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Distribution of Hemoglobin Phenotypes in Four Different Districts of Porto Velho, Rondônia, Brazil

A. ANDRADE-CASSEB,^{1,2} A. KRAUZE,^{1,3} R. M. LAFONTAINE,^{1,2} M. S. TADA,³ W. A. SILVA JR.,⁴ A. L. SIMÕES,⁴ AND V. ENGRACIA^{1,2}

Abstract Hemoglobin profile studies have been carried out in four samples from different districts of Porto Velho (Rondônia State) in the western Amazonian region of Brazil: Candelária, Bate Estaca, Hemeron (at the State Blood Bank), and São Carlos. Samples from 337 unrelated individuals were collected during medical and paramedical team visits by professionals from the Instituto de Pesquisa em Patologia Tropical and the Centro de Pesquisa em Patologias Tropicais (both research institutes in tropical diseases). The aim of this study is to assess the frequency of alleles in the hemoglobin system, mainly alleles *HB*A*, **S*, and **E*. The overall phenotype frequencies were *HB A,S* = 0.025, *HB A,E* = 0.006, and *HB A,A* = 0.969. Samples from the blood bank subjects and samples from the homogeneous areas of São Carlos and Candelária plus Bate Estaca have a chi-square of heterogeneity of 6.383 ($p = 0.041$) and 8.406 ($p = 0.015$), respectively. The allele frequencies (*HB*A* = 0.984, *HB*S* = 0.012, and *HB*E* = 0.003) do not significantly differ from frequencies found in other Brazilian regions.

One of the most important challenges in population genetics has been to understand the processes that maintain genetic polymorphism. The association of malaria infection and hemoglobin genotypes is a rare example of selection involved in the fixation of deleterious genes, as found by modeling human adaptive phenotypes. Malaria is an endemic tropical disease in different areas of northern Brazil. Because there is a complex relationship between hemoglobin S (*HB*S/*S*, *HB*A/*S*) or C (*HB*A/*C*, *HB*C/*C*) genotypes and malaria susceptibility (Allison 1964; Modiano et al. 2001), in this study we have focused our attention on the analysis of phenotype frequency departures from Hardy-Weinberg equilibrium in four mixed samples of urban, peripheral, and riverine populations of

¹Instituto de Pesquisa em Patologia Tropical (IPEPATRO), BR 364, Km 3.5, 78900-970 Porto Velho, RO, Brazil.

²Universidade Federal de Rondônia (UNIR), Br 364, Km 9.5, CEP 78900-500 Porto Velho, RO, Brazil.

³Centro de Pesquisa em Patologias Tropicais (CEPEM), BR 364, Km 4.5, CEP 78900-000 Porto Velho, RO, Brazil.

⁴Departamento de Genética da Faculdade de Medicina (FMRP), University of São Paulo (USP), Av. Bandeirantes 3, 900 Bairro Monte Alegre, CEP 14049-900, Ribeirão Preto, SP, Brazil.

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Porto Velho (Rondônia State) in the western Amazonian region of Brazil, as a first step of studies involving *HB* phenotypes and malaria. The contribution of European, African, and Amerindian genes to current Amazonian populations has been heterogeneous. Understanding the occurrence of characteristics presented in the ancestry and Amazonian groups is crucial because the pressure they are subjected to from environmental conditions may be different in the two populations (Salzano et al. 1986). The tri-ethnic composition of the Brazilian population (Engracia 1972; Krieger et al. 1965) that is present in Rondônia (Ferreira et al. 2002) occurred through extensive migration waves, such as European colonization (around 1723), the rubber boom (internal migration), the construction of the Madeira-Mamoré railroad (with workers from both northeastern Brazil and the Caribbean), and immigrants from eastern and southeastern Brazilian regions (20th-century immigrants). These migrations introduced different markers into the hemoglobin locus. Because the description of genetic variability is, first, a process of identifying genetic resistance to diseases, in the present study we describe *HB* polymorphism in a Brazilian mixed population from a malaria-endemic area.

Subjects and Methods

Population. Four samples of unrelated individuals (322 subjects) are considered in this study. Candelária and Bate Estaca are riverine districts of Porto Velho, and the subjects from Candelária (88) are descended from workers who had been involved in the construction of the Madeira-Mamoré railroad. Bate Estaca (34 individuals) presents a large number of migrating inhabitants. São Carlos (56 individuals) is located 34 km from Porto Velho, and the collected samples are from riverine people who sought medical help during periodic visits made by workers from the Instituto de Pesquisa em Patologia Tropical (IPEPATRO) or the Centro de Pesquisa em Patologias Tropicais (CEPEM) (both IPEPATRO and CEPEM are research institutes in tropical diseases located in Porto Velho). All the individuals from these three localities belong to the adult population (older than 18 years old) of a larger familial sampling. Hemeron is a Rondonian state hospital in Porto Velho, also in a malaria-endemic area, and its sample is different from the other three because the hospital is a blood bank (Figure 1). The individuals are examined for hematological diseases before being admitted as blood donors. This sample was composed of 159 adult individuals, before their selection as donors.

The estimates of ethnic admixture for Bate Estaca and Candelária (Krauze de França 2005) indicate that European genes are the most frequent (62% and 58%, respectively), followed by African genes (22% and 37%) and Amerindian genes (16% and 5%). The same can be observed in the other samples, a consequence of the peopling processes (migratory waves) in Rondônia. See Heckmann et al. (2005) for more information about the sample. Table 1 shows the precise location of these samples.

This research was approved by the Council of Ethic Procedures of the Brazilian Government (CONEP study 3349).

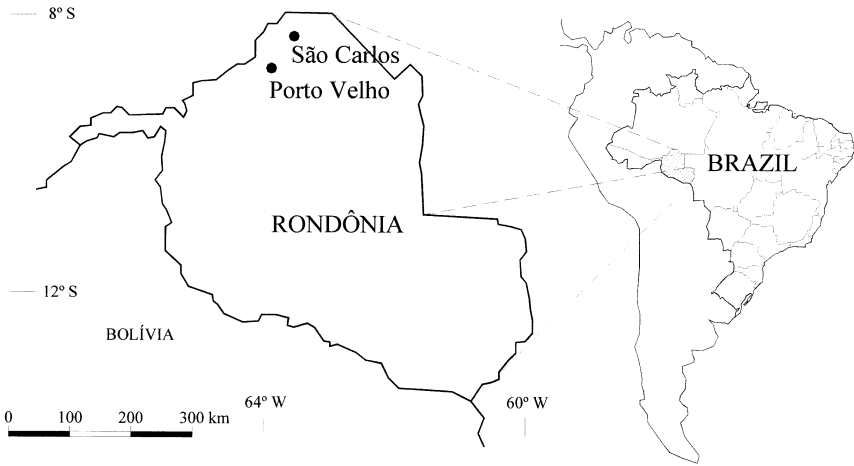


Figure 1. Study area showing locations where samples were collected.

Collection and Storage of Blood Samples. In Candelária, Bate Estaca, and São Carlos, blood samples were collected during periodic visits to those places, organized by one of us (M. S. Tada) and carried out by the CEPEM and IPEPATRO teams. The collection at Hemeron followed the routine of the blood bank over an 18-month period.

Blood samples (10 ml) were collected with anticoagulant, sent to the laboratory, and processed within 10 hr after collection, using traditional hematological laboratory procedures. Samples were maintained in Styrofoam boxes during transportation.

Analytical Methods. Hemoglobin was typed using PCR-RFLP according to the method of Modiano et al. (2001).

Table 1. Characterization of the Studied Groups from Rondônia State, Brazil

Characteristic	Sample			
	Candelária	Bate Estaca	São Carlos	Hemeron
Geographic coordinates	63°54'14"W, 8°45'43"S	63°54'14"W, 8°45'43"S	63°49'14"W, 8°39'44"S	63°54'14"W, 8°45'43"S
Socioeconomic status	Urban/riverine	Urban/riverine	Riverine	Urban
Year of collection	2001–2004	2002–2004	2000	2000–2001
Number of individuals	88	34	56	159

Allele frequencies were computed with the maximum-likelihood method using the FREGEN software program, part of the Genioc library (Cabello and Krieger 1997). A chi-square test for goodness of fit was used to verify the conditions of Hardy-Weinberg equilibrium and heterogeneity between samples, using the SPSS 11.0 package program.

Results and Discussion

Rondônia State has received a great number of migrants from other Brazilian states since the 1980s. The state capital, Porto Velho, has been chosen by most migrants because of the mining opportunities in the region and also because of the BR-364 road opening, which connects the western Amazon to southern states (thus enabling migration flow). During the 1990s, as a result of federal government investments in housing, new migration flow was seen in the region. From 1971 to 1989, mining exploitation was the main economic source, corresponding to almost 70% of total national production. At the beginning of the 20th century, Porto Velho was a village with about 300 inhabitants, almost all of whom were white men with some black women. There was only one white woman (Ferreira 1987). The region was originally populated by Indian natives, such as Torá, Mura, Caripuras, Pamas, and other Indian nations. These populations were exterminated by Jesuit missions and also by the Portuguese, who conquered the Madeira river region at the end of the 18th century (Heckmann et al. 2005; Silva 2001). In 1873, because of the influx of migrants to harvest the latex, the village developed, and in 1912, it was elevated to the “city” category. In 1907, when the Madeira-Mamoré railroad was constructed, the railroad company built a hospital, named Candelária. The region in which the hospital was built is currently the most ancient district of Porto Velho, and its inhabitants are mostly descendants of the railroad workers. From 1907 to 1912 the railroad company contracted 21,783 men from diverse nationalities, mostly Brazilians from other Brazilian regions. Other nationalities included Antilleans, Barbadians, Spaniards, Portuguese, Italians, and Colombians (Ferreira 1987). Currently, the population of Porto Velho is estimated at 310,000 inhabitants.

We have analyzed four samples of the Porto Velho population for the *HB* gene. This study is part of a large scientific project aimed at investigating the various aspects of the types of malaria infection in the region. The study is also aimed at studying how the human genome may influence the medical and epidemiological practices for malaria prophylaxis. The samples from Candelária and Bate Estaca are still under longitudinal follow-up, and they may provide further evidence on the association between malaria and hemoglobin in this region.

Analyses of the four samples show that the frequency of *HB***S* carriers is 2.50%. However, according to the heterogeneity tests, the samples from the blood bank (Hemerón) cannot be analyzed in conjunction with the ones from Candelária and Bate Estaca ($\chi^2_2 = 8.443$, $p = 0.01467$) and São Carlos ($\chi^2_2 = 6.322$, $p = 0.04238$). When the Hemeron fraction was removed from the sample, the medium

Table 2. Phenotype and Allele Frequencies in a Malaria-Endemic Area in Rondônia, Brazil

Hemoglobin Phenotype or Allele	Sample			
	Candelária	Bate Estaca	São Carlos	Hemeron
Observed phenotype:				
Number (and frequency)				
HB A,A	85 (0.966)	28 (0.824)	55 (0.982)	143 (0.993)
HB A,E	1 (0.011)	1 (0.029)	0 (0.000)	0 (0.000)
HB A,S	2 (0.023)	5 (0.147)	1 (0.018)	1 (0.007)
Total (322)	88	34	56	144
χ^2	0.206	4.738	0.335	9.257
<i>P</i>	1	1	1	1
χ^2 of heterogeneity) ^a		0.00061		
Allele frequency				
<i>HB</i> *A	0.983	0.912	0.991	0.997
<i>HB</i> *E	0.006	0.015	0.000	0.000
<i>HB</i> *S	0.011	0.074	0.009	0.003

a. Candelária, Bate Estaca, and São Carlos.

frequency of the HbS heterozygote individuals increased to 6.20%. The genotype frequencies of *HB**S heterozygote individuals were relatively high in Candelária, Bate Estaca, and São Carlos (0.023, 0.147, and 0.018, respectively) (Table 2).

The falcemic trait affects between 6% and 10% of Brazilian black individuals, about 5%–6% of individuals morphologically classified as having African admixture, and about 1% of the population in general. Hemoglobinopathy is one of the most frequent genetic conditions in the Brazilian population (Salzano et al. 1986). In the community of Portuchuelo, located on the right bank of Madeira River, 34 km from Porto Velho, the frequency of the *HB**S allele is 0.018 (Ferreira et al. 2002). The ethnic composition of Portuchuelo presents a high percentage of the Amerindian component (44%), 35% European genes, and 21% African genes (Ferreira et al. 2002). In Monte Negro, an agricultural settlement 260 km from Porto Velho whose population has come mainly from southern Brazil and has a high degree of European admixture (62%), we have verified that the frequency of the *HB**S gene (0.021) (Ferreira et al. 2002) is lower than the frequencies observed in this study, but the frequency surpasses the frequencies from Portuchuelo.

Our results, with the exception of the results obtained from the Hemeron sample, also have relatively higher frequencies of *HB**S than those reported for some African-derived black communities in Piauí State, Brazil, with 61%–71% black ancestry and a frequency of *HB**S varying from 0.025 to 0.032 (Arpini-Sampaio et al. 1999). An interethnic estimate performed in Bate Estaca and Candelária using four STR loci has revealed that the European component is the most frequent in both samples: approximately 60% and 58%, respectively (Krauze de França 2005).

Because our four sample districts are located in malaria-endemic regions, malaria could be one of the explanations for the high frequency of individuals bearing *HB*S*. Natural selection is a delayed process, but malaria provokes a differential migration in these studied localities. The individuals who more frequently suffer the effects of *Plasmodium* infection are the ones who more frequently miss work because of illness and who suffer more side effects from the metabolization of antimalarial drugs. The fact that 60% of these populations present European genes mainly reflects the migrations in the 20th century. The presence of two *HB*E* heterozygous individuals, a common gene in southeastern Asia, reflects the evidence of multiple independent origins of the beta E mutation in human populations (Kazazian et al. 1984) and may be a consequence of migratory procedures as well as of the presence of Amerindian mixture among the urban population of Rondônia.

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Literature Cited

- Allison, A. C. 1964. Polymorphism and natural selection in human populations. *Cold Spring Harbor Symp. Quant. Biol.* 24:137–149.
- Arpini-Sampaio, Z., M. C. B. Costa, A. A. Melo et al. 1999. Genetic polymorphisms and ethnic admixture in African-derived black communities of northeastern Brazil. *Hum. Biol.* 71(1):69–85.
- Cabello, P. H., and H. Krieger. 1997. *Genioc: Sistema de Análises de Dados de Genética*. Technical Department of Genetics, Oswaldo Cruz Institute, Rio de Janeiro, Brazil.
- Engracia, V. 1972. *Mistura racial em uma população rural brasileira*. Master's thesis, Universidade de São Paulo, São Paulo, Brazil.
- Ferreira, M. R. 1987. *A Ferrovia do Diabo*, 4th ed. São Paulo: Comp. Melhoramentos.
- Ferreira, R. G. M., M. M. F. Moura, V. Engracia et al. 2002. Ethnic admixture composition of two Amazonian populations. *Hum. Biol.* 74(4):607–614.
- Heckmann, M. I. O., C. T. Mendes-Junior, M. G. Santos et al. 2005. *CFTR* haplotype distributions in the Brazilian western Amazonian region. *Hum. Biol.* 77(4):499–508.
- Kazazian, H. H., Jr., P. G. Waber, C. D. Boehm et al. 1984. Hemoglobin E in Europeans: Further evidence for multiple origins of the beta E-globin gene. *Am. J. Hum. Genet.* 36(1):212–217.
- Krauze de França, A. 2005. *Frequências alélicas de quatro locos SRTs: HUMCSF1PO, HUMTH01, HUMTPOx e vWA—Em duas populações do município de Porto Velho, RO*. Master's thesis, Universidade Federal de Rondônia, Porto Velho, Brazil.
- Krieger, H., N. E. Morton, M. P. Mi et al. 1965. Racial admixture in northeastern Brazil. *Ann. Hum. Genet.* 29(2):113–125.

- Modiano, D., G. Luoni, B. S. Sirima et al. 2001. Haemoglobin C protects against clinical *Plasmodium falciparum* malaria. *Nature* 414(6861):305–308.
- Salzano, F. M., H. Gershowitz, H. Mohrenweiser et al. 1986. Gene flow across tribal barriers and its effect among the Amazonian Icana River Indians. *Am. J. Phys. Anthropol.* 69(1):3–14.
- Silva, E. G. 2001. *História de todo mundo*. Porto Velho, Brazil: Maia.