Mitochondrial DNA Polymorphisms in Chilean Aboriginal Populations: Implications for the Peopling of the Southern Cone of the Continent

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ABSTRACT The mitochondrial DNAs (mtDNAs) from individuals belonging to three Chilean tribes, the Mapuche, the Pehuenche, and the Yaghan, were studied both by RFLP analysis and D-loop (control region) sequencing. RFLP analysis showed that 3 individuals (1.3%) belonged to haplogroup A, 19 (8%) to haplogroup B, 102 (43%) to haplogroup C, and 113 (47.7%) to haplogroup D. Among the 73 individuals analyzed by D-loop sequencing, we observed 37 different haplotypes defined by 52 polymorphic sites. Joint analysis of data obtained by RFLP and sequencing methods demonstrated that, regardless of the method of analysis, the mtDNA haplotypes of these three contemporary South American aborigine groups clustered into four main haplogroups, in a way similar to those previously described for other Amerindians. These results further revealed the absence of haplogroup A in both the Mapuche and Yaghan as well as the absence of haplogroup B in the Yaghan. These results suggest that the people of Tierra del Fuego are related to tribes from south-central South America. Am J Phys Anthropol 113:19–29, 2000.

The analysis of mitochondrial DNA variation has been extensively used in the recent past, for genetic characterization of contemporary aboriginal populations of the Americas. The primary goals of this analysis have been to determine the origins, relationships, and migrational patterns of New World populations. In this respect, the study of the frequency distribution of the four founding Amerindian haplogroups, suggested by Schurr et al. (1990) and defined by Torroni et al. (1992), has proven useful.

The majority of these studies have focused on aboriginal populations from North and Central America. Of those studies conducted with South American groups, almost all have involved populations from the northern region of the continent (Torroni et al., 1993; Easton et al., 1996; Santos et al., 1996). Most of the data generated from these studies were obtained through RFLP

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mapping, rather than by D-loop sequencing, although some D-loop sequence data from these populations are available for analysis. Similarly, the mtDNAs taken from southern South American groups were primarily characterized by RFLP analysis (Merriwether et al., 1995; Lalueza et al., 1997). Thus, there remains limited D-loop sequence information from groups inhabiting the southern part of South America.

Studies of South Amerindians using RFLP and D-loop sequence analysis have revealed a concordance between the distribution of haplogroups A–D, and haplogroups (lineage clusters) I–IV as defined by Horai et al. (1993), based on the nucleotide polymorphisms occurring in the D-loop of each haplogroup (Torroni et al., 1993; Easton et al., 1996; Santos et al., 1996). However, Santos et al. (1996) found that 7% of individuals belonging to tribes from the Brazilian Amazon Region comprise unusual haplogroups because of their lack of correspondence between RFLP and sequencing analysis. There is also extensive information referring to RFLP analysis in these populations. In addition, Bailliet et al. (1994) described a subdivision of the classical Amerindian haplogroups A, C, and D on the basis of the presence or absence of the *Hae*III np 16517 site. This division has led to the creation of haplotypes A1/A2, C1/C2, and D1/D2. However, the *Hae*III np 16517 site has been shown to be highly polymorphic in African (Chen et al., 1995), European (Torroni et al., 1996), and Asian (Ballinger et al., 1992) populations, indicating that it may not be useful for distinguishing within Native American haplogroups A, C, and D. Similarly, Forster et al. (1996) also postulated subdivisions of founding haplotypes, using D-loop sequence data. In this case, they divided haplotypes A1 and A2 by a C→T transition at np 16111 and defined D1 and D2 haplotypes by the presence or absence of the 16271 T→C transition, with this transition being present only in North American Indian mtDNAs. Hence, there would appear to be specific nucleotide changes in the D-loop, which distinguish between North-Central and South American populations.

To expand our knowledge about the extent of mtDNA variation in southern South America and to test the hypothesis of a single wave of migration to the southern part of South America, we performed RFLP and sequence analysis on three Chilean aboriginal populations (the Pehuenche, the Mapuche, and the Yaghan).

MATERIALS AND METHODS

Population samples

The samples include three different Chilean aboriginal populations: 105 Pehuenche from the community of Trapa Trapa in the Province of Bio Bio, 8th region (37° 43′ S; 71° 16′ W), 111 Mapuche from the Huapi Island, Province of Valdivia, 10th region (40° 15′ S; 72° 25′ W), and the 21 last surviving Yaghan from Puerto Williams, Navarino Island, Province of Antartica, 12th region (55° S; 67° 40′ W). The three groups analyzed correspond to Amerinds showing a percentage of admixture between 2–13% (Llop et al., 1993, 1994). The geographic locations of the subjects are shown in Figure 1.

DNA extraction and PCR amplification

Total DNA was prepared from peripheral blood lymphocytes (Gustincich et al., 1991). DNA amplification of the four polymorphic mtDNA regions, defining the four Amerindian haplogroups, was carried out by using the following specific primers:

Haplogroup A:
L604 GTAGCTTACCTCCTCAAAGCAA
H708 AGGGTGAACTCACTGGAACG
Haplogroup B:
L8214 CACAGTTTCATGCCCATCGT
H8294 ATGCTAAGTTAGCTTTACAGTGG
Haplogroup C:
L13232 CGCCCTTACACAAAATGACATCAA
H13344 GGAGCACATAAATAGTATGGC
Haplogroup D:
L5120 CCTAACTACTACCGAATTCCTA
H5255 ATTCTTCGATAATGGCCCATTTG

PCR was performed in a final volume of 50 μl, containing 300 ng genomic DNA, 1 U Taq DNA polymerase (Promega), 25 pmoles of each primer, 200 nM of each deoxinucleotide, and the appropriate buffer. Samples

Fig. 1. Map of Chile, showing the geographic location of the three Chilean tribes included in the present study.

were processed under the following PCR conditions: 1 cycle at 95°C for 5 min, followed by 30 cycles at 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and finally one cycle at 72°C for 5 min. Haplotypes A, C, and D were analyzed by restriction digestion, using *Hae*III for haplotype A, *Hinc*II for haplotype C, and *Alu*I for haplotype D.

The resulting restriction fragments and the PCR product including region V, defining haplotype B, were analyzed by electrophoresis on 3% NuSieve-Agarose (2:1) (FMC BioProducts) gels. PCR amplification of the D-loop region was carried out with two specific primers, spanning a region of 1,021 bp: L15997, CACCATTAGCACCCAAAGCT; and H408, CTGTTAAAAGTGCATACCGCCA.

Direct sequencing of PCR products

DNA sequencing was carried out by using the PCR dsDNA Cycle Sequencing System (Life Technologies), with the primers L29, GGTCTATCACCCTATTAACCAC; and H16401, CACCATCCTCCGTGAAATCA, labeled at the 5′ end with γ-^{32}P-ATP. DNA sequence analysis was performed by electrophoresis on high-resolution 6% polyacrylamide gels, followed by autoradiography.

Data analysis

Sequence alignment was performed by using the Clustal W computer program (Thompson et al., 1994). Substitutions and deletions were inferred from direct sequence comparisons. The phylogenetic trees were obtained through two different methods: neighbor joining (Saitou and Nei, 1987), using the Clustal W program, and parsimony analysis (Swofford and Olson, 1990). Maximum parsimony trees were generated through random addition of sequences using the tree bisection and reconnection (TBR) algorithm, with the PAUP (Swofford, 1993) computer program.

RESULTS

RFLP analysis

All individuals analyzed showed one of the characteristic haplogroups, A, B, C, or D. As shown in Table 1, haplogroups C and D were the most frequent in the three populations, appearing at very similar frequencies in all of them. Haplogroup A was present in the Pehuenche at a very low frequency (2.8%), while both haplogroups A and B were absent in the Yaghan group. We tested the gain of the *Hae*III site at position 16517 only in individuals belonging to haplogroup B, since this is a specific marker to differentiate Amerindians from non-Amerindian B individuals (Torroni et al., 1992, 1993). The gain of the *Hae*III site was present in all individuals exhibiting haplogroup B, including 8 Mapuche and 11 Pehuenches.

A 3 $\times$ 4 contingency table analysis suggests that in terms of haplogroup frequencies, these three populations cannot be statistically distinguished (chi-square = 6.78, exact P-value = 0.32 with 10,000 permutations). However, in terms of haplotype diversity (Nei, 1978) defined by these four haplogroups, the Yaghan have the lowest diversity (52.4%), and the Pehuenche the

TABLE 1. MTDNA haplogroup distribution in three Chilean aboriginal populations

Populations	N	Haplogroups (%) A	B	C	D
Mapuche	111	0.0	7.2	44.1	48.7
Pehuenche (Trapa-Trapa)	105	2.8	10.5	41.0	45.7
Yaghan	21	0.0	0.0	47.6	52.4
Total	237	1.3	8.0	43.0	47.7

highest (61.7%), with the Mapuche being intermediate (56.8%), the differences being statistically insignificant ($P = 0.11$).

Sequence analysis

The two hypervariable regions of the D-loop were analyzed by direct sequencing of the DNA from 73 individuals from the three native populations, which were representative of the four haplogroups described previously. As shown in Table 2, 37 different haplotypes were observed, defined by 52 polymorphic sites. It is important to note that two haplotypes shared by individuals from different populations are shown separately. This gives a total of 40 lines in Table 2, corresponding to only 37 haplotypes. In haplotype C, lineages PE3C, YA4C, and HU4C are the same, as well as YA2C and HU1C.

Only nucleotide changes at np 73 and np 263 are shared by all haplogroups. One of these, transition A→G at position 73, has also been described in all individuals from an Argentinean Mapuche group (Ginther et al., 1993), as well as in half of the individuals from a Huétar sample of Costa Rica (Santos et al., 1994). Two types of adenine deletions, an A at np 248 and an AA at 286–291, were found at hypervariable region II (HVS-II). These A deletions have been found in all haplogroup C individuals from the three Chilean aboriginal populations, as well as the Argentinean Mapuche sample (Ginther et al., 1993). Since these A deletions have not been described for other populations (Handt et al., 1998), they may be characteristic nucleotide changes at least for South American aboriginal populations.

In these three Chilean groups, as with haplogroup diversity, we found the highest sequence diversity in the Pehuenche sample, showing 38 out of 52 total polymorphic sites at the D-loop, amounting to 93.2% sequence diversity. The Yaghan, likewise, showed the smallest sequence diversity of 91.4%, although this was not significantly smaller than the others.

Disregarding the phylogenetic relationships of the observed sequences, at the sequence level, the Yaghan appear to be almost 3–5 times as distant from the Pehuenche and the Mapuche, (the standard distance of Nei (1978) being 0.31 between the Pehuenche and the Mapuche, 1.55 between the Pehuenche and the Yaghan, and 1.09 between the Mapuche and the Yaghan). The contingency table analysis of sequence-lineage frequency differences suggests that the genetic distances among these three populations are statistically significant (chi-square = 15.12, $P < 10^{-5}$, with 10,000 permutations).

Haplogroup D was the most diverse at the sequence level, revealing 30 polymorphic sites, in contrast to haplogroups A with 13 changes, B with 14 changes, and C with 16 changes. We also found that, from all individuals classified in the four haplogroups A–D (Torroni et al., 1992), only haplogroups A–C corresponded perfectly to 3 of the 4 clusters described by Horai et al. (1993). The T nucleotide at position 16290 and A 16319 define haplogroup A/cluster III. Haplogroup B/cluster I is defined by C 16189 and C 16217. Haplogroup C/cluster IV is defined by a C 16298 and T 16327.

Regarding haplogroup D/cluster II, C nucleotides at position 16325 and 16362 have been described by Horai et al. (1993) as characteristics of cluster II, since they were shared by 72 native Americans, including 45 Chilean aborigines. From our study, one Mapuche individual lacked the 16362 T→C transition, and the other 13 individuals belonging to the three Chilean populations did not show the characteristic 16325 T→C transition. Hence, our results do not corrob-

TABLE 2. Polymorphic mtDNA D-loop sites in a sample of Chilean aborigines[1]

16086	16092	16111	16129	16153	16187	16189	16207	16209	16213	16217	16218	16223	16234	16241	16249	16270	16286	16290	16291	16298	16301	16304	16311	16319	16325	16327	16342	16343	16362	53	55	56	64	73	77	94	129	143	146	150	152	153	195	207	215	234	248	258	263	286–291		Anderson: Individuals	Anderson: Lineages
T	T	C	G	G	C	T	A	T	G	T	C	C	C	A	T	C	C	C	C	T	C	T	T	G	T	C	T	A	T	G	T	A	C	A	A	G	T	G	T	C	T	A	T	G	A	A	A	C	A	A	A		
·	·	T	A	·	·	·	·	·	·	·	·	T	·	·	·	·	·	T	·	·	·	·	·	A	·	·	·	·	C	·	·	·	T	G	·	·	·	·	C	·	·	G	C	·	·	G	·	·	G	·	·	3	PE1A
·	·	·	·	·	·	C	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	A	·	·	·	·	G	·	·	2	PE6B
·	·	·	·	·	·	C	·	·	·	C	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	A	·	·	·	·	G	·	·	1	PE5B
·	·	·	·	·	·	C	·	·	·	C	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	C	·	·	A	·	·	·	·	G	·	·	1	PE4B
·	·	·	·	·	·	C	G	·	·	C	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	C	·	·	·	·	·	·	G	·	·	1	PE1B
·	·	·	·	·	·	C	G	·	·	C	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	4	HU1B
·	·	·	·	·	·	C	G	·	·	C	·	·	·	·	C	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	3	HU3B
·	·	·	·	·	·	C	G	·	·	C	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	G	·	·	·	·	·	·	·	·	·	·	·	G	·	·	1	HU2B
·	·	·	·	·	·	C	·	·	A	C	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	1	PE2B
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·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	C	·	·	C	·	C	T	·	G	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	—	T	G	—	—	1	YA3C
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·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	C	·	·	·	·	C	T	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	—	T	G	—	—	1	**YA4C**
·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	C	·	·	·	·	C	T	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	—	T	G	—	—	7	**HU4C**
·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	C	·	·	·	·	C	T	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	—	·	G	—	—	2	**YA2C**
·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	C	·	·	·	·	C	T	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	—	·	G	—	—	3	**HU1C**
·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	C	·	·	·	·	C	T	·	·	·	·	·	·	·	G	·	·	·	·	·	T	·	·	·	·	·	·	·	—	·	G	—	—	1	HU2C
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·	C	·	·	·	T	C	·	·	·	·	·	T	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	G	·	·	·	A	·	·	·	·	·	·	·	·	·	·	G	·	·	3	HU5D
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·	·	·	·	A	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	C	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	2	HU8D
·	·	·	·	·	T	C	·	·	·	·	·	T	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	1	HU3D
C	·	·	·	·	T	C	·	·	·	·	·	T	·	·	·	·	T	·	·	·	·	·	·	·	C	·	·	·	C	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	4	YA2D
C	·	·	·	·	T	C	·	·	·	C	·	T	·	·	·	·	T	·	·	·	·	·	·	·	C	·	·	·	C	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	1	YA1D

[1] The number of individuals for each lineage is shown. In the right column each lineage is named by its corresponding population (PE, Pehuenche; HU, Mapuche (Huapi Island); YA, Yaghan) the sample number, and the RFLP haplogroup. Individuals highlighted in bold share the same sequence and belong to different groups (PE3C, YA4C, HU4C, and YA2C, HU1C). The sequence published by Anderson et al. (1981) was used as a reference.

orate the classification of haplogroup D/cluster II on the basis of these mutations, but are in agreement with the results of Ginther et al. (1993), who also observed the absence of the 16325 T→C mutation in haplogroup D mtDNAs from the Argentinean Mapuche group.

It is interesting to note that one of these nucleotide changes, C 16325, is present in all individuals of haplogroup C/cluster IV from our study, in the Argentinean Mapuche, and in Amazonian individuals (Santos et al., 1996). Also, in our study, C in position 16362 is present in individuals from cluster III/haplogroup A. Therefore it seems that there are no characteristic and exclusive nucleotide changes in either hypervariable region defining haplogroup D, at least for these aboriginal populations from South America. However, we found a C→T change at nucleotide 16187 which is present in the majority of South Amerindians of haplogroup D, which is absent from haplogroups A–C. This change is also present in 6/10 Argentinean Mapuche (Ginther et al., 1993), and in 14/18 native Americans (Horai et al., 1993). From this analysis, we suggest that this change is the most characteristic one for South Amerindian populations.

Phylogenetic analysis

The D-loop DNA sequences, spanning hypervariable regions I and II from the 37 Chilean lineages, were aligned and submitted to phylogenetic analysis through neighbor-joining (NJ) (Fig. 2) and parsimony methods (data not shown). In the NJ tree, we can see that all lineages fall into four differentiated branches, including haplogroups A–D. Nevertheless, it is noteworthy that three D lineages, HU8D, HU9D, and YA4D, cluster close to the C branch, while HU10D and YA3D cluster close to branch A. All the other D lineages fall into one clade.

The majority rule consensus (data not shown) shows that two branches B and C cluster independently in 100% of maximum parsimony trees. Seven out of 19 lineages corresponding to haplogroup D cluster in one branch, with 74 % consensus from a total of 500 maximum parsimony trees. The remaining lineages, also corresponding to haplogroup D, appeared unresolved in this analysis. D-loop sequences from the 3 Pehuenche individuals leading to haplogroup A correspond to one lineage, clustering obviously in one branch of 100% consensus.

DISCUSSION

In the present paper we report on the results of a mtDNA RFLP and sequence polymorphism analysis of three Chilean aboriginal populations. The RFLP analysis showed that all individuals belong to one of the previously defined Amerindian haplogroups, A, B, C, or D. In terms of haplogroup frequencies, the three Chilean aboriginal groups studied here cannot be genetically distinguished. However, as shown in Table 3, combining the present data with other published haplogroup frequencies of South American aborigines, it is noteworthy that the frequencies of haplogroups A and B decrease from north to south in meridian South America, whereas haplogroups C and D tend to increase. This trend is most likely the result of founder effect, which occurred during the initial peopling of the southern cone of the continent. As Paleoindians moved south, new territory was probably colonized by small bands that were carriers of a subsample of genes from the ancestral populations they left behind. Haplogroups A and B probably got lost as a consequence of this process. An alternative hypothesis, e.g., gene flow from migrant populations carrying A and B into more ancient groups possessing C and D, would imply that more than one migration event occurred during the peopling of the southern cone of South America. This hypothesis is not supported by recently published gene geography maps of South America (Rothhammer and Silva, 1992; Rothhammer et al., 1997).

Turning now to the sequence data, we note that the three Chilean aboriginal groups, can be genetically distinguished at the D-loop sequence level, making the Yaghan most distant (as well as least diverse) from the Pehuenche and the Mapuche.

Further, the nucleotide changes described by Forster et al. (1996), including one base substitution at nucleotide 16111 leading to subhaplogroups A1/A2, and another substitution at 16271 defining subhaplogroups D1/D2, have been tested in the present

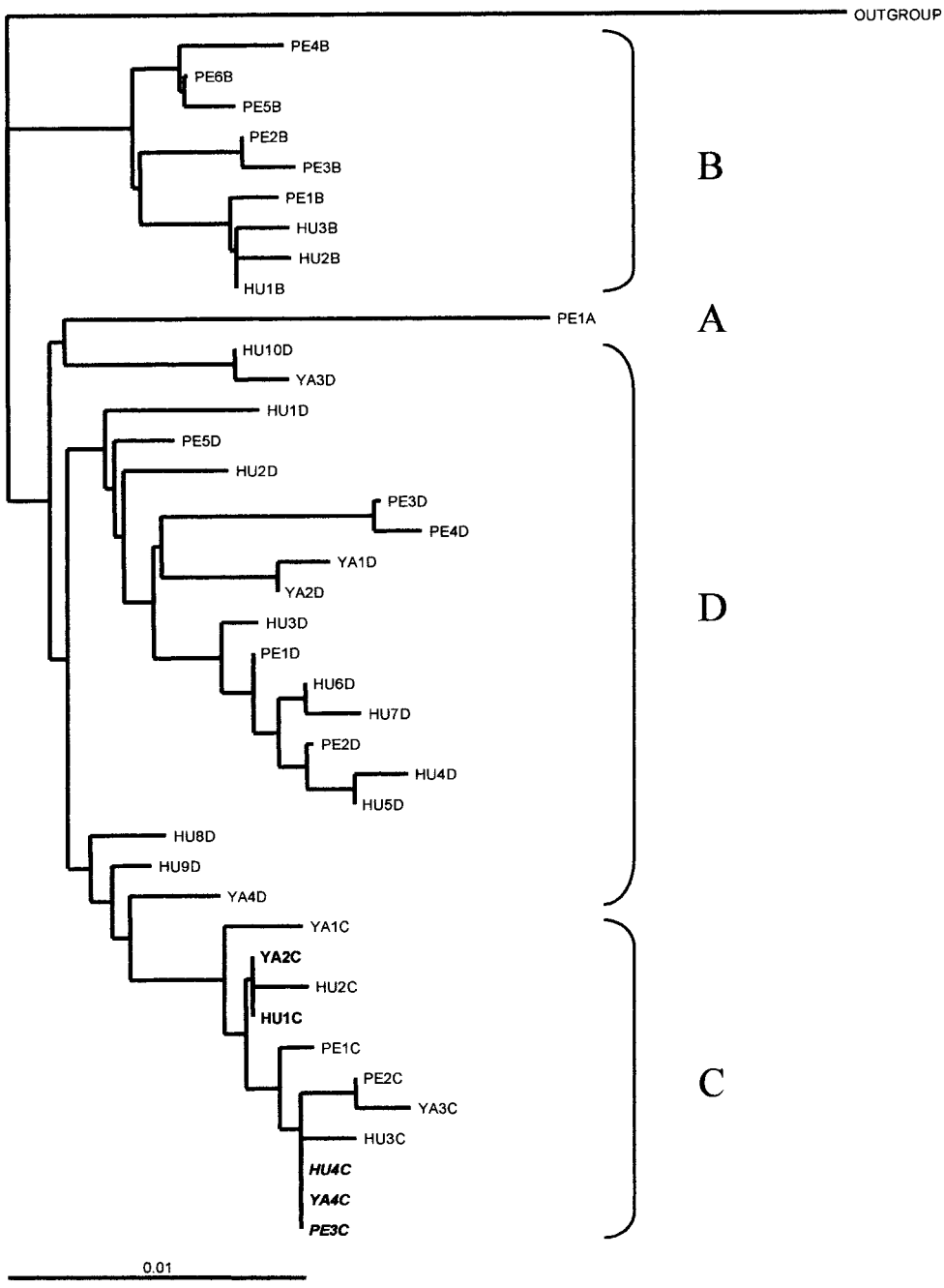

Fig. 2. Tree obtained by the neighbor-joining method for 37 Chilean haplogroups. Individuals highlighted in bold share the same sequence and belong to different groups (PE3C, YA4C, HU4C, and YA2C, HU1C). An African sequence was used as an outgroup in order to root the tree. The alignment was performed over 549 bp, including hypervariable regions I and II. Letters on the side indicate respective RFLP haplogroups.

study (Table 2). All individuals from haplogroup A did have the C→T transition at 16111, and therefore are subhaplotype A2 of Forster et al. (1996). Indeed, all haplotype D individuals lacked the T→C transition and, therefore, are haplotype D1.

TABLE 3. Frequency distribution of founding haplogroups in aboriginal populations of Chile and Argentina[1]

Populations	N	Haplogroups A	B	C	D	Others	References
Aymara	172	0.06	0.68	0.12	0.14		Merriwether et al., 1995
Atacameño	50	0.12	0.72	0.10	0.06		Merriwether et al., 1995
Whichi	72	0.06	0.63	0.03	0.26	0.03	Bravi et al., 1995
Chorote	20	0.15	0.40	0.30	0.15		Bravi et al., 1995
Mapuche 1	58	0.05	0.31	0.21	0.30	0.14	Ginther et al., 1993
Mapuche 2	111		0.07	0.44	0.49		This study
Pehuenche 1	100	0.02	0.09	0.37	0.52		Merriwether et al., 1995
Pehuenche 2	105	0.03	0.10	0.41	0.46		This study
Huilliche	80	0.04	0.28	0.19	0.49		Merriwether et al., 1995
Tehuelche	29		0.21	0.24	0.55		Bravi (pers. Comm.)
Fueguian	45			0.42	0.56	0.02	Lalueza et al., 1997
Yaghan	21			0.48	0.52		This study

[1] Populations are listed from top to bottom in order of distribution along Chile and Argentina, from northernmost to southernmost.

Comparison of the two hypervariable D-loop regions reveals a high variability of haplogroup D, in comparison to the other three haplogroups, and especially with haplogroup C, including a similar number of individuals, as shown in Table 2. This variability is expressed by 19 different lineages defined by 30 polymorphic sites. We were unable to find any nucleotide change shared by 100% of D individuals, as described previously by Horai et al. (1993) in a similar sample. Nevertheless, our results indicate a C→T 16187 change which is present in the majority of D individuals, and is absent in haplogroups A–C (Table 2), concordant with lineages found by Ginther et al. (1993) in an Argentinean Mapuche sample, as well as Chilean lineages described by Horai et al. (1993). However, this change seems to be exclusive to Chilean and Argentinean southern aborigines, since it is not present in other populations from South America.

As shown in Figure 3, it is noteworthy that 26/26 individuals from this study belonging to haplogroup C, two lineages from Chilean aborigines described by Horai et al. (1993), and 7/8 Argentinean aborigine individuals (Ginther et al. 1993), are grouped in one cluster. This extreme low diversity within haplogroup C for southern Chilean and Argentinean groups could also be explained by a founder effect.

Comparing our findings with other Amerindian mtDNA sequences from South America, we found that the retrieved sequences of the three Chilean groups cluster into the four basic mtDNA haplogroups, together with the Amazonian tribes and the Argentinean Mapuche (Fig. 3). These findings are noteworthy in relation to unconventional speculations concerning the origin of the aborigines of Tierra del Fuego. More than half a century ago, it was proposed that people of Tierra del Fuego had direct ancestral links to Australoid populations (Frenguelli, 1963; Imbelloni, 1938). This hypothesis was based on the unusual morphological characteristics, particularly the cranial robustness (supposedly a sign of antiquity), of the aborigines of Patagonia and Tierra del Fuego, that is to say the Ona (Selk'nam), Yaghan (Yamana), and Alacaluf (Kaweskar).

In the interim, a consensus among anthropologists was reached in the sense that Amerinds were derived from Mongoloids who, moving from Asia, crossed the Bering Land Bridge as suggested by Hrdlicka (Frenguelli, 1963). There are, however, still anthropologists with different views. Recently, on the basis of the statistical manipulation of craniometric means, Neves and Pucciarelli (1991) suggested the existence of a pre-Mongoloid migration to South America, coming close to reviving the old ideas of Imbelloni (1938) in a more current context.

In contrast, our results indicate that the Yaghan do not exhibit divergent D-Loop sequences, clustering together with the corresponding haplogroup sequences from other South Amerindian populations (Fig. 3), and presenting similar haplogroup frequencies compared to the Mapuche and Pehuenche. In other words, our data do not support the hypothesis that the Yaghan descend from a different Paleoindian migration lacking haplogroups A and B, as suggested by Lalu-

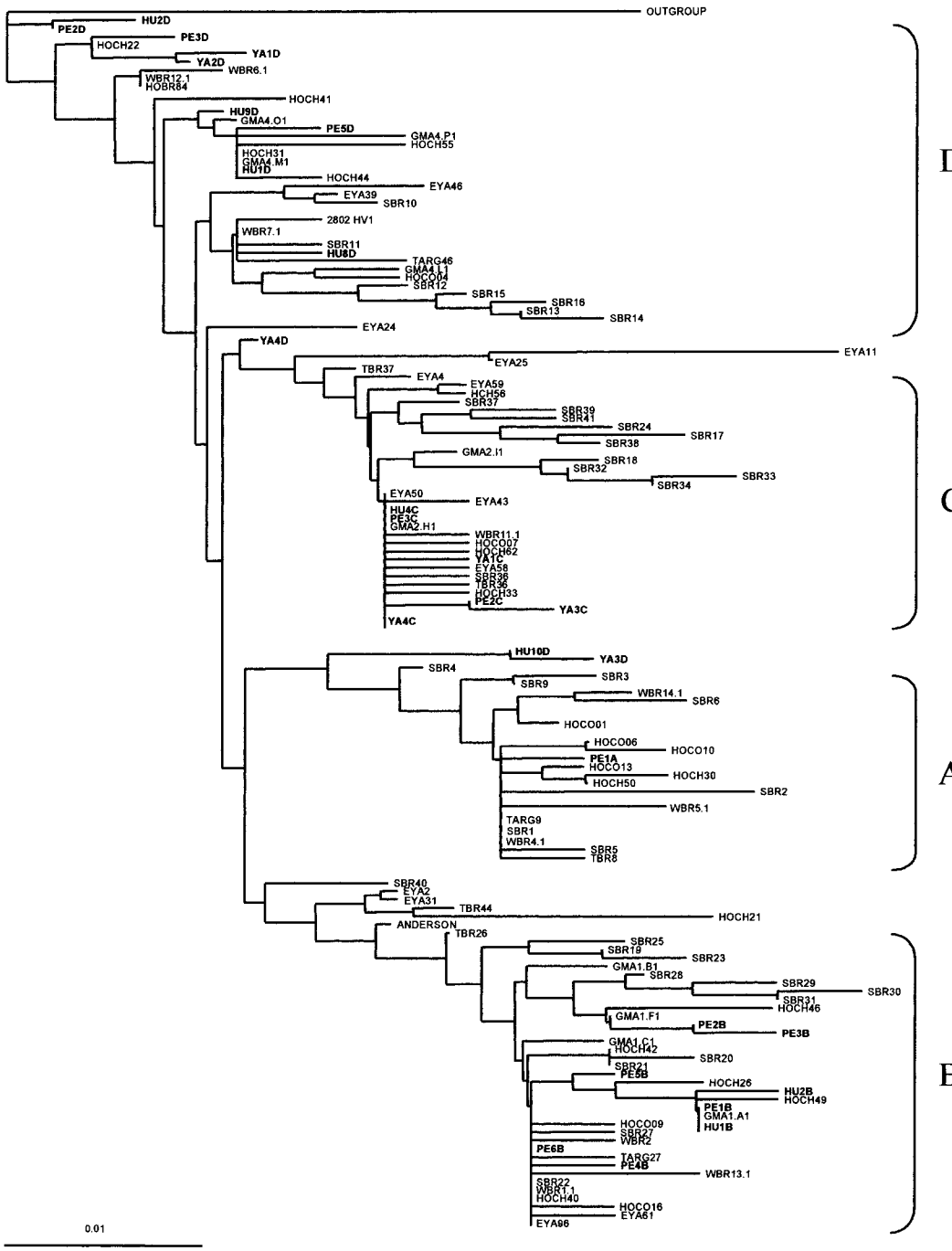

Fig. 3. Twenty-seven distinct Chilean haplogroups, as well as 105 previously published Amerindian lineages from 9 different South American populations, were analyzed using the neighbor-joining method with a 254-bp fragment, including only the hypervariable region I. An African sequence was used as an outgroup in order to root the tree. Letters on the side indicate respective RFLP haplogroups. PE, Pehuenche; HU, Mapuche (Huapi Island); YA, Yaghan; HOCH, Chilean aborigine; HOCO, Colombian aborigine; HOBR, Brazilian aborigine (Horai et al., 1993); WBR, Brazilian aborigine (Handt et al., 1998); GMA, Argentinean Mapuche (Ginther et al., 1993); SBR, Brazilian aborigine (Santos et al., 1996); TARG, Argentinean aborigine; TBR, Brazilian aborigine (Torroni et al., 1993); EYA, Yanomama (Easton et al., 1996).

eza et al. (1997). Our results are rather in agreement with the view that the Yaghan are related to tribes from south-central Chile and Argentina such as the Huilliche, Pehuenche, and Tehuelche, as previously suggested by Rothhammer et al. (1986) and Llop (1996). Furthermore, we note that the haplogroup distribution of the Yaghan is similar to the distribution obtained by Lalueza Fox (1996) for extinct groups from Tierra del Fuego-Patagonia. All samples from these geographic areas lack haplogroups A and B and are therefore most probably descendants of one common ancestral Paleoindian population. In this context, it is interesting to note that Bennett and Bird (1964) reported a complete archeological sequence linking the first Paleoindian migrants to contemporary Ona (Shelknam) populations.

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