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New Studies on the Esterase D Polymorphism in South American Indians

M.A. MESTRINER, A.L. SIMÕES, AND F.M. SALZANO
Departamento de Genética, Faculdade de Medicina, Universidade de São Paulo, 14100 Ribeirão Preto, SP, Brazil (M.A.M., A.L.S.) and Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, 90000 Porto Alegre, RS, Brazil (F.M.S.)

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ABSTRACT A total of 2,490 South American Indians affiliated with 10 tribes were studied for esterases A_1, A_2, A_3, B, D, and carbonic anhydrases 1 and 2. Wide variation was observed in the prevalence of *ESD*[1], the extreme values being 0.44, encountered among the Xikrin, and 0.95, found among the Cashinawa. Seven of the 10 frequencies fell within the interval of 0.66–0.87. These results were integrated with those of earlier surveys. In a general way we observe lower values in the eastern and Atlantic Coast groups, but this is mainly due to the low frequencies found among the Gê tribes and the Caingang, who speak a language which has many affinities with Gê. High prevalences were observed among the tropical forest tribes. A fair amount of intervillage, intratribal variation was found among the Ticuna and Caingang. No variability was detected in the A_1, A_2, A_3, B esterases. In the carbonic anhydrases we confirmed the presence of a "private polymorphism" among the Baniwa.

The esterase D polymorphism, discovered six years ago (Hopkinson et al., '73) has already proved to be of considerable interest for the genetic delineation of human populations. We have published the first information about its distribution in South American Indians (Mestriner et al., '76); subsequently Goedde et al. ('77), Neel et al. ('77), Salzano et al. ('78), and Tchen et al., ('78, '79) obtained additional data. We hereby report results about it on 2,490 South American Indians affiliated with 10 different tribes. They were integrated with these earlier studies and the distribution pattern thus obtained compared with the geographical and linguistic variation. Tests of genetic equilibrium were performed for all groups and the intratribal, intervillage variation tested in eight populations of Ticuna, two of Katukina and four of Caingang Indians. The generally invariant A_1, A_2, A_3 and B esterases, as well as carbonic anhydrases CA_1 and CA_2 were studied simultaneously and these observations will also be presented here.

MATERIALS AND METHODS

Information about the tribes studied can be summarized as follows:

1. *Warao.* Until recently their language was classified as independent, but attention has lately been drawn to Chibchan elements in it. The 11,000–12,000 members of this tribe occupy the Orinoco Delta of Venezuela and the adjacent swampy regions of Guiana (59°–62°W, 8–10°N). Data about their culture and blood groups can be found in Layrisse and Wilbert ('66) and Suarez ('68). The material described here was collected in the village of Winikina by a group of investigators from the Instituto Venezolano de Investigaciones Científicas and kindly made available to us by Dr. Zulay Layrisse.

2. *Baniwa.* They speak an Arawak language and number some 1,500 persons who live in the area drained by the Içana river, a tributary of the Rio Negro, in the extreme northwestern corner of Brazil and adjacent Colombia. A synthetic account of their culture and present degree of acculturation can be found in Oliveira and Galvão ('73). The samples studied were collected at a small station of the New Tribes Mission on the Upper Içana river, at lat. 1°33′N, long. 68°44′W, named Jandu Cachoeira. Representatives from six nearby villages were bled there.

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3. *Ticuna.* Probably the largest group of descendants from a single tribe still living in Brazil, the 11,000 Ticuna speak an independent language and are distributed along the Solimões river between 2° and 5°S and from 66° to 71°W. Demographic and cultural information about the eight populations sampled, as well as their precise location can be found in Salzano et al. ('79a).

4. *Kanamari.* The 800 members of this tribe live in the basins of the Juruá and Purus river, in the southwestern region of the Brazilian State of Amazonas. Their language, Katukina, shows no close affiliation with the others spoken in this area. We could not locate a good ethnographic study of them. Collections were made at a station of the New Tribes Mission called Três Unidos, on the Mamoré Creek, a tributary of the Juruá (6°37'S, 69°32'W).

5. *Marubo.* These Indians number about 400, living in the headwaters of the Itui and Curuçá rivers in the Javari basin, not very far away from the Kanamari. They speak a Pano language and their culture was described by Melatti and Melatti ('75) and Melatti ('77). Bloods were collected at the New Tribes station of Vida Nova (6°47'S, 72°8'W).

6. *Cashinawa.* Also speaking a Pano language their number is estimated at about 2,000, and they are distributed along the high Embira river and its tributaries. Three Peruvian groups have been extensively studied from the genetic and demographic point of view by Johnston and coworkers (references in Johnston and Kensinger, '71). Brief ethnographic information and bibliographic references about them and other Pano tribes can be found in Schultz and Chiara ('55) and Trujillo-Ferrari ('60). The specimens were obtained at the New Tribes mission of Cana Brava (8°7'S, 70°19'W).

7. *Jaminawa.* Spread in the same general area as that occupied by the Cashinawa and Katukina, a recent estimate of the Brazilian government Indian Service (FUNAI) indicates that there are 410 individuals of this Central Pano tribe presently living in the Brazilian State of Acre (Anonymous, '78). They were sampled near Sete Estrelas, another New Tribes mission (8°17'S, 71°34'W).

8. *Katukina.* Also a Central Pano tribe, their number in the State of Acre was estimated in 700 (Anonymous, '78). The collections among them were performed at the mission of Sete Estrelas (see above) and at Morada Nova, a village two km up the Embira river from the town of Feijó.

9. *Cayapo (Xikrin).* There are at present some 2,400 Cayapo Indians, a Gê-speaking tribe whose members live in northern Mato Grosso and southern Pará, reaching from latitude 5° to 10°S and from longitude 51° to 54°W. The number of persons in the Xikrin subgroup is estimated as 350. Demographic and historical information about this tribe can be found in Salzano ('71) and Verswijver ('78). The bloods

TABLE 1. Esterase D phenotypes and gene frequencies in several South American Indian tribes

Tribe	Locality	Phenotypes			Total	ESD[1] frequency
		1-1	2-1	2-2		
Warao	Winikina, Orinoco Delta, Venezuela	20	41	24	85	0.48
Baniwa	Jandu Cachoeira (39A-D) Amazonas State, Brazil	231	130	14	375	0.79
Ticuna	Eight localities, Amazonas State, Brazil	573	566	154	1293	0.66
Kanamari	Três Unidos (42A, B), Amazonas State, Brazil	58	20	0	78	0.87
Marubo	Vida Nova (34A-E), Amazonas State, Brazil	66	41	8	115	0.75
Cashinawa	Cana Brava (40A, B), Amazonas State, Brazil	83	10	0	93	0.95
Jaminawa	Near Sete Estrelas (35B), Acre State, Brazil	30	15	1	46	0.82
Katukina	Two localities, Acre State, Brazil	59	26	2	87	0.83
Cayapo (Xikrin)	Near Caeteté river, Pará State, Brazil	17	54	29	100	0.44
Caingang	Four localities, Rio Grande do Sul, Brazil	107[1]	86	25	218	0.69

[1]The data obtained in Guarita (see Table 3) were already reported in Mestriner et al. ('76).

studied were obtained in the village located near the Caeteté river, an affluent of the left margin of the Itacaiunas. Data about the acculturation process that is occurring in this population were given by Frikel ('63). Vieira-Filho ('70) described the reactions observed when they were vaccinated against smallpox.

10. *Caingang*. They speak an independent language that has many similarities with Gê and are distributed in the southern Brazilian States of São Paulo, Paraná, Santa Catarina and Rio Grande do Sul (21°30′–28°30′S; 49°30′–54°W). Population estimates place their number in 4,000. Four groups were studied (Nonoai, Guarita, Cacique Doble and Ligeiro) in the State of Rio Grande do Sul. Historical, demographic, cultural and genetic information about them can be found in Salzano ('61) and Becker ('76).

The A_1, A_2, A_3, B, and D esterases, as well as carbonic anhydrases 1 and 2 were determined using the tris-maleate buffer system in the conditions specified by Hopkinson et al. ('73). Staining for the A_1, A_2, A_3, and B esterases was done with alpha-naphthyl acetate (Tashian, '69); for esterase D with acetate of 4-methyl-umbelliferone (Hopkinson et al. '73); and for the carbonic anhydrases with fluorescein diacetate (Hopkinson et al., '74). Some samples from the Baniwa tribe were also typed for the carbonic anhydrases using the tris-citrate-borate-lithium hydroxide buffer in the conditions described by Neel et al. ('77) but with horizontal runs.

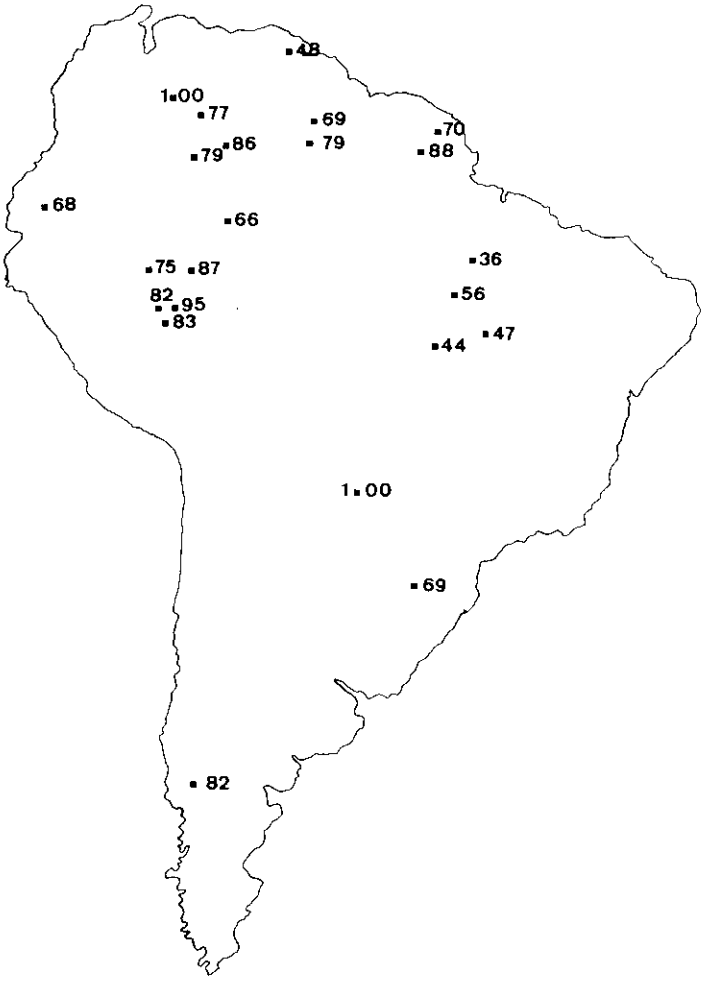

Fig. 1. The distribution of the *ESD*^{1} allele in several South American Indian tribes.

TABLE 2. The distribution of the ESD[1] allele in South American tribes classified by language

Language classification[1]	Tribe	No. of indiv. studied	*ESD*[1] frequency	Reference	Weighted averages[2]
Paleo-american tribes					0.645
Chaco division					
Zamuco stock	Ayoreo	141	1.00	(1)	
Central Brazil division					
Kaingán stock	Caingang	218	0.69	(2)	
Gê stock	Gorotire	163	0.56	(3)	0.498
	Krahô	146	0.47	(3)	
	Xikrin	100	0.44	(2)	
Tropical forest tribes					0.747
North central division					0.734
Tupi stock	Emerillon	55	0.70	(4)	0.792
	Waiampi	238	0.88	(4)	
	Parakanan	37	0.36	(3)	
Arawak stock	Cuiva	104	1.00	(5)	0.812
	Wapishana	567	0.79	(6)	
	Baniwa	375	0.79	(2)	
Tucuna stock	Ticuna	1293	0.66	(2)	
Jibaro stock	Shuara	90	0.68	(7)	
South central division					0.834
Pano stock	Marubo	115	0.75	(2)	
	Cashinawa	93	0.95	(2)	
	Jaminawa	46	0.82	(2)	
	Katukina	87	0.83	(2)	
Central division					0.870
Catuquina stock	Kanamari	78	0.87	(2)	
Northeastern division					0.745
Karaib stock	Makiritare	69	0.77	(3)	0.700
	Macushi	498	0.69	(6)	
Yanoama stock	Yanomama	419	0.86	(3)	
Uarao stock	Warao	85	0.48	(2)	
Andean tribes					
Southern division					
Mapuche stock	Mapuche	51	0.82	(3)	

[1]According to Loukotka ('68). [2]Weighted considering the sample sizes of the tribes studied. *References:* (1) Salzano et al. ('78); (2) Present investigation; (3) Mestriner et al. ('76); (4) Tchen et al. ('78); (5) Tchen et al. ('79); (6) Neel et al. ('77). Results from 46 admixed individuals mistakenly included in this report have been deleted in this tabulation. See Salzano et al. ('79b); (7) Goedde et al. ('77).

TABLE 3. Intervillage variation in the frequency of ESD[1] among the Ticuna, Katukina and Caingang

Tribe and village	Phenotypes 1-1	2-1	2-2	Total	*ESD*[1] frequency
Ticuna					
Bom Jardim (37I)	33	39	4	76	0.69
Umariaçu (37A-E)	136	110	29	275	0.69
Marajá (37F)	32	42	15	89	0.60
Feijoal (37G, H)	63	36	15	114	0.71
Belém (43A-D)	30	45	8	83	0.63
Vendaval (37J-N)	124	113	32	269	0.67
Campo Alegre (37T-X)	114	120	18	252	0.69
Nova Italia (37P, R)	41	61	33	135	0.53
Katukina					
Morada Nova (41A)	30	15	0	45	0.83
Sete Estrelas (35A)	29	11	2	42	0.82
Caingang					
Nonoai	32	10	0	42	0.88
Guarita[1]	32	33	9	74	0.65
Cacique Doble	19	17	0	36	0.76
Ligeiro	24	26	16	66	0.56

[1]Data already reported in Mestriner et al. ('76).

RESULTS AND DISCUSSION

Data about the *ESD*[1] frequency are presented in Table 1. A total of 2,490 individuals were studied, distributed among 10 tribes. As can be seen there is wide variation in the prevalence of this allele, the extreme values being 0.44, observed among the Xikrin, and 0.95, found among the Cashinawa. Seven of the 10 frequencies fell within the interval of 0.66–0.87.

This information was integrated with those of earlier surveys and the results are shown in Figure 1 and Table 2. To date, 5,068 South American Indians were studied for esterase D, the *ESD*[1] frequencies varying from 0.36, encountered in the Parakanan, to 1.00, reported among the Ayoreo and Cuiva; the total weighted average is 0.732. In a general way we observe lower values in the eastern and Atlantic Coast groups. This is mainly due to the low frequencies found among the Gê-speaking tribes and the Caingang, who speak a language which has many affinities with Gê.

Further comparisons can be made if we adopt the language classification of Loukotka ('68) and distribute the tribes according to it. A gradation can be seen in the frequencies of the Paleo-American (weighted average 0.64), Tropical Forest (0.75) and Andean (0.82) tribes. But there is wide variation in the values found among the groups classified as Paleo-American, suggesting that this may be an artificial unit of classification. The Tropical Forest tribes on the other hand show more uniformity, 13 of the 17 frequencies falling in the interval between 0.66 and 0.88. Within linguistic stocks, considering those with at least three tribes studied, the Gê tribes proved to be the less variable, followed by the Arawak and Pano, the Tupi showing the widest interval.

How does the intervillage, intratribal variation compare with the intertribal one? Among the Ticuna, Katukina and Caingang we sampled more than one village and the results are shown in Table 3. Only two communities were studied among the Katukina, and the *ESD*[1] frequencies found were almost identical. The interval of variation observed among the Ticuna (eight localities sampled) was of 0.18 (from 0.53, found in Nova Italia, to 0.71 encountered in Feijoal). These populations are distributed in a linear array along the Solimões river, Bom Jardim being the most westernly located and Nova Italia the most easternly. No clear gradients in gene frequency appear, however. This is understandable if we consider that these groups are of recent formation, being the result of a general trend towards the migration to the margins of this river of people who until recently lived scattered over a large area, along smaller streams of water (see Salzano et al., '79a for details). Only four communities were studied among the Caingang, but the interval of variation was almost two times as high (0.32) as that of the Ticuna. The lowest frequency was found in Ligeiro (0.56) and the highest in Nonoai (0.88). Previously (Salzano, '61) we have found the clustering of some genes, suggesting a dichotomy in relation to these four populations (Nonoai-Guarita on one hand and Cacique Doble-Ligeiro on the other). As can be observed in Table 3, the *ESD*[1] results do not fit this pattern.

Comparative data for these findings can be found in Mestriner et al. ('76) and Neel et al. ('77). They reported the intratribal variation for *ESD*[1] among nine Yanomama and eleven Wapishana villages, respectively. The Yanomama showed the smallest interval of variation (0.12) of all compared and the Wapishana an intermediate one (0.23).

Mestriner ('76) has found in a sample of 2,234 individuals from Northeastern Brazil a significant ($P<0.05$) lack of heterozygotes at the esterase D locus, considering the frequency expected under Hardy-Weinberg equilibrium. We tested the possibility of such a departure through Wright's ('21, '69) formula $F = 1 - (H_0/2pq)$, where H_0 is the observed frequency of heterozygotes, and p and q are the allele frequencies. A positive F would indicate a lack of heterozygotes; a negative one, an excess. In none of the 21 samples that permitted such a comparison was the F significantly different from zero. Moreover, its sign did not deviate significantly in one direction or the other (13 positives, 8 negatives, $P>0.20$). Whatever the causes that lead to the disequilibrium in the Northeastern sample studied by Mestriner, they are not found in the populations reported here.

As for the esterases A_1, A_2, A_3, and B, no variability was observed. The number of samples in which they were clearly visible is somewhat less than that examined for esterase D, being as follows: Baniwa: 371; Ticuna: 1,263; Kanamari: 75; Marubo: 113; Cashinawa: 89; Jaminawa: 43; Katukina: 84; Caingang: 218. The Warao and Xikrin were not studied in relation to esterases A_1, A_2, A_3, B.

As was indicated in the materials and methods section, all samples have been examined for the carbonic anhydrases using the tris-maleate buffer system. In these conditions we have been

able to detect three variant specimens only, one each among the Ticuna of Umariaçu, Jaminawa and Caingang of Nonoai. The pattern observed was typical of a $CA_2$2-1 blood, phenotype that occurs with a certain frequency among Blacks. The simplest explanation for these findings, therefore, is that this allele was introduced in these populations through racial admixture, although no other Black traits have been observed in these individuals.

Using the tris-citrate-borate-lithium hydroxide buffer our colleagues at the Department of Human Genetics of the University of Michigan Medical School at Ann Arbor were able to detect among the same Baniwa samples studied here a "private polymorphism" in the carbonic anhydrase 2 system. The corresponding allele CA_2^{BAN-1} occurs there with a frequency of five per cent. A detailed report about these results can be found in Mohrenweiser et al. ('79). Some of the variant samples were also run by us using this system, and we confirmed the results of these investigators.

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LITERATURE CITED

Anonymous (1978) Os cinco mil índios do Acre. Rev. Atual. Indígena (Brasília), *2*(13):42–45.

Becker, I.I.B. (1976) O índio Kaingáng no Rio Grande do Sul. Pesquisas (Antropologia 29):1–331.

Frikel, P. (1963) Notas sobre a situação atual dos índios Xikrin do Rio Caeteté. Rev. Museu Paulista, *14*:145–158.

Goedde, H.W., H.G. Benkmann, D.P. Agarwal, and A. Kroeger (1977) Genetic studies in Ecuador: Acetylator phenotypes, red cell enzymes and serum protein polymorphisms of Shuara Indians. Am. J. Phys. Anthropol., *47*:419–425.

Hopkinson, D.A., J.S. Coppock, M.F. Mühlemann, and Y.H. Edwards (1974) The detection and differentiation of the products of the human carbonic anhydrase loci, CA_I and CA_{II}, using fluorogenic substrates. Ann. Hum. Genet., *38*:155–162.

Hopkinson, D.A., M.A. Mestriner, J. Cortner, and H. Harris (1973) Esterase D: a new human polymorphism. Ann. Hum. Genet., *37*:119–137.

Johnston, F.E., and K.M. Kensinger (1971) Fertility and mortality differentials and their implications for microevolutionary change among the Cashinahua. Hum. Biol., *43*:356–364.

Layrisse, M., and J. Wilbert (1966) Indian Societies of Venezuela. Editorial Sucre, Caracas.

Loukotka, C. (1968) Classification of South American Indian Languages. Latin American Center, University of California, Los Angeles.

Mellatti, D.M., and J.C. Melatti (1975) Relatório sobre os índios Marubo. Trabalhos de Ciências Sociais, Série Antropologia Social (Brasília), *13*:1–162.

Melatti, J.C. (1977) Marúbo. Rev. Atual. Indígena (Brasília). *1*(2):64.

Mestriner, M.A. (1976) Estudo Populacional de Dois Polimorfismos Enzimáticos no Homem: Esterase D e Anidrase Carbônica CAII. Privat Docent Dissertation, Faculdade de Medicina, Universidade de São Paulo, Ribeirão Preto.

Mestriner, M.A., F.M. Salzano, J.V. Neel, and M. Ayres (1976) Esterase D in South American Indians. Am. J. Hum. Genet., *28*:257–261.

Mohrenweiser, H., J.V. Neel, M.A. Mestriner, F.M. Salzano, E.C. Migliazza, A.L. Simões, and C.Y. Yoshihara (1979) Electrophoretic variants in three Amerindian tribes: the Baniwa, Kanamari and Central Pano of western Brazil. Am. J. Phys. Anthropol., *50*:237–246.

Neel, J.V., R.J. Tanis, E.C. Migliazza, R.S. Spielman, F.M. Salzano, W.J. Oliver, M. Morrow, and S. Bachofer (1977) Genetic studies of the Macushi and Wapishana Indians. I. Rare genetic variants and a "private polymorphism" of esterase A. Hum. Genet., *36*:81–107.

Oliveira, A.E., and E. Galvão (1973) A situação atual dos Baniwa (Alto Rio Negro) - 1971. In: O Museu Goeldi no Ano do Sesquicentenário. Museu Paraense Emílio Goeldi, Belém, pp. 27–40.

Salzano, F.M. (1961) Estudos genéticos e demográficos entre os índios do Rio Grande do Sul. Bol. Inst. Ciênc. Nat. (Porto Alegre), *9*:1–161.

Salzano, F.M. (1971) Demographic and genetic interrelationships among the Cayapo Indians of Brazil. Soc. Biol., *18*:148–157.

Salzano, F.M., S.M. Callegari-Jacques, and J.V. Neel (1979a) Genetic demography of the Amazonian Ticuna Indians J. Hum. Evol. (in press).

Salzano, F.M., Callegari-Jacques, and J.V. Neel (1979b) Demographic and genetic relationships among Brazilian Wapishana Indians Ann. Hum. Biol. (in press).

Salzano, F.M., F. Pagés, J.V. Neel, H. Gershowitz, R.J. Tanis, R. Moreno, and M.H.L.P. Franco (1978) Unusual blood genetic characteristics among the Ayoreo Indians of Bolivia and Paraguay. Hum. Biol., *50*:121–136.

Schultz, H., and V. Chiara (1955) Informações sobre os índios do Alto Rio Purus. Rev. Museu Paulista, *9*:181–201.

Suarez, M.M. (1968) Los Warao. Departamento de Antropología, Instituto Venezolano de Investigaciones Científicas, Caracas.

Tashian, R.E. (1969) The esterases and carbonic anhydrases of human erythrocytes. In: Biochemical Methods in Red Cell Genetics. J.J. Yunis, ed. Academic Press, New York, pp. 307–336.

Tchen, P., E. Bois, J. Seger, P. Grenand, N. Feingold, and J. Feingold (1978) A genetic study of two French Guiana Amerindian populations. I. Serum proteins and red cell enzymes. Hum. Genet. *45:*305–315.

Tchen, P., F. Fonval, E. Bois, L. Rivat, and J. Seger (1979) Genetic study of the Cuiva Indians of Venezuela. Ann. Hum. Biol., *6:*231–240.

Trujillo-Ferrari, A. (1960) Análisis del comportamiento económico de los Kashibo frente a los efectos aculturativos. Rev. Museu Paulista, *12:*199–309.

Vieira-Filho, J.P.B. (1970) Vacinação dos indios Xikrin, Suruí e Gaviões contra a varíola. Rev. Assoc. Med. Brasil., *16:*357–360.

Verswijver, G. (1978) Os Kayapó-separações e junções dos grupos do norte. Rev. Atual. Indígena (Brasília), *2*(12):9–16.

Wright, S. (1921) Systems of mating. Genetics, *6:*111–178.

Wright, S. (1969) Evolution and the Genetics of Populations. Vol. 2. The Theory of Gene Frequencies. The University of Chicago Press, Chicago.