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GENETIC EPIDEMIOLOGY IN PEDIGREES: KINSHIP AND PATH ANALYSIS*

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ABSTRACT

Current advances in two aspects of genetic epidemiology are outlined.

INTRODUCTION

Genetic epidemiology has been defined as a science "concerned with etiology, distribution, and control of disease in relatives and with inherited causes of disease in populations" (Morton and Chung, 1978). Substantial progress has been made in nuclear families, but pedigrees interpose a *pons asinorum* to further advances. This paper sketches two approaches to exploit pedigrees for genetic epidemiology.

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KINSHIP

If i, j are two individuals or populations, their *kinship* ϕ_{ij} is defined as the probability that a random gene from i be identical by descent with a random allele from j (Malécot, 1948). Associated with this concept are three *identity coefficients* C_I, C_T, C_O giving the probability that 2, 1 or 0 genes be identical by descent, where $C_I + C_T + C_O = 1$ (Li and Sacks, 1954). In the absence of inbreeding and mutation

$$\phi = C_I/2 + C_T/4 = (\tfrac{1}{2})^{n+1} \tag{1}$$

where n is the degree of relationship (Table I). Inbreeding introduces 6 other identity coefficients (Gillois, 1966), but kinship is readily generalized to include inbred common ancestors. The inbreeding coefficient for a child of i and j is defined as $F = \phi_{ij}$.

Because kinship is a relation between random pairs of genes, in a complex pedigree it is easier to calculate than genotypic probabilities. However, kinship ignores phenotypic information on pedigree members, whose interdependence makes tests of significance on pairs of relatives approximate. The advantage of simplicity may be considered in at least several cases.

1) *Is a disease familial?*

Cotterman (1940) gave conditional frequencies in relatives for a gene with frequency q and allele frequency $p = 1 - q$:

$$\begin{aligned} P(aa/aa) &= C_I + C_T q + C_O q^2 \\ P(Aa/Aa) &= C_I + C_T/2 + 2C_O pq \end{aligned} \tag{2}$$

which for unilineal relatives reduce to

$$\begin{aligned} P(aa/aa) &= 4\phi pq + q^2 \\ P(Aa/Aa) &= 2\phi(1 - 4pq) + 2pq \end{aligned} \tag{3}$$

Edwards (1968) suggested for polygenic disease a conditional risk $e^{b\phi}$, which

has been verified to an extremely close approximation (Smith, 1970). If frequencies in controls and relatives are fitted to the equation

$$P_\phi = \begin{cases} P_O + b\phi & \text{if monogenic} \\ P_O e^{b\phi} & \text{if polygenic} \end{cases} \qquad (4)$$

then the null hypothesis of no familial concentration implies $b = 0$ (Morton, 1979). Age standardization, dependence of relatives, and inaccuracy of data on remote relatives are possible sources of error. Since every model of inheritance, genetic or cultural, provides a test of the null hypothesis, kinship analysis is preliminary.

2) *Can different genetic and cultural modes of inheritance be resolved by data on pairs of relatives?*

Formally this is an extension of path analysis to recurrence risks (Morton and Rao, 1978). Power may be low, but pilot studies are worthwhile to determine what information can be recovered from empiric risks, if data permitting segregation analysis are not accessible.

3) *Is there one or more significant founders?*

Pedigree studies are often oriented by anticipation or discovery of a founder responsible for a considerable number of affected descendants. In the most extreme case all affected members of a population are a monophylon descended from a single couple (Table II). Logic to determine principal founders has been illustrated (Morton, 1973) and discussed (Morton, 1979). Pedigree likelihoods give slightly greater precision but are extremely laborious even in a small data set (Thompson, 1978). Monophyla are suggestive of a major locus, which is inferred to be recessive if both parents of all affected trace to the same founder. In this instance kinship suffices to test recessivity, without recourse to the coefficient of double identity by descent C_I. The discovery of a small monophylon guides pedigree extension, inducing ascertainment bias in segregation analysis.

4) *What are recurrence risks in recessive disease?*

Inbreeding due to a common ancestor within a pedigree poses no obstacle to segregation analysis (Lange and Elston, 1975). An elective simplification when there are common ancestors outside the pedigree is to take the frequency of homozygotes as

$$P(aa) = q^2 + pq\phi \tag{5}$$

where ϕ is an estimate of kinship from bioassay of phenotypes or by extrapolation from migration and genealogy as a function of the pair of populations or their distance (Morton, 1973). This allows geographic propinquity to be taken into account when founders antedate the genealogy. If there is inbreeding within the pedigree (measured by F) and outside the pedigree (measured by ϕ) the resultant is $F + (1 - F)\phi$ by the hierarchical principle of Wright (1943). For mating between two populations with gene frequencies q_i, q_j, the appropriate value of kinship is

$$\phi = - \frac{(q_i - q_j)^2}{4q\,(1 - q)} \tag{6}$$

where

$$q = \frac{q_i + q_j}{2}$$

This satisfies the equation

$$P(aa) = q_i q_j \tag{7}$$

5) *Does population structure contribute to analysis of etiology?*

Equation (5) expresses the relation between homozygosity and inbreeding that is pathognomonic of rare recessive disease. If isolated probands are significantly less inbred than familial probands, a proportion of sporadic cases due to mutation or phenocopies is inferred and estimated (Morton, 1959).

Consanguinity analysis led to genetic load theory, in which the affection probability is taken as

$$P_F = 1 - e^{-(A + BF)} \tag{8}$$

where F is the inbreeding coefficient, A is the panmictic load, and B is the inbred load, defined as

$$A = X + \Sigma q^2 t$$
$$B = \Sigma q(1 - q)t \qquad (9)$$

Here X is the contribution of nonrecessive cases, t is the penetrance in homozygotes, and the summation is over n contributary loci (Morton, Crow, and Muller, 1956). If Q is the mean gene frequency per contributary locus, we have

$$A \doteq X + nQ^2$$
$$B \doteq nQ(1 - \dot{Q}) \qquad (10)$$

and so

$$n \geqslant B^2/A$$
$$Q \leqslant A/B \qquad (11)$$

One extension of genetic load theory gives mutation rates, which have been estimated in this way for a number of recessive genes (Dewey et al., 1965). Another extension compares inbreeding effects on the mean of a quantitative trait and on the frequency of extreme deviants for that trait to determine whether dominance deviations make an important contribution to phenotypic variance, with negative results for IQ (Morton, 1978).

Geographic clustering within a region is approximated by

$$\phi(\mathrm{d}) = (1 - L)ae^{-bd} + L \qquad (12)$$

where d is the distance between populations (Malécot, 1973). This definition of conditional kinship gives $\phi(d) = 0$ for random pairs within a region. The parameters a, b, and L have been estimated from segregation and phenotype bioassay for many populations (Morton, 1977), but there has been only one application to rare deleterious genes (Morton et al., 1973). This used the values of a and b from bioassay of ABO blood groups, since other studies show that weak systematic pressure is dominated by migration. More generally, let m_e be the effective migration rate and N_e be the evolutionary size of a local population, and

$$\Delta q = -k(q-Q) + 0(q-Q)^2 \tag{13}$$

be the rate of selective gene frequency change per generation, where Q is the equilibrium gene frequency. Then the linearized systematic pressure is

$$M_e = m_e - \frac{\partial \Delta q}{\partial q} \mid Q \tag{14}$$

$$= m_e + k$$

If a, b are the Malécot parameters for a neutral gene, then appropriate values for a deleterious gene with linear systematic pressure k are

$$a_k = a\, m_e/M_e$$
$$b_k = b\sqrt{M_e/m_e} \tag{15}$$

(Morton, 1977).

The distribution of distance among parents of affected children for a rare disease with a recessive component is

$$r(d) = \frac{[A + B\phi(d)]\,\mu(d)}{A + B\phi} = \frac{[C + ae^{-bd}]\,\mu(d)}{C + \phi} \tag{16}$$

where A, B are the genetic loads, $C = A/B + L$, $\mu(d)$ is the distribution of marital distance in the general population, and $\phi = \sum_d \phi(d)\,\mu(d)$, (Morton et al., 1968). The same equation holds for random pairs (excluding mates), with $\mu(d)$ appropriately defined, and this is useful to detect geographic clustering of any origin, including dominant as well as recessive genes.

When this analysis was applied to parental pairs for retinal degeneration (retinitis pigmentosa and macular degeneration) in Switzerland, the value of C was significantly less for isolated than familial probands, supporting evidence from segregation and consanguinity analyses that a large proportion of isolated cases are nonrecessive sporadics (Morton et al., 1973). All three

methods give consistent estimates of this fraction. The mean gene frequency per contributary locus was estimated as

$$Q = C - L \tag{17}$$

and the number of loci with recessive alleles for retinitis pigmentosa as

$$n > B/Q \tag{18}$$

with mutation rate

$$\mu = kQ/\text{locus/generation} \tag{19}$$

Since $k = .0145$ for recessive retinal degeneration, but $m_e = .132$ (Morton, 1977), no adjustment is necessary in the values of a and b.

Random pairs were made within and between diagnostic groups of retinal degeneration, using pairs between etiologically unrelated diseases as a control. The estimate of mean gene frequency within diagnostic groups agreed with its overall value, but between diagnostic groups the estimate was preposterously large, as if there were no appreciable identity by descent between diagnostic categories. This proves that each contributing allele tends to produce a characteristic diagnostic type.

The utility of population structure analysis for genetic epidemiology has just begun to be explored.

PATH ANALYSIS

The theory of path analysis was developed before 1920 and applied to family resemblance during the next decade (Wright, 1978). Haldane (1964) considered that path analysis "may replace our old notions of causation", but impact on human genetics was slight until the last few years when environmental indices, maximum likelihood estimation, and likelihood ratio tests of hypotheses were introduced (Rao et al., 1974). The model of genetic and cultural inheritance has been extended to include phenotypic and social homogamy (Rao et al., 1979), and even reciprocal interaction has been explored

(Cloninger, 1979). Limits of resolution have not yet been determined, but I think that adaptive evolution of models for genetic and cultural inheritance is near a plateau, and that emphasis is shifting to sampling design and statistical analysis.

Whereas early studies used data collected for other purposes, the need for systematic samples is apparent. What relationship (twins, half-sibs, adoptions, etc.) best complement nuclear families, which are the object of greatest interest for interpretation and risk prediction? Three pedigree structures are especially promising (Figure 1). A *lineage* is a three-generation pedigree fanning upwards *(U)* or downwards *(D)*. A *colony* is a three-generation pedigree including all first degree relatives of a proband. A *sector* is a two--generation pedigree containing at least two sibships in the same generation and at most one in the preceding generation. A *half-sector* is a two-generation pedigree containing one sibship in each of two successive generations.

Several of these sampling designs (twin half-sector, twin sector, half-sibship) involve special relationships whose parameters lead to no certain inference about nuclear families. For example, the correlation of multiple spouses may not be predictable from marital correlation, and twins may share a common environment different from sibs. Three-generation sampling designs require extra parameters for age-dependence of genetic and cultural heritability, and with cousinships they raise a possibility of sampling bias for geographic or social propinquity which invalidates extrapolation to nuclear families.

On balance it seems that uncleships may be the best extension of nuclear families. They involve only two generations and no unusual relationships, they give both juvenile and adult sibs and both first and second degree relatives, and they provide resolution of maternal effects. More detailed examination of the robustness and power of these sampling designs to resolve genetic and cultural inheritance in nuclear families is desirable, but it is reassuring that environmental indices provide overdetermination even in the simplest of these structures (Rao et al., 1974, 1979).

DISCUSSION

This short review has omitted many aspects of genetic epidemiology

Table I - Kinship and identity coefficients for autosomal genes under panmixia.

Relationship	Kinship ϕ	Identity Coefficients C_I	C_T	C_O	Degree of relationship n
Identical twins	1/2	1	0	0	0
Sibs	1/4	1/4	1/2	1/4	1
Double first cousins	1/8	1/16	6/16	9/16	2
Parent-child	1/4	0	1	0	1
Grandparent-grandchild (= uncle-niece = half sibs)	1/8	0	1/2	1/2	2
First cousins (= great grandparent-great grandchild = great uncle-niece)	1/16	0	1/4	3/4	3
First cousins once removed	1/32	0	1/8	7/8	4
Second cousins	1/64	0	1/16	15/16	5
Unilineal	$(1/2)^{n+1}$	0	4ϕ	$1 - 4\phi$	n

Table II - Kinship of parents of achromatopsia sibships to founder sibship 1067 in Micronesia (Hussels and Morton, 1972).

	Kinship to 1067	
	No	Yes
Not known carrier	363	727
Known carrier	0	117

Table III - The ITO matrices with mutation.

$$I = \begin{bmatrix} (1-\mu)^2 & 2\mu(1-\mu) & \mu^2 \\ 0 & 1-\mu & \mu \\ 0 & 0 & 1 \end{bmatrix}$$

$$T = \begin{bmatrix} p(1-\mu) & q(1-\mu)+p\mu & \mu q \\ \frac{p(1-\mu)}{2} & \frac{1+p\mu-q\mu}{2} & \frac{q(1+\mu)}{2} \\ 0 & p & q \end{bmatrix}$$

$$O = \begin{bmatrix} p^2 & 2pq & q^2 \\ p^2 & 2pq & q^2 \\ p^2 & 2pq & q^2 \end{bmatrix}$$

Table IV - Segregation Frequencies With Mutation

Mating	Frequency	Children		
		AA	Aa	aa
AA X AA	p^4	$(1-\mu)^2$	$2\mu(1-\mu)$	μ^2
AA X Aa	$4p^3q$	$(\frac{1-\mu}{2})^2$	$(1-\mu)(\frac{1+2\mu}{2})$	$\frac{\mu(1+\mu)}{2}$
AA X aa	$2p^2q^2$	0	$1-\mu$	μ
Aa X Aa	$4p^2q^2$	$(\frac{1-\mu}{2})^2$	$2(\frac{1-\mu}{2})(\frac{1+\mu}{2})$	$(\frac{1+\mu}{2})^2$
Aa X aa	$4pq^3$	0	$\frac{1-\mu}{2}$	$\frac{1+\mu}{2}$
aa X aa	p^4	0	0	1

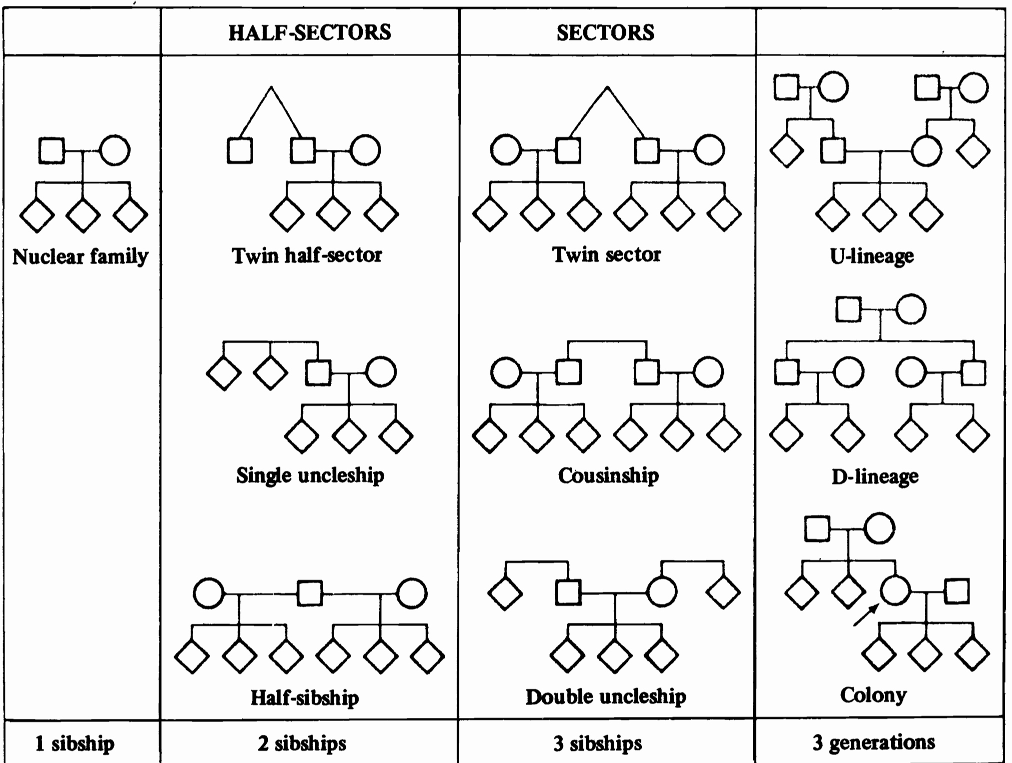

Figure 1 - Special pedigree structures.

yet to be solved. For example, the best estimators of familial correlations in random samples, with attribute data, and under incomplete ascertainment have not been established. Computer programs are being written to solve the problems sketched here, and the first applications will be made this year.

Numerical computation has increased in genetic epidemiology, and the end is not in sight. At least for the next few years many problems will require heavy computation for their solution. With modern computers this presents no insuperable, nor indeed costly, difficulty.

REFERENCES

Cloninger, C.R. (1979). Resolution of polygenic and cultural inheritance from reciprocal phenotype-environment interaction. 1. Introduction and description of the general dynamic model. Submitted to *Genet. Res.*

Cotterman, C.W. (1940). *A calculus for statistico-genetics.* Ph. D. Thesis, Ohio State Univ., Columbus. Reprinted in: *Genetics and social structure* (Ballanoff, P., Ed.), Dowden, Hutchinson & Ross, Inc., Stroudsburg (1974), pp. 157-272.

Dewey, W.J., Barrai, I., Morton, N.E., and Mi, M.P. (1965). Recessive genes in severe mental defect. *Am. J. Hum. Genet., 17:* 237-256.

Edwards, J.H. (1968). The interpretation of pedigree data. In: *Record linkage in Medicine* (Acheson, E.D., Ed.), Williams & Wilkins, Baltimore, pp. 282-295.

Gillois, M. (1966). Le concept d' identité et son importance en génétique. *Ann Génét. 9:* 58-65.

Haldane, J.B.S. (1964). A defense of beanbag genetics. In: *Perspectives in biology and medicine, Vol. VII.* pp. 343-360.

Hussels, I.E. and Morton, N.E. (1972). Pingelap and Mokil atolls: achromatopsia. *Am. J. Hum. Genet., 24:* 304-309.

Lange, K., and Elston, R.C. (1975). Extensions to pedigree analysis. I. Likelihood calculations for simple and complex pedigrees. *Hum. Hered., 25:* 95-105.

Li, C.C., and Sacks, L. (1954). The derivation of joint distribution and

correlation between relatives by the use of stochastic matrices. *Biometrics, 10:* 347-360.

Malécot, G. (1948). *Les mathématiques de l'hérédité.* Masson, Paris.

Malécot, G. (1973). Isolation by distance. In: *Genetic structure of populations* (Morton, N. E., Ed.). U. Press of Hawaii, Honolulu, pp. 72-75.

Morton, N.E. (1959). Methods of study in human genetics. In: *Genetics and Cancer.* University of Texas Press, pp. 391-407.

Morton, N.E. (1973). Population structure and historical genetics of isolates. *Isr. J. Med. Sci., 9:* 1299-1307.

Morton, N.E. (1977). Isolation by distance in human population. *Ann. Hum. Genet. (Lond.), 40:* 361-365.

Morton, N.E. (1978). The effect of inbreeding on IQ and mental retardation. *Proc. Natl. Acad. Sci., U.S.A., 75:* 3906-3908.

Morton, N.E. (1979). Genetic epidemiology of isolates. In: *Population genetic studies on isolates,* Academic Press (in press).

Morton, N.E., and Chung, C.S. (1978). *Genetic Epidemiology.* Academic Press, New York, 558 p.

Morton, N.E., and Rao, D.C. (1978). Quantitative inheritance in man. In: *Yearbook of Physical Anthropology, Vol. 21,* (Bennett, K.A., Ed.), American Assn. of Physical Anthropologists, Wash. D.C., pp. 12-41.

Morton, N.E., Crow, J.F., and Muller, H.J. (1956). An estimate of the mutational damage in man from data on consanguineous marriage. *Proc. Natl. Acad. Sci., 42:* 855-863.

Morton, N.E., Miki, C., and Yee, S. (1968). Bioassay of population structure under isolation by distance. *Am. J. Hum. Genet., 20:* 411-419.

Morton, N.E., Klein, D., Hussels, I.E., Dodinval, P., Todorov, A., Lew, R., and Yee, S. (1973). Genetic structure of Switzerland. *Am. J. Hum. Genet., 25:* 347-361.

Rao, D.C., Morton, N.C., and Cloninger, C.R. (1979). Path analysis under generalized assortative mating. I. Theory. *Genet. Res.* (in press).

Rao, D.C., Morton, N.E. and Yee, S. (1974). Analysis of family resemblance. III. Complex segregation of quantitative traits. *Am. J. Hum. Genet., 26:* 489-503.

Smith, C. (1970). Heritability of liability and concordance in monozygous twins. *Ann. Hum. Genet., 34:* 85-91.

Thompson, E.A. (1978). Ancestral inference. II. The founders of Tristan

da Cunha. *Ann. Hum. Genet.* (in press).
Wright, S. (1943). Isolation by distance. *Genetics, 28:* 114-138.
Wright, S. (1978). *Evolution and the genetics of population, Vol. 4. Variability within and among natural populations.* The Univ. Chicago Press, Chicago & London, 580 p.

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KARYOTYPIC STUDIES OF TWO ALLOPATRIC POPULATIONS OF THE GENUS *HOPLIAS* (PISCES, ERYTHRINIDAE)

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ABSTRACT

Cytogenetic studies were carried out on two allopatric populations of a fish of the genus *Hoplias* (Erythrinidae), from two lakes in the Doce River Valley (State of Minas Gerais, Brazil) and from the Juquiá River (State of São Paulo, Brazil). Indications of XX/XY chromosomal mechanisms were found in both populations, along with male heterogamety. The diploid number (2n) is 42 and the chromosomes are essentially homomorphic. However, some chromosomal differences can be detected between the two populations. These findings are discussed in terms of their implications for local geographic variation and for the speciation process. Specimens from the Doce River Valley showed some chromosomal variation in the size of specific chromosome pairs, possibly indicating the presence of variant chromosomes.

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INTRODUCTION

Among the Osteichthyes, the teleost fishes are the dominant group. The number of living bony fish species is estimated to be 20,000 to 40,000 (Greenwood et al., 1966). The frequent descriptions of new species and new genera demonstrate that we are still far from a comprehensive knowledge of the number of species of living fishes. The marine fauna from the ocean depths and the diverse fresh-water fauna of tropical America will no doubt yield many new species of fish (Greenwood et al., 1966).

Chromosome numbers have been determined for about 600 species of fishes, while the karyotypes are known for only approximately 300. Although it is accepted that the fundamental karyotype is composed of approximately 48 acrocentrics, the diploid number is quite variable, from 2n = 16 in some tropical species to 2n = 174 in lampreys (Denton, 1973 a). Large differences in chromosome numbers are found, not only between higher-ranking taxonomic groups, but also among orders, families, genera and even closely-related species (Kirpichnikov, 1973). According to Kosswig (1973), no other vertebrate group shows as much chromosomal variability as fishes.

The most common chromosome alteration is translocation, particularly a Robertsonian translocation. This and other types of rearrangements account for the occurrence of polymorphisms in chromosomal number and structure (Denton, 1973 b). Chromosomal alterations, such as tandem fusions (Campos and Hubbs, 1971) and pericentric inversions (Chen, 1971), have also been detected in fishes. In the genus *Fundulus,* Chen (1971) found variations in chromosome number, from 32 to 48 chromosomes, possibly due to Robertsonian translocations, pericentric inversions, and loss of some chromosome segments.

In the evolutionary process, the gene loci responsible for important functions are generally not subject to alterations. Based on this premise and on the consideration that speciation is many times followed by changes in karyotype, Ohno (1974) is inclined to accept the hypothesis that the karyotype, in itself, does not have a large significance in evolution. Thus, alterations in karyotype may be neutral changes which occur during speciation, and which do not confer any advantage. Ohno (1974) Believes that the role of chromosomal alterations has been overestimated as a mechanism for

creating sterility barriers between incipient species, and that behavioral and other types of barriers are probably more important in speciation.

On the other hand, Roberts (1970) considers it unlikely that a diversity of karyotypes should all have the same adaptative value. Thus, karyotypic as well as genetic variability could have a considerable influence on phenotypic variability, in such a way that their effects could not be easily identifiable.

Few karyotypic studies have been undertaken on Brazilian fishes. Thus, one objective of this study is to provide karyotypic information on Brazil's fresh water fishes, specifically of the genus *Hoplias.* These data may contribute to a future revision of this genus, which is now taxonomically confused. Furthermore, since this genus has a wide geographical distribution, two populations were studied: one from the Juquiá River, in the Ribeira River Valley (State of São Paulo) and the other from the Dom Helvécio and Carioca lakes, in the Doce River Valley (State of Minas Gerais). The 150 lakes of the Doce River Valley are ecologically interesting. They vary in size from small ponds about 3.0 m deep to large lakes of more than 20.0 m in depth. They are of quaternary origin, probably having been formed by blockage of the Doce River resulting in the isolation of the lakes from the river (Pflug, 1968, 1969). These peculiarities, in addition to the isolation among the lakes, make these environments particularly interesting for biological studies. Limnological studies of this series of lakes are also in progress (Tundisi et al., 1978).

MATERIALS AND METHODS

Eleven specimens (5 females and 6 males) from the Doce River Valley and 9 specimens (3 females and 6 males) from the Juquiá River were studied. These fishes were provisionally identified as *Hoplias malabaricus,* but, due to taxonomic problems in this genus, the two populations were labeled *H. malabaricus* and distinguished by their place of origin. Some morphological variations can be seen between the two populations, but the present taxonomy of this genus does not permit these differences to be interpreted at the level of species, subspecies, or geographical variations.

Specimens from this study have been deposited in the "Museu de Zoologia" of the University of São Paulo.

The animals were injected with an aqueous solution of 0.2-0.5% colchicine, 1 ml per 50g body weight. After 2 to 3 hours they were sacrificed and the kidneys removed and placed in a hypotonic 0.075 M KC1 solution. The tissue was fragmented, transferred to an incubator at 36 to 37° C for 20 min and then centrifuged. The sediment was fixed in methanol-acetic acid (3:1). After two additional centrifugations with changes of fixative, 2 or 3 drops of the cell suspension were placed on slides and air-dried. The preparations were stained with 5% Giemsa. Meiotic chromosomes from the testes were studied by the same method and also by the technique of Kligerman and Bloom (1977), slightly modified.

Chromosome measurements were made by the method of Yonenaga (1972), using the classification proposed by Levan et al. (1964): metacentrics (arm ratio - R = 1.00 to 1.70), submetacentrics (R = 1.71 to 3.00), subtelocentrics (R = 3.01 to 7.00) and acrocentrics (R $>$ 7.01). The fundamental number (FN) was found for each karyotype, counting each metacentric, submentacentric and subtelocentric as 2 chromosome arms.

RESULTS

Hoplias malabaricus from the Doce River Valley

A total of 423 female and 348 male cells were analyzed. The frequency distribution of diploid numbers (2n) in males and females is shown in Fig. 1. The diploid number is 42 for both sexes.

Fig. 2 represents the karyotype, with the chromosomes shown in order of decreasing size. The fundamental number is 84 for both males and females. Fig. 3 shows the meiotic chromosomes, with 42 chromosomes in spermatogonial metaphase, 21 bivalents in metaphase I and 21 chromosomes in metaphase II.

Some probable cases of variant chromosomes, with respect to pairs 5 and 6, are shown in Fig. 4.

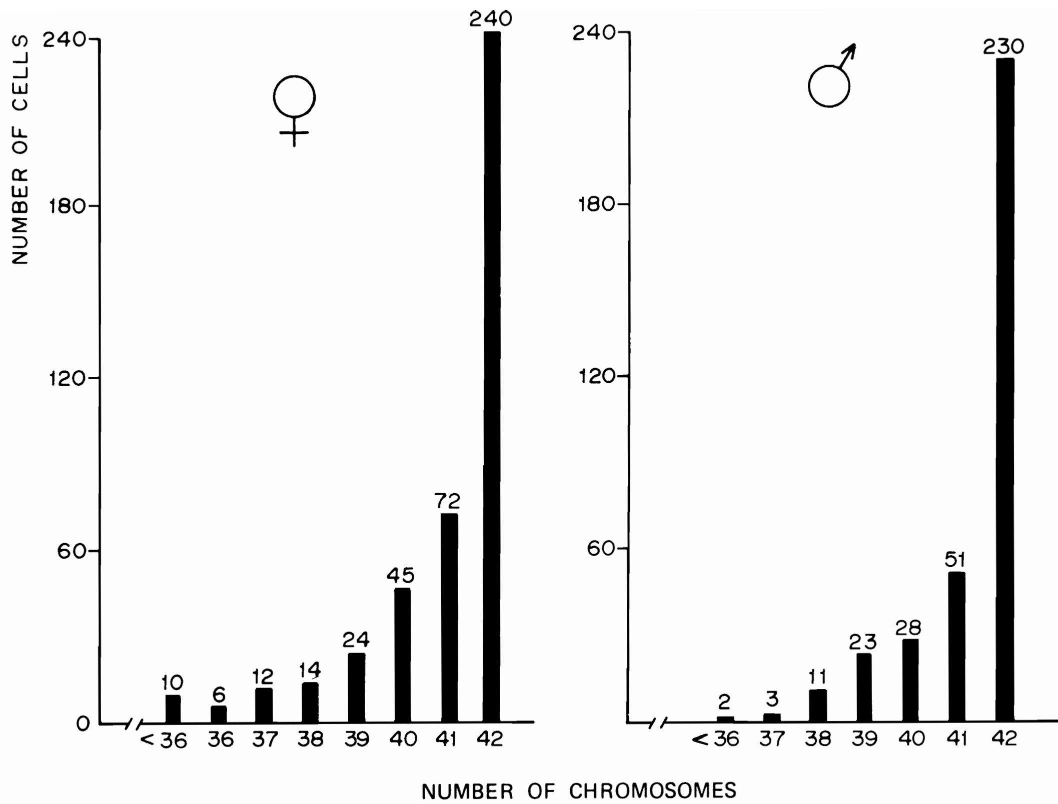
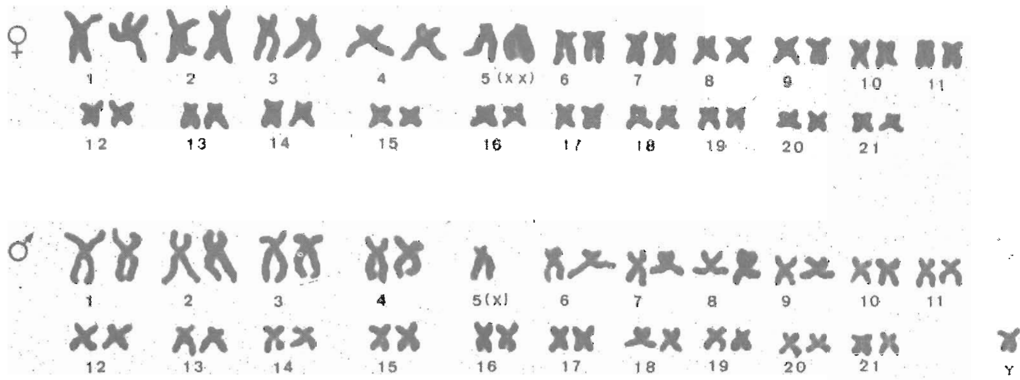


Figure 1 - Frequencies of diploid numbers (2n) for female and male *H. malabaricus* from the Doce River Valley.



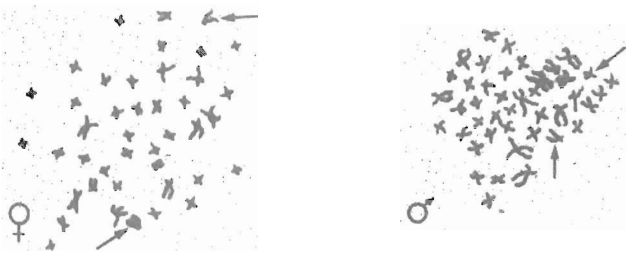


Figure 2 - Karyotype and somatic metaphase of female and male *H. malabaricus* from the Doce River Valley. Arrows indicate the female X - chromosome, and the male X – and probable Y - chromosomes. (2n = 42 and FN = 84).

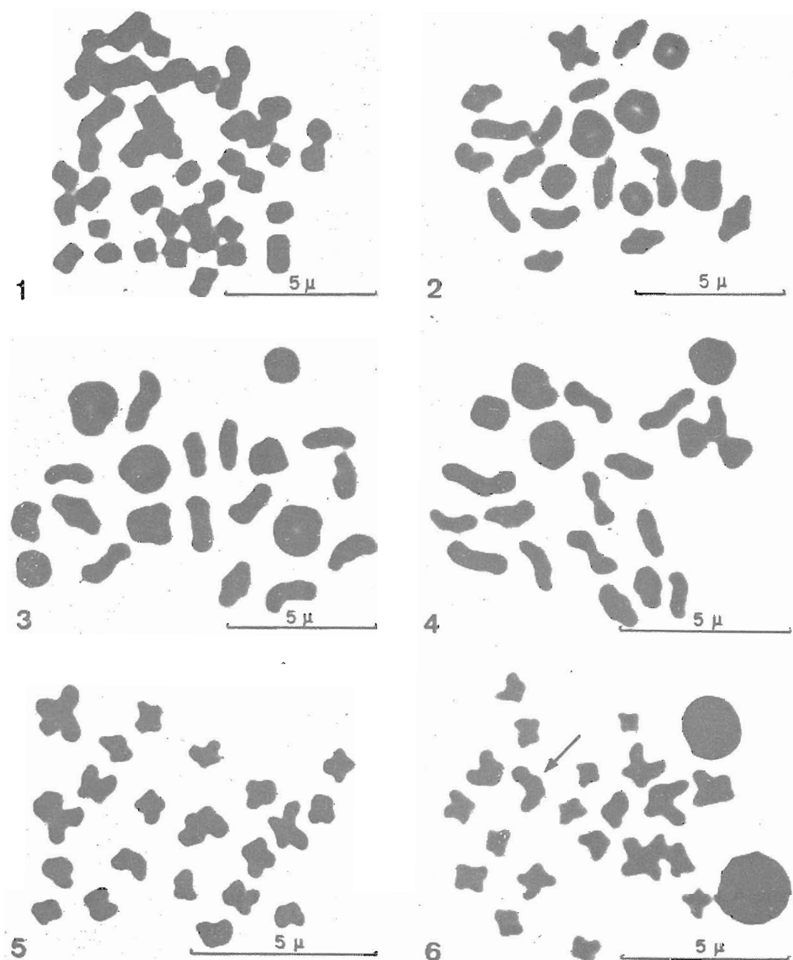


Figure 3 - Meiotic chromosomes of *H. malabaricus* from the Doce River Valley. (1) metaphase in the spermatogonium (2n = 42); (2, 3, 4) metaphase I (21 bivalents); (5, 6) metaphase II (21 chromosomes). The arrow indicates the probable X– chromosome.

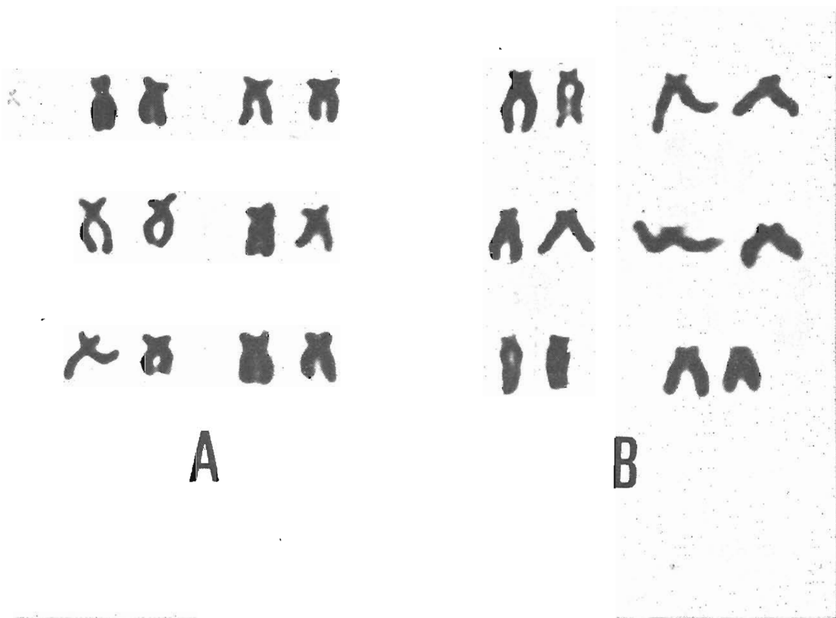


Figure 4 - Probable cases of variant chromosomes in pairs 6 (A) and 5 (B), in six somatic metaphases in *H. malabaricus* from the Doce River Valley.

Hoplias malabaricus from the Juquiá River

A total of 218 female and 158 male cells were analyzed. The frequency distribution of diploid numbers (2n) in males and females is shown in Fig. 5. The diploid number is 42, the same as in the Doce River Vallay population.

Fig. 6 represents the karyotype with the chromosomes shown in order of decreasing size. The fundamental number (FN) is 84 in both sexes. Unfortunately, the male testes were only slightly developed, and it was not possible to analyze meiotic chromosomes.

Female karyotypes from the two populations are compared in Fig. 7. The chromosomes are shown in order of decreasing size for the Doce River Valley females, and an attempt is made to indicate the corresponding chromosomes in the Juquiá River females.

Table I summarizes the results obtained with the chromosomal measurements in specimens of the two populations analyzed.

DISCUSSION

General considerations on the karyotypes

The karyotype of *H. malabaricus* females from the Doce River Valley has 42 chromosomes: 17 metacentric pairs, 3 submetacentric pairs, and 1 subtelocentric pair (Figs. 1, 2 and Table I). The karyotype of the male is similar, except that pair number 5 is represented by a univalent and there is an extra relatively small metacentric (Fig. 2). In this manner, the male is characterized by a heteromorphic pair, composed of a subtelocentric, number 5 of the karyotype, held to be the X-chromosome, and by a small metacentric, held to be the Y-chromosome but not completely identifiable among others of similar size and shape. Thus, an XX/XY sex-determination mechanism with male heterogamety is proposed, giving 40 autosomes plus XY in the male, and 40 autosomes plus XX in the female. The finding of 42 chromosomes in metaphase in the spermatogonium, 21 bivalents in metaphase I, and 21 chromosomes in metaphase II supports the diploid number observed

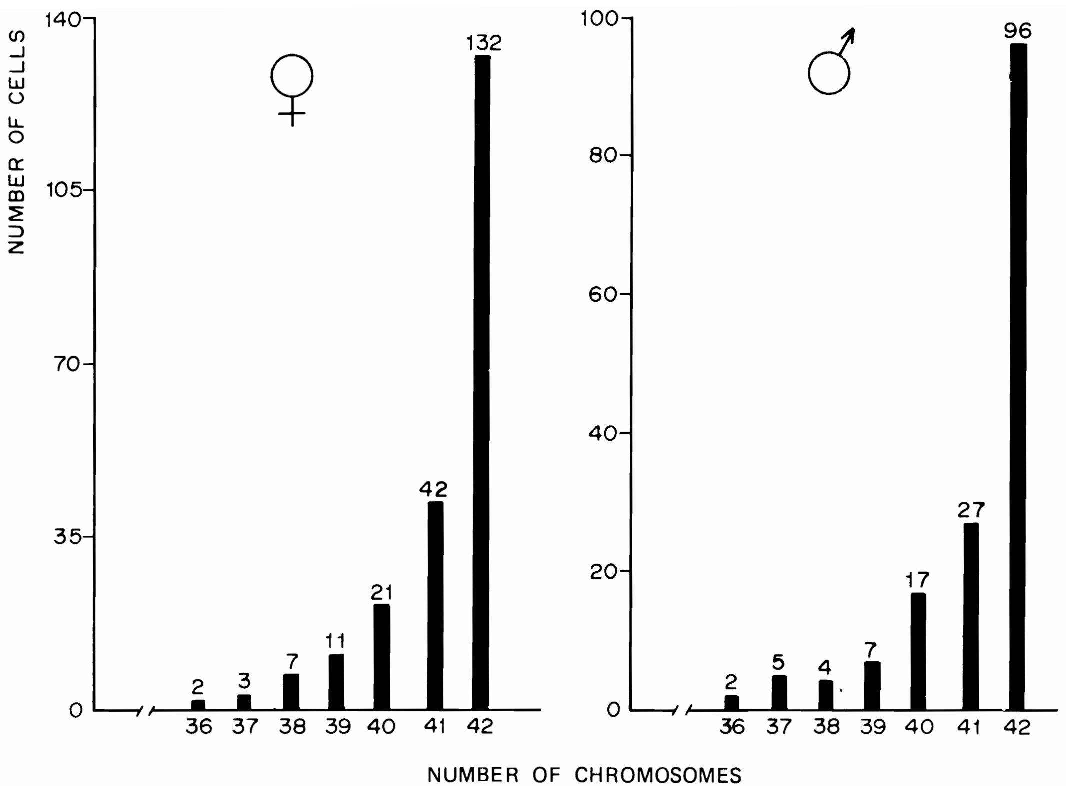
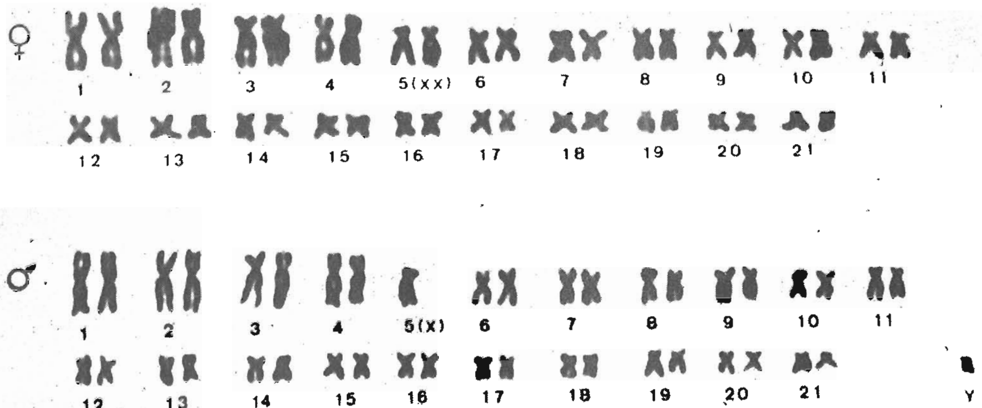


Figure 5 - Frequencies of diploid numbers (2n) for female and male *H. malabaricus* from the Juquiá River.



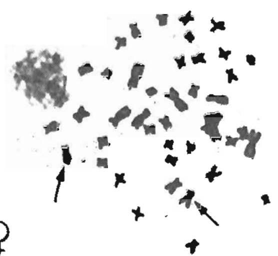


Figure 6 - Karyotype and somatic metaphase of female and male *H. malabaricus* from the Juquiá River. The arrows indicate the probable female X – chromosome and the male X – and Y – chromosomes. (2n = 42 and FN = 84).

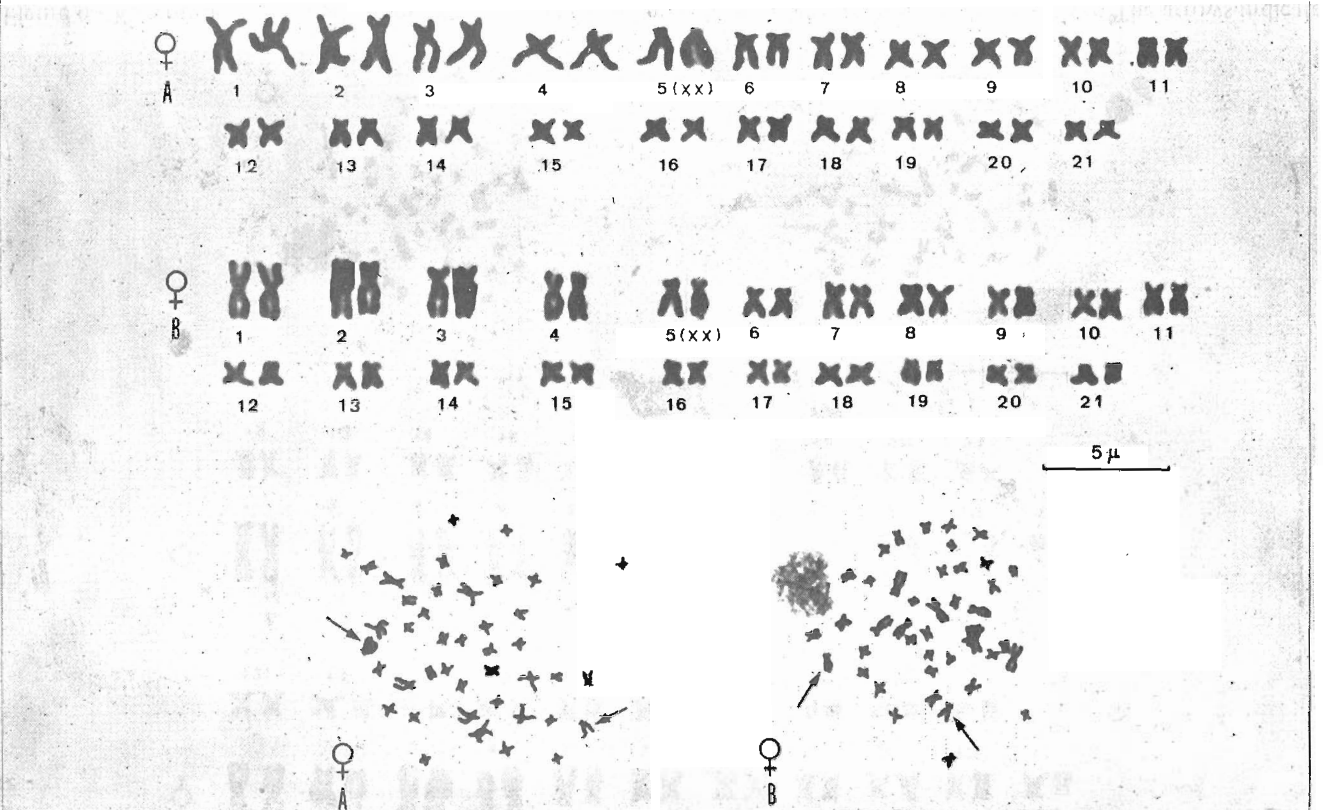


Figure 7 - Comparison of the female *H. malabaricus* karyotypes from the Doce River Valley (A) and the Juquiá River (B), shown with their respective somatic metaphases. ($2n = 42$ and $FN = 84$).

Table I - Average chromosome data for male and female *H. malabaricus* specimens from the Doce River Valley and Juquiá River.

CHROMOSOME	Doce River Valley			Juquiá River		
Chromosome	RL %[a]	R	TYPES	RL %	R[b]	TYPES
1	7.45	1.27	M[c]	7.86	1.09	M
2	7.29	1.50	M	7.57	1.39	M
3	6.74	2.06	SM[d]	7.11	1.91	SM
4	6.30	1.67	M	6.29	1.73	SM
5 (X)	5.28	3.19	ST[e]	5.38	2.15	SM
6	5.04	2.05	SM	5.15	1.26	M
7	4.69	1.38	M	4.92	1.13	M
8	4.69	1.13	M	4.92	1.44	M
9	4.54	1.25	M	4.58	1.73	SM
10	4.41	1.15	M	4.32	1.14	M
11	4.30	1.53	M	4.27	1.84	SM
12	4.22	1.14	M	4.15	1.15	M
13	4.22	1.88	SM	4.02	1.14	M
14	4.17	1.41	M	4.02	1.47	M
15	4.11	1.21	M	3.81	1.40	M
16	3.98	1.24	M	3.81	1.06	M
17	3.98	1.20	M	3.68	1.26	M
18	3.75	1.27	M	3.68	1.15	M
19	3.62	1.56	M	3.64	1.92	SM
20	3.62	1.14	M	3.50	1.28	M
21	3.59	1.40	M	3.30	1.57	M
Y	3.77	1.18	M	3.42	1.22	M

[a]RL = relative length; [b]R = arm ratio; [c]M = metacentric; [d]SM = submetacentric; [e]ST = subtelocentric

in somatic cells (Fig. 3). In some better preparations of metaphase II the X-chromosome can be identified by the position of the centromere, whereas in other metaphase preparations it is absent, as expected in an XX/XY mechanism.

The karyotype of the Juquiá River population is similar to that of the Doce River Valley population (Figs. 5 and 6), with 15 metacentric pairs and 6 submetacentric pairs in the female (Table I). The chromosomes of the male differ from the female's by the presence of an unpaired chromosome, number 5, submetacentric, and the presence of an extra, small metacentric (Fig. 6).

The male is thus characterized by a heteromorphic pair composed of one submetacentric from pair 5, held to be the X-chromosome, and a small, undistinguished metacentric, held to be the Y-chromosome. Thus, the male is the heterogamic sex of a probable XX/XY sex mechanism, with 40 autosomes plus XY.

The presence of cytogenetically differentiated sex chromosomes is not held to be the rule in fishes. However, various cases of XX/XY, XX/XO, ZZ/ZW mechanisms and multiple sex chromosomes have been found (Almeida Toledo, 1978; Bertollo, 1978; Chen, 1969; Chen and Ebeling, 1966, 1968; Ebeling and Chen, 1970; Foresti, 1974; Michele and Takahashi, 1977; Michele et al., 1977; Rishi, 1973, 1976; Uyeno and Miller, 1971, 1972). Thus, the present cases support the proposal of Ebeling and Chen (1970) that cytogenetic heterogamety among fishes is more common than generally thought.

Variant chromosomes

Two possible cases of variant chromosomes were found in the Doce River Valley population (Fig. 4). Autosome pair number 6 shows a small difference in size between its members, as do the two chromosomes of pair number 5, considered here to be the X-chromosomes (Fig. 4 A and B). Forty--eight percent of the analyzed metaphases showed this variant of chromosome 6, and 38 percent showed the variant of chromosome 5. Various cases of size variations in chromosomes are known for human and other vertebrate populations. Many of these cases are considered to be polymorphisms because of the high frequencies of the variants, their association with normal pheno-

types, and their transmission from one generation to the next (Lubs and Ruddle, 1970). The chromosome size differences in pairs 5 and 6 in the Doce River Valley population could be cases of chromosome variants, whose effects are not clear, or they could be cases of chromosomal polymorphisms for which confirmatory studies are necessary. These cases may be explicable by a small deletion or duplication in one of the members or by heterogeneity in the packing of the chromosomal material.

According to Brito da Cunha (1966), "the species and populations with a greater degree of chromosome polymorphism possess more integrated genomes and at the same time maintain the ability to create new adaptative types by combination and mutation. They are also better adapted to present environments and better able to survive temporal changes. As a genotype can never be the best in all ecological niches, polymorphism represents a mechanism which permits populations to best utilize their habitat". However, it is not simple to establish the relationship between habitat and polymorphism, mainly because of the physiological complexity of organisms and the ecological complexity of the environments.

Comparisons of karyotypes

Both populations have the same basic karyotype, with small differences (Fig. 7). The diploid number (2n = 42), the fundamental number (FN = 84), and the sex-determination mechanism (XX/XY) are the same. The same chromosomes are present in the karyotypes of the two populations, with the principal differences found in chromosomes 4, 5 to 13, 19 and Y. Pair number 5, considered to be the X-chromosome, is subtelocentric, at times submetacentric, in the Doce River Valley population, whereas in the Juquiá River population it is always submetacentric. Pair number 19 is metacentric in the Doce River Valley population and submetacentric in the Juquiá River population. The Y-chromosome is not unambiguously identifiable in either population, but was considered to have a more medial centromere in the Doce River Valley specimens.

The position of the centromere is the primary factor in the other interpopulation differences, notably in pairs 4, 6, 7, 8, 9, 11, and 13 (Figs. 2 and 6). In *H. malabaricus* from the Doce River Valley, as in *H. malabaricus* from the Juquiá River, pair 4 is almost borderline between the metacentric

and submetacentric classifications (arm ratio - R = 1.67 and 1.73 respectively). Thus we cannot exclude the possibility of technical problems in the measurements, leading to differences in chromosomal classification. As to chromosome 5 in *H. malabaricus* from the Doce River Valley and chromosome 9 in *H. malabaricus* from the Juquiá River, although they are almost borderline between submetacentric and subtelocentric chromosomes (R = 3.19) and between metacentric and submetacentric chromosomes (R = 1.73), respectively, there is a clear difference in the centromeric position when the chromosome of one specimen is compared with that of the other.

The attempt to correlate the chromosomes of the two populations (Fig. 7) leads to changes in size order, as can be observed in the karyotype of *H. malabaricus* from the Juquiá River (Fig. 7B). A karyotypic difference does not always characterize a species, nor does a similar karyotype guarantee that a single species is included. So, despite the chromosomal similarities of the two populations studied, it is difficult to establish if they belong to a single species, especially in view of the taxonomic difficulties of this genus. Because of the geographical separation between the two populations in two different drainage basins and because of the quiet nature and sedentary life of these fishes (Azevedo and Gomes, 1943), it is possible that the two groups studied may have developed characteristic chromosomal peculiarities. In addition, according to some authors, the family Erythrinidae may represent an old, if not the oldest, phylogenetic group of existent Cypriniforms, as suggested by their osteology and by the canine teeth which are present throughout all stages of life, including the larval stage (Azevedo and Gomes, 1943; Godoy, 1970, 1975). Thus, characteristics of the environment, such as those of the lakes of the Doce River Valley, of quaternary origin and consisting of isolated bodies of water, could have led to a diversification of the karyotype of the animals. In this manner, if the populations studied belong to one species, the karyotype differences may indicate incipient chromosomal evolution with the habitats acting as selective agents, as seems to be the case for some populations of *Fundulus diaphanus* (Arcement and Rachlin, 1976). A comparative study with animals from the Doce River itself would be of particular interest. If, on the other hand, the two populations constitute different species, this does not imply that the chromosomal alterations which lead to divergence in the karyotypes participated directly in speciation. Karyotypic differences can be more a consequence of speciation than a cause (Dobzhansky, 1973; Stebbins, 1974).

Meanwhile, chromosomal reorganization can play an important part in speciation as some authors have recently postulated. According to Avise and Gold (1977), chromosomal rearrangements could establish reproductive isolation and thus speciation. Besides, rearrangements in the position of genetic material in chromosomes cam be an effective method of acquiring new modes of gene regulation. In turn, such alterations in the gene regulating apparatus can play a crucial role in evolution. In speciation, gene rearrangements rather than changes in the structural genes can lead to true "genetic revolutions".

Wilson et al. (1974) suggest that gene rearrangements can be a more important evolutionary force than point mutations. The studies of Prager and Wilson (1975) are also in agreement with the hypothesis that modifications in genetic regulatory systems constitute the basis of anatomical evolution and the loss of potential hybridization. Bush (1975), in his fine study of the modes of animal speciation, states that "genetic revolution and geographical isolation may not be necessary for speciation in many animals. A single genetic substitution or chromosomal rearrangement can initiate speciation, if it has a drastic and permanent effect on the gene flow between the divergent populations".

As far as the present study is concerned, where slight karyotypic differences are seen between two allopatric populations, it is tempting to assume that such differences are the result of small chromosomal rearrangements which may be reflected in some genetic alterations and which may be implicated in a speciation process in these populations.

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REFERENCES

Almeida Toledo, L. F. de (1978). Contribuição à citogenética dos Gymnotoidei - (Pisces, Ostariophysi). Doctoral Thesis. Universidade de São Paulo. Instituto de Biociências. 128 p.

Arcement, R.J. and Rachlin, J.W. (1976). A study of the karyotype of a population of banded killifish (*Fundulus diaphanus*) from the Hudson River. *J. Fish Biol.* 8:119-25.

Avise, J.C. and Gold, J.R. (1977). Chromosomal divergence and speciation in two families of North American fishes. *Evolution* 31: 1-13.

Azevedo, P. and Gomes, A.L. (1943). Contribuição ao estudo da traíra *Hoplias malabaricus* (Bloch, 1794). *Bol. Ind. Anim.*, São Paulo 5:15-64.

Bertollo, L.A.C. (1978). Estudos citogenéticos no gênero *Hoplias* Gill, 1903 (Pisces, Erythrinidae). Doctoral Thesis. Universidade de São Paulo. Faculdade de Medicina de Ribeirão Preto. Departamento de Genética e Matemática Aplicada à Biologia. 164p.

Brito da Cunha, A. (1966). As bases genéticas da evolução. In: *Elementos de Genética* (Pavan, C. e Brito da Cunha, A., org.) Companhia Editora Nacional e Editora da Universidade de São Paulo, São Paulo, pp. 344-75.

Bush, G.L. (1975). Modes of animal speciation. *Annu. Rev. Ecol. Sys.* 6: 339-64.

Campos, H.H. and Hubbs, C. (1971). Apud Toledo, V. (1975). Contribuição ao estudo citogenético da família Pimelodidae (Pisces). Masters thesis. Universidade de São Paulo. Faculdade de Madicina de Ribeirão Preto. Departamento de Genética e Matemática Aplicada à Biologia. 84 p.

Chen, T.R. (1969). Karyological heterogamety of deep-sea fishes. *Postilla* (Yale University) 130: 1-29.

Chen, T.R. (1971). A comparative chromosome study of twenty killifish species of the genus *Fundulus* (Teleostei: Cyprinodontidae). *Chromosoma* 32: 436-53.

Chen, T.R. Ebeling, A.W. (1966). Apud Chen, T.R. (1969). Karyological heterogamety of deep-sea fishes. *Postilla* (Yale University) 130: 1-29.
Chen, T.R. and Ebeling, A.W. (1968). Apud Chen, T.R. (1969). Karyological heterogamety of deep-sea fishes. *Postilla* (Yale University) 130: 1-29.
Denton, T.E. (1973 a). The fish karyotype. In: *Fish Chromosome Methodology*. Charles C. Thomas Publisher, Illinois, pp. 3-17.
Denton, T.E. (1973 b). Evolution of the fish karyotype. In: *Fish Chromosome Methodology*. Charles C. Thomas Publisher, Illinois, pp. 129-48.
Dobzhansky, Th. (1973). Apud Toledo, V. (1975). Contriuição ao estudo citogenético da família Pimelodidae (Pisces). Masters thesis. Universidade de São Paulo. Faculdade de Medicina de Ribeirão Preto. Departamento de Genética e Matemática Aplicada à Biologia. 84p.
Ebeling, A.W. and Chen, T.R. (1970). Heterogamety in teleostean fishes. *Trans. Am. Fish. Soc.* 99: 131-8.
Foresti, F. (1974). Heteromorfismo cromossômico em peixes: evidências de um novo mecanismo. *Cienc. Cult.* Sup. 26: 248.
Godoy, M.P. de (1970). Estudos osteológicos da traíra *Hoplias malabaricus* (Bloch, 1794). *Rev. Brasil. Biol.* 30: 447-60.
Godoy, M.P. de (1975). Família Erythrinidae. In: *Peixes do Brasil.* Sub-ordem Caracoidei. Bacia do Rio Mogi Guassu, v.3. Editora Franciscana, Piracicaba, pp. 400-44.
Greenwood, P.H., Rosen, D.E., Weitzman, S.H. and Myers, G.S. (1966). Phyletic studies of teleostean fishes, with a provisional classification of living forms. *Bull. Amer. Mus. Nat. Hist.* 131: 339-455.
Kirpichnikov, V.S. (1973). On karyotype evolution in Cyclostomata and Pisces. *Ichthyologia* 5: 55-77.
Kligerman, A.D. and Bloom, S.E. (1977). Rapid chromosome preparations from solid tissues of fishes. *J. Fish. Res. Bd. Canada* 34: 266-9.
Kosswing, G. (1973). The role of fish in research on Genetics and Evolution. In: *Genetics and Mutagenesis of Fish* (Schroder, J. H., ed.) Springer--Verlag, Berlin, pp. 3-16.
Levan, A., Fredga, K. and Sandberg, A.A. (1964). Nomenclature for centromeric position in chromosomes. *Hereditas* 52: 201-20.
Lubs, H.A. and Ruddle, F.H. (1970). Apud Jin, S.M. (1976). Contribuição ao estudo da evolução cariotípica na ordem Carnivora. Doctoral thesis. Universidade de São Paulo. Faculdade de Medicina de Ribeirão Preto.

Uyeno, T. and Miller, R.R. (1972). Second discovery of multiple sex chromosomes among fishes. *Experientia* 15: 223-6.

Wilson, A.C., Sarich, V.M. and Maxson, L.R. (1974). Apud Jin, S.M. (1976). Contribuição ao estudo da evolução cariotípica na ordem Carnivora. Doctoral Thesis. Universidade de São Paulo. Faculdade de Medicina de Ribeirão Preto. Departamento de Genética e Matemática Aplicada à Biologia. 184p.

Yonenaga, Y. (1972). Chromosomal polymorphism in the rodent *Akodon arviculoides* ssp. (2n = 14) resulting from two pericentric inversions. *Cytogenetics* 11: 488-99.

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INHERITANCE OF PHOTOSYNTHETIC AREAS ABOVE THE FLAG LEAF NODE IN SPRING WHEAT*

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ABSTRACT

Five cultivars of spring wheat (*Triticum aestivum* L.) were crossed in a diallel system to study heterosis and combining ability for photosynthetic areas above the flag leaf node (flag leaf blade, flag leaf sheath, extrusion, and head areas). No heterosis was found for any of the photosynthetic areas in the F_1 hybrids. Analysis for general combining ability indicated that a large part of the total genetic variation observed for all areas except the extrusion area were associated with genes which were additive in their effects. A good prediction of hybrid performance could be made on the basis of the average parental performance. Selection for these characters should be successful.

INTRODUCTION

The importance of the photosynthetic areas above the flag leaf node in wheat (*Triticum aestivum* L.) i.e. flag leaf blade, flag leaf sheath, peduncle,

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and head, for yield of grain has been emphasized by Thorne (1966) and Walton (1969) and supported by autoradiographic studies of Rawson and Hofstra (1969). Because of these findings, several investigations have been conducted to determine the degree of association between the photosynthetic areas and wheat grain yield. Photosynthetic areas above the flag leaf node in wheat account for 85% of variations in yield (Voldeg and Simpson, 1967; Simpson, 1968; Hsu and Walton, 1971; Barriga and Fuentealba, 1976).

The results of Hsu and Walton (1970) showed that the inheritance of the photosynthetic areas in wheat germplasm was associated mainly with additive gene effects with different degrees of dominance. Walton (1971) also showed that inheritance in these areas in mainly controlled by genetic action of an additive type.

Reports by Yap and Harvey (1972) and Barriga (1976) indicated that the photosynthetic areas of the flag leaf node are highly heritable. It has been postulated that selection for green structures located above the flag leaf node in wheat would be effective to obtain genotypes having higher productivity (Smocek, 1969; Hsu and Walton, 1970; Lupton, et al. 1974; Barriga and Fuentealba, 1976).

The objective of this study was to obtain additional information on heterosis and on the effects of combining ability involved in inheritance of the blade, sheath, extrusion, and head areas located above the flag leaf node in the F_1 generation from a diallel crossing between five genotypes of spring wheat.

MATERIALS AND METHODS

The germplasm under study included five commercial varietes of spring wheat (Intermedio, Naofén, Express, Loncofén, and Toquifén) that were crossed by hand in a diallel system without reciprocals. The five parents and ten F_1 hybrids were sown at the "Santa Rosa" Experimental Center, Valdivia (latitude 30° 48' South and longitude 73° 14' West), Universidad Austral de Chile, in randomized complete block design with three replications. Each genotype was sown in a six-seed hill plot with 30 cm spacing between hill plots. One hill plot, or the surface area equivalent to one hill

plot, constituted the experimental unit. After emergence, a reseeding was made to standardize the population in those experimental units where a shortage of seedlings was observed.

Data to estimate the photosynthetic areas above the flag leaf node were obtained by measuring the main tiller of each plant during the second week after anthesis. Preliminary observations showed that these characters had their greatest growth at that time.

The phostosynthetic areas studied were characterized by the following parameters:

Flag leaf blade area (cm^2): length of the blade x maximum width of leaf x 0.80 (Voldeng and Simpson, 1967).

Flag leaf sheath area (cm^2): length of the sheath, measured from the auricles to the leaf node x diameter of the peduncle, including the sheath x 3.1416.

Extrusion of the head area (cm^2): length of the peduncle, from auricles of flag leaf to the rachis insertion x diameter of peduncle x 3.1416.

Head area (cm^2): length of the head, excluding the awns, x width of the head x 3.1416.

Initially, the data obtained for all photosynthetic areas were analyzed by analysis of variance for randomized complete blocks. The significance of the differences between means of genotypes was established by least significant differences (L.S.D.). The analysis of variance of the diallel, proposed by Gardner and Eberhart, analysis II (1966) and statistical procedure by Vencovsky (1970) were used to estimate the degree of variability of the heterotic response, utilizing the mean of each of the five parents and the ten F_1 hybrids.

To obtain additional information concerning genetic systems controlling the photosynthetic areas, general combining ability (GCA) and specific combining ability (SCA) also were analyzed with method 4, model I of Griffing (1956). The GCA/SCA ratio for each area was obtained as an estimate of the ratio between additive and non-additive gene effects.

RESULTS AND DISCUSSION

The comparisons of mean values of parents and F_1 hybrids for each of the photosynthetic areas are shown in Table I. Among the parents, Express

exhibited a greater photosynthetic area above the flag leaf node, while Toquifén had the smallest area. In this respect, according to Barriga and Funtealba (1976), the interrelationship between these areas is positive and high, so that an increase in the blade area implies an increase in the sheath and head areas. On the other hand, genotypes with a larger sheath area tend to produce a larger extrusion and head area.

Table I shows that hybrids (Naofén x Loncofén) and (Express x Loncofén) had the highest values for photosynthetic areas, and that (Intermédio x Toquifén) and (Naofén x Toquifén) had the lowest. All hybrids, however, showed photosynthetic areas similar to those of the parents.

Heterosis

The mean squares and their significance, for the photosynthetic areas of the diallel crosses, provide information on the genetic basis of these areas (Table II). The mean squares for genotypes were highly significant for all areas, except the extrusion area, indicating that there was variability among these genotypes for most of the photosynthetic areas under study. When analyzing the effects of genotypes for the blade, sheath, and head areas, only the variety mean squares (v_j parameters) were highly significant. These results clearly show differences among parents but no evidence of heterosis. Apparently, the general combining ability of each parent was independent of heterosis effects. Nevertheless, the non-significant values of heterosis for the areas considered in the present study do not allow us to conclude that there is an absence of dominance due to the possible cancelling of positive and negative genetic effects.

Vencovsky (1970) postulates that if heterosis is not significant in dialleles of varieties, it must be concluded that variety means constitute the main information regarding general combining ability or that experiments with dialleles have not been accurate enough to detect the importance of heterosis in general combining ability. Although the results obtained here must be considered within the restrictions inherent to this type of experiments, they can be used as a reference for future studies of this kind.

All sources of variation were non significant for the extrusion areas (Table II). Hence, the varieties included must constitute a homogeneous group for this trait due to a possible small genetic diversity among the parents for this photosynthetic area.

Combining Ability

An analysis of general combining ability (GCA), specific combining ability (SCA), and the relationship between additive and non-additive gene effects (GCA/SCA) is shown in Table III. GCA mean squares for the blade and head areas were highly significant, while only significant for the sheath area. SCA mean squares for these three areas were non-significant. For the extrusion area, all mean squares were non-significant, since the parents constituted a homogeneous group for this area, as shown in the diallel variance analysis (Table II).

The information obtained from Tables I and II and the estimated relationship of GCA/SCA (Table III) for all photosynthetic areas, except the extrusion area, suggest that the genetic variation observed is mainly due to additive genetic effects. These results are in agreement with those of Hsu and Walton (1970) and Walton (1971) for wheat, and those of Yap and Harvey (1972) and Singh *et al.* (1973) for barley (*Hordeum vulgare* L.). All evidence supports the hypothesis that selection should be effective for these photosynthetic areas to obtain plants with maximum photosynthetic efficiency and, therefore, with higher productivity.

Apparently none of the parental lines showed outstanding expression of SCA effects for any particular area, which explains the general lack of heterotic effects. Clearly, in the present material, SCA effects could be of limited use in practical application and improvement in these agronomic characters must depend on the exploitation of their GCA or additive gene action.

Loncofén had the greatest positive GCA effects for the blade, sheath, and head areas (g_i, Table III). Conversely, Toquifén had the greatest negative GCA effects for the three photosynthetic areas. Thus, Toquifén tends to diminish the values of the photosynthetic areas of the F_1 hybrids and, therefore, should not be used as a parent for obtaining hybrids of larger photosynthetic areas above the flag leaf node.

Vencovsky (1970) points out, however, that a significant mean square for GCA does not indicate if the differences in GCA are due to the behavior of varieties per se, to their behavior in crosses, or to both factors. Nevertheless, since only the mean squares of the variety effects were significant in the diallel analysis it can be concluded that the GCA of these varieties was only a function of their own behavior. On the basis of the

Table I - Mean values of parents and F_1 hybrids, for photosynthetic areas above the flag leaf node (cm^2).

Genotype	Blade area	Sheath area	Extrusion area	Head area
Intermédio	27.57	29.50	15.66	37.44
Naofén	23.22	31.00	18.54	37.58
Express	34.95	31.60	16.18	43.12
Loncofén	28.71	37.14	17.59	44.34
Toquifén	17.45	21.80	13.22	27.72
Intermedio x Naofén	23.50	30.42	15.91	38.27
x Express	26.13	28.98	17.32	40.11
x Loncofén	26.60	30.72	13.30	37.24
x Toquifén	23.59	26.61	20.69	33.54
Naofén x Express	28.44	31.56	21.66	41.46
x Loncofén	30.80	33.79	20.36	48.03
x Toquifén	18.99	26.08	14.45	31.99
Express x Loncofén	32.86	35.12	23.03	47.08
x Toquifén	28.37	32.43	21.36	42.34
Loncofén x Toquifén	26.53	30.08	22.55	39.94
L.S.D. .05	7.49	5.66	7.42	8.57

Table II - Mean squares values from analysis of variance for 5 x 5 diallel table by the Gardner-Eberhart method.

Source of variation	D.F.	Mean squares			
		Blade area	Sheath area	Extrusion area	Head area
Genotypes	14	22.1647**	14.0312**	11.0638	30.2934**
Varieties (v_j)	4	63.8012**	41.0057**	7.0087	81.8205**
Heterosis (h_{jj}')	10	5.5101	3.2414	12.6858	9.6826
Heterosis					
Average ($\bar{h}$)	1	0.1347	0,4588	26.6021	12.8053
Variety (h_j)	4	7.1168	3.6200	10.8006	6.1545
Specific (s_{jj}')	5	5.2998	3.4951	11.4107	11.8806
Error	28	6.6754	3.8104	6.5656	8.7313

** significant at the 1% level.

Table III - Mean squares for combining ability analysis, the GCA/SCA ratio and cultivars with the highest and lowest general combining ability effect (g_i).

Photosynthetic areas	Mean squares values			General combining ability effects (g_i) cultivars showing:	
	GCA	SCA	GCA/SCA	Highest value	Lowest value
Blade area	28.4129**	5.2997	5.3612	Loncofén	Toquifén
Sheath area	14.1723*	3.4950	4.0550	Loncofén	Toquifén
Extrusion area	13.6660	11.4107	1.1976	Express	Intermédio
Head area	44.8323**	11.8806	3.7735	Loncofén	Toquifén

* , ** significant at the .05 and .01 levels, respectively.

above evidence, the differences observed in the GCA of the five parents were independent of heterosis and can be evaluated on the basis of the variety performance per se, for these photosynthetic areas.

In general, the highly significant GCA estimates and non significant SCA estimates suggested that additive gene action played a predominant role and that selection for these photosynthetic areas should certainly be easier. Development of genotypes with larger photosynthetic areas above the flag leaf node should aid in the identification of genotypes with greater productivity. It should also be emphasized that valid inferences can be made only about the particular set of parents used in this study, since any conclusions regarding spring wheat as a whole could be misleading. Consequently, it might be worthwile to undertake a similar study in the future using a larger number of cultivars and testing in more locations and over a number of years. Despite the limitations, results obtained in this study are consistent with those of other studies of similar scope.

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REFERENCES

Barriga, P. (1976). Variabilidad y heredabilidad de algunos caracteres cuantitativos en trigo de primavera. *Agro Sur (Chile) 4(2):* 71-75.

Barriga, P. and Fuentealba, J. (1976). Relaciones entre rendimiento, componentes del rendimiento y caracteres morfológicos sobre el nudo de la hoja bandera en trigo de primavera. *Agro Sur (Chile) 4(2):* 65-70.

Gardner, C.O. and Eberhart, S.A. (1966). Analysis and interpretation of the variety cross diallel and related populations. *Biometrics 22:* 439-452.

Griffing, B. (1956). Concept of general and specific combining ability in

relation to diallel crossing systems. *Aust. J. Biol. Sci. 9:* 463-493.

Hsu, P. and Walton, P.D. (1970). The inheritance of morphological and agronomic characters in spring wheat. *Euphytica 19:* 54-60.

Hsu, P. and Walton, P.D. (1971). Relationship between yield and its components and structures above the flag leaf node in spring wheat. *Crop Sci. 11:* 190-193.

Lupton, F.G.H., Oliver, R.H. and Ruckenbauer, P. (1974). An analysis of the factors determining yields in crosses between semi-dwarf and taller wheat varieties. *J. Agric. Sci., Camb. 82:* 483-496.

Rawson, H.M. and Hofstra, G. (1969). Translocation and remobilization of C^{14} assimilates at different stages by each leaf of the wheat plant. *Aust. J. Biol. Sci. 22:* 321-331.

Simpson, G.M. (1968). Association between grain yield per plant and photosynthetic area above the flag leaf node in wheat. *Can. J. Plant Sci. 48:* 253-254.

Singh, V.P., Singh, M. and Tripathi, I.D. (1973). Combining ability analysis in barley. *Indian J. Genet. and Plant Breeding 33:* 189-196.

Smocek, J. (1969). A contribution to the analysis of association between economic yield components and four morpho-physiological subcharacters in winter wheat. *Biología Pl. 11:* 260-269.

Thorne, G.N. (1966). Physiological aspects of grain yield in cereals. In: *The growth of cereals and grasses.* (Milthorpe, F.L. and Ivins, J.D., eds.) Butherworthe, London, pp. 88-105.

Vencovsky, R. (1970). Alguns aspectos teóricos e aplicados relativos a cruzamentos dialélicos de variedades. Tese de Livre-Docência. ESALQ. Piracicaba, S.P. Brasil. 59 p.

Voldeng, H.D. and Simpson, G.M. (1967). Relationship between photosynthetic area and grain yield per plant in wheat. *Can. J. Plant Sci. 47:* 359-365..

Walton, P.D. (1969). The inheritance of morphological characters associated with yield in spring wheat. *Can. J. Plant Sci. 49:* 587-596.

Walton, P.D. (1971). Heterosis in spring wheat. *Crop Sci. 11:* 422-424.

Yap, T.C. and Harvey, B.L. (1972). Inheritance of yield components and morpho-physiological traits in barley, *Hordeum vulgare* L. *Crop Sci. 12:* 283-286.

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HERITABILITY AND CORRELATIONS OF ONION BULB TRAITS (*Allium cepa* L.). I*

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ABSTRACT

Heritability and genetic plus phenotypic correlations of onion bulb traits were estimated for 54 half-sib progenies of a C-5 cycle from a crossing of Baia X Red Creole cultivars. The bulb traits considered were: weight, diameter, and soluble solid content determined by refractive index measurements of extracts of outer scales. The genetic parameters were estimated using variance and covariance components. Based on individual data from 10 bulb samples, high heritability was found for all traits: 39.09 % for bulb weight; 24.34 % for diameter and 40.37 % for soluble solid content. All phenotypic correlations were negative: weight versus diameter (-0.1930), weight versus soluble solids (-0.8561), and diameter versus soluble solids (-0.1032). All genetic correlations were negative: weight versus diameter (-0.9622), weight versus soluble solids (-0.6983), and diameter versus soluble solids (-0.1401).

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INTRODUCTION

Knowledge of the heritability of a trait can be useful to the breeder for predicting the effectiveness of selection and for choosing a breeding method. Correlations show association between traits, which may be of genetic or environmental origin. Genetic correlations are the most important for breeding because they can be used to predict the responses to selection.

Few estimates of genetic parameters related to long-day onion groups are found in the literature (McCollum, 1966, 1968 and 1971). Estimates of heritability and of correlations between soluble solid content and other traits have been reported in only one study (McCollum, 1968) and these parameters would be expected to be specific for the population and environment studied. Soluble solid content is important because it is directly related to bulb conservation (Jones and Mann, 1963).

The objective of this study was to estimate the heritability and genetic and phenotypic correlations of onion bulb traits in a population of short-day onions grown at a single site.

MATERIAL AND METHODS

The germplasm studied consisted of 54 half-sib progenies obtained from a C-5 population resulting from crossing Baia periforme precoce X Red Creole short-day cultivars, and left unselected for the traits studied. They were grown in Piracicaba (State of São Paulo), 22° 41' 30" latitude, in red soil, by transplanting. The seeds were sown in beds (5 g per m^2) on May 12, 1977, and the seedlings transplanted on July 6, 1977. Forty vigorous seedlings were selected at random from each bed and transplanted as one plot. The soil was fertilized with 50 g of 4:30:20 NPK formula per linear row meter plus two time side dressing nitrogen during the cycle. The progeny test was carried out by a random block design in four replications, with 40 plants per plot in rows 0.50 m apart and spaced 0.10 m apart in each row. Culturing was carried out by standard methods. The plants were harvested when ripening, indicated by falling of the stalk and beginning of leaf dryness, had occurred in 80 % of the plants in each plot. Each plot was harvested separately and the bulbs placed in a well-ventilated storage shed, where the following traits were measured

and recorded for 10 samples from each plot: bulb weight in g after removing leaves and roots; bulb diameter in cm, expressed as the largest distance between opposite sides of the bulb at right angles with the height measurement; soluble solid content, in brix degrees, obtained by readings in a field refractometer of the extract obtained from a squashed 2 cm-thick slice taken from the widest diameter region of the bulb and containing one or two outer scales.

These parameters were analyzed by using variance and covariance components according to Vencovsky (1969) and Kempthorne (1966). Tables I and II show the models of variance and covariance analysis, and the respective expected mean squares of the products.

Heritability was estimated in the narrow sense, i.e. as $h^2 = \frac{var_A}{var_P}$, where var_A = total additive genetic variance of the trait at the individual level, and var_P = total phenotypic variance, where $var_P = var_E + var_D + var_{HS}$, and $var_A = 4\ var_{HS}$ according to Falconer (1964)[1]. Phenotypic correlations between two y and y traits were estimated as follows:

$$\hat{r}_P = \frac{c\hat{o}v_P\,(x,y)}{\sqrt{v\hat{a}r_P\,(x) \quad v\hat{a}r_P\,(y)}}, \tag{1}$$

where $cov_P\ (x,y) = cov_E\ (x,y) + cov_D\ (x,y) + cov_{HS}\ (x,y)$[2]; and genetic correlations were estimated as:

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[1] var_E = environmental variance among plots within blocks.
var_D = phenotypic variance among bulbs within plots.
var_{HS} = half-sib progeny variance.

[2] $cov_E\ (x,y)$ = environmental covariance among plots for traits x and y.
$cov_D\ (x,y)$ = phenotypic covariance among bulbs within plots for traits x and y.
$cov_{HS}\ (x,y)$ = genetic covariance among half-sib progenies for traits x and y at the individual level.

$$\hat{r}_A = \frac{\hat{cov}_A(x,y)}{\sqrt{\hat{var}_A(x) \quad \hat{var}_A(y)}}, \tag{2}$$

where $cov_A(x,y) = 4\, cov_{HS}(x,y)$ (Kempthorne, 1966).

Table I – Analysis of variance and expected mean squares for onion bulb traits. Piracicaba, 1977.

Variation Source	Degrees of Freedom	Expected Mean Squares
Blocks	3	–.–
Progenies	53	$var_D + 10var_E + 40var_P$
Error between plots	159	$var_D + 10var_E$
Error within plots	1944	var_D

Table II – Analysis of covariance and expected mean squares of the products for onion bulb traits. Piracicaba, 1977.

Covariance Source	Degrees of Freedom	Expected Mean Squares of the Products
Blocks	3	–.–
Progenies	53	$cov_D(x,y) + 10cov_E(x,y) + 40cov_P(x,y)$
Error between plots	159	$cov_D(x,y) + 10cov_E(x,y)$
Error within plots	1944	$cov_D(x,y)$

RESULTS AND DISCUSSION

The estimates of the heritability coefficients in the narrow sense are given in Table III. In this study bulb weight has high heritability (39.09%), in contrast to the values previously reported in the literature (McCollum, 1966, 1968 and 1971) which ranged between 0 and 28%. Because of the high heritability value, our population seems to offer good possibilities for successful selection for bulb weight even by using simple breeding methods such as mass selection. The value for heritability of bulb diameter, 24.34%, may also be considered high in comparison to 0-27% obtained by McCollum for 17 long-day onion populations (1966, 1968 and 1971). However, the possibility of selecting for a wider diameter seems to be limited. Estimated heritability of soluble solid content in our study was 40-43%, a high value indicating that this trait could also be selected for by simple breeding methods.This value differs significantly from those obtained by McCollum (1968), which were 78 and 81% for a long-day onion population.

Table IV shows the phenotypic and genetic correlations. All phenotypic correlations are negative. Thus high-weight bulbs have smaller diameters and a lower amount of soluble solids than lighter bulbs. Also, wide-diameter bulbs have a lower amount of soluble solids. The high negative value of phenotypic correlation between bulb weight and soluble solid content obtained in this study must be emphasized. In contrast, McCollum obtained positive and elevated phenotypic correlations between weight and diameter (1966, 1971), between weight and soluble solids, as well as between soluble solids and diameter (1968).

Additive genetic correlation between bulb weight and diameter was also negative and large (-0.9622), indicating that selection for increased weight may result in decreased bulb diameter. The value obtained was not in agreement with those reported in the literature (McCollum, 1966, 1968 and 1971). Additive genetic correlation between weight and soluble solid content was also negative (-0.6983). If confirmed, this negative correlation would indicate that selection for larger, and therefore heavier, bulbs may result in decreased soluble solid content, with consequent decreased storage life.

In conclusion, the weight, diameter and soluble solid content traits exhibited high heritability coefficients in the narrow sense, indicating easy modification by selection and also the possibility of successful mass selection

for population improvement for each trait separately. All phenotypic and genetic correlations between the three traits are negative. We emphasize the negative genetic correlation between bulb weight and soluble solid content which may represent an obstacle in the selection for a productive onion with large bulbs and a high soluble solid content, which would guarantee longer storage life.

Table III – Heritability estimates for onion bulb traits in the narrow sense. Piracicaba, 1977.

Trait	h^2 (%)
Weight	39.09
Diameter	24.34
Soluble solid content	40.73

Table IV – Estimates of phenotypic and genetic correlations for onion bulb traits. Piracicaba, 1977.[a]

Traits	Diameter	Soluble Solid Content
Weight	-0.1930	-0.8561
	(-0.9622)	(-0.6983)
Diameter		-0.1032
		(-0.1401)

[a] Genetic correlations are shown in parentheses.

REFERENCES BIBLIOGRÁFICAS

Falconer, D. S. (1964). *Introduction to Quantitative Genetics.* 3. ed. Oliver and Boyed, London.

Jones, H. and Mann, L. K. (1963). *Onions and Their Allies* - Leonard Hill, New York.

Kempthorne, O. (1966). *An Introduction to Genetic Statistic.* John Willey, New York.

McCollum, G.D. (1966). Heritability and genetic correlation of some onion bulb traits – Estimates from S_1 offspring - on - parent regression. *J. Hered.* 57 : 105-110.

McCollum, G.D. (1968). Heritability and genetic correlation of soluble solids, bulb size and shape in white sweet Spanish onions. *Can. J. Genet. Cytol.* 10 : 508 - 514.

McCollum, G. D. (1971). Heritability of onion bulb shape and size estimating from half-sib families. *J. Hered.* 62: 101-104.

Vencosky, R. (1969). Genética Quantitativa. In: *Melhoramento e Genética.* (Kerr, W. E. Org.). Melhoramentos Ed. USP, São Paulo, pp. 17-38.

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ABO BLOOD GROUPS AND SALIVARY SECRETION OF ABH ANTIGENS IN PATIENTS WITH CONGENITAL HEART DEFECTS

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ABSTRACT

ABO blood groups and salivary secretion of ABH antigens were studiea in 170 individuals with congenital heart defects and in their mothers (153 women).The results showed no statistically significant differences when compared to those obtained for control groups, thus showing that the presence of these types of antigens in the embryo has no detectable effect on the triggering of congenital heart malformations. Two aberrant secretors were found among the mothers group. Both are mothers of propositi with Tetralogy of Fallot. Two aberrant secretors were also found among the propositi. The expected frequency of incompatible mother-child pairs for the ABO blood groups did not deviate significantly from the frequency found in our sample, thus showing that this incompatibility is not an important factor in the etiology of congenital heart defects.

INTRODUCTION

The distribution of the ABO blood groups and the secretion of ABH antigens in body fluids vary in different populations and almost always

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represent genetic polymorphism, but their adaptive values are not known. A way to study this adaptability is to try to establish whether determined blood groups predispose an individual to disease or malformations more than others. This concept stems from the fact that blood group substances are already present in the erythrocytes of 6-week-old embryos, and in some tissues of 8-week-old embryos. They will persist throughout life in some tissues, while in others, such as the glandular epithelium of the thyroid, they are formed starting on the eighth week of intrauterine life and disappear four weeks later when the organ begins to show the first signs of morphological and functional maturity (Szulman, 1966). On this basis, these antigens are believed to play a role in the formation of various organs.

Blood group substances are already present in the plasma of 6-week old embryos and play an important role in cases of maternal-fetal incompatibility (Szulman, 1966).

Since the circulatory apparatus begins to be formed during the second week of intrauterine life and is complete by the eighth week of fetal life (Nadas, 1959), it is possible that the presence of blood group antigens in tissues and fluids influences its formation. Accordingly, the objective of this research was to verify whether the frequency of ABO erythrocytic groups and the frequency of secretion of ABH substances in body fluids is different in patients with heart defects when compared to normal individuals.

MATERIAL

The sample of patients with congenital heart defects was selected by the physicians of the Congenital Heart Disease Group, 2nd Clinic of the Hospital das Clínicas, University of São Paulo. The patients numbered 170 (94 males and 76 females), 68 of which were submitted to corrective surgery. The diagnoses of the types of congenital heart defects involved are shown in Table I. The ages of the propositi varied between 3 and 61 years. No patients less than 3 years of age were selected due to the difficulty in obtaining saliva samples from children younger than this age. The patients' age distribution is shown in Table II.

Table I – Number of patients in our study per each type of congenital heart defect diagnosed.

Diagnosis	Cases Nr.	%
Intraventricular communication	42	24.70
Tetralogy of Fallot	32	18.82
Interatrial communication	21	12.35
Persistence of arterial channel	15	8.82
Valvular pulmonary stenosis	13	7.64
Transposition of great vessels	8	4.70
Coarctation of the aorta	7	4.11
Aortic stenosis	6	3.52
Mitral insufficiency	4	2.35
Atrio Ventricularis Communis	4	2.35
Miscellaneous[a]	18	10.58

[a] This group includes several heart defects observed only once in the sample.

Table II – Distribution of patients with congenital heart defects by age groups

Sex	Age group						Total
	3-10	11-20	21-30	31-40	41-50	51 or +	
Male	39	28	9	-	-	-	76
Female	37	28	19	4	4	2	94
Total	76	56	28	4	4	2	170

The racial composition of the sample was as follows: White, 133 (78.23%); Negro, 6 (3.52%); dark-skinned Mulatto, 6 (3.52%); light-skinned Mulatto, 12 (7.05%), and Japanese, 13 (7.64%). Due to the heterogeneity of origin of white São Paulo inhabitants, and also due to the fact that a large number of the white propositi do not live in the city of São Paulo but come from other cities and states with racial compositions differing from those existing in São Paulo, we studied the origin of the grandparents of each white propositus. Of these, 63.25% are Brazilians, consisting of north-easterners (41.55%) and of inhabitants of the State of São Paulo or of southern states; 21.7% are Italian; 10.1% Portuguese, and 5.65% Spanish. For the sake of simplicity, all individuals coming from the State of Minas Gerais or North of this state were considered to be north-easterners. The ages of the patients' mothers (153 women) were between 15 and 46 years at the time of the propositi's birth. Of the mothers, 124 (81.04%) are White, 16 (10.45%) are Mulatto, 4 (2.56%) are Negro, and 9 (5.88%) are Japanese.

METHODS

The ABO tests were carried out with anti-A and anti-B sera (Johnson & Johnson), and with anti-A_1 lectin prepared with Crotalaria seeds by the technique of Palatnik (personal communication). Erythrocyte typing was performed by the method described by Stratton and Renton (1958). Erythrocytes classified as A or AB were tested with anti-A_1 lectin in order to be further classified as A_1 or A_2.

Saliva samples were collected, placed in a double boiler for 15 minutes at 100° C, and later centrifuged at 2000 rpm. The supernatants were stored frozen at - 20° C until testing time. The secretor character was determined by the technique of hemagglutination inhibition, qualitatively. In the case of aberrant secretors, however, quantitative measurements were carried out. Qualitative measurements were done by the " simple dilution " technique described in Dunsford and Bowley (1967, p. 381).

The anti-A and anti-B sera, both 98 titre, were used at 1:16 dilution. We searched the sera for the presence of immune antibodies of the anti-A or anti-B type, since saliva only neutralizes natural antibodies, and immune antibodies, when present, react with standard erythrocytes, thus making it

difficult to interpret the results. This search was carried out by the technique of agglutinin neutralization in saline, followed by an indirect Coombs test and by the thermostability test, both described in Dunsford and Bowley (1967). No immune antibodies occurred, at least at the dilution used. The anti-H serum was prepared with *Ulex europaeus* seeds by the method of Palatnik (personal communication) and used at 1:8 dilution.

The erythrocytes used were always obtained from the same donor; group 0 erythrocytes were obtained from two donors, group A_2 from one donor, and group B from two donors. All erythrocytes were washed 3 times, and 10% suspensions in isotonic saline were prepared.

RESULTS

The results of the salivary secretion tests, the frequency of nonsecretors and of the *se* gene in the propositi and their mothers are shown in Table III. The differences in the totals of mothers and patients are due to problems in obtaining saliva and, in some cases, to loss of saliva. Sex differences in frequency of nonsecretors are not statistically significant ($\chi^2_{(1)}$ = 0.003). Comparison between mothers and propositi also showed no statistically significant differences ($\chi^2_{(1)}$ = 0.460).

Table III - Distribution of salivary secretion and frequency of the *se* gene in patients with congenital heart defects and their mothers, in the city of São Paulo.

Sample	Sex	Number of individuals	Secretors (Nr.)	Nonsecretors (Nr.)	χ^2 (1.g.1.)	Secretors (%)	Nonsecretors (%)	*se* gene frequency
Propositi	M	66	56	10	0.003	84.66	15.34	0.391
	F	84	71	13				
	Total	150	127	23				
Mothers		125	102	23	0.460	81.60	18.40	0.429

The heterogeneity of the sample of patients and mothers (which included several racial groups such as Japanese, Negro and Mulatto) made it difficult to compare them with other samples studied in the Brazilian population. Only Caucasian patients, therefore, were used for comparison with other populations. These comparisons, shown in Table IV, did not reveal any significant differences.

Table IV – Frequency of nonsecretor Caucasian patients with congenital heart defects as compared to the general population of Brazil.

Comparison	Nr. of individuals	Nonsecretors (%)	χ^2 (1.g.1.)	Reference
Caucasian propositi	127	14.17		This paper
X			0.12	
Normal São Paulo caucasians	448	16.51		This paper
Caucasian propositi	127	14.17		This paper
X			0.09	
Normal Porto Alegre Caucasians	267	14.3		Palatnik et al., 1969.
Caucasian propositi's Mothers	107	16.82		This paper
X			0.06	
Normal São Paulo Caucasians	448	16.51		This paper
Caucasian propositi's Mothers	107	16.82		This paper
X			0.40	
Normal Porto Alegre Caucasians	267	14.3		Palatnik et al., 1969.

The distribution of ABO blood groups in patients and mothers is shown in Table V. No statistically significant differences were found between

samples. The genic frequencies of ABO blood groups were calculated according to the formula of Wellisch and Thomsen (cf. Race and Sanger, 1968, p. 16) and are presented in Table VI. These samples were compared with others studied in Brazilian populations, once more excluding all black, Mulatto and Japanese individuals for the reasons already stated. The Caucasian patients and mothers were compared to normal Caucasians from the city of São Paulo and also to a sample of 3697 north-eastern individuals coming for the most part from the States of Bahia and Minas Gerais, and, to a lesser extent, from other states such as Ceará, Sergipe and Alagoas, and studied by Cabello (1972) in São Paulo, Hospedaria dos Imigrantes. Since this particular group includes individuals from several states of north-eastern Brazil, we found it to be adequate for a comparison with our sample, in which about 40% of the Caucasian patients and their mothers come from several north-eastern states. The results of these comparisons are shown in Table VII.

Table V – Distribution of frequency of ABO blood groups in propositi with congenital heart defects and their mothers.

Group	Number of individuals	Blood Groups						χ^2(3.g.1.)
		0	A_1	A_2	B	A_1B	A_2B	
Propositi	170	48.23	28.23	6.47	14.12	2.35	0.59	2.31
Mothers	127	44.88	33.28	5.51	16.53	0.79	–	

Table VI – Gene frequency of A_1, A_2, B, and 0 blood groups in propositi with congenital heart defects and their mothers

Group	Number of individuals	Gene frequency			
		r	p_1	p_2	q
Propositi	170	0.694	0.182	0.090	0.095
Mothers	127	0.669	0.200	0.044	0.144

Table VII – Comparison of ABO blood groups between Caucasian propositi and mothers, and other samples of normal Caucasians.

Comparisons	Number of individuals	Blood groups (%) 0	A	B	AB	χ^2_c	Reference
Caucasian propositi	133	49.62	36.09	11.28	3.00		This paper
X						5.705	
São Paulo City Caucasians	429	43.82	42.18	9.32	3.96		This paper
Caucasian propositi	133	49.62	36.09	11.28	3.00		This paper
X						0.682	
North-easterners	3599	48.87	35.06	12.78	3.27		Cabello (1972)
Caucasian mothers	98	45.36	37.11	16.49	1.03		This paper
X						6.988	
São Paulo City Caucasians	429	43.82	42.18	9.32	3.96		This paper
Mothers of Caucasian patients	98	45.36	37.11	16.49	1.03		This paper
X						2.681	
North-easterners	3599	48.87	35.06	12.78	3.27		Cabello (1972)

$\chi^2_{(3.5\ \%)} = 7.81$

Four aberrant secretors (2 propositi and 2 mothers) were found in our study and classified according to the criterion of McNeil et. al. (1957). both mothers are Caucasian, have children with Tetralogy of Fallot, and belong to the B group; one only secretes the B antigen at the 1:256 level, and the other only secretes the H antigen at the 1:96 level. The latter's son, besides having Tetralogy of Fallot, has a karyotype of the 47, XYY type. His sister, aged 17 years, was born with spina bifida, clubfoot and absence of sphyncter in the bladder. Of the two propositi who are aberrant secretors, one is a White female with mitral insufficiency, while the other is a light-skinned Mulatto male with Atrio Ventricularis Communis. Both belong to the A_2 group and only secrete the H antigen at the 1:64 level.

In an attempt to verify whether maternal-fetal incompatibility in the ABO system is related to a higher frequency of congenital heart defects, the frequency of genetic mother-child incompatibility encountered in these families (classified according to Levine, 1943) was compared to the expected frequency. These calculations, based on the genic frequencies of the ABO blood groups in the mothers and the propositi, according to formulas cited by Li (1955, p. 50), are shown in Table VIII. According to the frequencies of

Table VIII – Expected frequencies of incompatible mother-child pairs in populations in Hardy-Weinberg equilibrium (p = 0.24; q = 0.14, and r = 0.61), with Bernstein's correction, according to Li (1965) (n = 114).

Mother-child pairs	Theoretical frequencies (Li, 1965)	Expected relative frequencies %	Expected frequencies in absolute numbers
0 x A	pr^2	0.092256	10.52
0 x B	qr^2	0.053816	6.14
B x A	pqr	0.020832	2.37
A x B	pqr	0.020832	2.37
B x AB	pq(q+r)	0.025536	2.91
A x AB	pq(p+r)	0.028896	3.29
Total	$r^2(1-r)+pq(1+3r)$	0.242168	27.60

ABO groups encountered in the propositi's mothers, 27.60 incompatible mother-child pairs would be expected in our sample. The actual number of incompatible pairs was 24 out of a total of 114, with no statistical difference ($\chi^2_{(1)} = 0.62$) between expected and actual figures. This shows that maternal-fetal incompatibility in the ABO system is not an important factor in the etiology of congenital heart defects.

DISCUSSION

The control sample of Caucasian individuals consists of students of the Faculties of Medicine and Nursing, University of São Paulo. No significant differences were found between patients and controls, although socioeconomic and ethnical differences exist amongst individuals of both groups. Actually, more than 40 % of the Caucasian propositi and mothers are from states in the Northeast of Brazil. Saldanha (1962 a, 1962 b) assumes that the composition of north-eastern populations consists of miscegenètion of Indians, Whites of Portuguese origin, and African Negroes, each group contributing with 18 %, 48 % and 34 % admixture, respectively. In an extensive study of north-eastern populations initiated by Morton (1964) in the Hospedaria dos Imigrantes, Krieger et al. (1965), using the maximum likelihood method, calculated the racial components of north-eastern populations as being 40.1 % Negro, 11.3 % mixed Indian, and 58.6 % mixed White. Thus, some individuals classified as White are actually mixtures of these 3 groups.

A larger amount of group 0 was found in the group of Caucasian propositi and mothers, probaly due to indigenous influence, since non miscegenated individuals only exhibit this group (Salzano, 1965); a lower amount of group A and increased quantities of group B were also found, probably due to Negro influence (Faria and Ottensooser, 1951). Despite these differences, comparison between the two Caucasian groups (patients and mothers x normal caucasoids from São Paulo) in terms of ABO groups and salivary secretion of ABH substances revealed no statistically significant differences at the 5 % level of probability (cf. Tables IV and VII). The same

is true for the comparisons with north-eastern samples (cf. Table VII), so that it may be concluded that the ABO groups and the presence of ABH substances in body fluids have no detectable effect on the appearance of congenital heart defects. These results confirm the data of Sartor (1964) and of Gershowitz and Neel (1965), but are in disagreement with those of Lev et al. (1967), who found excess group B in Negro children with congenital heart defects.

REFERENCES

Cabello, P.H.A. (1972). Frequência génica e coeficiente de endocruzamento em uma população rural brasileira. Masters thesis. Instituto de Biociências, University of São Paulo.

Dunsford, I., and Bowley, C.C. (1967). *Tecniques in Blood Grouping.* 2nd edition. Charles C. Thomas, Springfield.

Faria, R., and Ottensooser, F. (1951). Grupos ABO e tipos Rh em pretos e mulatos de São Paulo. *Arquivos de Biologia 35*: 68-73.

Gershowitz, H., and Neel, J.V. (1965). The blood groups and secretor types in five potentially fatal diseases of Caucasian children. *Acta Genet. 15*: 261-308.

Krieger, H., Morton, N.E., Mi., M.P., Azevedo, E., Freire-Maia, A., and Yassuda, N. (1965). Racial admixture in Northeastern Brazil. *Ann. Hum. Genet. 29*: 113-125.

Lev, M., Okada, R., Kerstein, M.D., Paiva, R., and Rimoldi, H.J.A. (1967). Blood groups and congenital heart disease. *Dis. Chest 52*: 616-620.

Levine, P. (1943). Serological factors as possible cause in spontaneous abortions. *J. Hered. 34*: 71-80.

Li, C.C. (1955). *Population Genetics*. University of Chicago Press, Chicago.

McNeil, C., Trentelman, E.F., Kreutzer, V.O., and Fullmer, C.D. (1957). Aberrant secretion of salivary A, B and H group substances in human beings. *Am. J. Clin. Pathol, 28*: 145-151.

Morton, N.E. (1964). Genetics studies of North-Eastern Brazil. *Cold Spring Harbor Symp. Quant. Biol. 29*: 69-79.

Nadas, A.S. (1959). *Cardiologia Pediátrica.* Libreria y Editorial Bernardes, S. R.L., Buenos Aires.

Palatnik, M., Sá e Benevides, M.J.F., and Salzano, F.M. (1969). ABH salivary secretion and White/Negro gene flow in a Brazilian population. *Hum. Biol. 41*: 83-96.

Race, R.R., and Sanger, R. (1968). *Blood Groups in Man.* Blackwell Scientific Publications, 5th Ed., Oxford.

Saldanha, P.H. (1962 a). Os componentes raciais das populações nordestinas. *Ciência e Cultura 14*: 115-117.

Saldanha, P.H. (1962 b). Race mixture among Northeastern Brazilian populations. *American Anthropologist 64*: 751-759.

Salzano, F.M. (1965). *Genética de populações humanas brasileiras. Homenagem a Juan Comas,* Vol. II, Mexico.

Sartor, V. and Fraser, R.S. (1964). ABO blood groups in patients with congenital and rheumatic valvular heart disease. *Can Med. Assoc. J. 90*: 428-429.

Stratton, F.W., and Renton, P.H. (1958). *Practical Blood Grouping.* Charles C. Thomas, Springfield.

Szulman, A.E. (1966). Chemistry, distribution and function of blood group substance. *Annu. Rev. Med. 17*: 307-322.

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DETECTION OF HAEMOGLOBIN M BOSTON IN A PATIENT WITH SEVERE HAEMOLYTIC ANAEMIA*

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ABSTRACT

An abnormal haemoglobin was detected in a 55-year-old Caucasian woman of Italian origin. The haemoglobin variant resulted from an amino acid Substitution of $\alpha^{His^{58}\text{-}Tyr}$, similar to haemoglobin M. Boston. Severe haemolytic anemia and Heinz bodies in the erythrocytes were observed. This is the first case of such a variant to be reported in Brazil.

INTRODUCTION

Haemoglobins M are a heterogeneous group of disorders characterized by chronic cyanosis due to the occurrence of methaemoglobinaemia. These

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abnormal haemoglobins are transmitted as a co-dominant character and must be distinguished from the congenital methaemoglobinaemia caused by the deficiency of erythrocyte diaphorase (Scott, 1960; Lehmann and Hunstman, 1974).

Five different molecular species of haemoglobin M have been chemically characterized. Four of them, M Boston (Gerald and Efron, 1961), M Saskatoon (Gerald and Efron, 1961), M Iwate (Miyaji *et al.*, 1963) and M Hyde Park (Heller *et al.*, 1966) involve the substitutions of a tyrosyl residue for the proximal or distal histidyl residue of either the alpha or beta chain. The fifth, M Milwaukee (Gerald and Efron, 1961) is due to the substitution of the neutral residue valyl, located near the haeme group, by the negatively charged glutamyl residue.

This paper reports the structural identification of a haemoglobin M foud in association with severe anaemia in a Caucasian woman of Italian ethnic backgroud.

MATERIAL AND METHODS

Blood was collected into 5% sodium EDTA. Haematological studies were carried out by standard methods (Dacie and Lewis, 1968). A haemoglobin solution, prepared from washed red cells by lysis with distilled water and carbon tetrachloride, was submitted to electrophoresis on cellulose acetate using Tris- EDTA-boric acid buffer, pH 8.9 (Marengo-Rowe, 1965) and the relative proportions of the haemoglobin components were determined by spectrophotometry after elution. Fetal haemoglobin was measured by the alkali denaturation method of Betke *et al.* (1966). The haemoglobin instability test was performed by incubating a haemoglobin solution at 37° C in 0.05 M isopropanol/Tris-HCL buffer, pH 7.4 (Carrell and Kay, 1972). Haemoglobin, 0.2 to 0.3 mg/ml in 0.1 M sodium phosphate buffer, pH 6.5, was used for the determination of methaemoglobin. The ratios of the optical densities,500:600 nm and 630:600 nm, were compared to those of a solution containing 100%

oxyhaemoglobin and 0% methaemoglobin (Evelyn and Malloy, 1938). Globin was precipitated from the whole haemolysate by acid acetone treatment (Ingram and Stretton, 1962), and analysed by electrophoresis on cellulose acetate with urea-barbital buffer, pH 8.0 (Traverse *et al.*, 1969). For structural studies, the haemoglobin variant was isolated by paper electrophoresis (Cradock-Watson *et al.*, 1959) and the globin prepared as described. An aqueous solution of the globin (10 mg/ml) was adjusted to pH 8.4 with 0.5 M NH_4HCO_3, and 0.1 ml trypsin solution (2 mg/ml) was added (globin:enzyme ratio 50:1). After incubation at 38° C for 2 hours the pH was lowered to 6.4 by addition of 0.5 M acetic acid. Insoluble peptides were precipitated by heating the digestion mixture at 90° C for 5 minutes and separated by centrifugation. The supernatant containing soluble peptides was lyophilized. Two-dimensional peptide maps (fingerprints) were prepared using paper electrophoresis at pH 6.4, at 55 Volts per cm, for 18 hours (Baglioni and Ingram, 1961). The peptides were stained with 0.2% (w/v) ninhydrin in acetone and also submitted to the usual colour tests for tyrosine, histidine, arginine and methionine (Baglioni, 1962).

For the amino acid composition studies, the abnormal peptide was eluted and hydrolysed with constant boiling 6 N HCL in an evacuated tube at 108° C for 18 hours. The amino acid composition was determined with a Locarte amino acid analyser using the two-column system of Spackman *et al.* (1958).

CASE REPORT

The proposita, F.F.S., a 55-year-old Caucasian woman from Botucatu, SP, Brazil, was admitted to the hospital for haematological studies in August, 1975, complaining of weakness, palpitation and dyspnoea. She had been splenectomized 16 months earlier due to severe haemolytic anaemia. Physical examination revealed slight cyanosis, especially of the lips and nailbeds.

Table I - Haematological data of the patient

	Patient	Normal Values (women)
Haemoglobin g/dl	8.8	11.5 - 15.0
Red Blood Count $\times 10^{12}/1$	2.7	4.0 - 5.0
Haematocrit	0.25	0.38 - 0.47
Reticulocytes %	16.0	0.2 - 2.0
Mean Corpuscular Volume fl	92.0	80.0 - 92.0
Mean Corpuscular Haemoglobin pg	32.0	27.0 - 33.0
White Blood Count $\times 10^{9}/1$	8.2	4.0 - 10.0
Platelets $\times 10^{9}/1$	155.0	150.0 - 400.0
Anisocytosis	+ + +	0
Poikilocytosis	+ + +	0
Ortho and Polychromatic Erythroblasts %	15.0	0

The results of the haematological tests are given in Table I. The presence of an abnormal haemoglobin component, with mobility between haemoglobins F and A, was detected on electrophoresis of the patient's haemoglobin, performed because of evidence of haemolysis and the presence of Heinz bodies in the erythrocytes.The gray appearance of this band and the presence of two other minor components (Figs. 1 and 2) led us to suspect a methaemoglobinaemia resulting from a defect in the alpha chain. The relative proportions of the haemoglobins are reported in Table II. The isopropanol instability test was weakly positive. Methaemoglobin concentrations of 2 and 12% were obtained on the basis of the ratios of the absorbance at 500:600 nm and 630:600 nm, respectively.

Electrophoretic separation of polypeptide chains at pH 8.0 on cellulose acetate demonstrated that the abnormal slow moving haemoglobin had an abnormal alpha chain (Fig. 3).

Table II - Relative proportions of the haemoglobin components of the patient and normal values from our laboratory (Naoum *et al.,* 1979)

	Patient	Normal Values
Hb A %	85.0	90.04 - 96.28
Hb A_2 plus M_2 %	4.0	1.96 - 3.72
Hb M %	11.0	
Hb F %	0.2	0.00 - 0.44

Table III - Amino acid composition of α Tp VII (α57-60)

Amino acid	Patient	(moles/mole peptide)	Normal
Gly	2.1		2
Tyr	0.9		0
His	0.0		1
Lys	1.0		1

Table IV - Amino acid sequence of the tryptic peptide VII from normal haemoglobin (Hb A) and haemoglobin M Boston

Residue number	57	58	59	60
Helical number	E6	E7	E8	E9
Hb A	Gly	His ↓	Gly	Lys
Hb M Boston	Gly	Tyr	Gly	Lys

The fingerprint of the trypsin digest of the abnormal haemoglobin showed that αTp VII from normal haemoglobin, or Hb A, which consists of the residues 57-60 of the alpha chain (*a* in Fig. 4), was replaced by a new, more negatively charged peptide (*b* in Fig. 4), which showed a positive reaction only with tyrosine stain and was negative for histidine. The amino acid composition of the new peptide (Table III) differed from αTp VII by the presence of one residue of tyrosine and by the absence of histidine. Since αTp VII from Hb A contains histidine at position 58 (Table IV) we conclude that the mutation in this abnormal haemoglobin is $\alpha^{\text{His}^{58}\text{-Tyr}}$, which is the same as described for Hb M Boston.

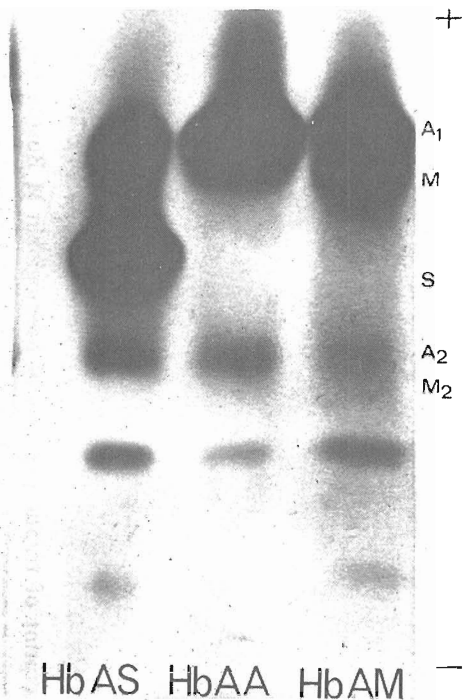

Figure 1 - Electrophoresis on cellulose acetate, pH 8.6, of haemoglobins AA, AS and AM_{Boston}. The high concentration of the haemolysate (about 12 g per cent) permits detection of two minor components of haemoglobins (A_2 and M_2) in the haemolysate from the proposita.

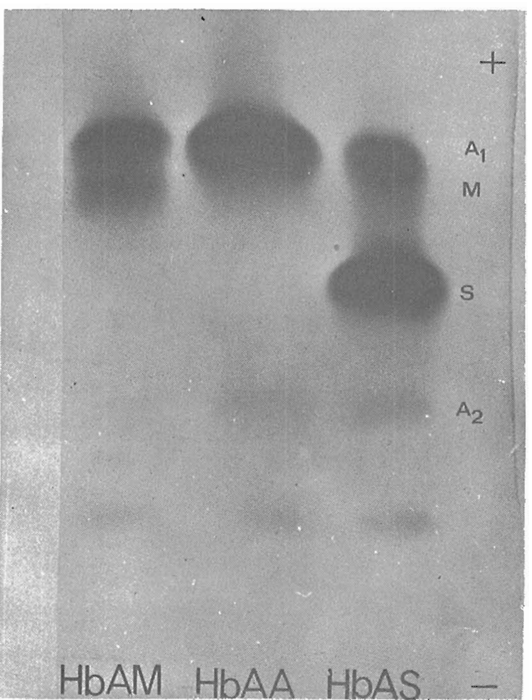

Figure 2 - Electrophoresis on cellulose acetate, pH 8.6, of haemoglobins AA, AS and AM_{Boston}. The low concentration of the haemolysates (about 5 g per cent) permits the efficient separation of the haemoglobins A and M Boston. However, haemoglobins A_2 and M_2 are not visible in the haemolysate from the proposita.

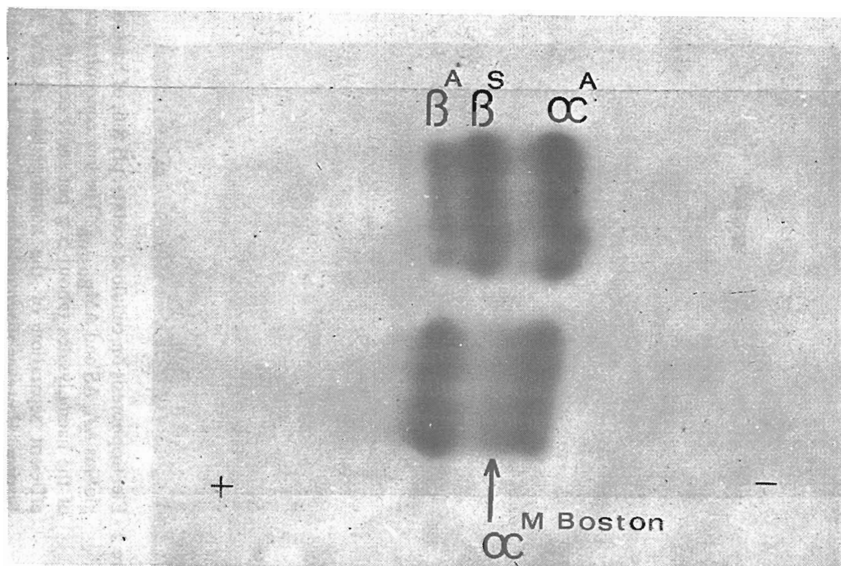

Figure 3 - Electrophoresis on cellulose acetate, pH 8.0, of whole globins AS and AM_{Boston}. The abnormal alpha chain from haemoglobin M Boston is between the normal alpha and beta chains.

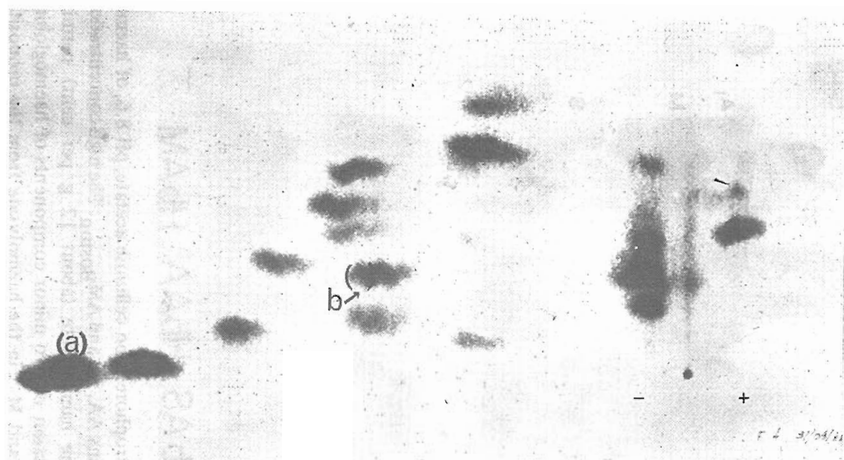

Figure 4 - Fingerprint of tryptic peptides of haemoglobin M Boston (alpha and beta chains).(a) Area where α Tp VII from normal haemoglobin is missing.(b) A new tyrosine-containing peptide α Tp VII from haemoglobin M Boston.

DISCUSSION

Variants of haemoglobin M have been observed in virtually every part of the world and are recognised as perfect examples of molecular abnormalities (Lehmann and Hunstman, 1974).

It is interesting to note that the molecular basis of a rare genetic disorder like Hb M is not the same in every case . In this disorder, an internal complex is formed between the haeme iron and a reactive side chain of an amino acid. This interaction is not possible in normal haemoglobin. An amino acid substitution in close proximity to the iron atom, especially the replacement of histidine by tyrosine with its phenolic group, apparently provides a favorable situation for its formation. This complex is also an unsuitable substrate for the normal erythrocyte enzyme concerned with the reduction of ferrihaemoglobin. The heterogeneity of haemoglobin M indicates that several point mutations along the alpha or beta chains can facilitate the formation of such an internal complex, resulting in a poorly reducible ferrihaemoglobin (Heller *et al.*, 1962; Perutz, 1976).

The occurrence of the severe haemolytic anaemia observed in this case, indicated by the high reticulocyte count, the morphological alterations of the erythrocytes, the presence of Heinz bodies, and the slight instability of the abnormal haemoglobin when submitted to incubation at 37° C, has been observed in other patients with a similar disorder (Pisciotta *et al.*, 1959; Gerald and Efron, 1961). The discovery of haemoglobin M in a Caucasian woman of Italian origin, which has been characterized on the basis of structural studies as a Hb M Boston, represents the first example of such variant found in Brazil.

REFERENCES

Baglioni, C. and Ingram, V.(1961). Abnormal human haemoglobin. V. Chemical investigation of Hb A, G, C, X from one individual. *Biochim. Biophys. Acta 48* : 253 -265.

Baglioni, C. (1962). Abnormal human haemoglobin. VI. Chemical studies of haemoglobin D. *Biochim. Biophys. Acta 49* : 437- 449.

Betke, K., Marti, H.R., and Schicht, I. (1966). Estimation of small percentages of foetal haemoglobin. *Nature 212* : 262-264.

Carrell, R.W. and Kay, R. (1972). A simple method for the detection of unstable haemoglobin. *Br. J. Haematol. 23* : 615-619.

Cradock-Watson, J.E., Fenton, J.C.B. and Lehmann, H. (1959). Tris buffer for the demonstration of Hb A_2 by paper electrophoresis. *J. Clin. Pathol. 12* : 372-375.

Dacie, J. V. and Lewis, S. (1968). *Practical Haematology*. 4th. ed., Churchill, London, 486p.

Evelyn, K. A. and Malloy, H. T. (1938). Microdetermination of oxyhaemoglobin, methemoglobin and sulphemoglobin in single sample blood. *J. Biol. Chem. 126* : 655 - 670.

Gerald, P.S. and Efron, M.L. (1961). Chemical studies of several varieties of Hb M. *Proc. Natl. Acad. Sci.* (Wash.), *47* : 1758-1767.

Heller, P., Coleman, R.D. and Yakulis, V. (1966). Hemoglobin M Hyde Park: a new variant of abnormal methemoglobin. *J.Clin. Invest. 45* : 1021 - 1027.

Heller, P., Hyman, G. W., Yakulis, V.J. and Rosenthal, I. M. (1962). Hemoglobin M Kankakee, a new variant of hemoglobin M. *Blood* 20 : 287-301.

Ingram, V. M. and Stretton, A. O. W. (1962). Human Haemoglobin A_2. II. The chemistry of some peptides peculiar to Hb A_2. *Biochim. Biophys. Acta 63* : 20-23.

Lehmann, H. and Hunstman, R.G. (1974). *Man's haemoglobins.* 2nd. ed., Amsterdam-Oxford. North Holland. 478p.

Marengo-Rowe, A. J. (1965). Rapid electrophoresis and quantitation of haemoglobin on cellulose acetate. *J. Clin. Pathol. 18* : 790 - 792.

Miyaji, T., Takeda, I. and Shibata, S. (1963). The new abnormal hemoglobin, Hb Tokuchi discovered in Hofu city. *Acta Haematol. Japn. 26* : 45-50.

Naoum, P. C., Machado, P. E. A., Michelim, O. C., Cury, P. R. and Pio da Silva, M. (1979). Concentration of haemoglobin A_2 and Fetal in Brazilian Indians: relationship between these haemoglobins and malaria. *Ciência e Cultura 31* : 188-190.

Perutz, M. F. (1976). Structure and mechanism of haemoglobin. *Br. Med. Bull. 32* : 195-208.

Pisciotta, A. V., Ebbe, S. N. and Hinz, J. E. (1959) . Clinical and laboratory features of two variants of methemoglobin M disease. *J. Lab. Clin. Med. 54* : 73-78.

Scott, E. M. (1960). The relation of diaphorase of human erythrocytes to inheritance of methemoglobinemia. *J. Clin. Invest. 39* : 1176-1180.

Spackman, D. H., Stein, W. H., and Moore, S. (1958). Automatic recording for use in the chromatography of amino acids. *Anal. Chem. 30* : 1190-1206.

Traverse, P. M., Coquelet, M. L. and Pechery, Cl. (1969). *Détection des anomalies de 1' hémoglobine en pratique courante.* CDU Place de la Sorbonne, Paris.

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SHORT COMMUNICATION

HEMOGLOBIN VARIANTS IN BRAZILIAN ZEBU CATTLE*

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Since Cabannes and Serain (1955) originally determined three haemoglobin phenotypes in cattle, attributing them to two codominant alleles (Hb^A and Hb^B), many papers have been published on this topic and at least 7 alleles have been identified at the Hb locus (Crockett et al., 1963; Carr, 1964; Naik et al., 1969; Schwellnus and Guérin, 1977, and others). Silva (1973), Suzuki and Amano (1973), and Schneider and Schneider (1978) found only Hb^A and Hb^B in Brazilian Zebu cattle.

Usings starch gel electrophoresis (12% potato starch in 100 ml Tris-citrate buffer, as described by Silva, 1973) we determined 15 haemoglobin phenotypes for Nelore cattle, attributed to 5 possible alleles: Hb^A, Hb^I, Hb^C, Hb^{B_1}, and Hb^{B_2}. Hb^A, corresponding to the slowest band, was the same as described by the authors mentioned above. Hb^C corresponded to that described by Naik et al. (1969), Osterhof and VanHeerden (1965), Braend (1971) and Schwellnus and Guérin (1977). Hb^I was discovered by Schwellnus and Guérin (1977), who advanced the hypothesis that this haemoglobin may be a variant specific to native African cattle. Its mobility is intermediate between that of Hb^A and Hb^C. This hypothesis was invalidated by the fact that we found Hb^I in Brazilian Nelore cattle, which has no known African source of genes.

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However, since Hb^{I} was found in one herd only and with very low frequency, it could possibly be a mutant.

The two fastest Hb bands, which we called Hb^{B_1} and Hb^{B_2}, had not been previously reported. They may represent a decomposition of Hb^{B}. A two-dimensional electrophoretic run confirmed them as two different bands, although very close.

At the moment, we have typed 570 blood samples and found the following gene frequencies: Hb^{A} 0.540 ± 0.022, Hb^{B_1} 0.193 ± 0.013, Hb^{B_2} 0.233 ± 0.014, Hb^{C} 0.020 ± 0.004, Hb^{I} 0.013 ± 0.004.

The sampling is being continued in several Nelore herds, and we expect to obtain at least 2000 samples in the near future.

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REFERENCES

Braend, M.(1971). Haemoglobin variants in cattle. *Anim.Blood Groups Biochem. Genet.* 2 : 15-21.

Cabannes, R. and Serain, C. (1955). Hétérogénéité de l'hémoglobine des bovidés. Identification électrophoretique de deux hémoglobines bovines. *C.R. Soc. Biol. (Paris)* 149 : 7 -10.

Carr, W.R. (1964). The haemoglobins of indigenous breeds of cattle in Central Africa. *Rhod. J. Agric.Res.* 2 : 93-94a.

Crockett, J.r., Koger, M. , and Chapman Jr., H.L. (1963). Genetic variations in haemoglobins of beef cattle. *J. Anim.Sci.* 22 : 173-176.

Naik, S.N., Sukumaran, P.K., and Sanghvi, L.D. (1969). Haemoglobin polymorphism in Indian Zebu cattle. *Heredity* 24 : 239-247.

Osterhoff, D.R. and VanHeerden, J. A.H. (1965). Haemoglobin polymorphism in cattle. *Proc. South African Soc. Anim. Prod.* 4: 274-277.

Schneider, M.P.C. and Schneider, H. (1978). *Anais da 30a. Reunião Anual da Sociedade Brasileira para o Progresso da Ciência, pg.540.*

Schwellnus, M. and Guérin, G. (1977). Difference between the HbC variants in Brahman and in indigenous Southern African cattle breeds. *Anim. Blood Groups Biochem.Genet.* 8 : 161-169.

Silva, R.G. (1973). Estudos básicos para seleção de gado de corte para regiões tropicais visando ganho de pêso e resistência ao calor. *Dissertação de Mestrado, Universidade de São Paulo, Campus de Ribeirão Preto.*

Suzuki, S. and Amano, T. (1973). Serological observations on Brazilian Zebu cattle. *Anim.Blood Groups Biochem. Genet.* 4 : 233-235.

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