mtDNA Microevolution in Southern Chile's Archipelagos

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ABSTRACT The genetic variability of four predominantly Indian populations of southern Chile's archipelagos was examined by determining the frequencies of four mitochondrial DNA haplogroups that characterize the American Indian populations. Over 90% of the individuals analyzed presented Native American mtDNA haplogroups. By means of an unweighted group pair method with arithmetic mean (UPGMA) dendrogram, a principal component analysis (PCA) derived from a distance matrix of mtDNA, and the exact test of population differentiation, we are able to prove the existence of a North-South cline. The populations in the northern part of the archipelagos are genetically similar to the Huilliche tribe, while the groups from the South are most closely related to the Fueguino tribe from the extreme South of Chile, and secondarily to the Pehuenche and Mapuche, who are found to the North and East of Chiloé archipelago. These results are consistent with a colonization of the southern archipelagos from Tierra del Fuego. We evaluate the evolutionary relationships of the population of the Chiloé area to groups from other geographic areas of Chile, using analysis of molecular variance (AMOVA). Three Amerindian clusters are identified: one formed by the Aymará and Atacameño, a second by the Huilliche, and a third including the Mapuche, Pehuenche, and Fueguino tribes, and the population inhabiting the South of the Chiloé arcipelago. These groups exhibit a North-South gradient in the frequency of haplogroup B, confirmed by F_{ST} tests.

During the last decade, a number of laboratories have examined and tried to explain the great diversity of polymorphisms present in the sequence of human mtDNA. Several haplogroups proved to be limited to, or concentrated on, one continent (Torroni et al., 1996; Chen et al., 1995). In Africa, almost all of the sub-Saharan mitochondrial genome belongs to haplogroup L. Haplogroups H, I, J, and K account for over half of the European genome, while over half of the genome in East Asia, including Siberians, harbors haplogroup M. Within this haplogroup, two variants were recognized, the C and D subgroups, which later were identified as two of the four most common haplogroups present in the founders of the New World.

The peopling of America began possibly 22,000 years before present with the crossing of the Bering Strait by one or several populations from northeastern Asia which carried polymorphisms for four common haplogroups called A, B, C, and D (Torroni et al., 1992; Schurr et al., 1990). In southern South America, the frequencies of haplogroups A and B decrease from North to South, while haplogroups C and D increase, due to either founder effects early in the colonization process, or to two or more independent migrations from East Asia (Merriwether et al., 1995; Moraga et al., 2000).

Chile harbors a large number of indigenous groups (Fig. 1), party due to its peculiar geography. The heterogeneity of these groups is reflected in their genetic components. In the extreme North, the Aymará and Atacameño present a high frequency of haplogroup B, whereas in the South, the Pehuenche and Mapuche have mainly haplogroups C and D (Moraga et al., 2000). In the extreme South, haplogroups A and B are completely absent (Lalueza, 1996) in tribes such as the Yámana and Fueguino. The Huilliche tribe falls more or less between these two tendencies, their most common alleles being B and D (Merriwether et al., 1995).

The Chiloé archipelago is an important 500-mile-long corridor (Fig. 1) which is the home of several indigenous groups (Latcham, 1911; Munizaga, 1966; Llop et al., 2000). According to the first chroniclers, during colonial times, the indigenous population was divided into two groups: one in the northern part of the archipelago, the area where the present communities of Carelmapu, Quetalmahue, and Detif are located, and the other in the southern part of the area, which includes Laitec island.

According to these authors, the majority of tribes in the northern group, which most authors have identified as the Veliches or Huilliches of Chiloé (Llop, 1996; Llop et al., 2000; Moreno et al., 2000), were descendants of the sedentary Mapuche tribes established to the North of the Toltén River. In contrast, the southern group was the Chono tribe, which spoke a different language, according to ethnohistorical information (Cárdenas et al., 1991). The small amount of cultural and bioanthropological material recovered from this now extinct tribe has raised doubts as to The origin of the Chonos for over a century. Their use of navigation parallels that of the Qawáshqar and Alacalufes of the southernmost archipelagos.

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Fig. 1. Map of Chile and Argentina, showing distribution of Chilean tribes on eve of Spanish conquest. Geographical locations of characterized populations in Chiloé archipelago are also shown: 1, Carelmapu; 2, Quetalmahue; 3, Detif; 4, Laitec.

Although there is no consensus on whether the native groups that the European conquerors found were descendents of the first inhabitants or belonged to later migrations, several authors proposed that Chiloé was occupied by Huilliche tribes approximately during the 13th century, which obliged the previous inhabitants to migrate farther South. According to this hypothesis, tribes from the Carelmapu area (Fig. 1) occupied the northern and central parts of the archipelago (Latcham, 1928, Cárdenas et al., 1991), while the Chonos moved farther South, reaching the Golfo de Penas (Fig. 1) and occupying the Chonos archipelago, where the Spaniards found them three centuries later.

Due to the prolonged Spanish occupation of Chiloé, having considered it a strategic position for the military control of the Strait of Magellan, and to the later waves of occupation by European colonists (Munizaga, 1978), the native population was gradually confined to the extremes of the archipelago. Similarly, the exploitation of cypresses from the archipelago led to the displacement of indigenous groups that lived in the conglomeration of small islands in the southern part of the Chiloé archipelago (Grebe, 1998) (Fig. 1).

In the middle of the 16th century, the Jesuits obliged most of the Chono tribe to move to the southern part of Chiloé, where they finally became extinct at the beginning of the 19th century, possibly due to an epidemic introduced by the "mestizos" or the Chiloé Huilliches (Hidalgo et al., 1996a).

Not far from these territories lived other human groups with which the people of Chiloé had some contact (Hidalgo et al., 1996b). Among these were Picunche ("people of the North"), conquered by the Incas in the middle of the 15th century, the Huilliche ("people of the South"), and Pehuenche ("people of the pehuén," the fruit of the *Araucaria* tree) on the Andean foothills to the East; all three groups were gatherers (Menghin, 1952). Although the generic term Mapuche ("people of the earth") has been used to refer to all these groups, in this paper we will use the classification of Latcham (1928) that restricts the term only to those groups of the South-Central part of Chile, including the Picunche (Fig. 1).

In contrast to these tribes of gatherers, in the archipelagos located from Chiloé to Tierra del Fuego lived the tribes collectively called Fueguinos, hunting groups who may have descended from the first colonizers of America, and who were subdivided into Yámanas or Yaganes, Alacalufes or Qawáshqar, and Selk'nam or Onas (Steward and Faron, 1959). The archaeological record of these populations indicates that they were hunters or maritime hunter-gatherers.

The purpose of this study is to investigate the ethnic origin of the populations in the southern archipelagos, working back from present populations, and to detect possible migration routes of the ancestral populations that gave rise to the contemporary ethnic structure.

MATERIALS AND METHODS

Samples

The present study includes 47 unrelated residents of Carelmapu (41°45′ South, 73°44′ West), 42 from the village of Quetalmahue (41°45′ South, 73°55′ West), 27 from Detif (42°40′ South, 73°35′ West), and 42 individuals from Laitec (43°24′ South, 73°38′ West). All subjects were informed of the objectives of this study and consented to donate blood samples to be used anonymously in the execution of this nonprofit scientific investigation. The Carelmapu sample represents 1.75% of the population of the locality, the Quetalmahue and Detif represent around 7% each, and the Laitec sample represents 16.5%.

The information gathered in the field regarding the birthplaces of subjects and their parents indicates that the inhabitants of these villages present moderate mobility, limited to their region of origin. Thus we may assume that the four populations represent, in general terms, the population structure of the Chiloé archipelago and the proposed regionalization based on ethnohistoric information (Munizaga, 1978).

DNA extraction and polymerase chain reaction amplification

Ten milliliters of blood were obtained from each of the volunteers by venipuncture. DNA was extracted from peripheral blood lymphocytes (Gustincich et al., 1991). Amplification of the polymorphic mtDNA regions defining the four Amerindian haplogroups was carried out using the primers described by Moraga et al. (2000). Polymerase chain reaction (PCR) was performed in a final volume of 50 μl containing 300 ng genomic DNA, 1 U Taq polymerase (Promega), 25 pmoles of each primer, 200 nM of each deoxynucleotide, and the appropriate buffer. Samples were processed under the following PCR

conditions: 1 cycle at 95°C for 5 min, followed by 35 cycles at 95°C for 45 sec, 55°C for 1 min, 72°C for 1 min and finally one extension cycle at 72°C for 5 min. Haplogroups A, C, and D were analyzed by restriction digestion, using *Hae*III for haplogroup A, *Hinc*II for haplogroup C, and *Alu*I for haplogroup D.

The resulting restriction fragments and the PCR product including region V, which defines haplogroup B, were analyzed by electrophoresis on 3% NuSieve-Agarose (2:1) (FMC Bioproducts) gels.

Statistical analyses

Several statistical analyses were carried out so as to characterize the genetic variability of the groups studied. Diversity for each population was calculated using the method of Tajima (1989), $H = (n/n-1)(1-\Sigma x^2)$, where n is population size, and x the frequency of each haplogroup found.

To evaluate whether certain haplogroups were principally responsible for the genetic differentiation between populations, we calculated F_{ST} value for each haplogroup according to Weir and Cockerham (1984), using the TFPGA program (Miller, 1997).

Since the mitochondrial haplogroup frequencies of the Fueguinos used in this study were obtained from skeletal remains from the 19th century (Lalueza, 1996), we treated this sample separately from the contemporary Yámana population.

The genetic affinities among populations were evaluated from the haplogroup frequencies obtained, using the genetic distances of Nei (1978) and modified Rogers distance (Wright, 1978). Although all four distances gave essentially the same results, we report the modified Rogers distance, considering that it allows for differences in population sizes and that it is more sensitive in the study of recent demographic events (Rogers, 1991). Standard errors (SE) of these estimates were obtained using a bootstrap approach with 1,000 replications.

The distance matrix was represented by means of an unweighted group pair method with arithmetic mean (UPGMA) tree (Saitou and Nei, 1987) built with the program MEGA 2.1 (Kumar et al., 2001) and also examined by means of a principal components (PC) analysis (Cavalli-Sforza et al., 1994). This technique has proved useful in the analysis of gene frequencies (Harpending et al., 1996; Stoneking et al., 1997), summarizing the distance matrix into a three-dimensional principal components map that retains most of the genetic variance.

AMOVA analysis

An analysis of molecular variance (AMOVA; Excoffier et al., 1992) was performed to estimate the degree of subdivision among putative groups, using the ARLEQUIN software package (Schneider et al., 2000). This analysis permits testing the hypothesis of a particular genetic structure by carrying out a hierarchical analysis that divides the total variance into covariance components that reflect differences among and between groups of populations. These covariance components are used to calculate an analogue of Wright's fixation index (F_{ST}) (Wright, 1921). The significance of these indices was tested using a nonparametric approach by permuting haplotypes, individuals, or populations between individuals, populations, or groups of populations. Ten thousand permutations were used to obtain the final probability of exceeding the observed value.

TABLE 1. Frequency distribution of mitochondrial DNA haplogroups in four populations of southern Chile

		Haplogroups (%)					
Populations	n	A	B	C	D	Others	H
Carelmapu	47	0.04	0.30	0.38	0.26	0.02	0.70 ± 0.03
Quetalmahue	42	0.0	0.31	0.36	0.26	0.07	0.69 ± 0.02
Detif	27	0.11	0.22	0.37	0.30	0.0	0.74 ± 0.04
Laitec	42	0.0	0.0	0.36	0.57	0.07	0.49 ± 0.04

Exact test of population differentiation

This test evaluates the hypothesis of a random distribution of $\boldsymbol{k}$ different haplotypes among $\boldsymbol{r}$ populations in a contingency table of $\boldsymbol{r} \times \boldsymbol{k}$, analogous to Fisher's exact test. All potential states of this table are explored, obtaining the probability of observing a table with the same marginal totals and which has a probability equal to or less than the observed table under the null hypothesis of panmixia.

The Bonferroni correction was not applied to determine the significance of pairwise comparisons among all pairs of populations. According to Rice (1989), this correction controls for type I errors on a table-wide basis and is suited to eliminating a few lone significant results in a table of nonsignificant results. As the number of comparisons in the table increases, the statistical power to detect differences decreases (Bland and Altman, 1995) and can lead to type II errors, or nonsignificant results, even when there are true differences among groups. The Bonferroni correction is therefore extremely conservative when the overall number of comparisons is large (Bender and Lange, 1999).

RESULTS

In total, 158 samples were characterized for the four haplogroups characteristic of American Indian populations; the observed frequencies for each population are given in Table 1. The results of the maternally inherited marker (mtDNA) showed that the aboriginal component was greater than 90%, revealing a low contribution of non-American Indian genes to the mitochondrial gene pool in these populations.

We found no compound haplotypes, and only seven individuals (4.4%) did not belong to one of the founder haplogroups, and were classified as "others." These may result from admixture with Caucasian populations or from mutations in diagnostic sites, or may belong to some rare American Indian haplogroup (Baillet et al., 1994).

mtDNA diversity

Table 1 also shows the values of the diversity indices of Tajima (1989). The values found for the three populations from the North of the archipelago were similar to those obtained by Bert et al. (2001) for all the American Indian populations ($H = 0.697$). However, a much lower value ($H = 0.554$) was found for the southern population of Laitec, which suggests the occurrence of microevolutionary processes different from those that affected the populations in the North of the archipelago.

Comparisons with other Chilean populations

The remainder of the analyses combine our results with previous estimations from seven Chilean indige-

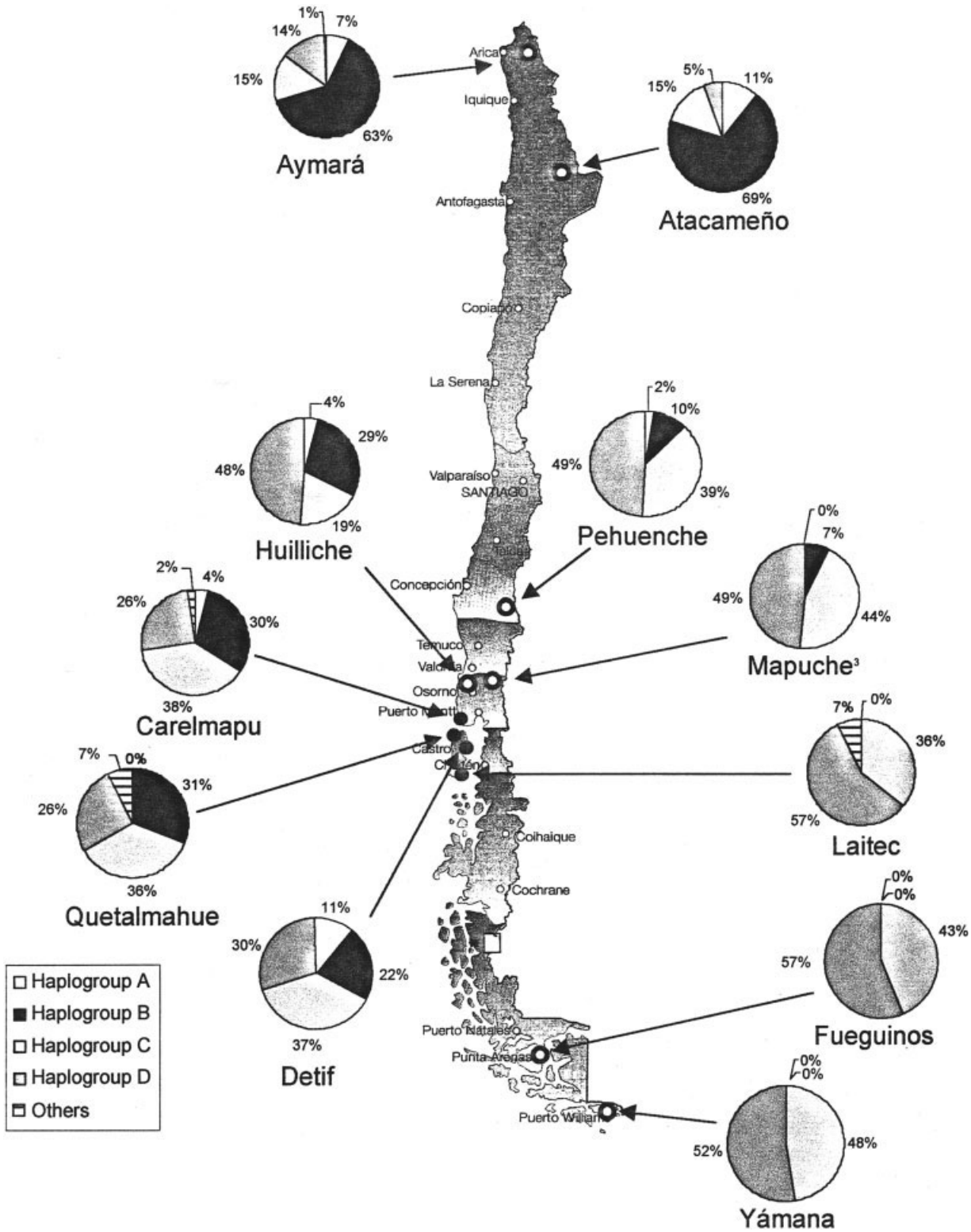

Fig. 2. Distribution of frequencies of four founding lineage haplogroups in Chile. Frequencies for region V deletion are shown in black.

nous groups, giving a total of 11 Chilean population samples (Fig. 2).

The fixation index F_{ST} allowed us to evaluate the weight of each haplogroup in the differentiation of populations. Results show a conclusive heterogeneity with respect to the differentiating role of each haplogroup. The value of F_{ST} for haplogroup B (0.1911) was four times greater than that for any of the other

TABLE 2. Exact differentiation tests of population differentiation based on mtDNA haplogroup frequencies: significant differences among all pairs of populations compared in study with significance level at 0.050[1]

	Atacameño	Huilliche	Carelmaput	Quetaimahue	Detif	Laitec	Pehuenche	Mapuche	Fueguinos	Yamána
Aymará[2]	–	+	+	+	+	+	+	+	+	+
Atacameño[2,3]		+	+	+	+	+	+	+	+	+
Huilliche[2]			+	+	–	+	+	+	+	+
Carelmapu[6]				–	–	+	+	+	+	+
Quetalmahue[5,6]					–	+	+	+	+	+
Detif[6]						+	–	+	+	–
Laitec[5,6]							–	–	–	–
Pehuenche[5,6]								–	–	–
Mapuche[3,4]									–	–
Fueguinos[4,5]										–

[1] Minus sign indicates that test was not significant. Shaded areas indicate tests within three groups postulated for aboriginal Chilean populations.
[2] Merriwether et al., 1995.
[3] Rocco et al., 2002.
[4] Moraga et al., 2000.
[5] Lalueza., 1996.
[6] This study.

haplogroups, which presented homogeneous values (average = 0.0249).

Exact test for population differentiation

The differentiation test applied to each pair of populations (Table 2) shows a clear separation of Chilean aborigines into three groups. All but two of the values among groups were significant the exceptions being Detif-Pehuenche ($P = 0.1906 \pm 0.0038$) and Detif-Yámana ($P = 0.0945 \pm 0.0038$). Congruently, only two of the within-group comparisons were significant; values for the Carelmapu-Huilliche pair ($P = 0.0138 \pm 0.0011$) and Quetalmahue-Huilliche pair ($P = 0.0260 \pm 0.0016$) are discussed below.

The differentiation tests also showed a clear North-South genetic segregation in the populations of Chiloé. The localities from the northern part of the archipelago, (Carelmapu, Quetalmahue, and Detif) group together with the Huilliche population (group B). All these populations show significant differences with the Laitec population, located in the southern part of the archipelago, which appears related to other aborigine populations located in the East (Pehuenche and Mapuche), as well as the extreme South of Chile (Yámana and Fueguinos) (group C, East-extreme South). Group A (North) is composed of the Ayamará and Atacameño populations in the extreme North of Chile.

Genetic distances

The UPGMA tree derived from the matrix of distances (Fig. 3) confirms the groups indicated above, and also shows the segregation between the populations of the North and the South of the archipelago. The populations of Carelmapu, Quentalmahue, and Detif are similar to the indigenous Huilliche population (Fig. 3), contrasting with the population of Laitec, which grouped with the Yámana and Fueguino populations first, and Pehuenche and Mapuche next.

This segregation between populations of the North and the South of Chiloé is supported by a bootstrap value of 100% (bootstrapping for each node of the dendrogram, Fig. 3), and corresponds to a major division observed in the aboriginal tribes of southern Chile, that appear grouped in two distinct conglomerates (Fig. 3):

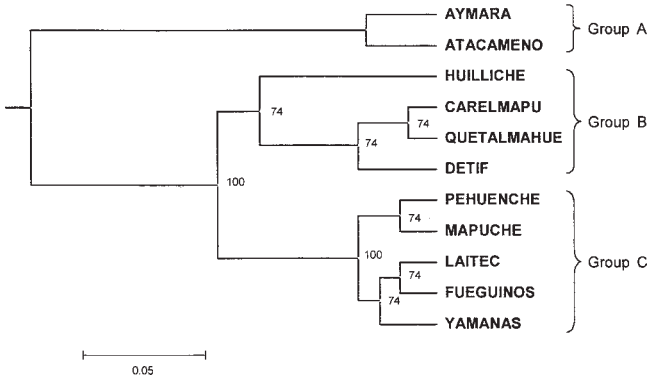

Fig. 3. UPGMA dendrogram based on mtDNA haplogroup frequencies. Aboriginal populations from northern Chile operate as outgroup. Numbers indicate bootstrap values.

the first is composed of populations affiliated with the Huilliche ethnic group, while the second is composed of Pehuenche and Mapuche populations from the East, Fueguino and Yámana from the extreme South of the continent, and the Laitec sample. The disjunction of groups that compose the latter conglomerate will be analyzed below.

The dendrogram also confirms group A, the Aymará and Atacameño from the Chilean highlands in the extreme North. Their genetic composition is sufficiently different from all the other samples to operate as an outgroup for the rest of the populations.

Principal component analysis

Since tree representation of the distance matrix forces a hierarchical structure, we performed a principal components analysis on the distance matrix (Cavalli Sforza et al., 1994). Figure 4 represents the three first eigenvectors extracted from the 11-dimensional distance matrix. Together, these components explain 98.1% of the variation in the four haplotypes. Consistent with the other analyses, the first component (accounting for 65.2% of the variance) clearly separates the analyzed populations into the same three groups. The second component

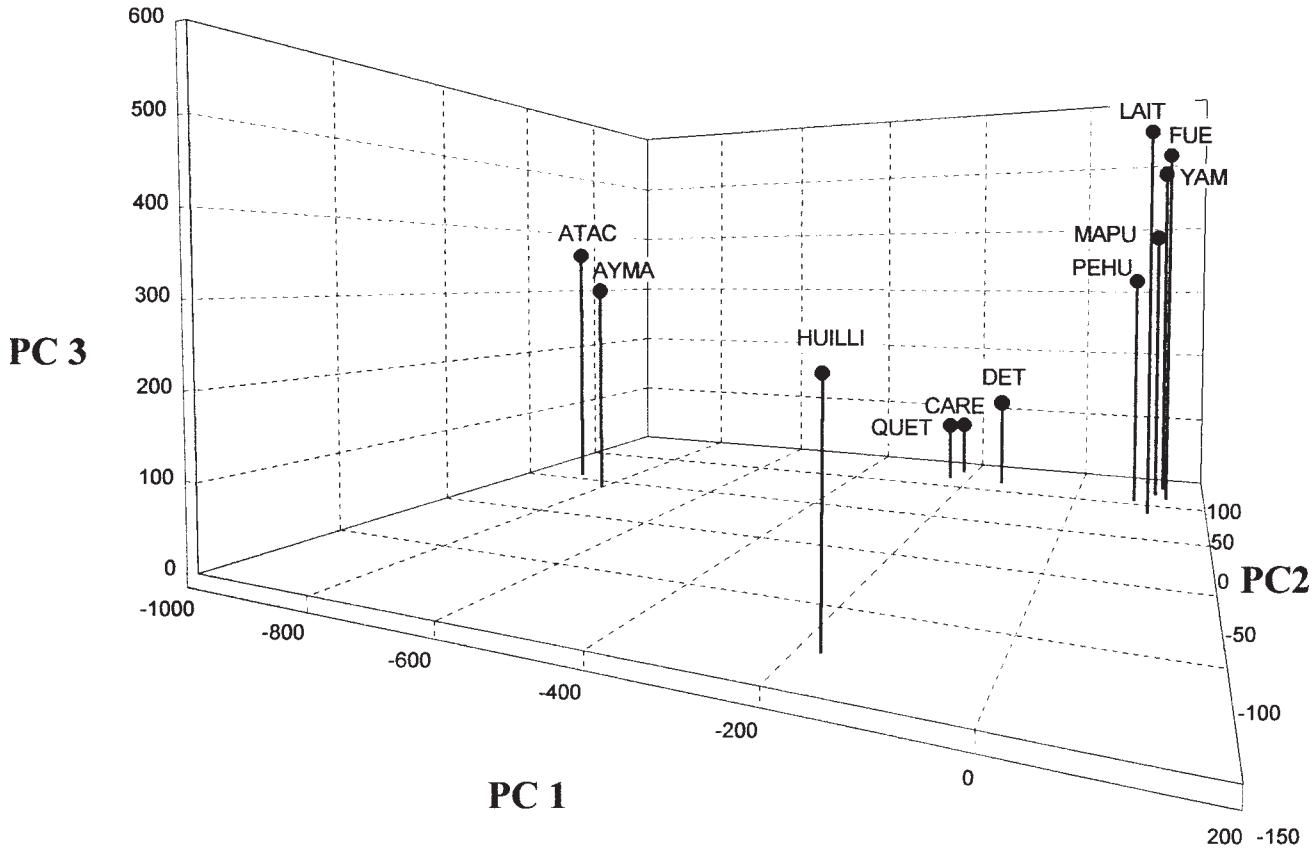

Fig. 4. Principal components analysis of distance matrix among 11 Chilean populations. ATAC, Atacameño; AYMA, Aymará; HUILLI, Huilliche; QUET, Quetalmahue; CARE, Carelmapu; DET, Detif; PEHU, Pehuenche; MAPU, Mapuche; YAM, Yámana; FUE, Fueguinos; LAIT, Laitec.

(27.9%) separates the Huilliche population from the northern Chiloé samples, due to the higher frequency of haplogroup B in the former.

Molecular variance analysis

In the first step of the analysis, we found that 76.6% of the total variance corresponded to differences within populations. If the Aymará-Atacameño (appearing as an outgroup in the dendrogram) we excluded from the analysis, the percentage of variance explained by differences within populations increases to 92.9%. This result is congruent with the results of Nei and Roychoudhury (1982) for total genetic variation within human populations.

Using the three groups indicated by the previous analyses, we found that 22.8% of the variance corresponded to differences among groups. If we exclude the Aymará-Atacameño group, this percentage drops to 6.3%. Finally, the proportion of variation explained by differences between populations within groups (0.59%) was much less than that explained by differences among groups, validating the groupings and indicating a strong genetic relation among populations of each group.

In agreement with the above, the permutation test of haplogroups among populations among groups was highly significant ($P = 0.0002 \pm 0.0001$), while the test among populations within groups was not significant ($P = 0.1264 \pm 0.0033$). These results strongly suggest the existence of a common ancestral population for the Mapuche and Pehuenche of the East, Fueguino and Yámana of the extreme South, and Laitec from Chiloé.

DISCUSSION

For the populations of the Chiloé archipelago, the analysis of mtDNA haplogroup frequencies allowed us to identify a North-South segregation that should be closely related to the ethnic origin of these populations. To the North, the populations of Carelmapu, Quetalmahue, and Detif are genetically related to the Huilliche, while in the South, the population of Laitec is most closely related to the Fueguino and Yámana tribes, and then to the Pehuenche-Mapuche. This division, which was confirmed by an exact test of differentiation and by both representations of the distance matrix (Figs. 3, 4), is congruent with the ethnohistorical record from the zone that indicates the historical presence of Chono groups in the South and "Veliches" (or Huilliches of Chiloé) in the center and North of the archipelago.

Gene flow may have occurred in historical times between the northern and southern parts of Chiloé; the Detif population, which is closest to the center of the archipelago, has a lower frequency of haplogroup B and perhaps a higher frequency of haplogroup D than the

other two North Chiloé localities. This makes the Detif sample a little more similar to the East-extreme South group, and reveals the existence of a North-South gradient in the distribution of haplogroups in Chiloé. Since populations of the North of Chiloé (e.g., the Huilliche also present a high prevalence of haplogroups B and D, it is impossible to discount genetic contributions from these populations, especially considering that Detif is located near a major urban center.

Figure 4 shows most clearly the differences between the North Chiloé populations and the Huilliche population reported by Merriwether et al. (1995), which is located 200 km north of the archipelago. There are several possible explanations for this difference. They are discrete groups, separated geographically, and thus have had independent effects of genetic drift, which may have changed them from their original genetic homogeneity. An alternative hypothesis, more in accordance with the ethnohistorical background of this region, is that the Chiloé-Huilliche are a distinct-cultural entity. The first chroniclers noted that the aborigines from this area called themselves Veliches (which was later changed to the Huilliches of Chiloé), distinguishing themselves from their neighbors, the Huilliche of continental Chile, by their way of life linked to the exploitation of marine resources of the archipelagos. Ethnolinguists described a dialect specific to Chiloé (called Tsesungún or simply Huilliche), which has an 85% homology with Mapudungún, the language of the Mapuche culture (Croese, 1980). Finally, we note that the Huilliche population characterized by Merriwether et al. (1995) does not present important cultural and linguistic differences with the Mapuche (Gissi, 1997), while the aboriginal populations of Chiloé do present such differences (Cárdenas et al., 1991).

In contrast, the population of Laitec is quite different from the Huilliche-North Chiloé group. Neither haplogroup A nor B was found in Laitec, and haplogroup D was the most frequent allele (Fig. 2); it has a much greater similarity to the ancestral populations that inhabited Tierra del Fuego in historical times. This similarity coincides with the ethnohistorical information, which indicates that, during Spanish colonial times, the southern part of Chiloé was occupied by native Chonos, who were distinguished from their Huilliche neighbors by having their own language, and by their canoe-based way of life (Etcheverry et al., 1967; Cárdenas et al., 1991).

It was suggested that the fixation of haplogroups C and D in the extreme South of the continent may be a result of a founder effect, which occurred during the peopling of the Southern Cone (Moraga et al., 2000). Based on craniometrical data, Guichón et al. (1991) indicated the existence of two genetic stocks in the Southern Cone, which remained in relative isolation from about 8,000 years ago, one on the Pacific side and one on the Atlantic side of South America.

It is worth noting at this point that, if the Chono population came from this "Pacific stock" and if the mitochondrial haplogroup frequencies observed in the Laitec population reflect those of the Chonos, it is possible to suggest a Fueguino origin for this group. The absence of archaeological remains revealing a settlement as far back as that of Tierra del Fuego (10,420 ± 100 ybp, Massone and Hidzlgo, 1981) in the Chiloé and Chono archipelagos, and the close genetic relationship found between southern Chiloé and Fueguino populations (Fig. 3), all support a peopling of the chain of archipelagos of the southern Pacific coast from Tierra del Fuego.

Considering these elements, we suggest that there were small bands of Paleo-Indian tribes (Pámpidos, following the terminology of Bórmida, 1953) carrying a subset of genes from ancestral populations, who crossed the Argentinean pampas, reaching Tierra del Fuego. Subsequent maritime adaptations of these groups would have permitted them to expand along the chain of archipelagos that borders the Pacific coast of southern Chile as far north as the Chiloé archipelago, to the extent allowed by decreasing glacial conditions (Bird, 1938).

In this context, the populations from the Chiloé archipelago clearly represent both tendencies found in the aborigines of southern Chile (Fig. 2). On the one hand, we have a group composed of populations that have a relatively large and homogeneous frequency of haplogroup B. In the three populations located in the North of the archipelago (Carelmapu, Quetalmahue, and Detif) and in the Huilliche group, over 25% of individuals present the deletion of the 9 base pairs which defines haplogroup B.

As we advance to the South of the archipelago, however, we find a gradient characterized by a low frequency of haplogroup B and a relative increase in haplogroups C and D. This North-South gradient also appears on a larger scale in the whole of Chile: in the populations of the extreme North (Aymará and Atacameño), two out of three individuals show the 9-base pair deletion; it becomes more infrequent toward the South-center of the country (29%, 10%, and 7%, in the Huilliche, Pehuenche, and Mapuche, respectively), and disappears completely in the ethnic groups of the extreme South (Fueguinos).

Due to their dependence on the natural resources of the ecogeographical zone that they inhabit, the populations of the archipelagos have maintained only moderate interpopulational mobility and gene flow. This leads us to infer that ancestral genetic relations among local indigenous populations gave rise to the contemporary populations.

Since these considerations are based on a marker with uniparental (maternal) inheritance without recombination, it has to be kept in mind that this type of inference may be less viable when based on markers with biparental or paternal inheritance, due to the unequal contribution of maternal and paternal genes to the American gene pool. This is due to the fact that Spanish women arrived in America much later and in much smaller numbers than Spanish men (Rothhammer and Cruz-Coke, 1983); thus the admixture was usually Spanish men and Amerindian women.

In recent years, the study of the peopling of the New World has become more complex, due to the difficulties in obtaining samples of pure populations. These difficulties include the continued abandonment of traditional localities by individuals who migrate to urban centers, and political pressures directed against taking samples in these traditional localities.

The advent of new molecular techniques has allowed the study of polymorphisms characteristic of Amerindian populations, which are detectable in contemporary populations. This, along with the low migration rate of some rural populations, makes it possible to study ancestral microevolutionary patterns based on contemporary populations. It will be a challenge to perform these studies before the large urban centers of each region provoke a restructuring of the genetic composition of the rural pop-

ulations that still maintain their maternal ancestral genetic pool.

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