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Genetic Population Structure of Two African-Ecuadorian Communities of Esmeraldas

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ABSTRACT The genetic structure of two African-Ecuadorian communities, Rio Cayapas and Viche (Esmeraldas province, northwest Ecuador), was studied on the basis of ACP1, ADA, AK1, CA2, ESD, GLO1, G6PD, PGD, and PGM1 subtypes and thermostability, PGM2, HBβ, F13A, F13B, ORM1, AHSG, C6, C7, and APOC2 gene frequency, and migration data on 255 individuals. The fixation index of Wright (F_{ST}), correspondence, and genetic distance analysis were applied to compare the genetic relationships between these communities and other American populations of African ancestry. F_{ST} values from the migration data and surname origins suggest that Rio Cayapas is genetically more isolated and shows less mobility and admixture than does Viche.

The genetic admixture estimates indicate a large contribution of African genes to the gene pool of both communities (74.3% to 58.4%), whereas the proportion of the Amerindian component differs significantly (14.5% in Rio Cayapas to 27.6% in Viche). Am J Phys Anthropol 109:159–174, 1999.

The Spanish colonies of the Caribbean and northern South America were among the principal centers of African slavery in the New World. By the middle of the 16th century, massive importation of black Africans from northern and western Africa (present day Sierra Leone, Gold Coast, Burkina Faso, Western Nigeria, Benin, Niger, Congo, and Angola) became necessary to fill the increasing need for cheap labor to work in the gold mining areas and on the plantations. High death rates among the Native American populations had led to labor shortages, as they steadily succumbed to the newly introduced diseases and the psychological shock of conquest. With the rapid decimation of the native Indian populations,

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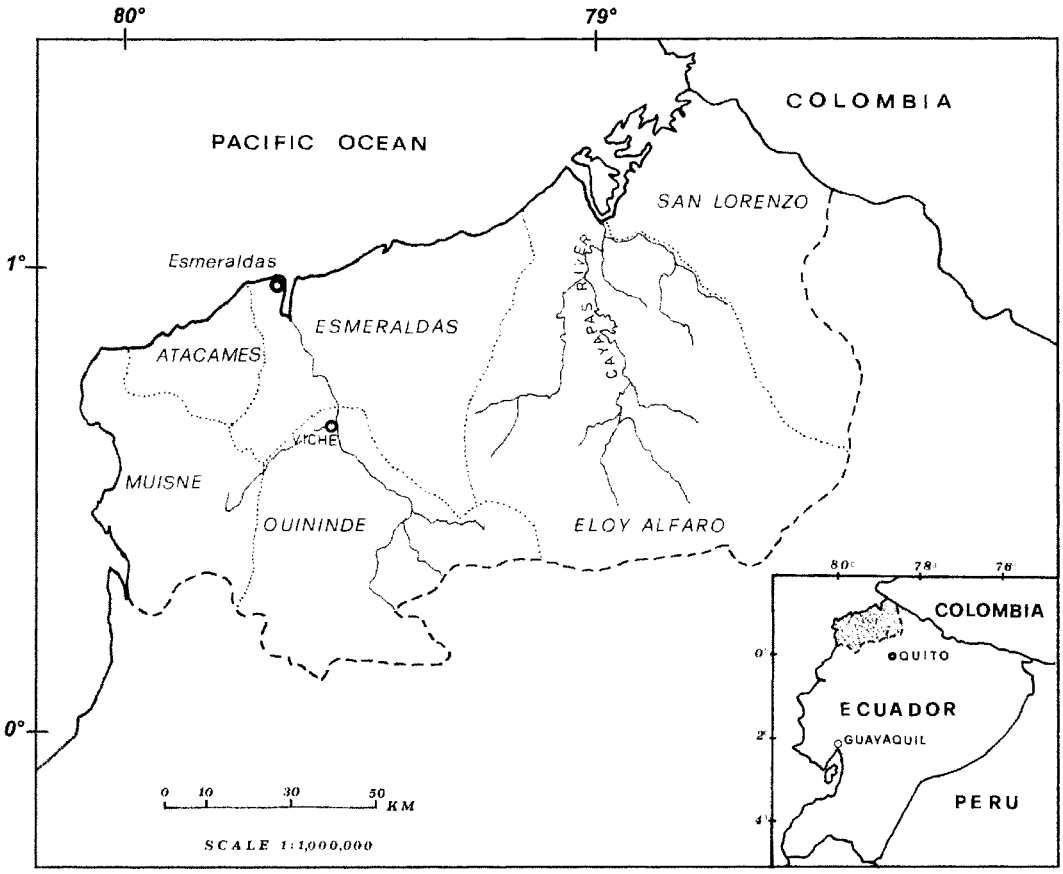

Fig. 1. Map of the Province of Esmeraldas, showing the location of the African-Ecuadorian communities of Rio Cayapas (established along the Cayapas river and its tributaries) and Viche. Dotted lines indicate boundaries of "cantones." (Each province is subdivided into "cantones," and each "cantón" is subdivided into parishes; the parish is the smallest administrative unit of Ecuador).

small African communities rose both in number and size over time (Whitten, 1974; Alcina Franch, 1976).

The existing black communities of Ecuador offer an excellent context for exploring the population dynamics and anthropological genetics of colonizing populations. As new elements come to light, the demographic, cultural, and genetic consequences of the extensive admixture between African, Indian, and to a lesser extent European peoples can be evaluated to better determine the genetic structure of northern South American black populations.

Here we report on the genetic and demographic structure of two African communities of northwestern Ecuador, Rio Cayapas and Viche (Fig. 1), which appear to differ substantially from one another. We studied the genetic relationships among various communities of African ancestry from North, Central, and South America by applying the fixation index of Wright (F_{ST}), correspondence, and genetic distance analysis, and their relatedness to African, Indian, and European parental populations. We then estimated the degree of admixture of the two communities and compared these values to the demographic data estimates to obtain an unbiased evaluation of the genetic contribution from the parental populations in the gene pool of the Rio Cayapas and Viche communities.

AFRICAN-ECUADORIAN COMMUNITIES

The first clearly documented evidence of African settlements in Ecuador dates back

to the middle of the 16th century, when a slave ship was reported to have run aground near the present town of Esmeraldas in 1553. During the shipwreck a group of 23 Africans (17 men, 6 women) from the Guinea Coast managed to flee their captors and settle in the Esmeraldas area, already inhabited by the Campaces and Niguas Indians. This first African colony grew rapidly and expanded its territory (Alcina Franch, 1976). Although Spanish law strictly prohibited mating between Africans and neighboring Indian populations, admixture has occurred since the earliest African settlements and involves much of the population. This African-Indian intermixture generated a new population called "Zambo" by the colonizers. At present these people identify themselves as *costeño*, "coastal," or *moreno*, "dark" (Whitten, 1974). At the end of the 19th century, 4,000 individuals arrived in Ecuador from Jamaica to build the Quito-Guayaquil railway. A migratory flow of people of African ancestry from Colombia as well is reported to have continued during the second half of the 19th and up through the 20th century. Frequently, the slaves who succeeded in running away from mines and plantations, called "Cimarrones," founded independent communities, mostly in the jungle and along the hills (West, 1957).

In Ecuador, populations of African ancestry have never constituted a large proportion of the population, and because of contacts with other populations the percentage of genetically unmixed people of African ancestry is small. The intermixture generally occurred with Indians, whereas the European contribution remained negligible because no direct ocean-going routes linked Ecuador with Europe. The population is dispersed throughout the country, with major concentrations in the capital city, Quito, and in Guayaquil (see Fig. 1); the density of the population of African ancestry is greater on the coast than in the highlands and greatest in Esmeraldas Province (51%, about 156,000 individuals).

BRIEF HISTORY OF THE COMMUNITIES OF ESMERALDAS

The Rio Cayapas community is a rural settlement located in the Eloy Alfaro "cantón" of Esmeraldas Province. This community, populated mostly by slaves who escaped from Colombia, was established in several settlements along the Cayapas river and its tributaries (see Fig. 1). After the abolition of slavery, the population expanded along these rivers, where they now live with little genetic admixture occurring with the neighboring Cayapa Indians (Pepe et al., 1994; Rickards et al., 1994; Scacchi et al., 1994; Rickards, 1995; Stinson, 1996).

The people living in the mangrove swamp-rain forest phenotypically resemble West Africans. They are a quite sedentary group, and rarely ascend the rivers or move along the coast. With a strong preference for nucleated villages, their settlements lack any significant pattern, so there are seldom any planned streets or paths. The rectangular houses have gabled, thatched roofs and are constructed on interwoven saplings filled in with mud. Their main activities are fishing, manufacturing nets, and repairing nets. Agricultural implements, including Indian-style digging sticks, are also made by the African-Ecuadorians. Except for some full-time fishermen, most of the population engages in agriculture and produces food crops for domestic consumption. The polygamic and patrilineal family structure is by far the most important social unit. Marriage is preferentially if not exclusively conducted within the community. All community members are nominally Catholic and speak Spanish (Erickson et al., 1966; Whitten, 1974).

The community of Viche, located in the Quininde "cantón" of Esmeraldas Province (see Fig. 1), dates back to the 1940s. It was established by populations of African ancestry immigrating here from other parts of the country and the continent. They so thoroughly intermixed with the American Indian and Mestizo/Ladino (combined Indian and European extraction) populations settled there that no Native American groups presently inhabit Viche. This African-Ecuadorian community resembles an urban nucleus more than Rio Cayapas does. Its population consists of individuals who are only slightly darkly pigmented and clearly display American Indian characteristics. The community members represent the economically poorer classes of Viche society. Their matrimonial structure is quite open, and intermarriages with other ethnic groups (e.g., Mulattos,

TABLE 1. Distribution of the total population and of the population of African ancestry together with each effective population size in the parishes of Eloy Alfaro and Quininde cantones of the two Ecuadorian communities (Instituto Nacional Estadistica y Censas, 1991)

Parish	Total population	Population of African ancestry	N_e[1]
Eloy Alfaro (Rio Cayapas community)			
Playa de Oro	215	183	61
Telembi	2,235	1,900	633
S. Domingo de Onzole	1,403	1,193	398
Camarones	1,589	1,351	450
Selva Alegre	1,266	1,076	359
Maldonado	1,772	1,506	502
Anchayacu	1,372	1,166	389
La Tola	3,600	3,060	1,020
Limones (Valdes)	6,500	5,525	1,842
Quininde (Viche community)			
Viche	3,026	605	202
Cube	6,807	1,361	454
Chura	3,400	680	227
Rosa Zarate	38,269	7,654	2,551
Malimpia	11,178	2,236	745

[1] Effective population number.

Ladinos) living in Viche are common. Most labor on the cacao and coffee estates or the banana, sugar, and rice plantations or weave textiles. They are Roman Catholic, and Spanish is the official language (Alcina Franch, 1976).

MATERIALS AND METHODS

Demographic analysis

Demographic data were collected by two of us (G.F.D.S. and C.V.d.V.) through interviews with 255 subjects coming from the African-Ecuadorian communities of Rio Cayapas (96 males, 81 females) and Viche (32 males, 46 females). The migration analysis was performed by subdividing the birthplaces of the subjects and their parents into the parishes of the two "cantones" (Table 1). The basic recurrence equation for predicting kinship between parish subdivisions in the th generation was computed according to Imaizumi et al. (1970) from the parent-offspring migration matrix and the subsequent column stochastic migration matrix. Then the conditional kinship (R matrix), which is kinship relative to the contemporary gene pool, was calculated according to Harpending and Jenkins (1974). Diagonal values of the R matrix are the predicted kinship within parish subdivisions, and the mean of this values weighted on the effective population number (Ne) is the observed inbreeding due to population subdivision R_{ST}, equivalent to the F_{ST} of Wright (1973). In addition, the expected F_{ST} in the absence of heterogeneity was calculated, taking into account the effective size of the population, i.e., one third of the population size of African ancestry reported in Table 1 (Imaizumi et al., 1970; Workman and Niswander, 1970; Cavalli-Sforza and Bodmer, 1971). In order to investigate the topology of population structure, both the kinship matrix and the matrix of geographical distances were analyzed by means of the multidimensional scaling method (MDS) (Torgerson, 1952; Kruskal, 1964; Lalouel, 1980). The first two eigenvectors of both matrices were rotated to maximum congruence by means of the least squares method.

Genetic analysis

Blood samples were collected by two of us (G.F.D.S. and A.E.B.G.) from the 255 apparently healthy interviewed adults of both sexes. They were tested for ACP1, ADA, AK1, CA2, ESD, GLO1, G6PD, PGD, and PGM1 subtypes and thermostability, PGM2 and HBβ red cell markers, and F13A, F13B, ORM1, AHSG, C6, C7, and APOC2 serum markers. Blood specimens were withdrawn by venipuncture and collected in sterile tubes with acid citrate dextrose (ACD) as anticoagulant. The tubes were kept at 4–6°C for no longer than 15 days until they could be taken to the University of Rome "Tor Vergata." Red cell lysates were carried out according to the standard technique and stored at -80°C pending analysis. Red cell and serum markers were typed as described elsewhere (Rickards et al., 1994; Scacchi et al., 1994; Biondi et al., 1996).

As our sample included some related couples, their frequencies were computed by counting two genes both for each unrelated subject and for one member of each related couple; the first served as the protocol number of sibships. The second member of each couple, sib of sibships, was counted as one gene if parent-son or brother, and 1.5 genes if half-brother, uncle-nephew, or grandpar-

TABLE 2a. Parent-offspring migration matrix for Rio Cayapas community (parishes of Eloy Alfaro "cantón")

	Parent's parish									
Offspring's Parish	PO	Te	SD	Ca	SA	Ma	An	LT	Li	Out[1]
Playa de Oro	2	3	0	0	0	2	0	0	1	0
Telembi	2	104	8	10	0	5	2	2	1	16
S. Domingo	0	1	2	2	0	0	0	0	0	0
Camarones	1	5	9	28	0	3	0	0	0	4
Selva Alegre	0	0	2	3	6	4	0	0	0	1
Maldonado	0	0	0	0	0	4	0	0	0	0
Anchayacu	0	0	1	1	0	0	2	0	0	1
La Tola	1	8	0	2	0	1	0	5	0	1
Limones	0	0	0	0	0	0	0	0	4	2

[1] Parents born outside Eloy Alfaro "cantón."

ent-grandson. The numbers N of "independent genes" computed in this way were then used for calculating gene frequencies and their standard errors.

The fixation index F_{ST} of Wright (1965, 1969) was used as a convenient measure of genetic differentiation among American populations of African ancestry. The contribution of the parental populations (Africans, Europeans, and Native Americans) to the gene pool of the African-Ecuadorians was computed according to two alternative tests, which utilize different admixture three-hybrid models, for comparisons (Krieger et al., 1965, MISTURA-pc package; Chakraborty et al., 1992, ADMIX3.FOR).

Correspondence analysis (Benzécri, 1973; Greenacre, 1984; Lebart et al., 1984) was applied to study the genetic relationships among the populations of Rio Cayapas and Viche, the other American populations of African ancestry, and the parental populations. It was carried out using the CORRESP and MXPLOT procedures of the NTSYS-pc package (Rohlf, 1988). Further methods based on genetic distances were applied (Cavalli-Sforza and Edwards, 1967; Reynolds et al., 1983), using the BIOSYS-1 pc, release 1.7 (Swofford and Selander, 1989) and PHYLIP version 3.5c (Felsenstein, 1993).

RESULTS

Demographic structure

A study on the origin of the African-Ecuadorian population performed by Estupiñan Tello (1983) gave us a list of "black" Colombian surnames. As expected from the history of the two communities in question, the population of Rio Cayapas shows higher frequencies of Colombian surnames than Viche: 73% vs. 29%. The latter is mainly inhabited by populations of African ancestry coming from different places in South America and the Caribbean Islands.

TABLE 2b. Parent-offspring migration matrix for Viche community (parishes of Quininde "cantón")

	Parent's parish					
Offspring's parish	Vi	Cu	Ch	RZ	Ma	Out[1]
Viche	21	1	1	1	1	18
Cube	0	5	0	2	0	1
Chura	0	0	2	0	0	6
Rosa Zarate	0	0	0	1	1	2
Malimpia	0	0	0	0	1	5

[1] Parents born outside Quininde "cantón."

The effects of population subdivision, which reduces the heterozygosity of a subpopulation due to random genetic drift, were studied by analyzing the parent-offspring migration matrices (Table 2a,b). In Rio Cayapas, the fixation index (F_{ST}), a measure of inbreeding and, therefore, of isolation of the parish populations, is much higher (0.08730) than the value expected under panmictic conditions (0.00024). In contrast, the observed and expected values in Viche are identical and very low (0.00016), indicating high gene flow during the ethnogenesis of this community.

The extent to which geographical factors like the geographical distances separating the parish populations affect marriage migration and hence kinship is displayed by MDS, where the pattern of ϕij matrices are compared with the matrices of geographical distances. In Rio Cayapas, the correlation between geographic and genetic distances is not statistically significant. In fact, the test of Mantel (1967) for comparing matrices gives a value of $Z = 3.3475$ ($P > 0.05$). This

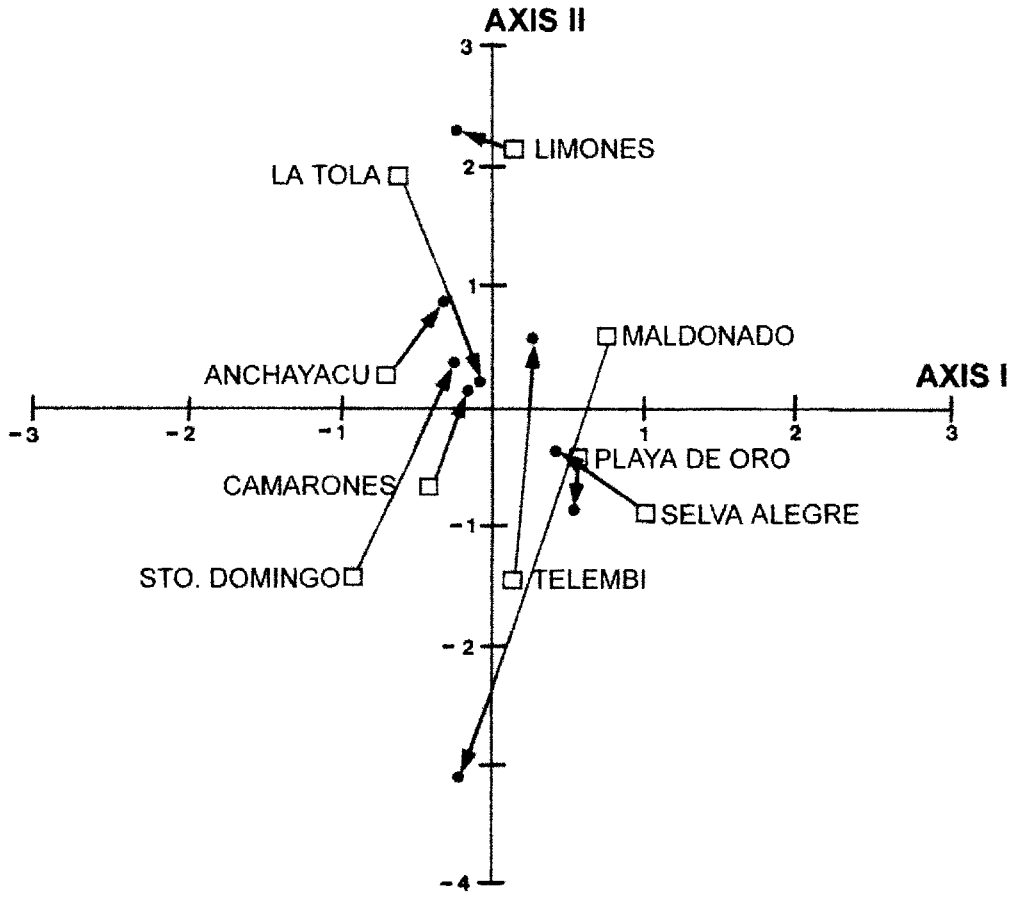

Fig. 2. Eigenvector representation of kinship in the parishes of Eloy Alfaro "canton" (Rio Cayapas) after normalization and rotation to maximum congruence with geography (MDS method). Solid circles, genetic locations; open squares, geographic locations.

means that the variation in the location predicted by kinship does not seem to be due to distance factors. The MDS analysis (Fig. 2) explains 99.9% of the variation described by the distance matrix, and the percentage of distortion of the bidimensional representation is 1.8. Excluding Limones and Maldonado, the parishes plot close together along the diagonal line representing the direction of the Cayapas River, the principal route of communication. This pattern indicates that mutual mate exchange occurred among the parishes. The genetic isolation of Limones and Maldonado from the main cluster is very likely due to the small effective size of African ancestry population present in these parishes.

In Viche, on the other hand, the MDS analysis (Fig. 3) explains 100% of the geographic variation, with a distortion equal to 0.2%. Also, in this case, the absence of a correlation between geographic distances and genetics (Mantel test, $Z = 0.0029$, $P > 0.05$) means that there is no genetic isolation due to geographic distances.

In conclusion, the joint F_{ST} and MDS analyses seem to indicate that although the parishes are very isolated from each other in both communities, Rio Cayapas also appears isolated from all the other populations of the area, whereas Viche represents a more panmictic unit. These results are consistent with the historical records that report that Viche was established at the cross-

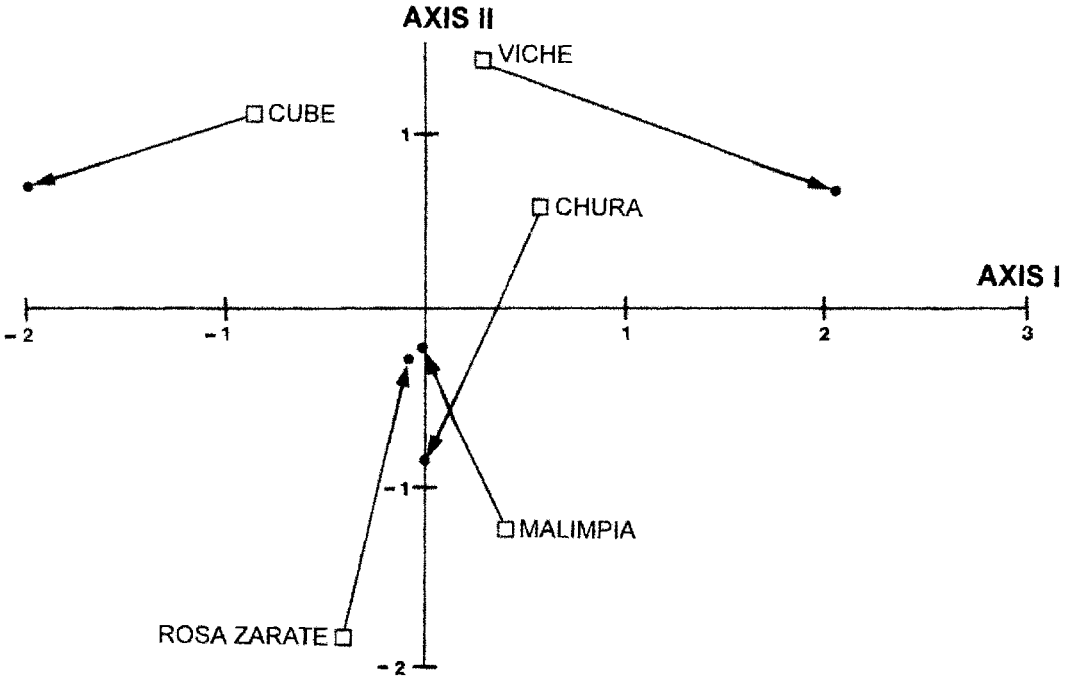

Fig. 3. Eigenvector representation of kinship in the parishes of Quininde "canton" (Viche) after normalization and rotation to maximum congruence with geography (MDS method). Solid circles, genetic locations; open squares, geographic locations.

roads of several communication routes in the Esmeraldas Province.

Genetic structure

The distribution of phenotype and allele frequencies in the two African-Ecuadorian populations is given in Table 3. The observed phenotype distributions agree with the distributions expected on the basis of the Hardy-Weinberg formula (only one significant deviation out of the 19 comparisons).

Two males showed G6PD variants with normal activity and an electrophoretic mobility slower (93%) than that of the B type. As the same electrophoretic variant has been found in Sicily, in southern Italy (Rickards et al., 1988), and in the Berba population of western Africa (Biondi et al., 1996), we propose an African origin for this allele, which moved to Sicily as a consequence of the uninterrupted gene flow from northern Africa and to the Americas during the slave trade.

PGM1 thermostability, studied for the first time in American populations of African ancestry, was found to be polymorphic as a result of the contribution of Europeans to the gene pool of these hybrid populations. In fact, sub-Saharan Africans and Native Americans are monomorphic for thermostability (Scozzari et al., 1984; Rickards et al., 1994; Biondi et al., 1996).

F13B frequencies in the Viche group were intermediate between those observed in parental populations, while in the Rio Cayapas sample they were more similar to those observed in sub-Saharan Africans. F13A*2, AHSG*2, and C6*B allele frequency values are indicative of an admixture with the neighboring native populations. A chi-square test for heterogeneity between the two communities showed a statistically significant variation ($P < 0.01$) in the frequencies of eight (ACP1, AK1, ESD, GLO1, HBβ, PGM1-IEF, F13B, and AHSG) out of the 17 markers examined. Such a genetic differentiation reflects their separate microevolutionary history, i.e., the fairly pronounced genetic isolation of the Rio Cayapas in respect to the Viche population, the latter being highly

TABLE 3. Phenotype and gene frequencies distribution in the African-Ecuadorian population of Esmeraldas

Genetic markers	Rio Cayapas				Viche			
	Frequencies		N and alleles[1]	Frequencies ± 1 SE	Frequencies		N and alleles[1]	Frequencies ± 1 SE
	Observed	Expected			Observed	Expected		
ACP1			N = 337.5				N = 148	
A	1	1.96	ACP1 ∗ A	0.112 ± 0.017	3	2.97	ACP1 ∗ A	0.209 ± 0.033
A-B	35	31.03	ACP1 ∗ B	0.888	22	22.48	ACP1 ∗ B	0.791
B	120	123.01			43	42.55		
Total	156				68			
		$\chi^2_1 = 1.503$; $0.20 < P < 0.30$				$\chi^2_1 = 0.015$; $P = 0.90$		
ADA			N = 337.5				N = 148	
1	154	153.20	ADA ∗ 1	0.991	67	67.05	ADA ∗ 1	0.993
1-2	1	2.79	ADA ∗ 2	0.009 ± 0.005	1	0.94	ADA ∗ 2	0.007 ± 0.007
2	1	0.01			0	0.01		
Total	156				68			
AK1			N = 337.5				N = 148	
1	156		AK1 ∗ 1	1.000–0.003[2]	64	64.38	AK1 ∗ 1	0.973
1-2					4	3.57	AK1 ∗ 2	0.027 ± 0.013
2					0	0.05		
Total	156				68			
CA2			N = 333.5				N = 148	
1	146	145.50	CA2 ∗ 1	0.972	59	58.81	CA2 ∗ 1	0.944
1-2	8	8.38	CA2 ∗ 2	0.028 ± 0.009	6	6.98	CA2 ∗ 2	0.056 ± 0.019
2	0	0.12			1	0.21		
Total	154				66			
ESD			N = 337.5				N = 148	
1	142	145.50	ESD ∗ 1	0.954	54	53.26	ESD ∗ 1	0.885
1-2	14	13.69	ESD ∗ 2	0.046 ± 0.011	13	13.84	ESD ∗ 2	0.115 ± 0.026
2	0	0.33			1	0.90		
Total	156				68			
G6PD			N = 90				N = 29	
B	67		G6PD ∗ B	0.744 ± 0.046	22		G6PD ∗ B	0.759 ± 0.079
A	13		G6PD ∗ A	0.145 ± 0.037	3		G6PD ∗ A	0.103 ± 0.056
A−	8		G6PD ∗ A−	0.089 ± 0.030	4		G6PD ∗ A−	0.138 ± 0.064
V (93%)	2		G6PD ∗ V	0.022 ± 0.015				
Total males	90				29			
GLO1			N = 337.5				N = 148	
1	28	30.75	GLO1 ∗ 1	0.444 ± 0.027	7	5.96	GLO1 ∗ 1	0.296 ± 0.037
1-2	81	77.02	GLO1 ∗ 2	0.556	24	28.34	GLO1 ∗ 2	0.704
1	47	48.23			37	33.70		
Total	156				68			
		$\chi^2_1 = 0.483$; $0.30 < P < 0.50$				$\chi^2_1 = 1.17$; $0.20 < P < 0.30$		
HBβ[3]			N = 337.5				N = 148	
A	104	102.95	HBβ ∗ A	0.815 ± 0.021	59	59.96	HBβ ∗ A	0.939 ± 0.020
A-C	31	34.36	HBβ ∗ C	0.136 ± 0.019	2	1.79	HBβ ∗ C	0.014 ± 0.009
A-S	12	12.38	HBβ ∗ S	0.049 ± 0.012	7	6.00	HBβ ∗ S	0.047 ± 0.017
C	5	2.86			0	0.01		
C-S	2[4]	2.06			0	0.09		
S	1[4]	0.37			0	0.15		
Total	155				68			
		$\chi^2_2 = 2.060$; $0.30 < P < 0.50$						
PGD			N = 337.5				N = 148	
A	142	141.68	PGD ∗ A	0.953	67	66.65	PGD ∗ A	0.990
A-C	14	13.56	PGD ∗ C	0.047 ± 0.012	1	1.34	PGD ∗ C	0.010 ± 0.008
C	0	0.35			0	0.01		
Total	156				68			
PGM1			N = 337.5				N = 148	
1	118	115.38	PGM1 ∗ 1	0.860	52	51.24	PGM1 ∗ 1	0.868
1-2	32	37.56	PGM1 ∗ 2	0.140 ± 0.019	15	15.58	PGM1 ∗ 2	0.132 ± 0.029
2	6	3.06			1	1.18		
Total	156				68			
		$\chi^2_1 = 3.700$; $0.05 < P < 0.10$				$\chi^2_1 = 0.060$; $0.80 < P < 0.90$		
PGM1-IEF			N = 337.5				N = 148	
1A	82	74.44	PGM1 ∗ 1A	0.693 ± 0.025	23	22.72	PGM1 ∗ 1A	0.578 ± 0.041
1A-1B	28	35.88	PGM1 ∗ 1B	0.167 ± 0.020	24	22.72	PGM1 ∗ 1B	0.289 ± 0.037
1B	7	4.32	PGM1 ∗ 2A	0.108 ± 0.017	5	5.68	PGM1 ∗ 2A	0.083 ± 0.023
1A-2A	18	23.20	PGM1 ∗ 2B	0.032 ± 0.010	4	6.53	PGM1 ∗ 2B	0.050 ± 0.018
1A-2B	2	6.87			4	3.93		
1B-2A	6	5.59			4[6]	3.26		
1B-2B	6	1.66			3[7]	1.96		
2A	4	1.81			1[6]	0.47		
2A-2B	2[5]	1.07			0[7]	0.56		
2B	0[5]	0.16			0[7]	0.17		
Total	155				68			
		$\chi^2_5 = 23.280$; $P < 0.001$				$\chi^2_3 = 1.570$; $0.50 < P < 0.70$		
PGM1-ter[8]			N = 124				N = 78	
1ATr-1BTr	26		1ATr	0.379 ± 0.043	22		1ATr	0.385 ± 0.055
1ATr-1BTs	1		1ATs	0.008 ± 0.008	0		1ATs	0.026 ± 0.018
1ATs-1BTr	1		1BTr	0.314 ± 0.042	2		1BTr	0.397 ± 0.055

TABLE 3. (continued)

Genetic markers	Rio Cayapas				Viche			
	Frequencies		N and alleles[1]	Frequencies ± 1 SE	Frequencies		N and alleles[1]	Frequencies ± 1 SE
	Observed	Expected			Observed	Expected		
1ATr-2ATr	18		1BTs	0.008 ± 0.008	4		1BTs	0.000 + 0.013[2]
1ATr-2BTr	2		2ATr	0.210 ± 0.036	4		2ATr	0.102 ± 0.034
1BTr-2ATr	6		2ATs	0.000 + 0.008[2]	4		2ATs	0.000 + 0.013[2]
1BTr-2BTr	6		2BTr	0.073 ± 0.023	3		2BTr	0.090 ± 0.032
2ATr-2BTr	1		2BTs	0.008 ± 0.008	0		2BTs	0.000 + 0.013[2]
2ATr-2BTs	1				0			
Total	62				39			
PGM2			N = 337.5				N = 148	
1	155	155.06	PGM2 ∗ 1	0.997	68		PGM2 ∗ 1	1.000–0.003[2]
1-2	1	0.93	PGM2 ∗ 2	0.003 ± 0.003			PGM2 ∗ 2	
2	0	0.01						
Total	156				68			
F13A			N = 240.5				N = 146	
1	62	64.20	F13A ∗ 1	0.771	42	43.20	F13A ∗ 1	0.803
1-2	43	38.14	F13A ∗ 2	0.229 ± 0.029	23	21.20	F13A ∗ 2	0.197 ± 0.034
2	3	5.66			2	2.60		
Total	108				67			
		$\chi^2_1 = 1.945$; $0.10 < P < 0.25$				$\chi^2_1 = 0.325$; $0.50 < P < 0.75$		
F13B			N = 240.5				N = 146	
1	5	7.13	F13B ∗ 1	0.257 ± 0.030	9	9.88	F13B ∗ 1	0.384 ± 0.042
1-2	29	33.36	F13B ∗ 2	0.601 ± 0.033	13	18.42	F13B ∗ 2	0.358 ± 0.041
2	44	39.01	F13B ∗ 3	0.134 ± 0.023	10	8.58	F13B ∗ 3	0.238 ± 0.037
1-3	12	7.44	F13B ∗ 6	0.008 ± 0.006	18	12.25	F13B ∗ 6	0.020 ± 0.012
2-3	15	17.40			12	11.42		
3	1	1.94			2	3.79		
1-6	2[9]	0.44			1[10]	1.03		
2-6	0[9]	1.04			2[10]	0.96		
3-6	0[9]	0.23			0[10]	0.64		
6	0[9]	0.01			0[10]	0.03		
Total	108				67			
		$\chi^2_3 = 5.471$; $0.10 < P < 0.25$				$\chi^2_3 = 5.526$; $0.10 < P < 0.25$		
ORM1			N = 240.5				N = 146	
1	47	47.76	ORM1 ∗ 1	0.665	18	22.31	ORM1 ∗ 1	0.577
1-2	49	48.12	ORM1 ∗ 2	0.335 ± 0.032	40	32.70	ORM1 ∗ 2	0.423 ± 0.043
2	12	12.12			9	11.99		
Total	108				67			
		$\chi^2_1 = 0.029$; $0.50 < P < 0.75$				$\chi^2_1 = 3.208$; $0.05 < P < 0.10$		
AHSG			N = 238.5				N = 146	
1	47	47.03	AHSG ∗ 1	0.663 ± 0.032	31	27.96	AHSG ∗ 1	0.646 ± 0.041
1-2	30	30.51	AHSG ∗ 2	0.215 ± 0.028	21	25.62	AHSG ∗ 2	0.296 ± 0.039
2	7	4.95	AHSG ∗ 3	0.042 ± 0.014	8	5.87	AHSG ∗ 10	0.051 ± 0.019
1-3	3	5.96	AHSG ∗ 10	0.080 ± 0.019			AHSG ∗ 11	0.007 ± 0.007
2-3	2	1.93						
3	1[11]	0.19						
1-10	15	11.35			4	4.41		
2-10	0	3.68			2	2.02		
3-10	1[11]	0.72						
10	1[11]	0.68			0[12]	0.17		
1-11					1[12]	0.61		
2-11					0[12]	0.28		
10-11					0[12]	0.05		
11					0[12]	0.01		
Total	107				67			
		$\chi^2_4 = 8.434$; $0.05 < P < 0.10$				$\chi^2_2 = 1.988$; $0.25 < P < 0.50$		
C6			N = 239.5				N = 146	
A	24[13]	20.91	C6 ∗ A	0.440 ± 0.034	16	14.93	C6 ∗ A	0.472 ± 0.043
A-B	48[13]	53.22	C6 ∗ B	0.558 ± 0.034	29	32.51	C6 ∗ B	0.514 ± 0.043
B	36[13]	33.87	C6 ∗ A3		20	17.70	C6 ∗ A3	0.014 ± 0.010
A-A3			C6 ∗ M1	0.002 ± 0.002[14]	1[15]	0.89		
B-A3					1[15]	0.96		
A3					0[15]	0.01		
Total	108				67			
		$\chi^2_1 = 1.103$; $0.25 < P < 0.50$				$\chi^2_1 = 0.765$; $0.25 < P < 0.50$		
C7			N = 131.5				N = 146	
1	108		C7 ∗ 1	1.000–0.008[2]	67		C7 ∗ 1	1.000–0.007[2]
Total	108				67			
APOC2			N = 240.5				N = 146	
1	107	107.14	APOC2 ∗ 1	0.996	67		APOC2 ∗ 1	1.000–0.007[2]
1-2	1	0.86	APOC2 ∗ 2	0.004 ± 0.004	0			
2	0	0.00			0			
Total	108				67			

[1] N refers to "independent alleles." We counted two alleles for both unrelated subjects and for one member of each related couple drawn at random. The second member of each related couple was counted as one allele for parents-offspring (12 couples in Rio Cayapas and 3 in Viche) and brothers/sisters (3 and 3, respectively); and 1.5 alleles for half-brothers/sisters (3 and 2, respectively), and uncle/aunt-nephew/niece (4 and 2, respectively). The numbers of "independent alleles" thus computed were used for calculating gene frequencies and their standard errors.

[2] SE was calculated according to Morpurgo et al. (1983).

[3] Data on HBβ distribution in a larger sample of Rio Cayapas will be reported elsewhere (De Stefano, in preparation).

[4,5,6,7,9,10,11,12,15] Phenotypes pooled for HW equilibrium.

[8] PGM1 thermostability allele frequencies were only calculated on the PGM1-IEF heterozygote phenotypes because the resistant allele (Tr) is dominant on the sensible allele (Ts), and Cayapa Indians and Africans are monomorphic for the resistant allele (Rickards et al., 1994; Biondi et al., 1996).

[13] Expected frequencies calculated after exclusion of C6 ∗ M1 allele. In this case, the frequency of the C6 ∗ B allele turned out to be 0.560.

[14] C6 ∗ M1 allele was found in a heterozygote B-M1 individual of a couple of relatives.

intermixed with the American populations of African ancestry coming from other areas, the Native Americans, and the Ladinos/Mestizos.

Historiography describes the American populations of African ancestry mainly as the result of a fusion of African, European, and American Indian peoples. It is easy to recognize that the genes deriving from these peoples were carried to the American populations of African ancestry through different migration patterns. This process generated distinct populations whose genetic makeup is very likely to be different. Several statistics were applied to test the extent of this differentiation and to evaluate the genetic contribution of parental populations to the gene pool of these communities.

The evaluation of the relative proportions of the parental contribution to the gene pool of the African-Ecuadorians was made by comparing the allele frequencies of present populations, assuming that a moderate genetic evolution has characterized these populations during the last four centuries. The African populations mainly involved in the slave trade came from West Africa (West, 1957; Whitten, 1974; Erickson et al., 1966; Curtin, 1975). The greater Spanish contribution to immigration to Central and South America came from Andalusia, Castile, and Galicia (Foster, 1960). Data from Madrid and Barcelona were also considered because of their genetic representativity of the whole Spanish population. Lastly, the Cayapa Indians of Ecuador were considered as the Native American component. The allele frequencies of the parental populations were reported elsewhere (blood groups and red cell markers in Martínez-Labarga, 1993, unpublished data available on http://www.uniroma2.it/biologia/lab/anthromol/amafrhybr.htm; serum markers in Corbo et al., 1994; Scacchi et al., 1994; Cameselle et al., 1993; Caeiro et al., 1992, 1993; Alonso et al., 1990, 1991; Montiel et al., 1988, 1990).

In order to analyze the genetic relationship among the American communities of African ancestry throughout the Americas (database reported in Martínez-Labarga, 1993, and available on http://www.uniroma2.it/biologia/lab/anthromol/amafrhybr.htm), the mean fixation index (F_{ST}) was calculated on a total of 16 alleles (0.0216) (Table 4). This value indicates that they are characterized by low genetic heterogeneity, and it is comparable to the results reported by Jorde (1980) for populations such as the Papago Indians (0.0208), New Guinean Fore (0.0201), and African Pygmies (0.0200).

TABLE 4. F_{ST} values in American communities of African ancestry[1]

Alleles	K	P	σ^2	F_{ST}
ACP1 ∗ A	23	0.1723	1.43E-4	0.01876
ACP1 ∗ B	23	0.8145	3.36E-3	0.02227
ACP1 ∗ C	23	0.0067	1.43E-4	0.02150
ACP1 ∗ R	23	0.0065	8.38E-5	0.01294
ADA ∗ 1	11	0.9855	2.16E-4	0.01512
AK1 ∗ 1	22	0.9895	8.53E-5	0.00821
CA2 ∗ 1	6	0.9475	1.88E-3	0.03771
ESD ∗ 1	21	0.8858	5.38E-3	0.05316
GLO1 ∗ 1	11	0.3516	4.40E-3	0.03771
PGD ∗ A	25	0.9682	4.17E-4	0.01355
PGM1 ∗ 1	24	0.7997	1.74E-3	0.01088
PGM1 ∗ 1A	14	0.5573	7.60E-3	0.03082
PGM1 ∗ 1B	14	0.2516	7.60E-3	0.04037
PGM1 ∗ 2A	14	0.1522	2.46E-3	0.01905
PGM1 ∗ 2B	14	0.0037	5.47E-4	0.01517
PGM2 ∗ 1	12	0.9966	2.16E-5	0.00636
Mean F_{ST}				0.02157

[1] K, number of populations; P, mean allele frequencies; σ^2, frequency variance.

In addition, we applied correspondence analysis to study the genetic relationships among Ecuadorians, other American populations of African ancestry, and their parental populations for which data on the genetic markers analyzed in the present paper were available. We reached a total of 11 alleles (ABO*A, ABO*B, RH*d, ACP1*A, ACP1*C, ACP1*R, AK1*1, ESD*1, GLO1*1, PGD*A, and PGM1*1) and 14 populations. Figure 4 displays the two-dimensional plot of the first two axes, which account for 84.6% of total variability. The goodness of this representation yields a characteristic pattern (absolute and relative contributions not reported). The parental populations appear unambiguously separated, largely along the first axis. Closely grouped together, the American communities of African ancestry are organized into a cluster with West Africans. The four Native American populations also form a tight cluster, and the population of Viche appears more genetically similar to the Cayapa Indians than that of Rio Cayapas does. Only the Brazilian community of Paredao appears sharply differentiated along both axes. The alleles that mostly contribute

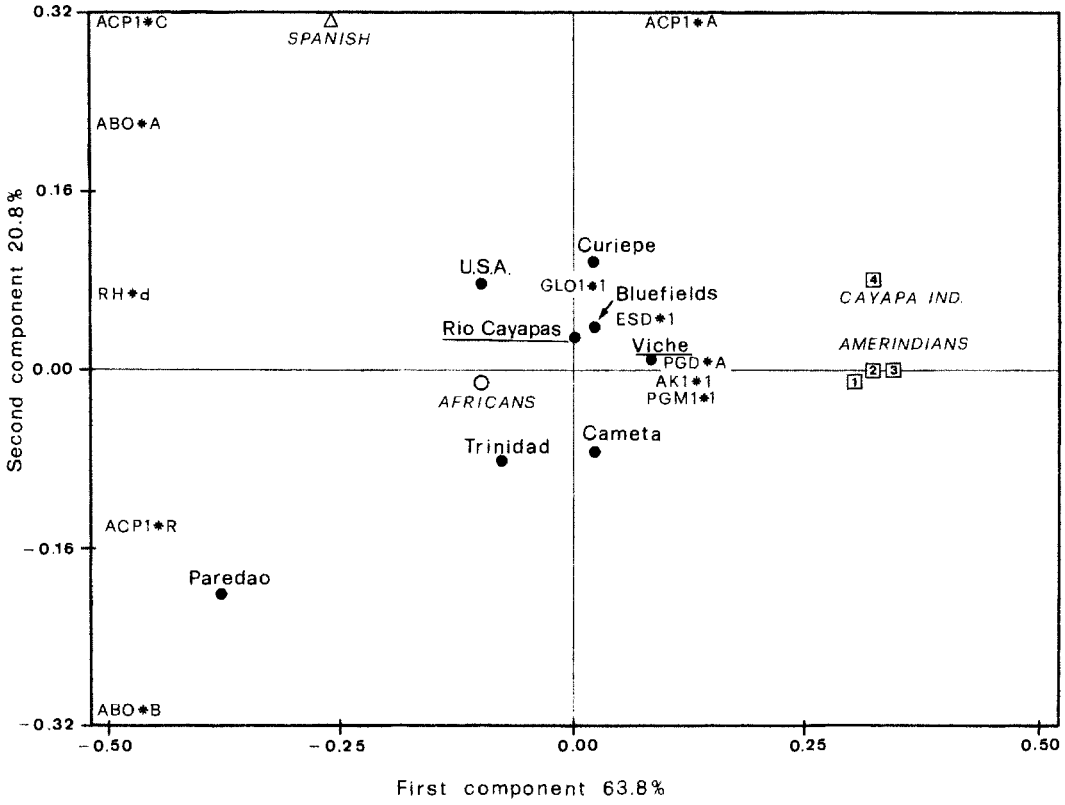

Fig. 4. Two-dimensional correspondence analysis of 14 populations and 11 alleles. Solid circles, American communities of African ancestry; open circle, West Africans; open triangle, Spaniards; open squares, Native Americans (1, Kaingang Indians; 2, Amazonian Indians; 3, Carib Indians; these Native populations are the Indian parental populations of the American communities of African ancestry of Paredao, Cameta, and Curiepe).

to the observed pattern of distribution are ABO*A, ABO*B, RH*d, ACP1*A, and ACP1*C. The projection of the populations on the second axis provides good, dependable evidence for the slight genetic contribution of Europeans to the gene pool of American communities of African ancestry.

The same clustering of American populations of African ancestry was obtained considering a different set of communities (Rio Cayapas, Viche, Trinidad, Black Caribs Livingstone, Black Caribs Belize, Black Caribs St. Vincent, Creoles St. Vincent, USA, Paredao, Curiepe, Africans, Spaniards, and Cayapa Indians) and 12 independent alleles (AK1*1, ESD*1, PGD*A, ACP1*A, ACP1*C, ACP1*R, PGM1*1B, PGM1*2A, PGM1*2B, ABO*A, ABO*B, and RH*d). The percentage of the total variability explained by the first two axes was 71.9%. Moreover, since some American communities of African ancestry have British and Portuguese populations in their European ancestry, we repeated both correspondence analyses using these European populations and obtained the same topology (data not shown). Different distance methods confirmed the genetic relationships highlighted by correspondence analyses. Figure 5 reports the tree obtained applying Reynolds' distance.

In the next step, we tried a quantitative estimation of the degree of admixture in the two Ecuadorian communities using 18 systems (ACP1, ADA, AK1, CA2, ESD, GLO1, G6PD, PGD, PGM-IEF, PGM2, HBβ, AHSG, F13A, F13B, ORM1, ABO, MNSs, and RH). The obtained admixture proportions (Krieger et al., 1965), together with the values from

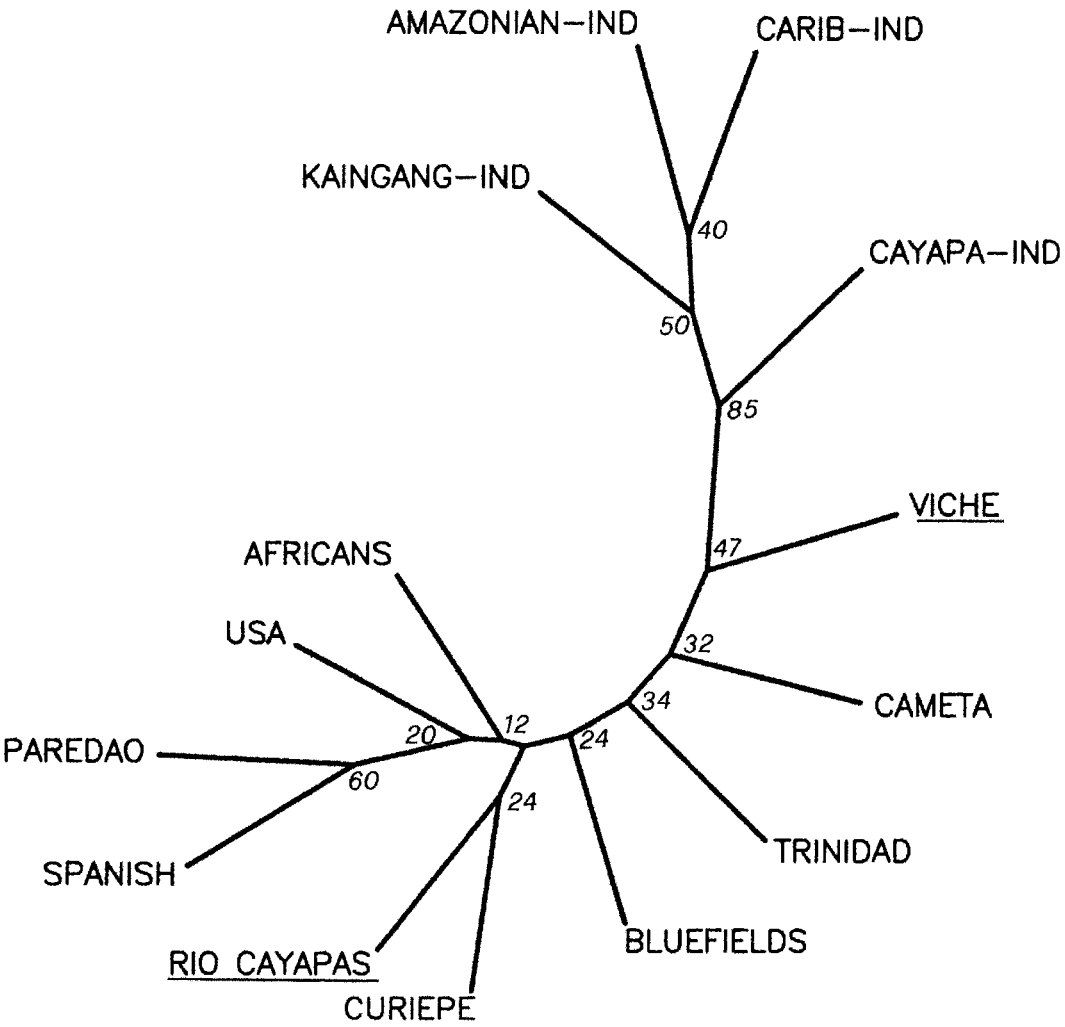

Fig. 5. Neighbor-joining tree of Reynolds genetic distances. Numbers in tree nodes represent the percentage of times that a certain node was found in 1,000 bootstrapped trees.

the literature for the other American populations of African ancestry, are reported in Figure 6. Viche and Rio Cayapas fall into the cluster of three-hybrid populations that are genetically more similar to those of West Africa. However, the relative contributions of the parental populations in the genes presently segregating in the populations of the two communities are quite different. In Viche, the admixture with the Native American component is twice that of Rio Cayapas (27.6 ± 10.4 vs. 14.5 ± 10.4), which exhibits one of the highest proportions of admixture with sub-Saharan populations compared to other American communities of African ancestry (74.3 ± 11.8). These figures confirm the biodemographic and cultural evidence of the marriage patterns of the two communities, i.e., the population of Rio Cayapas behaves as a more genetically isolated group. The present data do not allow a reliable estimate of the European contribution, if any, to the gene pool of American communities of African ancestry, since the standard errors associated with the proportion of European admixture come close to the esti-

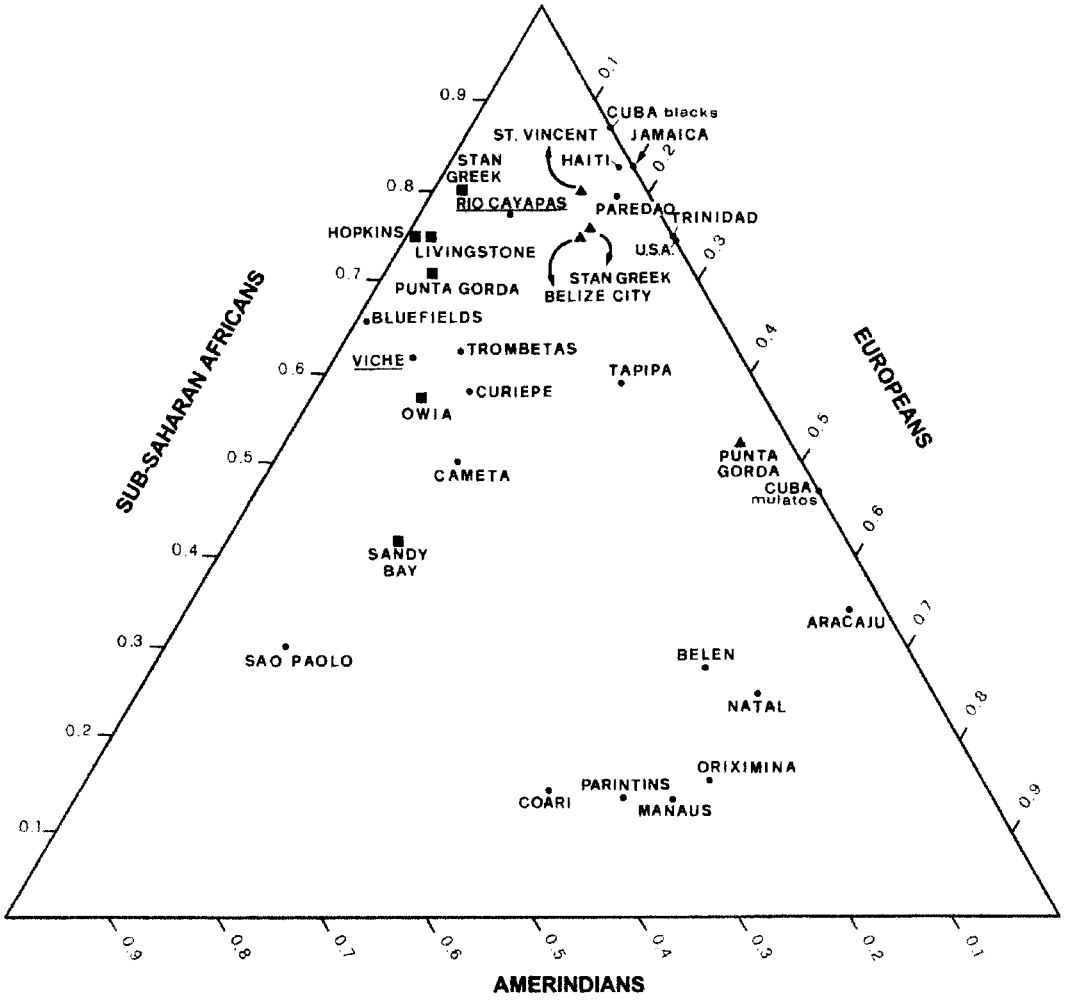

Fig. 6. Spatial representation of the contribution of African, European, and Native American populations to the gene pool of American communities of African ancestry. Squares indicate Black Caribs (Hopkins, Punta Gorda, and Stan Greek, Belize; Livingston, Guatemala; Owia and Sandy Bay, St. Vincent); triangles indicate Creoles (Belize City, Punta Gorda, and Stan Greek, Belize; St. Vincent); circles indicate other American communities of African ancestry (Aracaju, Belém, Cameta, Coari, Manaus, Natal, Oriximina, Paredao, Parintins, Sao Paulo, and Trombetas, Brazil; Blacks and Mulattos, Cuba; Rio Cayapas and Viche, Ecuador; Haiti; Jamaica; Bluefields, Nicaragua; Trinidad; USA; Curiepe and Tapipa, Venezuela). Database and the relative references are reported in Martínez-Labarga (1993) and are available on http://www.uniroma2.it/biologia/lab/anthromol/amafrhybr.htm.

mates (Viche, 14.0 ± 13.0; Rio Cayapas, 11.1 ± 12.8). Similar results (Viche, 53.07 ± 3.94, 25.11 ± 1.69, and 21.82 ± 4.81; Rio Cayapas, 71.16 ± 1.37, 12.05 ± 0.59, and 16.79 ± 1.68 for African, Indian, and Spanish relative contributions, respectively) were obtained using an alternative test for the estimation of admixture proportions (Chakraborty et al., 1992).

DISCUSSION

Admixture among Africans, Europeans, and Native Americans in the formation of the hybrid population of American communities of African ancestry varies widely by ethnicity, geography, and sex. Both European males and females greatly contributed to the gene pool of the US and Cuba popula-

tions of African ancestry, whereas the genetic contribution from Native Americans is only anecdotal (Reed, 1969; Gonzalez et al., 1976; Chakraborty et al., 1992; Hsieh and Sutton, 1992). Much the opposite occurred with the African slaves deported or who escaped to central-southern regions of the New World, whose main genetic partners were Native Americans (Battistuzzi et al., 1986; Crawford, 1986; Biondi et al., 1988).

African slaves first entered northwestern Ecuador during the 16th century. Although Spanish law strictly prohibited sexual contact, they intermixed with the Campaces and Niguas, two populations who have meanwhile become extinct. The new mixed population, called "Zambo" (Whitten, 1974), later increased when groups of African ancestry entered the area, mainly from Colombia. Despite their common ethnogenesis, the two very representative communities of African ancestry in Ecuador, Rio Cayapas and Viche, took different patterns of admixture over the centuries. Biodemographic analysis of parent-offspring migration reveals a very high level of inbreeding and, therefore, isolation in Rio Cayapas, a rural community. Since this genetic isolation was not due to geographic distances, it is likely that mutual mate exchange among nuclear families of African ancestry along the Rio Cayapas, the major communication route of the area, was common behavior. In contrast, the more recently established urban community of Viche behaved as a modern society open to intermarriage with neighboring populations of different ethnicity. Surname analysis confirmed the marriage patterns of the two groups. In fact, names of Colombian origin are common in Rio Cayapas, whereas in Viche there is a consistently higher percentage of foreign names, coming mainly from South America and the Caribbean Islands (Estupiñan Tello, 1983).

The evaluation of the genetic contribution of the three parental populations to the gene pool of the communities of African ancestry of Rio Cayapas and Viche suffered several limitations common to the studies of admixture. They concern uncertainty in defining the parental populations contributing to the mixed group and inexact knowledge of their gene frequencies. We considered populations from West Africa and Spain as representative of the Old World founders, and the Cayapa Indians of Ecuador as the Native American component. The two Ecuadorian communities clustered together with the other communities of African ancestry; the population of Rio Cayapas was closer to the West Africans. Quantitative evaluation of the genetic contribution of the parental populations showed that sub-Saharan Africans contributed about 74.3% and 58.4% to the genetic ancestry of the Rio Cayapas and Viche communities, respectively. The amount of gene flow from Native Americans clearly depicted the rural community as more genetically isolated than the urban population, with only 14.5% vs. 27.6% of Amerindian genes.

Further evidence of the genetic isolation of the Rio Cayapas community was seen in the unusually high frequency of the C allele of hemoglobin: about 13% vs. $<5\%$ in the other American communities of African ancestry. Besides this, mtDNA haplotypes associated with the region V deletion, characteristic of Asian and Asian-derived populations, provided a good estimate of maternal gene flow from Native Americans to the two communities. As reported in Rickards (1995), the difference between the incidence of the 9-bp mutation in Viche (21.3%) and Rio Cayapas (3.3%) was statistically significant ($\chi^2 = 8.718$, 1 d.f., $0.01 > P > 0.001$). This finding can be quantitatively expressed as a relative contribution of the Amerindians to the gene pool of the two hybrid populations. The fraction of mtDNA of Native American origin in the Rio Cayapas population was quite small ($8\% \pm 5\%$), whereas in Viche female Native Americans contributed the major portion ($51\% \pm 15\%$) of the gene pool.

In conclusion, the bulk of the present analyses shows that different patterns of gene flow from Europeans and Native Americans characterized the biological history of populations of African ancestry throughout northern and central-southern regions of the Americas. In the particular case of Ecuadorian communities, the genetic contribution of Native populations is rather consistent. However, while the Viche community received a high level of gene flow by its mainly exogamic marriage behavior, the

population of Rio Cayapas refused intermarriages with neighboring populations, to produce one of the "blackest" communities of African ancestry.

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