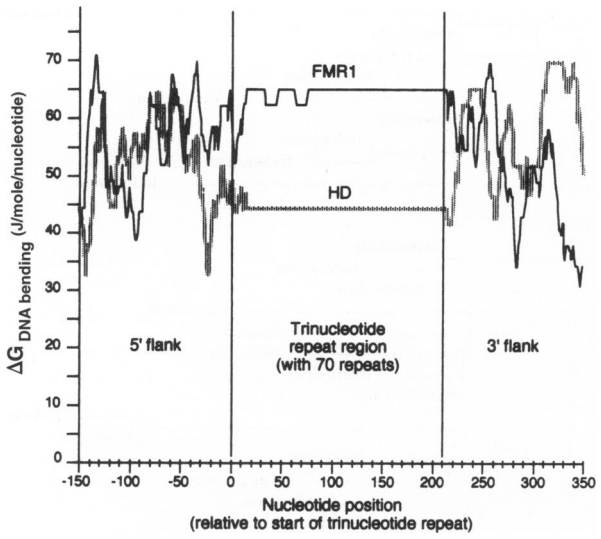

Figure 1 Free energy of DNA bending around a nucleosome core (radius 4.3 nm), calculated for 12-nt DNA segments of FMR1 (*unbroken line*) and HD (*stippled line*) loci, on the basis of formulas described in the text. The number of trinucleotide repeats was normalized to 70 in each case, with the FMR1 and HD repeat structures being $(CGG)_9AGG(AGG)_{10}AGG(CGG)_{49}$ and $(CAG)_{70}$, respectively (the modeled FMR1 trinucleotide repeat would be in allele class 9+10+ according to the nomenclature of Eichler et al. [1994]). Nucleotide position 0 on the *X*-axis represents the start of the trinucleotide repeat. Flanking DNA sequences were obtained from the GenBank database (accession numbers S65791 and L12392) and from Eichler et al. (1994).

latter case the CAG/CTG repeat has been shown to be the strongest known natural nucleosome-positioning element (Wang and Griffith 1995).

Zhong et al. (1995) and others have proposed that the "purity" of the CGG/CCG repeat may have a significant effect on its propensity to expand. Quite aside from the issue of repeat expansion, it is also interesting to consider the effect that these punctuating AGG/CCT triplets may have on nucleosome formation at normal alleles. The two depressions on the graph of ΔG of bending of FMR1 DNA (in fig. 1, see the unbroken line at nucleotide positions 30 and 63, relative to the start of the trinucleotide repeat) are due to AGG sequences at the 10th and 21st triplets, since the FMR1 allele was modeled by using a structure of $(CGG)_9AGG(CGG)_{10}AGG(CGG)_{49}$. These depressions are caused by the more flexible dinucleotides GA and AG in the sequence, which have a DNA-bending ΔG of 39 J/nmol/nt (note that the plotted value on the graph is 62 J/nmol/nt because that calculation is averaged over a window size of 12 nt). Further experiments may indicate whether these two "islands of flexibility" affect nucleosome formation in a normal FMR1 allele—and perhaps trinucleotide-repeat stability, as an indirect consequence.

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References

Eichler EE, Holden JJA, Popovich BW, Reiss AL, Snow K, Thibodeau SN, Richards CS, et al (1994) Length of uninterrupted CGG repeats determines instability in the FMR1 gene. Nat Genet 8:88–94

Hogan ME, Austin RH (1987) Importance of DNA stiffness in protein-DNA binding specificity. Nature 329:263–266

Satchwell SC, Travers AA (1989) Asymmetry and polarity of nucleosomes in chicken erythrocyte chromatin. EMBO J 8: 229–238

Sivolob AV, Khrapunov SN (1995) Translational positioning of nucleosomes on DNA: the role of sequence-dependent isotropic DNA bending stiffness. J Mol Biol 247:918–931

Wang Y-H, Griffith J (1995) Expanded CTG triplet blocks from the myotonic dystrophy gene create the strongest known natural nucleosome positioning elements. Genomics 25:570–573

Zhong N, Yang W, Dobkin C, Brown WT (1995) Fragile X gene instability: Anchoring AGGs and linked microsatellites. Am J Hum Genet 57:351–361

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0002-9297/96/5901-0032$02.00

Am. J. Hum. Genet. 59:253–256, 1996

Lack of Ancient Polynesian-Amerindian Contact

To the Editor:

Data from mtDNA sequences were recently used in two studies as evidence for ancient contact between remote Pacific Islanders and Native Americans directly across the Pacific Ocean. In the first study (Cann 1994), a Polynesian contribution to the Americas was raised as a possible explanation for the presence of the mtDNA B lineage (Torroni et al. 1993*a*) in both Amerindians and Polynesians. The B lineage is one of four common mtDNA lineages found in Amerindians and is defined mainly by a 9-bp deletion in the COII/$tRNA^{Lys}$ region. The B lineage is conspicuously absent in Amerindians from Beringia (Shields et al. 1993). In the second, two mtDNA Polynesian sequences not related to the B lineage matched sequences from Amerindians and were

postulated to be evidence of contact (Sykes et al. 1995). Here, we test the Polynesian-Amerindian contact hypothesis by using all mtDNA sequence data available from Pacific (Hagelberg and Clegg 1993; Hagelberg et al. 1994; Lum et al. 1994; Redd et al. 1995; Sykes et al. 1995), Amerindian (Ward et al. 1991, 1993, and in press; Ginther et al. 1993; Horai et al. 1993; Santos et al. 1994; Batista et al. 1995; Kolman et al. 1995), Mongolian (Kolman et al. 1996), and Siberian (Shields et al. 1993; Torroni et al. 1993*b*) populations. The Pacific sample of 452 persons includes individuals from contemporary western Pacific and several Polynesian populations as well as ancient samples from the Pacific (Hagelberg and Clegg 1993) and Easter Island (Hagelberg et al. 1994). The Native American sample consists of 654 individuals from a total of 18 populations from North, Central, and South America, including the Mapuche from Patagonia (Ginther et al. 1993) and coastal Chile (Horai et al. 1993) and unpublished data (S. L. Bonatto, F. M. Salzano, M. Stoneking, M. H. Hutz, C. E. A. Coimbra, Jr., and R. V. Santos) from the Surui and Wai Wai populations of Brazil. All individuals have been sequenced for the first hypervariable segment (HVS-I, ~360 bp) of the mtDNA control region (CR), but ~364 additional bp from the second hypervariable segment (HVS-II) are available from Indonesian, Polynesian (Redd et al. 1995), Huetar (Santos et al. 1994), Mapuche (Ginther et al. 1993), Ngöbé (Kolman et al. 1995), Surui, and Wai Wai populations only.

Among B lineage sequences, the CR variable sites in Polynesians are distinct from Amerindians. The suite of sites common in Polynesians (Hagelberg and Clegg 1993; Hagelberg et al. 1994; Lum et al. 1994; Redd et al. 1995; Sykes et al. 1995)—the Polynesian motif—includes three HVS-I substitutions: a C at position 16,217, a G at position 16,247, and a T at position 16,261 (relative to the reference [Anderson et al. 1981]). The Polynesian motif has been postulated to be of recent origin and to have increased in frequency together with the expansion of proto-Polynesian populations (Redd et al. 1995). In contrast, Amerindian CR sequences of the B lineage commonly have only one of these variable sites (C at 16,217), which is common in Southeast Asia (Melton et al. 1995). In our Amerindian sample no CR sequences have the Polynesian motif, although one individual from Amazonia has two of the variable sites but lacks the G in position 16,247. This Amerindian individual also lacks a C at position 146 in HVS-II, a polymorphism that is common in Polynesian motif sequences (Redd et al. 1995).

Two possible explanations for the sharing of identical sequences between Polynesians and Amerindians are (*a*) retention of an ancestral Asian sequence and (*b*) admixture (ancient or recent). Ancestral sequences more likely are in basal positions or in the nodes of the phylogenetic

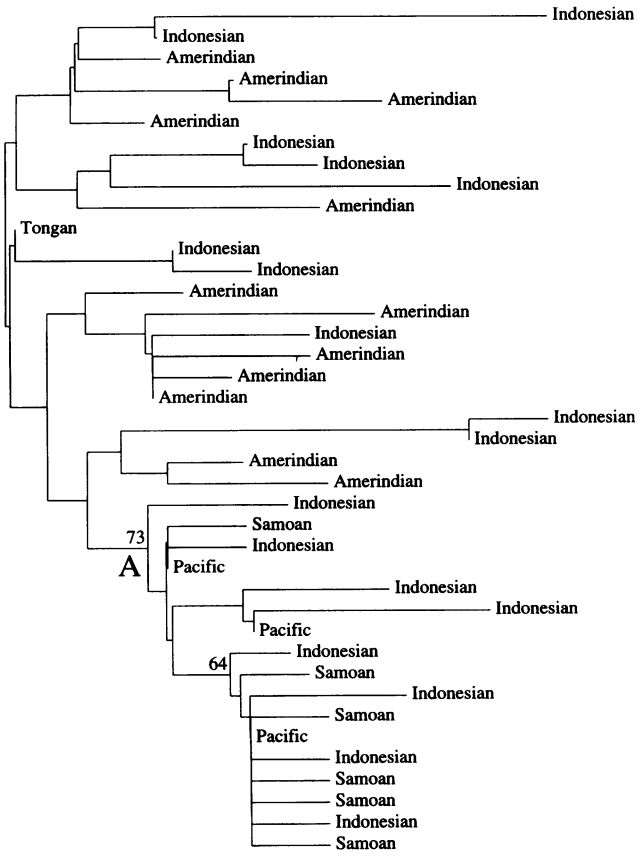

Figure 1 Neighbor-joining phylogenetic tree of Amerindian, Polynesian, and Indonesian sequences (B lineage only), by using both hypervariable segments of the mtDNA CR. "Pacific" indicates sequences shared between Polynesians and Indonesians. The tree was estimated from the distance matrix of the proportion of nucleotide differences. The letter A indicates the Polynesian/Pacific cluster. The numbers on the branches are confidence probability values (Kumar et al. 1993).

trees (Castelloe and Templeton 1994), while sharing of sequences located at the tips or the top of the trees is more likely explained by admixture (Shields et al. 1993). We performed phylogenetic analyses (neighbor-joining method with several distances [Kumar et al. 1993]) by using sequences from both data sets (HVS-I; HVS-I + HVS-II). We found one more case of a shared sequence between Polynesians and Amerindians, beyond the two already described (Sykes et al. 1995), and in all three cases the shared sequences fall in nodal (basal) positions in the phylogenetic trees, nodes that originate both Asian and Amerindian descendent sequences. The Tahitian lineage 47 (Sykes et al. 1995) is identical to an Argentine Mapuche (Ginther et al. 1993), a Central American Kuna (Batista et al. 1995), a Mongolian (Kolman et al. 1996), and a Siberian Chukchi (Shields et al. 1993). The B lineage Tongan 33 HVS-I sequence (Redd et al. 1995) is identical to one of the two most frequent Amerindian sequences and occurs in the three American

Table 1

Expansion and Divergence Time Estimates for Polynesians and Amerindians (B Lineage only), on the Basis of mtDNA Control Region Sequences: HVS-I, and HVS-I plus HVS-II Combined

Population(s) and mtDNA Region	*n*	Time before Present[a] (years)	95% Confidence Interval[b]
		Expansion	
Polynesians:			
HVS-I	25	7,900	400–15,400
HVS-I and HVS-II	25	7,200	2,100–12,400
Amerindians:			
HVS-I	46	21,000	8,900–33,300
HVS-I and HVS-II	24	21,000	7,900–34,000
		Divergence	
Amerindians vs. Polynesians:			
HVS-I	71	28,800	13,100–44,500
HVS-I and HVS-II	49	34,400	16,000–52,900

[a] Times were estimated assuming the following substitution rates: 15% (standard error [SE] 4%) per million years for HVS-I (Ward et al. 1991) and 11.5% (SE 3%) per million years for both HVS-I and HVS-II (Stoneking et al. 1992).

[b] The estimates of the 95% confidence intervals include the error in the estimates of the mutation rate and tau (Redd et al. 1995); the SE of tau was calculated by jackknifing (Efron 1982) the sequences.

subcontinents. However, the HVS-II sequence of this Tongan is different from all HVS-II Amerindian sequences described thus far. The last case of sharing is the Cook Island lineage 45 (Sykes et al. 1995). This sequence is different from all of the completely sequenced HVS-I Amerindians (~600 individuals) but is identical to a Chilean Mapuche (Horai et al. 1993), whose first 100 bp of the HVS-I were not sequenced. If we disregard these first 100 bases for all other known human sequences, this sequence is also identical to a North American Athapascan (Shields et al. 1993), two Siberians (Torroni et al. 1993*b*), a Mongolian (Kolman et al. 1996), and one Asian from Tibet (Torroni et al. 1993*a*). Therefore, all the shared sequences between Polynesians and Amerindians are much more likely explained as a retention of ancestral Asian sequences by both descendant populations than by an ancestral or recent post-divergence admixture.

If ancient contact between Polynesia and the Americas occurred, then a phylogenetic tree of mtDNA sequences should result in an intermingling of sequences from the Americas with those from Polynesia. In all phylogenies, the non-B lineage sequences of Amerindians clustered well apart from those of Polynesians, except for the three matching sequences discussed above. The B lineage sequences of Polynesians clustered in a separate exclusively Polynesian/Pacific cluster (see the A cluster in fig. 1). The Tongan sequence that did not cluster with the Polynesians in the HVS-I + HVS-II data set tree (see fig. 1) is likely an ancestral sequence, as explained above; or, alternatively, this Polynesian sample may be an example of recent Asian admixture. Although Easter Island is geographically closest to South America, Easter Islander sequences fell within the Pacific cluster (phylogeny not shown).

Mismatch distribution analysis (Sherry et al. 1994) of the CR sequences provides further evidence of a distant relationship between Polynesian and Amerindian populations. Population expansion and divergence times for the Polynesian and Amerindian samples (only B lineage sequences were considered) were calculated for the two data sets. The results (table 1) indicate that Polynesian populations have a much more recent origin than Amerindian populations and that the two diverged ~30,000 years ago. This divergence estimate is considerably older than the date of 3,500 years ago associated with early Polynesian archaeological sites (Bellwood 1989).

In conclusion, the presence of the B lineage and the matching of three other sequences between Polynesians and Amerindians probably reflect a shared Asian origin rather than direct contact. Nevertheless, these results do not rule out the possibilities of still-undetected minor contact and nonmaternal genetic exchange.

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Acknowledgments

This work was funded by Financiadora de Estudos e Projetos, Conselho Nacional de Desenvolvimento Científico e Tecnológico, and Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior in Brazil, as well as by National Science Foundation grant BNS 90-20567 in the United States.

References

Anderson S, Bankier AT, Barrrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, et al (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457–465

Batista O, Kolman CJ, Bermingham E (1995) Mitochondrial DNA diversity in the Kuna Amerinds of Panamá. Hum Mol Genet 4:921–929

Bellwood PS (1989) The colonization of the Pacific: some current hypotheses. In: Hill AVS, Sergeantson SW (eds) The colonization of the Pacific: a genetic trail. Oxford University Press, New York, pp 1–59

Cann, RL (1994) mtDNA and Native Americans: a southern perspective. Am J Hum Genet 55:7–11
Castelloe J, Templeton AR (1994) Root probabilities for intraspecific genes trees under neutral coalescent theory. Mol Phylogenet Evol 3:102–113
Efron B (1982) The jackknife, the bootstrap, and other resampling plans. Society of Industrial and Applied Mathematics, Philadelphia
Ginther C, Corach D, Penacino GA, Rey JA, Carnese FR, Hutz MH, Anderson A, et al (1993) Genetic variation among the Mapuche Indians from the Patagonian region of Argentina: mitochondrial DNA sequence variation and allele frequencies of several nuclear genes. In: Penna SDJ, Chakraborty R, Epplen JT, Jeffreys AJ (eds) DNA fingerprinting: state of the science. Birkhäuser, Basel, pp 211–219
Hagelberg E, Clegg JB (1993) Genetic polymorphisms in prehistoric Pacific islanders determined by analysis of ancient bone DNA. Proc R Soc Lond B Biol Sci 252:163–170
Hagelberg E, Quevedo S, Turbon D, Clegg JB (1994) DNA from ancient Easter Islanders. Nature 369:25–26
Horai S, Kondo R, Nakagawa-Hattori Y, Hayashi S, Sonoda S, Tajima K (1993) Peopling of the Americas, founded by four major lineages of mitochondrial DNA. Mol Biol Evol 10:23–47
Kolman CJ, Bermingham E, Cooke R, Ward RH, Arias TD, Guionneau-Sinclair F (1995) Reduced mtDNA diversity in the Ngöbé Amerinds of Panamá. Genetics 140:275–283
Kolman CJ, Sambuughin N, Bermingham E (1996) Mitochondrial DNA analysis of Mongolian populations and implications for the origin of New World founders. Genetics 142: 1321–1334
Kumar S, Tamura K, Nei M (1993) MEGA, version 1.01. Pennsylvania State University, University Park
Lum JK, Rickards O, Ching C, Cann RL (1994) Polynesian mitochondrial DNAs reveal three deep maternal lineage clusters. Hum Biol 66:567–590
Melton T, Peterson R, Redd AJ, Saha N, Sofro ASM, Martinson J, Stoneking M (1995) Polynesian genetic affinities with Southeast Asian populations as identified by mtDNA analysis. Am J Hum Genet 57:403–414
Redd AJ, Takezaki N, Sherry ST, McGarvery ST, Sofro ASM, Stoneking M (1995) Evolutionary history of the COII/tRNA^{Lys} intergenic 9 base pair deletion in human mitochondrial DNAs from the Pacific. Mol Biol Evol 12:604–615
Santos M, Ward RH, Barrantes R (1994) mtDNA variation in the Chibcha Amerindian Huetar from Costa Rica. Hum Biol 66:963–977
Sherry ST, Rogers AR, Harpending H, Soodyall H, Jenkins T, Stoneking M (1994) Mismatch distributions of mtDNA reveal recent human population expansions. Hum Biol 66: 761–775
Shields GF, Schmiechen AM, Frazier BL, Redd A, Voevoda MI, Reed JK, Ward RH (1993) mtDNA sequences suggest a recent evolutionary divergence for Beringian and northern North American populations. Am J Hum Genet 53:549–562
Stoneking M, Sherry ST, Redd AJ, Vigilant L (1992) New approaches to dating suggest a recent age for the human mtDNA ancestor. Philos Trans R Soc Lond B 337:167–175
Sykes B, Leiboff A, Low-Beer J, Tetzner S, Richards M (1995) The origin of the Polynesians: an interpretation from mitochondrial lineage analysis. Am J Hum Genet 57:1463–1475
Torroni A, Schurr TG, Cabell MF, Brown MD, Neel JV, Larsen M, Smith DG, et al (1993*a*) Asian affinities and continental radiation of the four founding Native American mtDNAs. Am J Hum Genet 53:563–590
Torroni A, Sukernik RI, Schurr TG, Starikovskaya YB, Cabell MF, Crawford MH, Comuzzie AG, et al (1993*b*) mtDNA variation of aboriginal Siberians reveals distinct genetic affinities with Native Americans. Am J Hum Genet 53:591–608
Ward RH, Frazier BL, Dew-Jager K, Pääbo S (1991) Extensive mitochondrial diversity within a single Amerindian tribe. Proc Natl Acad Sci USA 88:8720–8724
Ward RH, Redd A, Valencia D, Frazier B, Pääbo S (1993) Genetic and linguistic differentiation in the Americas. Proc Natl Acad Sci USA 90:10663–10667
Ward RH, Salzano FM, Bonatto SL, Hutz MH, Coimbra CEA Jr, Santos RV. Mitochondrial DNA polymorphism in three Brazilian Indian tribes. Am J Hum Biol (in press)

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0002-9297/96/5901-0033$02.00

Am. J. Hum. Genet. 59:256–258, 1996

Mitochondrial Myopia: Reply to Bonatto et al.

To the Editor:

Bonatto et al. propose a phylogenetic test that they claim definitively excludes the possibility that sharing of maternal genetic lineages between Pacific Islanders and some Native Americans, assayed by mtDNA sequencing, could be due to direct contact. Their analysis rests on (1) an arbitrarily narrow identification of Polynesian maternal genotypes, which omits known (Lum et al. 1994; Sykes et al. 1995) authentic mtDNA lineages in Hawaii, Samoa, and the Cook Islands; (2) an overly optimistic expectation that the tree-building method chosen will, with statistical confidence, reveal the true genetic affinities of the lineages examined; and (3) an assumption that mismatch distributions include relevant populations in the Pacific and the Americas. Serious difficulties surround each of these conditions. Further, they admit that their analysis cannot exclude sex-biased dispersal, which could be tested with additional nuclear genetic markers (specifically, short-tandem-repeat or Y-chromosome haplotypes) and sequences from viral isolates, such as HTLV-1 (Miura et al. 1994).

The use of the "Polynesian" motif to represent Polynesians is misleading. Lineages including the three substitutions at nucleotides 16217, 16247, and 16261 are found