Permanence or change? The meaning of genetic variation

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This contribution is part of the special series of Inaugural Articles by members of the National Academy of Sciences elected on April 27, 1999.

Contributed by Francisco M. Salzano, March 17, 2000

Selected aspects of the evolutionary process and more specifically of the genetic variation are considered, with an emphasis in studies performed by my group. One key aspect of evolution seems to be the concomitant occurrence of dichotomic, contradictory (dialect) processes. Genetic variation is structured, and the dynamics of change at one level is not necessarily paralleled by that in another. The pathogenesis-related protein superfamily can be cited as an example in which permanence (the maintenance of certain key genetic features) coexists with change (modifications that led to different functions in different classes of organisms). Relationships between structure and function are exemplified by studies with hemoglobin Porto Alegre. The genetic structure of tribal populations may differ in important aspects from that of industrialized societies. Evolutionary histories also may differ when considered through the investigation of patrilineal or matrilineal lineages. Global evaluations taking into consideration all of these aspects are needed if we really want to understand the meaning of genetic variation.

Conservatives and Revolutionaries

In the interpretation of our existence two philosophies can be distinguished, one that places emphasis on stability, in the maintenance of the status quo, and another that emphasizes the importance of change. It is not unrealistic to relate these two positions to the biological constitution of the individuals who maintain them. Obviously, there are no specific hereditary factors for conservatism or revolutionary tendencies. But the psychic makeup of all of us is undoubtedly influenced by genes, through the structure and functioning of the neuro-endocrine system.

An example may be in order here: maybe there is not, in all human society, an institution as conservative as the Catholic church; but its officials always have had to worry about rebels, for instance, Pierre Teilhard de Chardin or the Brazilian Leonardo Boff. And although Saint Augustine (354–430 AD) cannot be classified as a rebel, he favored an allegoric interpretation of the Bible's book of Genesis and developed an evolutionary concept, which included organic and inorganic matter, as opposed to a special creation.

Changes: Nonbiological and Biological

The universe is not static. Edwin Hubble (1889–1953) was the person responsible for the definitive denial of such a monotonous idea. His observations led to the conclusion that we live in a world in expansion, with the galaxies moving away from each other.

A special type of change is the evolutionary modification. The word evolution derives from the Latin term *evolutio* and its literal meaning is an unrolling. It can be used in this and other senses that involve the idea of change. But not all change is evolutionary. There is ceaseless change in the ocean surface, but it is not an evolutionary process. Implicit in the evolution concept are those of: (*a*) continuous change, (*b*) divergence, (*c*) restriction of opportunities, and (*d*) in a large number of situations, irreversibility. Presently there are doubts whether there is a general direction for the organic evolutionary process or progress in it. In relation to this last point, the key problem is, of course, the definition of progress. To many it would be intimately connected with the adaptation concept, but there are discordant views. Those critics argue that the examples chosen constitute *a posteriori* explanations, incapable of predicting future tendencies (1).

The most accepted theory about the origin of the universe maintains that in the beginning all matter would be concentrated in a single point, with a density approaching infinity. Twenty billion years ago a marvelous explosion (the Big Bang) put in motion all of the evolutionary process.

The second chapter of the "Book of the Universe" has to do with the origin of life. Life in our planet should have begun some 3.5 billion years ago. Because our solar system, and with it the Earth, originated approximately 4.5 billion years ago, it is possible to conclude that life appeared relatively soon in our planet's history.

Evolutionary Transitions

Periodically, along the evolutionary process, a new event completely revolutionize the field, opening ample horizons for further changes. Some of these events, in the general history of the organisms, as well as more specifically in animals, are listed in Table 1. It is impossible here, because of space limitations, to consider each of them in detail. It is clear, however, that these developments simultaneously evoke new ways of change, but also restrict them to a certain direction.

As a matter of fact, evolution can be viewed as a series of opposing, dichotomic, contradictory processes (4), and a list of some of these dialectical relationships is provided in Table 2.

Biological Variation

The most conspicuous property of the variation found in living organisms is that it is structured. At least theoretically we could envisage a world in which all forms of life would be parts of an immense continuum and not separated in reproductively isolated species as is true now. There are reasons for such constraints. An evolutionary unit must be sufficiently cohesive, so that it could successfully meet the challenges of its biotic and physical environments. This can be accomplished in a variety of ways. For instance, the genetic material can be organized in semi-independent arrays. In plants no fewer than three classes of genomes can be characterized, arranged as nuclear, mitochondrial, and chloroplastic DNA. In animals only the two first genomes coexist (in humans they consist of 3,300 mega bp of nuclear DNA, and 16.6 kb of mtDNA). Variability at the nuclear DNA, mtDNA, and protein levels does not necessarily occur in parallel fashion (6).

With the development of molecular methods, it was possible to verify that the genetic material is highly heterogeneous, with coding and noncoding (regulatory) regions, segments of highly or moderate repetitive DNA, and remnants of past insertion

Abbreviation: PR, pathogenesis-related protein.

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Table 1. The evolutionary transitions

General stages, previous	General stages, subsequent
Replicating molecules	Populations of molecules in compartments
Independent replicators	Chromosomes
RNA as gene and enzyme	DNA + protein (genetic code)
Prokaryotes	Eukaryotes
Asexual clones	Sexual populations
Protists	Animals, plants, fungi (cell differentiation)
Solitary individuals	Colonies (nonreproductive castes)
Primate societies	Human societies (language)

Animal development

1. Multicellularity (cell layers, cell adhesion, spatially controlled patterns of differentiation)
2. Defined axes of symmetry (species-specific body shape, structural repetition, origin of neurons, inner and outer epithelial germ layers)
3. Conversion of a two-germ layer body plan into a three-germ layer body plan
4. Inversion of dorsoventral patterning systems
5. Origin of vertebrates, new strategies for deploying cells in development
6. Invention of migratory lateral mesodermal cells, origin of two sets of patterned paired appendages, and anteroposterior diversification of the cranial visceral arches

Sources: Maynard Smith and Szathmáry (2); Holland (3).

events. The variability to be expected in these dissimilar structures is highly diverse. In some regions change is partially or completely forbidden, because it would impair functions or would be incompatible with life. In others it could be inconsequential (although probably there is no such a thing as a completely neutral mutation); whereas in some limited cases it could be favored.

Common variants (polymorphisms) can occur in a number of ways. The most frequent, in the human genome, are single base pair differences, also called single-nucleotide polymorphisms (SNPs). It is estimated that the complete human sequence will reveal at least one million SNPs (7). But other types of sequence variation occur, such as copy number changes, insertions, deletions, duplications, and rearrangements. Each of them has specific dynamics related to the origin, maintenance, and eventual loss of variation, which should be taken into account in any balanced general evaluation. According to the methods of study these variants are labeled as (*a*) restriction fragment length polymorphisms, (*b*) insertion/deletion (*Alu*) polymorphisms, (*c*) variable number of tandem repeats or minisatellites, and (*d*) short tandem repeats or microsatellites.

From the 1950s to the 1970s much discussion occurred about the genetic structure of organisms (see, for instance, ref. 8). One key point was the proportion of heterozygous, as compared with homozygous, regions a given species could afford. Answers now can start to be made by using direct molecular techniques. Using a set of 8,000 short tandem repeats polymorphisms, Broman and Weber (9) verified in several families average largest homozygous segments greater than 10 cM, even among "outbred" individuals from Utah and Venezuela.

Another question relates to units of selection. As far as the human immune defenses mechanisms are concerned, the process of lymphocyte diversity generation and subsequent clonal selection is fundamentally Darwinian. Much can be learned, therefore, about evolutionary processes within individuals (10). Bosch *et al.* (11), on the other hand, considering 11 biallelic polymorphisms and seven short tandem repeat human Y chromosome markers, asserted that "A population may be better understood as an association of lineages from a deep and population-independent gene genealogy, rather than as a complete evolutionary unit."

From the bare DNA until the formation of a fully grown individual there is a long way; variability in the processing and translation of DNA into protein also should be taken into consideration when comparisons at different levels of the biological hierarchy are considered.

The genes' vehicles in the intergeneration transfer of genetic information are the chromosomes, and here again the coupling of molecular with traditional chromosome methods, plus comparative gene mapping, is opening new ways in the investigation of evolution. Present-day DNA probes allow a resolution of up to 10–15 mega bp for chromosome subregions, and using these techniques it was possible to establish that the equivalent to chromosomes 3 and 21 from *Homo sapiens* formed a syntenic block for all placental mammals, the fission between the two having occurred in Old World primates after the divergence of New World monkeys. Intrachromosomal rearrangements also could be identified (12).

Table 2. Dialectical relationships in the evolutionary process

1. Chaos and antichaos
2. Matter and energy
3. Inorganic and organic
4. Unicellularity and multicellularity
5. Immortality and mortality
6. DNA and protein
7. Nucleus and cytoplasm
8. Asexual and sexual reproduction
9. r and K selection
10. Nonsocial and social
11. Aggression and cooperation
12. Nonhumans and humans
13. Biology and culture
14. Ecological success and dominance
15. Nonethics and ethics
16. Freedom and organization

Source: Salzano (5).

Pathogenesis-Related Proteins (PRs)

As an example of the types of approach that can be developed in the area of genetic variation, I turn now to some unpublished results obtained by L.B. Freitas, S.L. Bonatto, and myself in a particular type of proteins determined by a multigenic family and related to defense mechanisms in plants, but that also occur in fungi and animals (invertebrates and vertebrates, including humans). We have examined the phylogenetic relationships in

Table 3. Beta-Hb variants observed in Latin América

Variant	Substitution	Abnormal characteristics	Country
Deer Lodge	2 His→Arg	↑ O_2 affinity	Venezuela
HbS-Antilles	6 Glu→Val + 23 Val→Ileu	Sickling	Martinique
G-San José	7 Glu→Gly	Slightly unstable	Mexico
Siriraj	7 Glu→Lys	—	Martinique
Rio Grande	8 His→Thr	—	Mexico
Porto Alegre	9 Ser→Cys	↑ O_2 affinity, tendency to polymerize	Argentina, Brazil, Cuba, Venezuela
J-Baltimore	16 Gly→Asp	—	Brazil, Colombia, Guadeloupe, Martinique, Mexico, Trinidad
Alamo	19 Asn→Asp	—	Cuba, Venezuela
D-Iran	22 Glu→Gln	—	Guadeloupe, Jamaica
E	26 Glu→Lys	—	Brazil, Costa Rica, Cuba, Guadeloupe, Jamaica, Martinique, Mexico, Surinam
Knossos	27 Ala→Ser	Affects synthesis	Martinique
Genova	28 Leu→Pro	Unstable	Cuba
Bucuresti	42 Phe→Leu	Unstable; ↓ O_2 affinity	Cuba
Willamette	51 Pro→Arg	Unstable; ↓ O_2 affinity	Cuba, Venezuela
Osu Christiansborg	52 Asp→Asn	—	Jamaica
Ocho Rios	52 Asp→Ala	—	Jamaica
Dhofar	58 Pro→Arg	—	Jamaica
Zürich	63 His→Arg	Unstable; ↓ O_2 affinity	Brazil
I-Toulouse	66 Lys→Glu	Unstable; Fe^{+3}	Cuba
Korle-Bu	73 Asp→Asn	↓ O_2 affinity	Colombia, Costa Rica, Cuba, Guadeloupe, Jamaica, Martinique, Panama, Venezuela
J-Chicago	76 Ala→Asp	—	Venezuela
Costa Rica	77 His→Arg	—	Costa Rica
Buenos Aires	85 Phe→Ser	Unstable; ↑ O_2 affinity	Argentina
Santa Ana	88 Leu→Pro	Unstable	Brazil, Cuba
Roseau-Pointe a Pitre	90 Glu→Gly	Slightly unstable ↓ O_2 affinity	Guadeloupe
Caribbean	91 Leu→Arg	Slightly unstable ↓ O_2 affinity	Jamaica
N-Baltimore	95 Lys→Glu	—	Brazil, Cuba, El Salvador, Guadeloupe, Martinique
J-Cordoba	95 Lys→Met	↓ O_2 affinity	Argentina
Köln	98 Val→Met	Unstable	Brazil
Mainz	98 Val→Glu	Unstable	Brazil
Camperdown	104 Arg→Ser	Unstable	Brazil
New York	113 Val→Glu	Slightly unstable ↓ O_2 affinity	Costa Rica
Fannin-Lubbock	119 Gly→Asp	Slightly unstable	Mexico
Riyadh	120 Lys→Asn	—	Mexico
D-Punjab	121 Glu→Gln	↓ O_2 affinity	Argentina, Brazil, Cuba, Guadeloupe, Jamaica, Martinique, Mexico, Venezuela
O-Arab	121 Glu→Lys	—	Jamaica, Martinique, Puerto Rico
Hofu	126 Val→Glu	Unstable	Peru, Venezuela
J-Guantanamo	128 Ala→Asp	Unstable	Chile, Cuba
K-Woolwich	132 Lys→Gln	—	Brazil, Dominica, Guadeloupe, Jamaica, Martinique
North Shore-Caracas	134 Val→Glu	Unstable	Venezuela
Hope	136 Gly→Asp	Unstable ↓ O_2 affinity	Cuba, Martinique

Sources: Refs. 31–65.

seven families of these proteins (classified in relation to serology, molecular weights, and similarity in amino acid sequences). Within-family comparisons involved 79 plant species, 166 amino acid sequences, and 1,791 sites. For 37 species, 124 different PR isoforms (that is, those which occur in the same species) have been identified (an average of 3.3 per species). Thirty-one (84%) of those investigated in the 37 species tended to cluster together. Of the 17 clusters distinguished in the seven phylogenetic trees 10 (59%) were in agreement with the species' taxonomic status, ascertained at the family level. The strong similarities among the intraspecific, as compared with the interspecific comparisons, argue for some kind of concerted evolution, but the rare occurrence of widely different isoforms also may suggest diversifying selection.

Species of *Passiflora* (the passionflower plants) also are being studied by my group considering Betv1 homologous proteins (PR family 10), an intergenic spacer (ITS) located between the *trnb* and *trnF* genes of chloroplast DNA, and the ITS1 and ITS2 of ribosomal DNA. As was found with humans (6), agreement between systems was only partial.

These proteins exemplify the dialectical relationship indicated in the title of this paper. Although they may vary widely even within a given plant, they maintain similarities that make possible their classification in a wide "PR protein superfamily." This set includes fungi (*Saccharomyces, Schizophylum*) and nematode (*Caenorhabditis*) proteins; antigen 5, one major vespid venom antigen, and an antigen 5-related protein from *Drosophila melanogaster*; helothermine, from a lizard venom; mammalian Tpx-1 testis-specific protein and sperm-coating glycoprotein Scg; and human-specific granule protein 28 from neutrophils, P25TI trypsin inhibitor of neuroblastoma and glioblastoma cells, and

glioma pathogenesis-related GliPr. The latter, specifically, shows a remarkable molecular similarity to tomato's PR14a protein (13).

β-Globin Changes in the Latin American Microcosm

No fewer than 485.85 million persons live in Latin America, distributed over an area of 21.25 million km^2. They present a dazzling morphological variability, conditioned by the varied contribution of their founders. Many studies were conducted in this area, and presently Maria Catira Bortolini and myself are preparing a book describing as comprehensively as possible all aspects related to the genetics and evolution of Latin Americans.

Table 3 lists 41 variants (besides the common S and C types) observed in the β chain of Hb in Middle and South America. The mutations found are distributed all over the molecule, from position 2 to position 136. Some of them lead to abnormal characteristics, as listed, and their geographical distribution is uneven. Some occur in one country only, whereas HbE (which is polymorphic in Asian populations) and D-Punjab are more widespread, having been found in subjects from eight nations.

One of these Hb types (Hb Porto Alegre) was discovered by my group, in a family of Portuguese descent living in the indicated city (14). In that first report it was suggested that the change in the molecule would lead to polymerization *in vitro,* but that this process would not occur *in vivo*, the mutation, therefore, resulting in a "silent" phenotype. Four years later the molecular change responsible for the new type was identified (15). Cysteine substitutes serine at the ninth residue of the chain; because this sulfhydryl group is on the surface of the molecule, intermolecular disulfide bonds are allowed to form. Subsequent studies identified this Hb type in four other Brazilian populations (Coari, Belém, Natal, Campinas), in Buenos Aires (two carriers of Spanish descent), and in Havana (one carrier of Portuguese and one of Spanish origin) (16–21). In Buenos Aires one of the carriers also had beta thalassemia, and in Campinas Hb Porto Alegre was found associated with Hb Santa Ana, an unstable Hb. These findings emphasize that the change in Hb Porto Alegre does not lead to clinical problems (because it can coexist with abnormal variants). The lack of polymerization *in vivo* is probably because of a compensatory synthesis of glutathion reductase (18, 22). Casemiro V. Tondo (23, 24) used Hb Porto Alegre as a model for different types of biophysical and biochemical investigations and was able to demonstrate that *in vitro* bloods of homozygotes form dodecamers and that the tetramer formed in the material from heterozygotes is asymmetric.

Tribal Life

Previous genetic work by our group in South American Indians has been reviewed at regular intervals (for instance, refs. 6 and 25) and therefore I am not going to re-examine it here except for one aspect. It refers to our demographic and genetic studies among the Xavante Indians. They are unusual in relation to other tribal groups, because one of their populations (São Domingos, also called Rio das Mortes or Etéñitépa) has been closely followed for nearly half a century. Genetic information was obtained both in the 1960s and 1990s, making possible integration with extended genealogies that were collected at regular intervals since 1957.

Tribal life differs from those of agricultural or industrial communities in various ways. The groups are small, behavior of their members is much more regulated by kinship rules, many of them practice polygamy (generally polyginy, one man having simultaneously more than one wife, who frequently are sisters), and mortality is caused mostly by infectious diseases.

It is not yet clear how much these patterns influence genetic variation. Present demographic methods generally are adapted for large populations, without concern to intergeneration effects in fertility or mortality. For evolutionary purposes, however, fitness, that is, the amount of genetic influence one individual contributes to the next generations, is an important parameter. In a polyginous society it would be expected that the average and variability in this parameter would be higher in males than in females. But unpublished analyses made by Sidia M. Callegari-Jacques, Nancy M. Flowers, Nara F.M. Laner, and myself indicates that among the Xavante this is not true. This result probably reflects the fact that in our species both males and females are needed for reproduction and that in this society generally all females are engaged in the reproductive process.

The Ultimate Dichotomy: Sex

Is the comparison between males and females really dimorphic? Blackless *et al.* (26) argued that 1.7% of all live births in our species do not conform to a platonic ideal of absolute sex chromosome, gonadal, genital, and hormonal dimorphism. Fausto-Sterling (27) even contended that there are five sexes in our species! Exaggerations apart, the fact is that the evolutionary histories among humans, as considered through mtDNA (maternaly inherited) or the Y chromosome (transmitted exclusively by males) are giving different patterns. One reason for these results is that Y chromosome variants tend to be more localized geographically than those of mtDNA and the autosomes. This discrepancy may be caused by the custom of patrilocality, namely, the tendency for a wife to move into her husband's natal household (28).

But there are other differences in the evolutionary histories of men and women. For instance, the mutation rate for the hemophilia B locus among males is 8.6 times higher than that observed among females (29), and the assessment we have made of interethnic admixture in 11 African-derived South American populations based on the mtDNA sequences of the first hypervariable region and one Y chromosome marker (DYS19) showed evidence for asymmetrical unions in relation to sex in nine (82%) of them (30).

This research is financed by Programa de Apoio a Núcleos de Excelência (PRONEX), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Financiadora de Estudos e Projetos (FINEP), and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS).

1. Nitecki, M. H. (1988) *Evolutionary Progress* (Univ. of Chicago Press, Chicago).
2. Maynard Smith, J. & Szathmáry, E. (1995) *The Major Transitions in Evolution* (Freeman, New York).
3. Holland, P. W. H. (1998) *Am. Zool.* **38,** 829–842.
4. Levins, R. & Lewontin, R. (1985) *The Dialectical Biologist* (Harvard Univ. Press, Cambridge, MA).
5. Salzano, F. M. (1995) *Evolução do Mundo e do Homem: Liberdade ou Organização?* (Editora da Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil).
6. Hutz, M. H., Callegari-Jacques, S. M., Bortolini, M. C. & Salzano, F. M. (1999) in *Genomic Diversity: Applications in Human Population Genetics*, eds. Papiha, S. S., Deka, R. & Chakraborty, R. (Plenum, New York), pp. 23–32.
7. Collins, A., Lonjou, C. & Morton, N. E. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 15173–15177.
8. Salzano, F. M. (1975) *The Role of Natural Selection in Human Evolution* (North–Holland, Amsterdam).
9. Broman, K. W. & Weber, J. L. (1999) *Am. J. Hum. Genet.* **65,** 1493–1500.
10. McDade, T. W. & Worthman, C. M. (1999) *Am. J. Hum. Biol.* **11,** 705–717.
11. Bosch, E., Calafell, F., Santos, F. R., Pérez-Lezaun, A., Comas, D., Benchemski, N., Tyler-Smith, C. & Bertranpetit, J. (1999) *Am. J. Hum. Genet.* **65,** 1623–1638.
12. Müller, S., Stanyon, R., Finelli, P., Archidiacono, N. & Wienberg, J. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 206–211.
13. Szyperski, T., Fernández, C., Mumenthaler, C. & Wüthrich, K. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 2262–2266.
14. Tondo, C. V., Salzano, F. M. & Rucknagel, D. L. (1963) *Am. J. Hum. Genet.* **15,** 265–279.
15. Bonaventura, J. & Riggs, A. (1967) *Science* **158,** 800–802.

16. Seid-Akhavan, M., Ayres, M., Salzano, F. M., Winter, W. P. & Rucknagel, D. L. (1973) *Hum. Hered.* **23,** 175–181.
17. Peñalver, J. A. & Miani, M. S. A. (1974) *Medicina (Buenos Aires)* **34,** 287–292.
18. Martínez, G., Lima, F., Wade, M., Estrada, M., Colombo, B., Heredero, L. & Granda, H. (1977) *J. Med. Genet.* **14,** 422–425.
19. Silva, M. I. A. F., Salzano, F. M. & Lima, F. A. M. (1981) *St. Phys. Anthropol.* **7**, 3–11.
20. Rosa, V. L., Salzano, F. M., Franco, M. H. L. P. & Freitas, M. J. M. (1984) *Rev. Bras. Genet.* **7,** 569–582.
21. Colombo, B. & Martínez, G. (1985) *Hemoglobin* **9,** 415–422.
22. Tondo, C. V. (1982) *Biochem. Biophys. Res. Commun.* **105,** 1381–1388.
23. Tondo, C. V. (1971) *An. Acad. Bras. Ciênc.* **43,** 651–669.
24. Tondo, C. V. (1976/77) *Hemoglobin* **1**, 195–210.
25. Salzano, F. M. & Callegari-Jacques, S. M. (1988) *South American Indians: A Case Study in Evolution* (Clarendon, Oxford).
26. Blackless, M., Cheruvastra, A., Derryck, A., Fausto-Sterling, A., Lauzanne, K. & Lee, E. (2000) *Am. J. Hum. Biol.* **12,** 151–160.
27. Fausto-Sterling, A. (1993) *Sciences* March/April, 20–24.
28. Seielstad, M. T., Minch, E. & Cavalli-Sforza, L. L. (1998) *Nat. Genet.* **20,** 278–280.
29. Green, P. M., Saad, S., Lewis, C. M. & Giannelli, F. (1999) *Am. J. Hum. Genet.* **65,** 1572–1579.
30. Bortolini, M. C., Silva, W. A., Jr., Castro de Guerra, D., Remonatto, G., Mirandola, R., Hutz, M. H., Weimer, T. A., Silva, M. C. B. O., Zago, M. A. & Salzano, F. M. (1999) *Am. J. Hum. Biol.* **11,** 551–563.
31. Arends, T., Garlin, G., de Perera, A. P. & Castillo, O. (1984) *Hemoglobin* **8,** 621–626.
32. Monplaisir, N., Merault, G., Poyart, C., Rhoda, M.-D., Craescu, C., Vidaud, M., Galacteros, F., Blouquit, Y. & Rosa, J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9363–9367.
33. Ruiz Reyes, G. (1983) *Hemoglobin* **7,** 603–610.
34. Monplaisir, N., Galacteros, F., Arous, N., Rhoda, M. D., Delanoe-Garin, J., Ouka-Montjean, M., Linval, J. C. & Rosa, J. (1985) *Nouv. Rev. Franç. d'Hématol.* **27,** 11–14.
35. Sáenz, G. F., Rodríguez Romero, W. & Chaves Villalobos, M. (1993) *Rev. Biol. Trop.* **41,** 393–403.
36. Arends, T. (1966) *New Zeal. Med. J.* **65,** Suppl., 831–844.
37. Fabritius, H., Millan, J., Blot, M., Rivat, P., Charles-Gervais, J., Lacroix, H. & Le Corroller, Y. (1980) *Nouv. Rev. Franç. d'Hématol.* **22,** 243–248.
38. Sáenz, G. F. (1988) *Rev. Biol. Trop.* **36,** 361–372.
39. Colombo, B. & Martínez, G. (1985) *Hemoglobin* **9,** 415–422.
40. Arends, T., Castillo, O., Garlin, G., Maleh, Y., Anchustegui, M. & Salazar, R. (1987) *Hemoglobin* **11,** 135–138.
41. Serjeant, G. R., Serjeant, B. E., Forbes, M., Hayes, R. J., Higgs, D. R. & Lehmann, H. (1986) *Br. J. Haematol.* **64,** 253–262.
42. Targino de Araújo, J., Ribeiro, V. S., Costa e Silva, M. S., de Bonis, J. C. & Targino de Araújo, R. A. (1982) *Rev. Inst. Med. Trop.* **24,** 229–233.
43. Galacteros, F., Garin, J. D., Monplaisir, N., Namoune, S., Arous, N., Blouquit, Y., Mamalaki, A., Tulliez, M., Ouka, M., Goossens, M. & Rosa, J. (1984) *Hemoglobin* **8,** 215–228.
44. Martínez. G., Carnot, J. & Hernandez, P. (1983) *Hemoglobin* **7,** 591–594.
45. Miranda, S. R. P., Kimura, E. M., Saad, S. T. O, & Costa, F. F. (1994) *Hemoglobin* **18,** 337–342.
46. Restrepo, A. (1971) in *Genetical, Functional, and Physical Studies of Hemoglobins,* eds. Arends, T., Bemski, G. & Nagel, R. (Karger, Basel), pp. 39–52.
47. Rodriguez Romero, W. E., Castillo, M., Chavez, M. A., Saenz, G. F., Gu, L.-H., Wilson, J. B., Baysal, E., Smetanina, N. S., Leonova, J. Y. & Huisman, T. H. J. (1996) *Hum. Genet.* **97,** 829–833.
48. Gonçalves, M. S., Sonati, M. F., Kimura, E. M., Arruda, V. R., Costa, F. F., Nechtman, J. F. & Stoming, T. A. (1994) *Hemoglobin* **18,** 337–342.
49. Merault, G., Keclard, L., Saint-Martin, C., Jasmin, K., Campier, A., Delanoe-Garin, J., Arous, N., Fortune, R., Theodore, M., Seytor, S., *et al.* (1985) *FEBS Lett.* **184,** 10–13.
50. Rabb, L. & Serjeant, G. R. (1982) *Hemoglobin* **6,** 403–405.
51. Arends, T. (1971) in *The Ongoing Evolution of Latin American Populations,* ed. Salzano, F. M. (Charles C. Thomas, Springfield), pp. 509–559.
52. Naoum, P. C. (1997) *Hemoglobinopatias e Talassemias* (Sarvier, São Paulo, Brazil).
53. Bardakjian, J., Kister, J., Rhoda, M. D., Marden, M., Arous, N., De Leon, J., North, M. L., Lacombe, C., Blouquit, Y., Castracane, C., *et al.* (1988) *Hemoglobin* **12,** 1–11.
54. Sonati, M. F., Kimura, E. M., Grotto, H. Z. W., Gervasio, S. A. & Costa, F. F. (1996) *Hemoglobin* **20,** 175–179.
55. Miranda, M. R. P., Fonseca, S. F., Figueiredo, M. S., Yamamoto, M., Grotto, H. Z. W., Saad, S. T. O, & Costa, F. F. (1997) *Braz. J. Genet.* **20,** 745–748.
56. Passos, L. N. M., Marques, H. O., Naoum, P. C., Carrel, R. W. & Williamson, R. T. (1998) *Bol. Soc. Bras. Hematol. Hemoter.* **20,** Suppl., 98.
57. Miranda, S. R. P., Kimura, E. M., Teixeira, R. C., Bertuzzo, C. S., Ramalho, A. S., Saad, S. T. O, & Costa, F. F. (1996) *Hemoglobin* **20,** 147–154.
58. Franco-Gamboa, E., Ibarra, B., Zuñiga, P., Figuero-Peña, C. L. & Cantú, J. M. (1981) *Proceedings 6th International Congress of Human Genetics,* eds. Tamir, B. B., Cohen, T. & Goodman, R. M. (Alan R. Liss, New York), 35.
59. Arends, T., Garlin, G., Pérez-Bández, O. & Anchustegui, M. (1982) *Hemoglobin* **6,** 243–246.
60. Arends, T. (1984) *Gac. Med. Caracas* **92,** 189–224.
61. Naoum, P. C., Machado, P. E. A. & Michelim, O. C. (1976) *Rev. Bras. Pesq. Med. Biol.* **9,** 273–278.
62. Naoum, P. C., Mattos, L. C. & Curi, P. R. (1984) *Bull. Pan Am. Health Org.* **18,** 127–138.
63. Zago, M. A. & Costa, F. F. (1988) *Hemoglobin* **12,** 399–403.
64. Martínez, G., Lima, F. & Colombo, B. (1977) *Biochim. Biophys. Acta* **491,** 1–6.
65. Sciarrata, G. V., Ivaldi, G. & Moruzzi, F. (1990) *Hemoglobin* **14,** 115–117.