mtDNA mutations. The 9-fold-higher mutation rate for the control region is mainly an artifact of extrapolation of the mutation rate of the entire control region from two hypervariable nucleotide positions in the second control-region segment (Ma-