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THE GENETICS OF *Acremonium chrysogenum*

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ABSTRACT

Protoplast fusion techniques have been used to hybridise closely related and divergent strains of *Acremonium chrysogenum*. No clear evidence for diploid formation was obtained and heterozygotes were rarely detected. Recombinants, presumed haploid, were recovered by plating the protoplast fusion mixture on to a variety of selective regeneration media. This enabled attempts at genetic analysis and the isolation of a recombinant which compared with the parent having the highest titre was improved in cephalosporin C titre, growth rate and sporulation.

INTRODUCTION

The strains of *Acremonium chrysogenum (Cephalosporium acremonium)* used in the industrial production of cephalosporin C are derived from the Brotzu strain. In the absence of any detailed reports of genetic analysis in this species, the aim of this work was to investigate the possibility of attempting such analysis. We have used, not only the conventional methods of crossing (Nüesch et al, 1973), but also the new technique of fungal protoplast fusion (Anné and Peberdy, 1976). The latter method enables parental nuclei to be brought into close proximity. In *A. chrysogenum* this is a particu-

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lar advantage because hyphal cells are mainly uninucleate and juxtapositioning of nuclei in such cells infrequent (Nüesch et al, 1973).

MATERIALS AND METHODS

Media

The complete medium (hereafter designated CM) contained per litre: Maltose (BDH) 40g, peptone (Oxoid) 10g, malt extract (Oxoid) 24g, Oxoid Nº 3 agar 20g. The pH was adjusted to 7.5 with sodium hydroxide before autoclaving at 121°C for 20 minutes. The minimal medium (hereafter designated MM) contained per litre: Sucrose 30g, sodium nitrate 2g, potassium dihydrogen phosphate 1g, magnesium sulphate septahydrate 0,5g, potassium chloride 0.5g, ferrous sulphate septahydrate 0.01g, Oxoid Nº 3 agar 20 g. The pH was adjusted to 6.8 with potassium hydroxide before autoclaving as above.

All media on to which protoplasts were plated for regeneration were osmotically stabilised with a solution containing 0.8M sodium chloride plus 2% sucrose (Fawcett et al, 1973).

Strains and Type of Cross

Three groups of genealogically related strains were used. These were designated A, B and C. Group C consisted of strains derived from M8650, a low-titre cephalosporin C producer closely related to the Brotzu strain. The other two groups were derived from Group C by many mutation and selection steps.

The strains within the groups differed by only a few mutation steps, mainly those involved in introducing auxotrophic and morphological markers, using ultra-violet light to give 1% spore/cell survival. The following markers are referred to, the group of origin being noted in brackets:

Requirement for arginine, *arg*-1 (C) *arg*-2 (C) *arg*-3 (B)
Requirement for proline, *pro*-1 (A)
Requirement for methionine, *met*-1 (C) *met*-2 (C) *met*-3 (A)
Requirement for aneurin, *ane*-1 (C) *ane*-2 (A) *ane*-3 (B)

Requirement for nicotinamide, *nic*-1 (A)
Requirement for leucine, *leu*-1 (C)
Requirement for thiosulphate, *thi*-1 (B)
Red mycelial pigment, *red*-1 (C) *red*-2 (A) *red*-3 (A)

Some markers, e.g. *arg*-1, were leaky and others, e.g. *arg*-2, were not leaky. Phenotypes were indicated by capital first letter, e.g. Arg for arginine--requiring phenotypes and Arg^+ for arginine-independent phenotypes.

Three general types of cross were carried out. There were sister--strain crosses of type A/A and C/C, divergent-strain crosses of type A/B and ancestral crosses of types A/C.

Crossing Techniques

All incubations were carried out at 25.5°C. The first method of crossing was the conventional one of Nüesch et al. (1973) and prototrophs were selected on MM. The second method of crossing using protoplast fusion also involved selecting prototrophs on MM.

Protoplasts were prepared according to the method of Fawcett et al. (1973) using L1 enzyme to remove the cell wall. Protoplasts were fused using the method of Anné and Peberdy (1976) and employing polyethylene glycol (PEG) as the fusogen. For both the conventional and protoplast fusion method of crossing the parent strains each carried et least one marker that was non-leaky on MM and did not back-mutate spontaneously at high frequency. Prototrophs recovered from crosses were classified by plating spores, cells and hyphal fragments on to CM an MM at different plating densities. Good agreement between the CM and MM plates would indicate that the strain was a stable prototroph. Poor agreement would indicate that the strain was an unstable prototroph. Certain types of unstable prototroph were found to be heterokaryons, others were classified as heterozygous for markers that segregated on CM.

Also various prototrophs so defined were tested to see if they segregated auxotrophs following treatment with the recombinogens parafluorophenylalanine (PFA) as described by Nüesch et al. (1973) or gamma-rays (γ) as described by Käfer (1963).

Cephalosporin C Titre Testing

A method based on that described by Nüesch et. al (1973) was used.

Electron Microscopy

Protoplasts were fixed in 4% glutaraldehyde in 0.8M mannitol for 1½ hours at room temperature and post fixed in 2% aqueous osmium tetroxide for 1 hour. After washing the fixed cells were dehydrated and embedded in Spurr's resin. Thin sections were stained with uranyl acetate and lead citrate and examined in the electron microscope at 80 Kv.

RESULTS

Comparison of Conventional and Protoplast Fusion Crosses

More than 40 different conventional crosses were carried out. These included 8 sister strains, 26 divergent strains and 6 ancestral crosses. Stable prototrophs, that did not segregate after treatment with PFA and γ, were recovered in 10 of these crosses. Unstable prototrophs, other than heterokaryons, were also detected but only in 2 of these crosses namely *arg*-1 *leu*-1/*arg*-2 *met*-1, a sister strains cross of type C/C and *arg*-2/*pro*-1 *red*-2, an ancestral cross of type C/A. The unstable prototrophs recovered from the first cross spontaneously produced arginine-requiring segregants and the unstable prototrophs from the second cross segregated arginine-requiring and arginine-independent segregants as well as segregating for the *red*-2 morphological marker. (Phenotype frequencies were Red^+ Arg 25; Red^+ Arg^+8; Red Arg 22; Red Arg^+1). None of these heterozygotes showed any change in the type of segregant it produced when treated with the recombinogens detailed previously.

The results from conventional breeding suggested that fusion between nuclei of different strains does not readily occur.

Protoplast fusion techniques were then adopted hoping to improve the chances of such fusion. Electron microscopy indicated that immediately after protoplast fusion up to 1% of the treated protoplasts exhibit nuclear fusion (Plate I). Nuclear fusion was not observed in protoplasts that had not been fused.

Table I shows the results from eight protoplast fusion crosses. In contrast with the results obtained by conventional crossing, stable prototrophs were always recovered together with certain types of recombinant

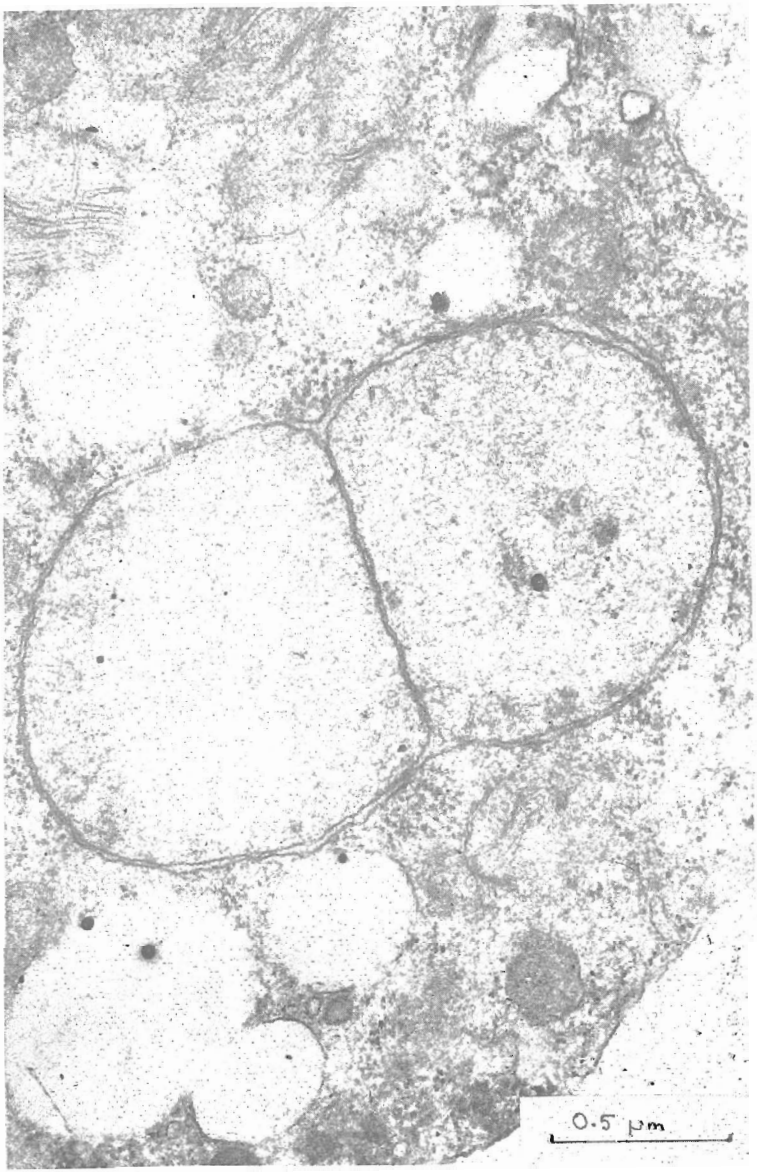

Plate 1. Electron Micrograph Illustrating Putative Nuclear Fusion following Protoplast Fusion. Following fusion, a small proportion of the protoplasts (< 1%) contained nuclei apparently fusing whereas no nuclear fusion was observed in unfused protoplast preparations.

Table I - Detection[a] of Prototrophs and Recombinant Auxotrophs Following Protoplast Fusion

Type of Cross	Heterozygotes	Stable Prototrophs	Recombinant Auxotrophs[b]
Sister Strains			
1. *arg*-1 *leu*-1 *red*-1/*arg*-2 *met*-1 (C/C)	+	+	+
2. Repeat of 1	+	+	+
3. *arg*-1 *leu*-1 *red*-1/*ane*-1 *met*-2 (C/C)	-	+	+
4. *ane*-2 *nic*-1 *red*-3/*met*-3 (A/A)	-	+	+
Divergent Strains			
1. *ane*-2 *nic*-1 *red*-3/*ane*-3 *arg*-3 (A/B)	-	+	+
2. Repeat of 1	+	+	+
3. *ane*-2 *nic*-1 *red*-3/*arg*-3 (A/B)	-	+	+
4. *ane*-2 *nic*-1 *red*-3/*thi*-1 (A/B)	-	+	+

a. + indicates detected, - indicates not detected.
The frequency of prototrophs was between 10^{-4} and 10^{-6} protoplasts in the fusion mixture.

b. Recombinant auxotrophs were detected on CM after platings from either heterozygotes or heterokaryons.

auxotrophs. Other recombinant auxotrophs were produced as spontaneous segregants from rare heterozygotes.

In the sister strain cross the heterozygotes recovered were unstable and identical to those discovered by conventional crossing. In the divergent line crosses the single heterozygote detected in cross *ane*-2; *nic*-1; *red*-3/*ane*-3; *arg*-3 was unstable and spontaneously segregated all the markers of the cross except *arg*-3 (The phenotypes were:

Red^+ Ane Nic-4; Red^+ Ane^+ Nic-6; Red^+ Ane Nic^+5; Red Ane Nic^+1; Red^+ Ane^+ Nic^+9; Red Ane Nic 8; Red Ane^+ Nic^+1; Red Ane^+ Nic 3)

There was no change in the types of segregant produced when the heterozygote was treated with recombinogens.

The stable prototrophs shown in Table I did not segregate after treatment with recombinogens. Also, no individual recombinants with auxotrophic requirements from each parent were recovered by any route but the consistent recovery of certain types of recombinant led us to use protoplast fusion in detailed genetic analysis and empirical breeding both of which we shall describe.

Genetic Analysis

The detection of recombinant auxotrophs and stable prototrophs from crosses in which heterozygotes were not recovered suggested that the diploid condition in *A. chrysogenum* is transient. Therefore, we plated out fused protoplasts from the sister cross *arg*-1; *leu*-1; *red*-1/*arg*-2; *met*-1 on to a range of selective media on which the parent strains could not grow. Random samples of colonies growing on such media were then purified on CM and classified. In many cases the original colonies were confirmed as pure auxotrophs. The total classification results are shown in Table II. The main features to be noted are the recovery of all the parental alleles and most of the possible recombinant phenotypes including the double auxotrophic recombinant phenotype Leu Met.

The bottom of Table II shows the ratios of the various marker phenotypes. Column 1 shows a bias in favour of Arg^+ as prototrophs were favoured by MM. In column 2 the 3:1 ratio of Arg: Arg^+ is as expected if the alleles *arg*-1 and *arg*-2 are freely recombining.

In other columns the ratio of Arg: Arg^+ is not as biased in favour

Table II - Classification[a] of Purified Colonies from Selective Regeneration Medium Following the Protoplast Fusion Cross *arg*-1 *leu*-1 *red*-1/*arg*-2 *met*-1

Strain Phenotypes	Selective Regeneration Medium					
	MM	MM+ Arginine	MM+ Methionine	MM+ Leucine	MM+ Methionine + Leucine	Total
Red Met^+ Leu Arg	1	1	1	8	7	18
Red^+ Met Leu^+ Arg		11	8		13	32
Red Met Leu Arg^+					2	2
Red^+ Met Leu Arg^+					3	3
Red Met Leu^+ Arg					2	2
Red^+ Met^+ Leu Arg				1	4	5
Red Met^+ Leu^+ Arg	3	4	2	1		10
Red^+ Met^+ Leu^+ Arg	1	7	13	2	3	26
Red Met^+ Leu Arg^+				5	3	8
Red^+ Met^+ Leu Arg^+				3	11	14
Red Met Leu^+ Arg^+		1			1	2
Red^+ Met Leu^+ Arg^+		1	1	1	4	7
Red Met^+ Leu^+ Arg+	3	1	1	1	2	8
Red^+ Met^+ Leu^+ Arg^+	18	6	12	8	15	59
Red Met Leu Arg						
Red^+ Met Leu Arg				1	1	2
Ratios						
Arg^+:Arg	21:5	9:23	14:24	18:13	41:30	
Met^+:Met	26:0	19:13	19:9	29:2	45:26	
Leu^+: Leu	25:1	31:1	37:1	13:18	40:31	
Red^+:Red	19:7	25:7	34:4	16: 15	54:17	

a. Colonies from each regeneration medium were purified on CM, and then classified, the phenotypes being shown in this table.

Phenotypes involving Arg. appear in all columns due to breakdown of heterozygotes (See Table III). Other anomalous phenotypes seen in various columns arose due to unavoidable transfer of mixed colonies (e.g. heterokaryons) from the regeneration media to CM purification medium.

of Arg^+ as in column 1 probably because *arg*-2 heterozygotes with extra requirements were allowed to grow on various selective media. Such heterozygotes break down on CM to give Arg phenotypes as shown in Table III.

In column 4 the ratio of Leu^+: Leu is as expected and there is no

Table III - Heterozygotes[a] Recovered on Selective Regeneration Medium[b] from Protoplast Fusion Cross *arg*-1 *leu*-1 *red*-1/*arg*-2 *met*-1

Phenotypes		Possible Aneuploid Genotype of Centre			
		Linkage Groups			
Centre	Sector	I	II	III	IV
$Red^+ Met^+ Leu^+ Arg^+$	$Red^+ Met^+ Leu^+$ Arg	+	+	+	*arg*-2 +
Red $Met^+ Leu^+ Arg^+$	Red $Met^+ Leu^+$ Arg	+	+	*red*-1	*arg*-2 +
Red^+ Met $Leu^+ Arg^+$	Red^+ Met Leu^+ Arg	+	*met*-1	+	*arg*-2 +
Red Met Leu^+ *Arg*$^+$	Red Met Leu^+ Arg	+	*met*-1	*red*-1	*arg*-2 +
$Red^+ Met^+$ Leu Arg^+	$Red^+ Met^+$ Leu Arg	*leu*-1	+	+	*arg*-2 +
Red Met^+ Leu Arg^+	Red Met^+ Leu Arg	*leu*-1	+	*red*-1	*arg*-2 +

a. The Arg sectors were all identified as non-leaky like *arg*-2.
The absence of Arg^+ sectors can be explained by invoking a translocation breakpoint involved in the determination of *arg*-2.

b. See Table II.

selection for or against the allele *leu*-1. However, the ratios in other columns suggest that the *met*-1 and *red*-1 alleles have been selected against possibly because strains carrying the *met*-1 marker are slow-growing on MM supplemented only with methionine and in the case of *red*-1 because the allele might be linked to *leu*-1. Allowance can be made for this selection when estimating possible linkage between certain markers recovered from each regeneration medium. When this is done the total data are compatible with free recombination between most of the alleles. This would be expected if segregation involves a high frequency of interchromosomal recombination (haploidisation) and/or intrachromosomal recombination (mitotic crossing over).

Further analysis might be aided by plating fused protoplasts on to CM before plating on to selective media since purification of colonies from these media would then not be necessary.

Improvement of Cephalosporin C Titre and other Characteristics using Hybridisation by Protoplast Fusion

The asporulating and slow-growing strain A could synthesise cephalosporin C from sulphate and had three times the cephalosporin C titre of the sporulating strain B while the latter had approximately four times the growth rate of strain A. An extensive breeding programme using protoplast fusion was carried out to recombine the merits of both strains. (See Table I - Crosses 1 and 2).

Most of the tests for these characteristics were made with colonies arising on CM which had been used in stability-testing prototrophs. From one prototroph a wide range of growth rates and morphological types were seen.

Aproximately 600 recombinants were titre tested (Figure 1). One isolate, on repeat testing consistently showed more than a 40% improvement in cephalosporin C titre compared with the prototrophic parent A, plus a faster growth rate and better sporulation than strain A when cultured on CM. It could also synthesise cephalosporin C from sulphate.

CONCLUSION

In these studies with *A. chrysogenum* heterozygotes were rarely

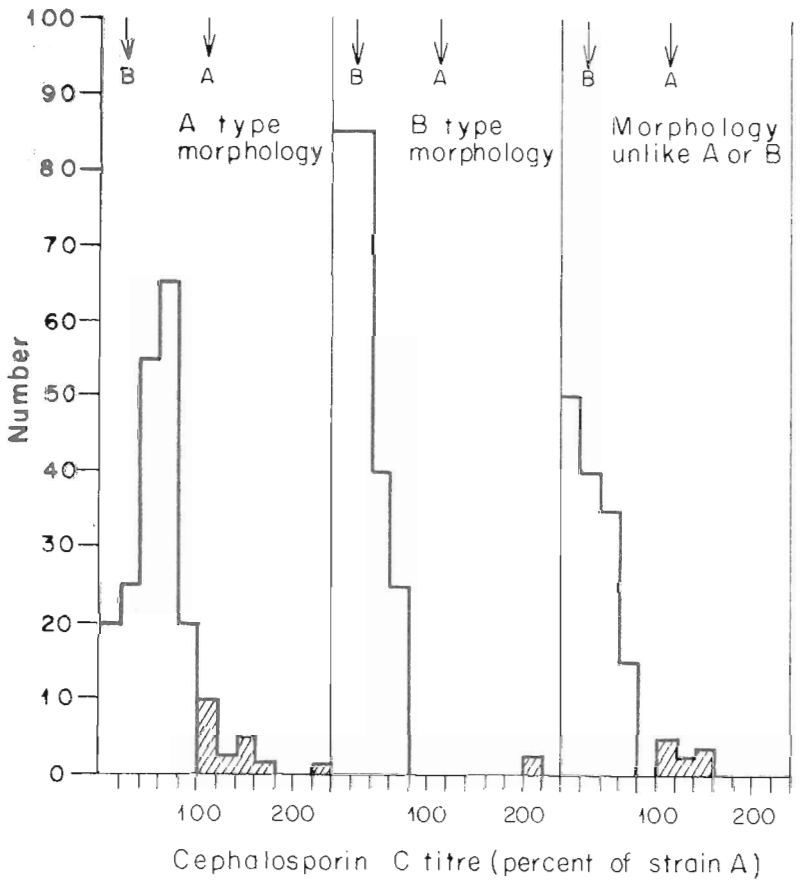

Figure 1. Cephalosporin C Production by Recombinants from Protoplast Fusion of Marked Derivatives of Strains A and B. Cross *ane*-2 *nic*-1 *red*-3/*ane*-3 *arg*-3 **(A/B)** (Table I). The marking of strain A caused a 30% reduction in titre. The recombinants were prototrophs or carried the *nic*-1 marker. Arrows indicate titres of prototrophic parents A and B. Shaded areas indicate improved titres.

recovered and multiple heterozygosity was detected only in crosses between strains separated by a large number of mutation steps. All heterozygotes were very unstable on CM.

Overall the results suggest that in *A. chrysogenum* the parasexual cycle could resemble a meiotic cycle in that diploidisation due to nuclear fusion is transient and is often followed by rapid chromosome segregation and possibly efficient intrachromosomal recombination, assuming that most of the segregants shown in Table II are haploid. Although this process may be typical only of hybridisation by protoplast fusion, the generality of the phenomenon is supported by our results from conventional crosses. The theory of a high frequency of parasexual recombination in *A. chrysogenum* is further supported by work with related organisms *Emericellopsis* (Fantini, 1962) and *Cephalosporium mycophyllum* (Tuveson and Coy 1961). With the latter organisms genetic recombination in supposed diploids occurs at high frequency.

Finally, our results following protoplast fusion indicate that fundamental genetic analysis of *A. chrysogenum* may be possible using selective media and the rationales of multi-factor analysis. Furthermore, following protoplast fusion we have succeeded in breeding a strain of *A. chrysogenum* showing a 40% improvement in cephalosporin C titre and also improved in growth rate and sporulation when compared with its highest titre parent.

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MECHANISM OF POLYPLOIDIZATION IN THE MALPIGHIAN TUBES OF BLOOD-SUCKING REDUVIID HEMIPTERANS

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ABSTRACT

The possibility of polyploidy arising by nuclear fusion in the Malphighian tubes of reduviid hemipterans was investigated by counting the number of nuclei of the epithelial cells in organs of adults and early and fully-grown nymphae of fully--nourished *Panstrongylus megistus* Burmeister. Constancy in number of nuclei was demonstrated, which indicates that nuclear fusion does not normally play a part in the polyploidization mechanism of these Malpighian tube nuclei. Polyploidy is therefore assumed to be attained in this case by endomitosis.

INTRODUCTION

High levels of ploidy are attained by nuclei of the epithelial cells of the Malpighian tubes of blood-sucking hemipterans as nymphal development progresses (Mello, 1971, 1975, 1978 a). The increase in ploidy degree stops at the late 5th nymphal instar (Mello and Raymundo, 1977). Feulgen - DNA classes (32C and 64C) are then demonstrable in epithelial cell nuclei all over the Malpighian tubes. Polyploidy in this case has been evaluated by

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cytophotometric methods and attributed to an endomitotic phenomenon (Mello, 1971, 1975, 1978 a). In the fat body cells of the blood-sucking hemipteran, *Rhodnius prolixus,* however, high ploidy degrees arise by nuclear fusion, a phenomenon which has been detected in specimens reared under starvation conditions (Wigglesworth, 1967). This fact led us to the supposition that nuclear fusion may well be playing a part in the process of polyploidization occurring in the epithelial cells of the Malpighian tubes of reduviids. It is worth mentioning that certain reduviid species display a change in frequency and distribution of heterochromatic bodies on part of their Malpighian tube nuclei after these attain increasingly higher ploidy degrees (Mello, 1978 a, b). Perhaps this could be a consequence of the nuclear fusion phenomenon.

In order to test the hypothesis of nuclear fusion contributing to polyploidy in the Malpighian tubes of fully-nourished blood-sucking hemipterans, we determined the total number of nuclei in the organs of nymphae and adults of a reduviid species.

MATERIALS AND METHODS

Fully-nourished adults and 5th and early 2nd instar nymphae of *Panstrongylus megistus* Burmeister were used. Whole mounts of their Malpighian tubes were stained with a lacto-acetic orcein solution and sealed. Observations were made with a Zeiss photomicroscope. An eyepiece graticule was used to count nuclei.

RESULTS AND DISCUSSION

The total number of epithelial cell nuclei in the Malpighian tubes of the fully-nourished specimens does not appear to change as development proceeds (Table I). In addition, the Malpighian tubes of both early and fully-grown nymphae of *P. megistus* exhibit binucleate epithelial cells (Figs. 1-3). This situation persists in adults (Fig. 4). Consequently, nuclear fusion is not responsible for inducing polyploidy in the nuclei of the Malpighian tubes of *P. megistus* at least under fully-nourished conditions. However,

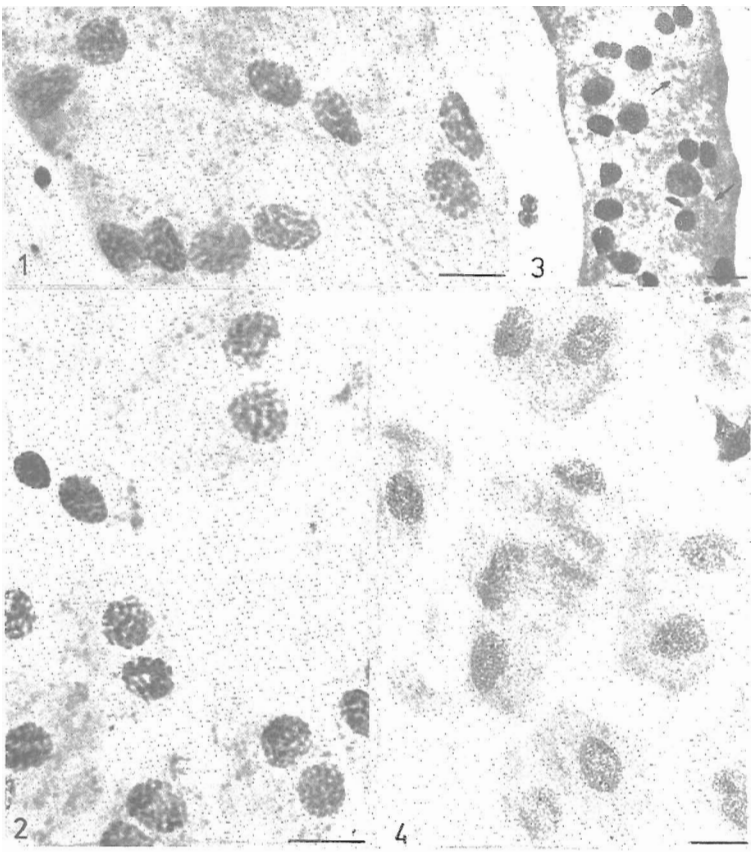

Figure 1. Several pairs of nuclei in a Malpighian tube of a 2nd instar nympha of *P. megistus*. λ = 550 nm. Bar = 20 μm.

Figure 2. Binucleate cells isolated from the Malpighian tubes of a 2nd instar nympha. λ = 550nm. Bar = 20 μm.

Figure 3. Whole mount of a Malpighian tube at the 5th nymphal instar showing pairs of nuclei which differ in size. Arrows indicate nuclei which are underfocused. λ = 550 nm. Bar = 20 μm.

Figure 4. Binucleate cells isolated from the Malpighian tubes of a male adult of *P. megistus*. λ = 530 nm. Bar = 50 μm.

Table I. - Number of epithelial cell nuclei counted in the Malpighian tubes of *P. megistus.*

Life stages	Number of nuclei ($\bar{X} \pm S$)	Coefficient of variation (%)	Number of insects
Early 2 nd nymphal instar	10 232 ± 198	1.9	5
5th nymphal instar	10 336 ± 162	1.6	4
adult	10 166 ± 181	1.8	4

some fusing nuclei have been detected in the Malpighian tubes of specimens subjected to prolonged starvation (Mello, 1978 b). The same is certainly valid for other reduviid species, such as *Triatoma infestans* and *Rhodnius prolixus.* These also display Malpighian tubes containing binucleate epithelial cells, the nuclei of which attain 32C and 64C Feulgen-DNA classes when compared with the Feulgen-DNA contents of their respective sperm line cell nuclei (Mello, 1975, 1978 a; Mello and Lima, 1978). Therefore, the mechanism of attaining polyploidy in the Malpighian tubes of fully-nourished blood-sucking hemipterans involves endomitosis and not nuclear fusion. On the other band, the changes in frequency, distribution, size and shape of the heterochromatic clumps which occur in part of the Malpighian tube nuclei of some reduviid species when nuclei attain high ploidy levels (Mello, 1971, 1978 a, b) do not appear to be a consequence of nuclear fusion. They are probably related to some modification in cell function, with heterochromatin being also affected.

Although two nuclear sizes were observed in the Malpighian tubes of 5th instar nymphae and adults of *P. megistus* (Fig. 3), the largest nucleus does not arise by fusion of two smaller ones. The cells are invariably binucleate. These nuclear sizes possibly correspond to the different ploidy degrees previously described for the Malpighian tube nuclei of *P. megistus* and *T. infestans* during the periods mentioned (Mello, 1975, 1978 a; Mello and Raymundo, 1977).

The variability in numbers of total nuclei in the various life stages

of P. megistus is assumed to be due to some occasional loss of tissue at the site of insertion of the Malpighian tubes in the hindgut-midgut connection, when the organs were removed from the insects, and/or to errors in the counts.

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CYTOTAXONOMIC CONSIDERATIONS ON *Hoplias lacerdae* (PISCES, ERYTHRINIDAE)

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ABSTRACT

Cytogenetic studies were performed on fish of the genus *Hoplias* (Pisces, Erythrinidae) obtained from the Limoeiro Hydroelectric Plant, São José do Rio Pardo (State of São Paulo, Brasil). These fish are considered to belong to the *Hoplias lacerdae* species, commonly known in Brazil as "trairão". The specimens showed a diploid number equal to 50 chromosomes for both males and females, with evidence of the existence of an XX/XY chromosomal mechanism, with male heterogamety. The karyologic data are analyzed in terms of the taxonomic aspects within this group, and the validity of its existence as a species chromosomally distinct from the *Hoplias malabaricus* species is emphasized.

INTRODUCTION

Cytotaxonomy, i.e. the correlation between cytology (cytogenetics

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in particular) and taxonomy, originated during the second half of the 19th century, when it was discovered that some animal and vegetal species may be classified acoording to their chromosomal characteristics. However, chromosomes began to be considered as useful tools in taxonomy only when comparisons between several species of the same genus were made on the basis of the number and morphology of chromosomes (Brown and Bertke, 1969).

Taxonomically, some groups of fish present serious difficulties leading to disagreements amongst classifiers on the identity of a given species. In many cases, cytogenetic studies may clarify this problem (Ojima, Ueno and Hayashi, 1976).

Kosswig (1973) emphasized the fact that many "biospecies", due to their phenotypical similarities, may be grouped under one same "morphospecies", with the possibility of demonstrating reproductive isolation in controlled experiments. Thus the study of fish cytogenetics and genetics is very promising in terms of the solution of these problems.

The Erythrinidae family, including the fish commonly known as "traíras", "trairões", "jejus", "morobás", etc. in Brazil, has a wide geographical distribution. According to Menezes (1972), this family is found in all Brazil, and is present in all four great Brazilian river basins, i.e. the Amazon, Paraná, São Francisco and East systems. Azevedo and Gomes (1943) consider this family to have three genera: *Hoplias, Hoplerythrinus* and *Erythrinus,* all of them present in South America. The genus *Hoplias* is the most widely distributed, and occurs at several latitudes. These investigators included four species in this genus: *H. microlepis* Günther, 1864, *H. macrophthalma* Pellegrin, 1902, *H. microphthalma* Pellegrin, 1908, *H. malabaricus* (Bloch, 1794), and *H. lacerdae* Ribeiro, 1908. Fowler (1950) only mentions two species for Brazil: *H. lacerdae* and *H. malabaricus*, the latter including two subspecies: *H. malabaricus malabaricus* and *H. malabaricus macrophthalmus.* According to Britski (1972), only two species occur in the State of São Paulo: *H. malabaricus,* the common "traíra", and *H. lacerdae*, the "trairão" (= big traíra). Azevedo and Gomes (1943) believe that all species described for the genus *Hoplias,* which are morphologically very similar, having only a few distinctive traits, could be included in one "singameon", i.e. a population whose components may cross and exchange genes. Accordingly, only very careful comparative studies could establish the specific or subspecific differences amongst these species. In a later study, Azevedo, Vaz and Parreira (1965) redescribed the "trairão" fish, which had initially been described

by Ribeiro in 1908, on the basis of a specimen obtained from the Ribeira river basin, State of São Paulo. According to these authors, at least *H. malabaricus* and *H. lacerdae* constitute distinct species in the genus *Hoplias*.

Acoording to Azevedo *et al.* (1965) and Britski (1972), the distinction between the *H. malabaricus* and *H. lacerdae* species can be made on the basis of the gular region, since in *H. malabaricus* the teeth lines converge, practically meeting in the anterior region in an inverted "V" shape, while in *H. lacerdae* these lines are almost parallel and do not meet in the anterior region (Fig. 1). Azevedo *et al.* (1965) also cite other important differential traits, such as the number of scales in the lateral line: 46-48 in *H. lacerdae*, and 38-42 in *H. malabaricus* (39-44 according to Azevedo and Gomes, 1943), and the number of scales above and below the lateral line: 7 and 9 in *H. lacerdae* and 5 and 6 in *H. malabaricus*, respectively.

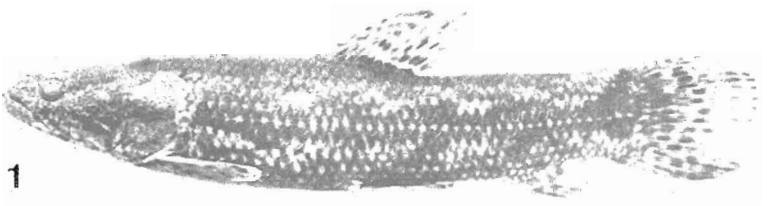
The objective of this research was to study the "trairão" fish cytogenetically and to compare the data obtained with those for other specimens of the genus *Hoplias*. We sought to provide additional data on the karyotypes of Brazilian fresh water fish, which have been the subject of relatively few studies so far, and to contribute, at the same time, with cytogenetic data which may be of help in future taxonomic revisions of the genus.

MATERIALS AND METHODS

The fish analyzed here were obtained from the Pisciculture Station of the Limoeiro Hydroelectric Plant, São José do Rio Pardo (State of São Paulo, Brazil), property of the Companhia Energética do Estado de São Paulo (CESP).

The specimens were identified by the methods of Azevedo *et al.* (1965) and Britski (1972). The aspect of the gular region agrees with that described for the *H. lacerdae* species (Fig. 1), and the number of scales in the lateral line (43 - 45) is close to that expected. In view of these facts, to avoid possible doubts as to the real "status" of the fish studied, we decided to use the name *Hoplias lacerdae* of the Limoeiro Hydroelectric Plant, thus identifying the fish by region of origin.

Twenty-six specimens, 12 females and 14 males, were studied. Each animal was injected intraperitoneally with colchicine (0.2 - 0.5%), 1 ml/50 g



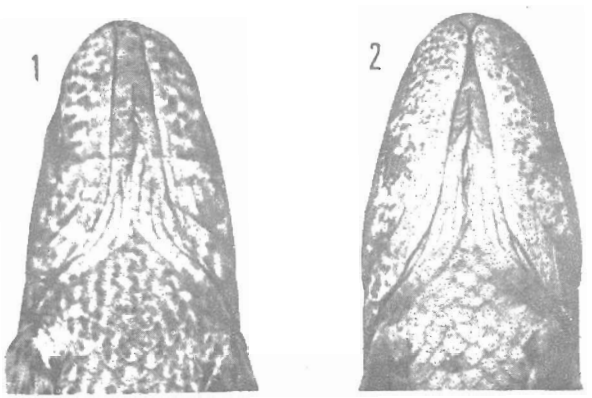


Figure 1. External morphology of specimens of the genus *Hoplias*. 1: specimen of *Hoplias lacerdae* of the Limoeiro Hydroelectric Plant (23.0 cm). View of the gular region, characteristic of this species. 2: View of the gular region typical of *Hoplias malabaricus*.

bodyweight, and sacrificed two or three hours later. The kidneys were obtained and placed in hypotonic potassium chloride 0.075 M. The material was fragmented, placed in an incubator at 36-37° C for 20 minutes, and centrifuged. The supernatant was discarded and the cell sediment was fixed with methanol-acetic acid (3:1). After two additional centrifugations with fresh fixing solution, drops of the cell suspension were placed on clean slides (kept at ice temperature), and air-dried. The slides were stained with 5% Giemsa.

Meiotic chromosomes from testicle material were prepared by the same procedure or by a small modification of the technique of Kligerman and Bloom (1977). Chromosome measurements were carried out by the method described by Yonenaga (1972), using the classification of Levan, Fredga and Sandberg (1964). The fundamental number (FN) was determined, considering the metacentric and submetacentric chromosomes having two chromosome arms.

RESULTS

Three-hundred-and-seven cells of female and 305 cells of male fish were analyzed. Fig. 2 shows the frequencies of diploid numbers found in the somatic metaphases of females and males. It is clear that the diploid number is 50 for both sexes.

Fig. 3 shows the karyotypes, illustrated in decreasing order of size. The fundamental number (FN) is 100 for both males and females. Meiotic chromosomes in testicular metaphases are shown in Fig. 4, with 50 chromosomes occurring in the spermatogonial metaphases, 25 bivalents in metaphases I, and 25 chromosomes in metaphases II.

Table I shows the data concerning the chromosomal measurements. Table II shows some karyotypic characteristics of different specimens of the genus *Hoplias*.

DISCUSSION

The karyotype of *Hoplias lacerdae* of the Limoeiro Hydroelectric

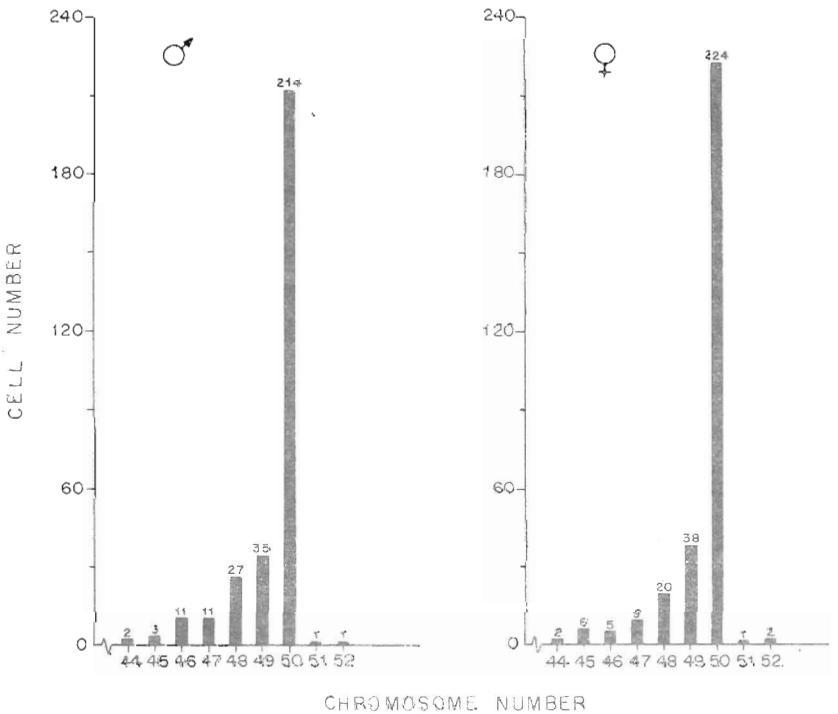


Figure 2. Frequency graphs for the diploid numbers of *Hoplias lacerdae* males and females of the Limoeiro Hydroelectric Plant.

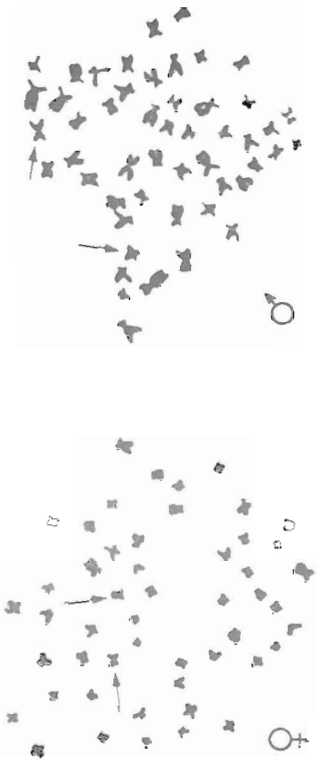


Figure 3. Karyotypes and somatic metaphases of a *Hoplias lacerdae* female and male of the Limoeiro Hydroelectric Plant. The arrows indicate the probable X chromosomes of the female, and X and Y chromosomes of the male. 2n = 50 and FN = 100.

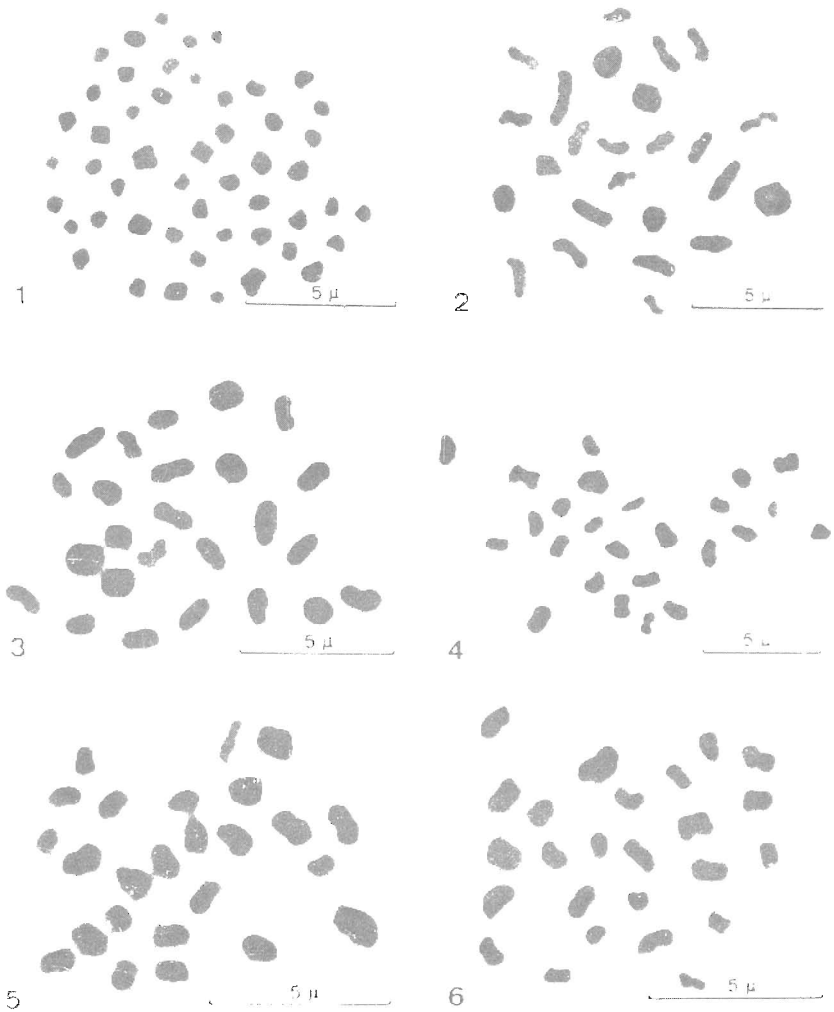


Figure 4. Meiotic chromosomes of *Hoplias lacerdae* of the Limoeiro Hydroelectric Plant, 1: spermatogonial metaphase (2n = 50); 2 and 3: metaphases I (25 bivalents); 4, 5 and 6: metaphases II (25 chromosomes).

Plant is characterized by 2n = 50 and by FN = 100 for both sexes. Eighteen pairs of metacentric and 7 pairs of submetacentric chromosomes occur in the female (Table I). The male's karyotype is similar to the female's, except for the fact that a heteromorphic pair occurs in the male, which is formed by a relatively large metacentric chromosome, presumably the X one (the sixth chromosome of the haploid genome in decreasing order of size) and by a small metacentric chromosome, presumably the Y, which is not perfectly identifiable amongst the other chromosomes of the complement with similar size and shape. It is possible to identify the X as pair number 6 because it is metacentric and because the pairs next in size (numbers 5 and 7) are submetacentric. On this basis, an XX/XY system can be postulated, where the male represents the heterogametic sex, with 48 autosomes + XY, and the female represents the homogametic sex, with 48 autosomes + XX. These results support the hypothesis of Ebeling and Chen (1970) that cytologic heterogamety in fish may be more common than generally thought. These authors described several cases exhibiting chromosomal mechanisms of the XX/XY, XX/XO, ZZ/ZW types, in contrast with the hypothesis that cytologic heterogamety is uncommon in fish, where sex determination is usually genetical (Denton, 1973; Ohno, 1974; White, 1973). Recent investigations have demonstrated chromosomal heterogamety in different fish species (Bertollo, 1978; Foresti, 1974; Michele and Takahashi, 1977; Michele, Takahashi and Ferrari, 1977; Rishi, 1973, 1976; Uyeno and Miller, 1971, 1972).

According to Denton (1973), meiosis in fish is similar to that of all other vertebrates. During male gametogenesis in *Hoplias lacerdae* of the Limoeiro Hydroelectric Plant (Fig. 4), it is possible to observe the occurrence of 50 chromosomes in spermatogonial metaphase, 25 bivalents in metaphase I, and 25 chromosomes in metaphase II, which confirms the diploid number found in somatic cells. On the basis of the figures obtained, the chromosomes considered as X and Y could not be demonstrated during the meiotic process.

In the genus *Hoplias* (Table II), the diploid number varies from 2n = 39 (in the Hoplias malabaricus males from the Lobo Dam, São Carlos, São Paulo State) to 2n = 50 (in the *Hoplias lacerdae* males and females of the Limoeiro Hydroelectric Plant). Nevertheless, among all these different forms it is evident that at least one species, *Hoplias lacerdae* of the Limoeiro Hydroelectric Plant, is karyotypically well characterized and constitutes a separate species. In the other forms the karyotypic resemblance is more marked, with a lower numerical variation, from 39 to 42 chromosomes

Table I - Mean chromosome values (in number of points) for *Hoplias lacerdae* males and females from the Limoeiro Hydroelectric Plant.

Chromosomes	Mean values between males and females					Types
	LA	SA	TL	RL%	AR	
1	23.5	15.5	39.0	5.98	1.52	M
2	20.8	11.8	32.6	5.00	1.76	SM
3	17.5	14.0	31.5	4.83	1.25	M
4	16.3	14.8	31.1	4.77	1.10	M
5	20.3	10.3	30.6	4.69	1.97	SM
6(X)	15.3	13.8	29.1	4.46	1.11	M
7	18.0	10.3	28.3	4.34	1.75	SM
8	18.3	9.3	27.6	4.23	1.97	SM
9	15.8	11.5	27.3	4.19	1.37	M
10	16.3	10.3	26.6	4.08	1.58	M
11	15.5	11.0	26.5	4.06	1.41	M
12	15.3	10.5	25.8	3.96	1.46	M
13	16.8	9.0	25.8	3.96	1.87	SM
14	16.8	8.5	25.3	3.88	1.98	SM
15	13.0	11.3	24.3	3.73	1.15	M
16	16.0	8.0	24.0	3.68	2.00	SM
17	12.3	11.5	23.8	3.65	1.07	M
18	14.0	9.8	23.8	3.65	1.43	M
19	12.0	11.0	23.0	3.53	1.09	M
20	13.0	9.8	22.8	3.50	1.33	M
21	11.8	10.3	22.1	3.39	1.15	M
22	13.5	8.5	22.0	3.37	1.59	M
23	13.0	8.3	21.3	3.27	1.57	M
24	10.5	9.0	19.5	2.99	1.17	M
25	10.5	7.5	18.0	2.76	1.40	M
Y	14.0	9.0	23.0	3.53	1.56	M

LA = long arm; SA = short arm; TL = total lenght; RL = relative length; AR = arm relationship; M = metacentric chromosome; SM = submetacentric chromosome.

Table II - Karyotypic characteristics of a few specimens of the genus *Hoplias*. The results marked with an asterisk were obtained by Bertollo (1978).

SPECIES	*Hoplias lacerdae* of the Limoeiro Plant (SP)		*Hoplias Malabaricus**							
			Lobo Dam (SP)		Aripuanã River (MT)		Rio Doce Valley (MG)		Juquiá River (SP)	
Sex	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
2n	50	50	40	39	40	41	42	42	42	42
NF	100	100	80	78	80	81	84	84	84	84

(Bertollo, 1978).

Thus the cytogenetic data demonstrate that the analyzed animals constitute a separate species within the genus *Hoplias*, whose morphology and chromosomes differ from those of other specimens belonging to the same genus. These results support the taxonomic data which consider the existence of at least two valid species in the genus *Hoplias: H. lacerdae* and *H. malabaricus* (Azevedo and Gomes, 1943; Azevedo *et al.*, 1965; *Britski*, 1972; Fowler, 1950; Godoy, 1970).

It would be particularly interesting to compare the karyotypes of *Hoplias lacerdae* of the Limoeiro Hydroelectric Plant and of *Hoplias lacerdae* from the Ribeira river basin (São Paulo), site of origin of the species described by Ribeiro in 1908. Such a study, together with the data presented here, could be very useful for a future taxonomic revision of the genus *Hoplias*.

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(B. J. Genetics)

CORRELATION BETWEEN POPULATION MEANS IN DIFFERENT GENERATIONS UNDER SELFING AND FULL-SIBBING. ONE POPULATION[1]

J.B. Miranda Filho[2] and Arnel R. Hallauer[3]

ABSTRACT

Maize (*Zea mays* L.) plants that produce two or more ears permit simultaneous selfing of one ear and crossing of another ear on the same plants. The technique has been used to test full-sib progenies (single-cross hybrids) in each generation of inbreeding. Evaluation of the full-sib progenies also permits the integration of inbred development with recurrent selection procedures for population improvement; i. e., reciprocal recurrent selection based on full-sib progenies. Several aspects of the full-sib procedure may be considered theoretically to maximize the potential use of the procedure. Our primary objective was to examine theoretically the relations between progenies in different generations by use of correlations and regressions.

Our reference populations were characterized by their variation in gene frequencies as intermediate ($\bar{p} = 1/2$), low ($\bar{p} = 1/3$), and high ($\bar{p} = 2/3$). Phenotypic and genotypic correlations followed the same trend, but the phenotypic correlations were smaller because of environmental effects. For low heritabilities, the differences

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among the three reference populations were not great. However, as the heritability increased all correlations increased, with the greastest increase expected in high gene frequency populations. Genetic correlations showed that selection among S_0 plants assures the best performance of S_1 progenies but not necessarily the best performing $S_0 \times S_0$ and $S_1 \times S_1$ full-sib families. This was particularly evident when a high level of dominance was assumed, which seems to be the case for grain yield of maize. When no dominance was assumed, the correlations were large among generations in all instances. Level of dominance had a greater effect on the correlations than did population structure.

INTRODUCTION

A technique was outlined by Hallauer (1967) and Lonnquist and Williams (1967) for the developing single-cross maize (*Zea mays* L.) hybrids from full-sib families (cryptic double crosses). The method has been shown to be effective (Hallauer, 1973; Hoegemeyer and Hallauer, 1976), but theoretical studies would provide a better understanding of the genetic implications and would provide guides for the maximization of selection.

Briefly, the full-sib method is initiated with crosses between pairs of selected two-eared S_0 plants (noninbred) giving rise to full-sib families. The selected plants that are crossed also are selfed by use of the second ear. Full-sib families are evaluated in replicated trials the next year and the pairs of S_1 (selfed) progenies are planted ear-to-row for continued crossing and selfing. The process continues until the desired degree of inbreeding has been reached and single crosses and pairs of inbred lines have been developed.

Several theoretical aspects can be considered about the full-sib procedure to maximize its potentialities. One such aspect deals with the expected correlations between means (pairs of S_0 plants or inbred families) in different generations. Our purpose is to investigate these relations theoretically for different genetic models. Results will be expressed in terms of expected values for different situations with respect to the genetic structure of populations and types of gene action.

THEORY

Genetic variance

Our theory is based on Fisher's (1918) model, as described by

Kempthorne (1957). The model includes only one locus with two alleles and only additive and dominant genetic effects. Assumptions required are: normal diploid segregation, no linkage, no preferential fertilization, no reciprocal effects, and population in equilibrium. According to this model, the following variances can be defined as function of gene frequencies and gene effects:

$$\sigma^2_A = 2\,p\,(1-p)\,[a + (1-2p)\,d]^2\,;$$

$$\sigma^2_D = 2p^2\,(1-p)^2\,d^2\,;$$

$$\sigma^2_{A_1} = 2p\,(1-p)\,[a + 1/2\,(1-2p)\,d]^2\,;\text{ and}$$

$$\sigma^2_{A_2} = 2p\,(1-p)\,[a^2 + 1/2\,(1-2p)^2\,d^2 + 3/2\,(1-2p)\,ad],$$

where p is the frequency of the favorable allele, a is half the difference between the two homozygotes, and d is the deviation of heterozygote from the mean of the two homozygotes; σ^2_A is the additive genetic variance defined as the variance due to regression of genotypic values on the number of favorable alleles in the genotypes; and σ^2_D is the variance due to deviations from the additive model. $\sigma^2_{A_1}$ and $\sigma^2_{A_2}$ are not specifically defined; they arise in the theory of covariances between relatives under selfing as remainders when σ^2_D is removed.

In the breeding procedure under consideration, the following means are of interest in the first generation:

$\overline{S}_0$: mean of a pair of two S_0 plants taken at random in the population;
$\overline{S}_1$: mean of two S_1 families resulting from selfing both S_0 plants in each pair;
$\overline{FS}_0$: mean of a full-sib family resulting from a cross between the two S_0 plants in each pair; and
$\overline{FS}_1$: mean of a full-sib family resulting from a cross between two S_1 plants, one from each S_1 family, in a given pair.

Thus, the following variances and covariances can be determined:

$$\sigma^2_0 = \sigma^2_{\overline{S}_0} = 1/2\,(\sigma^2_A + \sigma^2_D);$$

$$\sigma^2_1 = \sigma^2_{\overline{S}_1} = 1/2\,\sigma^2_{A_1} + 1/8\,\sigma^2_D;$$

$$\sigma^2_{00} = \sigma^2_{\overline{FS}_0} = 1/2\,\sigma^2_A + 1/4\,\sigma^2_D;$$

$$\sigma^2_{11} = \sigma^2_{\overline{FS}_1} = 3/4\ \sigma^2_A + 9/16\ \sigma^2_D$$

$$Cov_{0,1} = Cov_{(\overline{S}_0, \overline{S}_1)} = 1/2\ \sigma^2_{A_2} + 1/4\ \sigma^2_D;$$

$$Cov_{0,00} = Cov_{(\overline{S}_0, \overline{FS}_0)} = 1/2\ \sigma^2_A;$$

$$Cov_{0,11} = Cov_{(\overline{S}_0, \overline{FS}_1)} = 1/2\ \sigma^2_{A_2};$$

$$Cov_{00,1} = Cov_{(\overline{FS}_0, \overline{S}_1)} = 1/2\ \sigma^2_{A_2};$$

$$Cov_{00,11} = Cov_{(FS_0, FS_1)} = 1/2\ \sigma^2_A + 1/4\ \sigma^2_D;$$

$$Cov_{1,11} = Cov_{(S_1, FS_1)} = 1/2\ \sigma^2_{A_2}$$

The index in first notation for σ^2 and Cov is one digit when no cross is involved; thus we use 0 and 1 to denote S_0 and S_1 means, respectively. When crosses are involved we use two digits, i.e., 00 and 11 denoting means of full sibs between S_0's and S_1's, respectively. The expected genetic components of variance for the different variances and covariances can be summarized in matrix form as follows: Ab = c where:

$$A = \begin{bmatrix} 1/2 & 0 & 1/2 & 3/4 & 0 & 1/2 & 1/2 & 0 & 1/2 & 0 \\ 0 & 1/2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1/2 & 0 & 0 & 1/2 & 0 & 1/2 \\ 1/2 & 1/8 & 1/4 & 9/16 & 1/4 & 0 & 0 & 0 & 1/4 & 0 \end{bmatrix},$$

$$b = [\sigma^2_A \quad \sigma^2_{A_1} \quad \sigma^2_{A_2} \quad \sigma^2_D]$$

$$c = [\sigma^2_0 \quad \sigma^2_1 \quad \sigma^2_{00} \quad \sigma^2_{11} \quad Cov_{0,1} \quad Cov_{0,00} \quad Cov_{0,11} \quad Cov_{00,1} \quad Cov_{00,11} \quad Cov_{1,11}]$$

The expected values of each parameter were determined according to a defined distribution of gene frequencies in a population (i.e., as used by

Ho, 1973). It is assumed that genes controlling a quantitative character have their frequencies distributed from 0 to 1 in a fashion similar to the Beta distribution, whose density function is

$$\phi(p) = \frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\,\Gamma(\beta)} p^{\alpha - 1} (1 - p)^{\beta - 1}.$$

The simplest function to be used is obtained by taking $\alpha = \beta = 2$, with a density function $\phi(p) = 6p(1 - p)$, which is symmetrical around $\bar{p} = 1/2$ with a variance of 1/20. Another population was assumed to have a distribution of gene frequencies according to a Beta distribution taking $\alpha = 2$ and $\beta = 3$ with density function $\phi(p) = 12p(1 - p)^2$, which is asymmetrical, skewed to the left with an average gene frequency $\bar{p} = 0.4$, a maximum at $p = 1/3$, and with a variance of 1/25. According to this distribution, genes controlling the character are assumed to have frequencies from 0 to 1, but most of them have a frequency in the neighborhood of 1/3. A third population was assumed to have most of its genes at higher frequencies, around $p = 2/3$. This distribution was defined by Beta function with $\alpha = 3$ and $\beta = 2$, a density function $\phi(p) = 12p^2(1 - p)$, and an average gene frequency at $\bar{p} = 0.6$.

Using the populations and parameters as defined, the expected values were calculated and the coefficient of correlation determined using the expected values for each particular situation.† In each instance it was assumed that a quantitative character was controlled by a large number of genes (theoretically infinite) with equal effects. It must be emphasized that the expected value for each parameter does not mean a summation over all loci, but a weighted average when gene frequencies vary from 0 to 1. If the genes could be grouped into distinct classes, the weights would be the frequencies in each class. Linear regression coefficients also were calculated to express the average regression of means in any generation on means of preceding generations. The regression coefficient may give useful information, because it expresses the expected deviation for any individual mean after a unitary deviation had been observed in a preceding generation.

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† Expected value for any parameter (σ^2_X) was obtained by integranting:
$E(\sigma^2_X) = \int_0^1 \sigma^2_X \, \phi(p)\, dp$

Phenotypic variance:

Attention must be given to phenotypic estimates because they are the ultimate expression of the genotype and environment. Thus, phenotypic variances and covariances were considered to allow the calculation of phenotypic coefficient of correlation. For theoretical relations between means in different generations, all environmental (nongenetic) covariances were expected to be zero, because environmental effects were assumed to be uncorrelated between generations. All phenotypic covariances, therefore, were expected to be the same as genetic covariances.

We used a hypothetical value for heritability (h^2) to establish a relation between genetic and environmental effects. Our task was to determine the expected value of the phenotypic variance among progeny means to obtain the given value of h^2. For a reference we considered a full-sib progeny trial where the phenotype of any individual plant could be expressed by the following model:

$Y_{ijk} = u + f_i + b_j + e_{ij} + s_{ijk}$, where u is the mean;

f_i = family effect, i = 1, 2, ..., g;

b_j = block effect, j = 1, 2, ..., r;

e_{ij} = plot-to-plot error; and

s_{ijk} = phenotypic deviation within families and plots.

The corresponding analysis of variance (Table I) gives mean squares calculated on an individual-plant basis for one environment. Estimates of variance components can be obtained as follows:

$$\hat{\sigma}^2_f = \frac{1}{nr}(M_1 - M_2); \hat{\sigma}^2_e = \frac{1}{n}(M_2 - M_3); \text{ and } \hat{\sigma}^2_w = M_3.$$

The estimated variance among family means ($\hat{\sigma}^2_f$) is expected to estimate $(\frac{1+F}{2})\sigma^2_A + (\frac{1+F}{2})^2 \sigma^2_D$ (Kempthorne, 1957; Chapter 20). The estimates

Table I - General structure for analysis of variance of g full-sib maize families in r replications with n plants sampled in each plot for one enviroment.

Source of variation	Degrees of freedom	Mean squares	Expected mean squares[a]
Replications	r - 1		
Families	f - 1	M_1	$\sigma^2_w + n\,\sigma^2_e + n\,r\sigma^2_f$
Error	(r - 1) (f - 1)	M_2	$\sigma^2_w + n\,\sigma^2_e$
Within	f r (n - 1)	M_3	σ^2_w

[a] Components of variance, σ^2_f, σ^2_e, and σ^2_w, refer to the variance among families, experimental error, and within-plot error, respectively.

within plot variance also are expected to estimate

$(\frac{1 - F}{2})\ \sigma^2_A + (3/4 - 1/2F - 1/4F^2)\ \sigma^2_D + \sigma^2_w$, where σ^2_w, is the environmental within plot variance. The error term (σ^2_e) is a plot-to-plot environmental variance.

Phenotypic variance among pairs of S_0 plants:

The coefficient of heritability on an individual-plant basis is defined as:

$h^2 = \frac{\sigma^2_A}{\sigma^2_{P_f}} = \frac{\sigma^2_A}{\sigma^2_f + \sigma^2_e + \sigma^2_w}$. By rearranging terms we find that

$\sigma^2_e + \sigma^2_w, = (\frac{1 - h^2}{2})\ \sigma^2_A - \sigma^2_D$. Then we find that $\sigma^2_{P_f} = \frac{\sigma^2_A}{h^2}$. For pairs of S_0 plants the phenotypic variance is $\sigma^2_{\overline{P}_{S_O}} = \frac{\sigma^2_A}{2h^2}$.

Phenotypic variance among full-sib family means at any degree of inbreeding of the parents:

From the analysis of variance given in Table I, the phenotypic variance among family means is estimated as:

$$\sigma^2_{P_{\overline{FS}_k}} = \sigma^2_f + \frac{\sigma^2_e}{r} + \frac{\sigma^2_w}{nr} = \frac{M_1}{nr} = (\frac{nr + 1}{2nr})\ \sigma^2_A + (\frac{nr + 3}{4nr})\ \sigma^2_D +$$

$$+\frac{1}{r}(\sigma_e^2 + \frac{\sigma_{w'}^2}{n}), \qquad k = 1, 2, \ldots \infty.$$

If environmental variances are expressed in terms of genetic variances, another relation must be defined. Let t be the relation between the within plot phenotypic variance and the plot-to-plot environmental variance (σ_w^2 / σ_e^2). Such a relation can be estimated from variance analysis. Thus, the following sets of equations become available:

$$t\sigma_e^2 - \sigma_{w'}^2 = (\frac{1-F}{2})\sigma_A^2 + [1 - (\frac{1-F}{2})^2]\sigma_D^2; \text{ and } \sigma_e^2 + \sigma_{w'}^2 = (\frac{1-h^2}{h^2})\sigma_A^2 - \sigma_D^2.$$

Substitutions and algebraic transformations of the sets of equations give the following formula for phenotypic variance among family means:

$$\sigma^2_{P_{\overline{FS}}} = \sigma^2_{\overline{FS}_k} = \frac{1}{nr(t+1)}([(\frac{1+F}{2})[nr(t+1) - (n+t)] + (\frac{n+t}{h^2})]\sigma_A^2 + (\frac{1+F}{2})^2$$

$[nr(t+1) - (n+t)]\sigma_D^2$). This equation gives an estimate of $\sigma^2_{P_{\overline{FS}_k}}$ to obtain a given value for h^2 under fixed values for r, n, and t. Also, if a population is defined according to the distribution of gene frequencies then the expected value of σ_A^2 and σ_D^2 as well as of $\sigma^2_{P_{\overline{FS}_k}}$ can be calculated under the given circumstances.

RESULTS

Results are expressed in expected correlation and linear regression coefficients for populations defined according to their distributions of gene frequencies. Theoretical expected values for variances among means within generations and covariances between means in different generations are given in matrix notation (Y = XW) for each of the three distributions of gene frequencies, where

$Y = [\sigma_0^2 \quad \sigma_1^2 \quad \sigma_{00}^2 \quad \sigma_{11}^2 \quad Cov_{0,} \quad Cov_{0,00} \quad Cov_{0,11} \quad Cov_{1,00} \quad Cov_{1,11} \quad Cov_{00,11}]$;

$W = [a^2, ad, d^2]^1$;

$$X_1 = \frac{1}{140}\begin{bmatrix} 28 & 28 & 28 & 42 & 28 & 28 & 28 & 28 & 28 & 28 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 16 & 4 & 10 & 19.5 & 8 & 4 & 4 & 2 & 2 & 10 \end{bmatrix}'$$

$$X_2 = \frac{1}{140}\begin{bmatrix} 28 & 28 & 28 & 42 & 28 & 28 & 28 & 28 & 28 & 28 \\ 8 & 4 & 8 & 12 & 6 & 8 & 8 & 6 & 6 & 8 \\ 16 & 4 & 10 & 19.5 & 8 & 4 & 4 & 2 & 2 & 10 \end{bmatrix}'$$

$$X_3 = \frac{1}{140}\begin{bmatrix} 28 & 28 & 28 & 28 & 28 & 28 & 28 & 28 & 28 & 28 \\ -8 & -4 & -8 & -12 & -6 & -8 & -8 & -6 & -6 & -8 \\ 16 & 4 & 10 & 19.5 & 8 & 4 & 4 & 2 & 2 & 10 \end{bmatrix}'$$

where X_1, X_2, and X_3 correspond to the distributions Beta (2,2) Beta (2,3) and Beta (3,2), respectively.

Taking $c = d/a$ as a measure of the degree of dominance for each locus, then a^2, ad, and d^2 can be expressed as a^2, ca^2, and c^2 a^2, respectively. Tables II, III and IV give the expected correlation coefficients for each distribution of gene frequencies.

The regression coefficients using expected values of variances and covariances for each distribution of gene frequencies are shown in Tables V, VI, and VII. If there is no dominance, there is an expected unit change in the pairs of means in all instances, which would be expected. As the level of dominance increased, the expected change from the preceding generation was reduced, particularly when the average gene frequency was 0.6. The regression coefficients were the same for some pairs of generations for each of the three distributions considered; i.e., the regression coefficients were the same for $\overline{S}_0$, $\overline{FS}_0$, and $\overline{S}_0$, $\overline{FS}_1$ pairs in each distribution although they differed among distributions. The regression coefficients tended to be greater for the distribution of $\overline{p} = 0.3$, intermediate for $\overline{p} = 0.5$, and smallest for $\overline{p} = 0.6$ for the first three pairs of generations. For the pair, $\overline{FS}_0$, $\overline{S}_1$, greater regression coefficients were obtained for $\overline{p} = 0.6$, in the range of partial to complete dominance. On the other hand, greater values were obtained for population type ($\overline{p} = 0.4$) in the level of overdominance. A unit change was obtained in all distributions at all levels of dominance for $\overline{FS}_0$ and $\overline{FS}_1$.

Table II - Expected genetic correlations (r)[a] between means in different generations under selfing and full-sibbing, for several levels of dominance for the Beta function: $\alpha = \beta = 2$; $\emptyset(p) = 6p(1 - p)$.

Level of dominance	Means in different generations combined in pairs					
	$\overline{S}_0, \overline{FS}_0$	$\overline{S}_0, \overline{S}_1$	$\overline{S}_0, \overline{FS}_1$	$\overline{FS}_0, \overline{S}_1$	$\overline{FS}_0, \overline{FS}_1$	$\overline{S}_1, \overline{FS}_1$
0.0	1.000	1.000	0.816	1.000	0.816	.816
0.2	0.987	0.997	0.804	0.993	0.815	.819
0.4	0.952	0.990	0.771	0.973	0.810	.788
0.6	0.901	0.980	0.724	0.942	0.803	.756
0.8	0.843	0.969	0.670	0.903	0.797	.718
1.0	0.783	0.959	0.615	0.860	0.786	.676
1.2	0.726	0.952	0.564	0.816	0.778	.635
1.4	0.674	0.947	0.519	0.773	0.770	.595

[a] Calculated with expected values of variances and covariances.

Table III - Expected genetic correlations (r)[a] between means in different generations under selfing and full-sibbing, for several levels of dominance for the Beta function: $\alpha = 2$, $\beta = 3$; $\phi(p) = 12p(1-p)^2$.

Level of dominance	Means in different generations combined in pairs					
	$\overline{S}_0, \overline{FS}_0$	$\overline{S}_0, \overline{S}_1$	$\overline{S}_0, \overline{FS}_1$	$\overline{FS}_0, \overline{S}_1$	$\overline{FS}_0, \overline{FS}_1$	$\overline{S}_1, \overline{FS}_1$
0.0	1.000	1.000	0.816	1.000	0.816	.816
0.2	0.988	0.998	0.805	0.993	0.804	.809
0.4	0.957	0.992	0.776	0.975	0.791	.791
0.6	0.914	0.984	0.735	0.949	0.778	.764
0.8	0.866	0.977	0.691	0.918	0.767	.733
1.0	0.818	0.971	0.647	0.885	0.757	.700
1.2	0.772	0.966	0.606	0.850	0.748	.667
1.4	0.730	0.963	0.569	0.817	0.741	.636

[a] Calculated with expected values of variances and covariances.

Table IV - Expected genetic correlations[a] between means in different generations under selfing and full-sibbing, for several levels of dominance for the Beta function: $\alpha = 3$, $\beta = 2$; $\phi(p) = 12p^2(1-p)$.

Level of dominance	Means in different generations combined in pairs					
	$\bar{S}_0, \overline{FS}_0$	$\bar{S}_0, \bar{S}_1$	$\bar{S}_0, \overline{FS}_1$	$\overline{FS}_0, \bar{S}_1$	$\overline{FS}_0, \overline{FS}_1$	$\bar{S}_1, \overline{FS}_1$
0.0	1.000	1.000	0.816	1.000	0.816	.816
0.2	0.987	0.997	0.804	0.993	0.827	.809
0.4	0.947	0.988	0.766	0.970	0.834	.785
0.6	0.884	0.975	0.708	0.933	0.836	.747
0.8	0.809	0.960	0.639	0.884	0.835	.699
1.0	0.730	0.945	0.569	0.828	0.830	.645
1.2	0.655	0.933	0.503	0.768	0.824	.590
1.4	0.589	0.924	0.446	0.709	0.816	.537

[a] Calculated with expected values of variances and covariances.

Table V - Expected regression coefficients[a] involving means in different generations under selfing and full-sibbing, for several levels of dominance for the Beta function: $\alpha = \beta = 2$; $\phi(p) = 6p(1 - p)$.

Level of dominance	Means in different generations combined in pairs					
	$\overline{S}_0, \overline{FS}_0$	$\overline{S}_0, \overline{S}_1$	$\overline{S}_0, \overline{FS}_1$	$\overline{FS}_0, \overline{S}_1$	$\overline{FS}_0, \overline{FS}_1$	$\overline{S}_1, \overline{FS}_1$
0.0	1.000	1.000	1.000	1.000	1.000	1.000
0.2	0.983	0.989	0.983	0.989	1.000	0.997
0.4	0.937	0.958	0.937	0.957	1.000	0.989
0.6	0.872	0.915	0.872	0.909	1.000	0.976
0.8	0.799	0.866	0.799	0.851	1.000	0.958
1.0	0.727	0.818	0.727	0.789	1.000	0.938
1.2	0.661	0.774	0.661	0.728	1.000	0.915
1.4	0.604	0.736	0.604	0.671	1.000	0.891

[a] Calculated with expected values of variances and covariances.

Table VI - Expected regression coefficients[a] involving means in different generations under selfing and full-sibbing for several levels of dominance for the Beta function: $\alpha = 2, \beta = 3; \phi(p) = 12p(1-p)^2$.

Level of dominance	Means in different generations combined in pairs					
	$\bar{S}_0, \bar{FS}_0$	$\bar{S}_0, \bar{S}_1$	$\bar{S}_0, \bar{FS}_1$	$\bar{FS}_0, \bar{S}_1$	$\bar{FS}_0, \bar{FS}_1$	$\bar{S}_1, \bar{FS}_1$
0.0	1.000	1.000	1.000	1.000	1.000	1.000
0.2	0.984	0.976	0.984	0.976	1.000	1.011
0.4	0.943	0.938	0.943	0.937	1.000	1.016
0.6	0.888	0.894	0.888	0.888	1.000	1.015
0.8	0.828	0.849	0.828	0.835	1.000	1.009
1.0	0.769	0.808	0.769	0.783	1.000	1.000
1.2	0.715	0.770	0.715	0.732	1.000	0.988
1.4	0.667	0.738	0.667	0.686	1.000	0.973

[a] Calculated with expected values of variances and covariances.

Table VII - Expected regression coefficients[a] involving means in different generations under selfing and full-sibbing for several levels of dominance for the Beta function: $\alpha = 3, \beta = 2; \phi(p) = 12p^2 (1 - p)$.

Level of dominance	Means in different generations combined in pairs					
	$\bar{S}_0, \bar{FS}_0$	$\bar{S}_0, \bar{S}_1$	$\bar{S}_0, \bar{FS}_1$	$\bar{FS}_0, \bar{S}_1$	$\bar{FS}_0, \bar{FS}_1$	$\bar{S}_1, \bar{FS}_1$
0.0	1.000	1.000	1.000	1.000	1.000	1.000
0.2	0.982	1.003	0.982	1.003	1.000	0.982
0.4	0.930	0.982	0.930	0.982	1.000	0.959
0.6	0.851	0.942	0.851	0.937	1.000	0.929
0.8	0.759	0.889	0.759	0.874	1.000	0.895
1.0	0.667	0.833	0.667	0.800	1.000	0.857
1.2	0.583	0.780	0.583	0.722	1.000	0.818
1.4	0.512	0.733	0.512	0.646	1.000	0.778

[a] Calculated with expected values of variances and covariances.

The expected phenotypic correlations (Tables VIII, IX and X) were lower in all instances than the expected genotypic correlations (Tables II, III and IV). The expected phenotypic correlations also decreased as the level of dominance increased; but, as expected, they increased as the heritabilities increased in all instances. With no dominance, the expected phenotypic correlations were the same for each of the three heritabilities considered for each distribution. The differences among the expected phenotypic correlations were very small for a 10% heritability in all distributions. As the heritability increased, the expected phenotypic correlations showed a pattern similar to that of the expected genotypic correlations; the correlations decreased as the level of dominance increased. The largest correlations were obtained for $\bar{p} = 0.3$, smallest for $\bar{p} = 0.6$, and intermediate for $\bar{p} = 0.5$.

DISCUSSION

Correlations between relatives have been of interest since Fisher's first report in 1918 and since Wright's (1921) papers. Wright (1931) characterized the structure of Mendelian populations under evolutionary pressures by fitting gene distributions in populations according to a defined mathematical function. Wright (1931) stated that, with complete dominance, the total genetic variance for any distribution of gene frequencies ($\emptyset$ (p)) was given by $4a^2 \int_0^1 (1 - p)^2 \; p\,(2 - p)\,\emptyset\,(p)\,dp$. In the same way, in the absence of dominance this variance was $2a^2 \int_0^1 p\,(1 - p)\,\emptyset\,(p)\,dp$. In our case the distribution of gene frequencies was assumed to be defined by Beta Function, where $\emptyset$ (p) may be expressed by three different functions, according to values of α and β ($\alpha = \beta = 2$; $\alpha = 2, \beta = 3$; and $\alpha = 3, \beta = 2$).

The models and concepts discussed above were used to characterize populations and to study correlations between means in different generations produced by selfing and full-sibbing with the breeding procedure described by Hallauer (1967) and Lonnquist and Williams (1967). This procedure has been quite efficient in developing inbred lines and single crosses in maize, and theoretical considerations about its inherent properties can be used for breeding purposes. Wright (1921) stated that "in practical breeding one of the foremost considerations in the mind of the breeder is to obtain such control over the heredity of his stock that the characteristics of the progeny can be predicted from those of the parent. The most direct measure of the

Table VIII - Expected phenotypic correlations[a] between means in different generations under selfing and full-sibbing and under environmental conditions[b] for the Beta function: $\emptyset p = 6p(1 - p)$.

h^2	c	$\overline{S}_0, \overline{FS}_0$	$\overline{S}_0, \overline{FS}_1$	$\overline{FS}_0, \overline{FS}_1$
0.10	0.0	0.240	0.212	0.509
	0.5	0.236	0.207	0.515
	1.0	0.228	0.195	0.529
	1.2	0.224	0.190	0.535
0.20	0.0	0.385	0.331	0.636
	0.5	0.378	0.321	0.638
	1.0	0.362	0.299	0.642
	1.2	0.354	0.290	0.644
0.50	0.0	0.669	0.560	0.749
	0.5	0.655	0.541	0.745
	1.0	0.621	0.500	0.737
	1.2	0.606	0.482	0.734

[a] Calculated with expected values of variances and covariances.

[b] $n = 20$; $r = 4$ for FS_0 and FS_1; $t = 8$ for FS_0; and $t = 8$ for FS_1, where n is the number of plants, r is the number of replications, and t is the relation between the phenotypic variance within plot and the plot-to-plot environmental variance (σ^2_w / σ^2_e).

Table IX - Expected phenotypic correlations[a] between means in different generations under selfing and full-sibbing and under fixed environmental conditions[b] for the Beta function: $\emptyset\ p = 12p\ (1 - p)^2$.

h^2	Level of dominance	$\overline{S}_0, \overline{FS}_0$	$\overline{S}_0, \overline{FS}_1$	$\overline{FS}_0, \overline{FS}_1$
0.10	0.0	0.240	0.212	0.509
	0.5	0.237	0.208	0.514
	1.0	0.230	0.198	0.525
	1.2	0.228	0.194	0.530
0.20	0.0	0.385	0.331	0.636
	0.5	0.379	0.322	0.638
	1.0	0.366	0.305	0.641
	1.2	0.360	0.297	0.642
0.50	0.0	0.669	0.560	0.749
	0.5	0.656	0.543	0.745
	1.0	0.630	0.510	0.739
	1.2	0.618	0.497	0.736

[a] Calculated with expected values of variances and covariances.

[b] $n = 20$, $R = 4$ for FS_0 and FS_1, $t = 8$ for FS_0 and FS_1.

Table X - Expected phenotypic correlations[a] between means in different generations under selfing and full-sibbing for the Beta function: $\emptyset p = 12p^2 (1 - p)$.

h^2	Level of dominance	$\overline{S}_0, \overline{FS}_0$	$\overline{S}_0, \overline{FS}_1$	$\overline{FS}_0, \overline{FS}_1$
0.10	0.0	0.240	0.212	0.509
	0.5	0.236	0.206	0.516
	1.0	0.225	0.190	0.535
	1.2	0.219	0.183	0.544
0.20	0.0	0.385	0.331	0.636
	0.5	0.377	0.319	0.638
	1.0	0.355	0.290	0.644
	1.2	0.344	0.277	0.646
0.50	0.0	0.669	0.560	0.749
	0.5	0.653	0.538	0.744
	1.0	0.607	0.484	0.734
	1.2	0.585	0.459	0.730

[a] Calculated with expected values of variances and covariances.

success of a system of breeding in this respect would seem to be the correlation between parent and offspring, relative to general population".

Our study involved only the first generations of the full-sib breeding procedure used to develop single crosses and inbred lines. Our reference populations may be characterized by their variation in gene frequencies, as follows: 1) "A" populations, where most of the genes are at intermediate gene frequencies, around $p = 1/2$; composites synthesized from several heterogeneous varieties or populations are expected to be of this type. 2) "B" populations, where most of genes are at lower frequencies, around $p = 1/3$; this type of population may be represented by unimproved varieties. 3) "C" populations, where most of genes are at higher frequencies, around $p = 2/3$; this type is expected to occur with relatively improved varieties or populations.

When population type A is considered, the genetic correlation between means of S_0 plants and their corresponding full-sib families vary from 1.000 (no dominance) to 0.783 (complete dominance) or less (overdominance). The corresponding values are even higher for population type B (Table III). For complete dominance, Falconer (1960) has shown that total genetic variance reaches its maximum at $p = 0.297$. Hence population type B variances are expected to be greater than population A variances; however, the covariance also is greater, which leads to greater correlation values. On the other hand, correlations of population type C seem smaller relative to those of population type A. Genes of population type C occur at higher frequencies than those of population type A, with a decrease in covariance at a rate greater than decreases in variances.

Correlation coefficients between pairs of S_0 plants and their related S_1 family pairs were expected to be high for any degree of dominance and for all populations under consideration. The same rank was maintained for the three types of population relative to correlations between $\overline{S}_0$ and $\overline{FS}_0$.

Genetic correlations between S_0 pairs and their related S_1 x S_1 full-sib families ranged from 0.816 (no dominance) to 0.615 (complete dominance) or less (overdominance) in population type A. The correlations were slightly greater for population type B and slightly smaller for population type C with some level of dominance. The maximum value in every case was 0.816 instead of 1.000, because the inbreeding of the parents (S_1 families) caused an additional increase in variance among S_1 x S_1 means. Such an increase occurred at the rate of $(1 + F)$ for the additive variance and $(1 + F)^2$ for the dominance variance, where F is Wright's coefficient of

inbreeding.

The results presented here indicate that selection among S_0 plants may assure the best performance of S_1 families but not necessarily assure the best performance in $S_0 \times S_0$ or $S_1 \times S_1$ full-sib families. This conclusion was evident mainly when a high level of dominance was assumed, which seems to be the case for grain yield in maize (Gardner, et al. 1953; Robinson, Comstock and Harvey, 1949; Robinson, Comstock, and Harvey, 1955). Alternatively, selection for characters where dominance is of less importance (such as plant or ear height) may be more efficient in the first generations.

Selection pressure usually is greater when the selection unit is a full-sib family evaluated over replicated trials. Hence, expected correlations involving this kind of family are of special importance. Correlations between $\overline{FS}_0$ and $\overline{S}_1$ were relatively high, ranging from 1.000 (no dominance) to 0.860 (complete dominance) or less (over-dominance) when population type A was considered. For population types B and C, slightly greater or slightly smaller values were expected, respectively.

Expected correlations between $\overline{FS}_0$ and $\overline{FS}_1$ were in the range of 0.816 (no dominance) to 0.786 (complete dominance) or less (overdominance) for the A type populations. However, these values were not expected to change greatly for population type B. Thus, the decrease in correlation coefficients depended more on the level of dominance than on the structure of populations. Such results showed that selection among $S_0 \times S_0$ full-sib families assured a high probability of the best performance in related S_1 families and, to a lesser extent, in $S_1 \times S_1$ families, if invironmental effects are not considered. On the other hand, increasing the level of dominance did not seem to decrease seriously the relationship between $S_0 \times S_0$ and $S_1 \times S_1$.

The expected phenotypic correlations followed the same trend as the expected genotypic correlations, but, as expected, the correlations were smaller because of the environmental effects. The phenotypic correlations in the A, B and C types of populations were not expected to differ greatly if the heritability of the trait was low, which would be the case of a complex trait such as grain yield. An increase in the heritability showed an increase in the phenotypic correlations with the greatest correlations expected for the B type populations.

If the Beta distribution of gene frequencies in populations is assumed to represent a realistic situation, both phenotypic and genetic correlations are expected to decrease as some genes are increasing in frequency because of selection. If a recurrent full-sib selection is conducted

parallel with hybrid development from full-sib families, the distribution of gene frequencies is expected to increase toward a higher mean gene frequency, thereby changing the shape of the distribution. For example, starting from a population like type B ($\bar{p}$ = 0.4), selection will change it gradually toward type C ($\bar{p}$ = .6), passing from a distribution like type A ($\bar{p}$ = .5) and some intermediate ones. Continued selection leads to a more skewed distribution than that of type C, and the limit is a complete fixation of all genes. Because gene frequencies are increasing, the relationship between means in the method herein discussed decreses slightly, according to the results of this paper. However, as previously shown, the relationship between means in different generations depends more on the type of gene action and heritability than on differences in population structures. On the other hand, an increase in gene frequencies through recurrent selection offers better opportunities to develop outstanding hybrids than from unimproved populations.

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TESTOSTERONE SENSITIVITY OF LYMPHOCYTES FROM A PATIENT WITH TESTICULAR FEMINIZATION SYNDROME AND CONTROLS[1].

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ABSTRACT

Testosterone inhibits growth of PHA-stimulated lymphocytes. This effect is measurable by 3H - thymidine uptake and by mitotic activity evaluation. Lymphocytes from a patient with Testicular Feminization Syndrome exhibited the same pattern of growth depression in comparison with lymphocytes from normal male donors.

INTRODUCTION

Testicular Feminization Syndrome (TFS) is due to X-linked mutations, which cause defective androgen action. Affected individuals have normal male karyotype and their gonads differentiate as testes. However, since they are unable to respond to androgens (testosterone and dihydrotestosterone), they develop as phenotypic females (Wilson and Goldstein, 1975; Meyer III et al., 1975). There are at least two genetic variants of TFS, which

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are distinguishable from each other only through dihydrotestosterone binding by skin fibroblasts: the binding activity is present in one form and absent in the other (Amrhein et al., 1976).

Ohno (1971) proposed that it might be possible to detect two populations of lymphocytes in obligate TFS-heterozygotes, as a consequence of the Lyon effect. When cells are submitted to a high level of testosterone, about half of them presumably would be able to undergo blastic transformation, while the other half would not. As Opitz (1971) pointed out, "it is of obvious importance to develop such a test for TFS, not only for purposes of counseling women who have had one affected child about their recurrence risk, but also for possible prenatal diagnosis of the TFS".

A similar test-strategy was recently developed for heterozygous detection and prenatal diagnosis of Cystic Fibrosis (CF). It was based on the enhanced dexamethasone resistence of fibroblasts cultured from CF patients. The fibroblasts cultured from obligate heterozygotes showed an intermediate survival compared to normal and homozygous CF cells (Breslow et al., 1978).

In the present study the test proposed by Ohno has been submitted to experimental evaluation.

MATERIAL AND METHODS

Human lymphocytes from normal male volunteer donors and from a TFS patient were collected and cultured according to the methods described by Zackai and Mellman (1974). Bacto-phytohemagglutinin P (PHA-P, Difco Laboratories) was added to cultures containing 2 x 10^6 cells per ml, to a final concentration of 0.01 ml of the reconstituted PHA-P per ml of RPMI 1640 tissue culture medium (GIBCO), supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and 10% heat-inactivated human AB serum.

The effect of testosterone on the mitogenic response of lymphocytes was evaluated through the determination of both the mitotic index and the cellular uptake of tritium-labelled thymidine. Ethanol-soluble testosterone (Sigma Chemical Co.) was added to the cultures at a 2 x 10^{-5} M concentration, since lower doses showed no appreciable effects on mitogenic response (Fig. 1). The ethanol concentration was 0.1%, this amount having no effect on the mitotic index (Morgan and Perris, 1974). Control cultures, without testos-

terone, received an ethanol placebo.

The mitotic index was determined by microscopic analysis of coded slides prepared from cultures incubated for 72 hours. The number of dividing cells and blast cells, as well as the number of undifferentiated lymphocytes were recorded for a total of 250 cells per slide and 1000 per culture. A G-test of independence (Sokal and Rohlf, 1973) was used to compare the scores of testosterone-treated and control cultures.

Unless otherwise stated, tritiated thymidine ([^{3}H] TdR) uptake was measured in cultures incubated for 72 hours. After 48 hours in incubation each culture tube received 1.0 μCi per ml of ^{3}H TdR (spec. act. 7.0 Ci/m mole; Shwarz/Mann). Twenty-four hours later the cultures were terminated and the cells washed twice in normal saline and once in 2% acetic acid. The cell pellet was dissolved in Insta-Gel (Packard Instrument Co.) and the incorporated radioactivity was measured in a liquid scintillation counter.

The viability of the cells under test was cheked by measuring ^{3}H--Isoleucine incorporation after the initial 24 h of incubation. At this time, PHA-induced protein synthesis was on course, both in testosterone-treated and control cultures, and the incorporation values did not differ significantly (Table V). This test demonstrates clearly that PHA stimulation is not affected by the steroid presence.

The data were transformed from cpm to their natural logarithmic values, in order to make the variance independent of the mean. A one-way analysis of variance was performed cheking for differences in incorporated radioactivity between testosterone-treated and control cultures. The differences between means reported in Table III and Fig. 1 were tested using the Student-Newman-Keuls multiple range test (SNK). It was applied to the ranked means to detect where significant differences were located.

RESULTS

Mitotic activity analyses are reported in Tables I and II. Table I shows the suppressive effect of testosterone on mitogen-stimulated lymphocytes from two normal male donors. Table II shows that this effect also occurs on TFS patient lymphocytes and to the same extent verified in control cultures.

Table III shows the effect of testosterone on [^{3}H] TdR uptake by normal male lymphocytes incubated during different periods. The radioactive

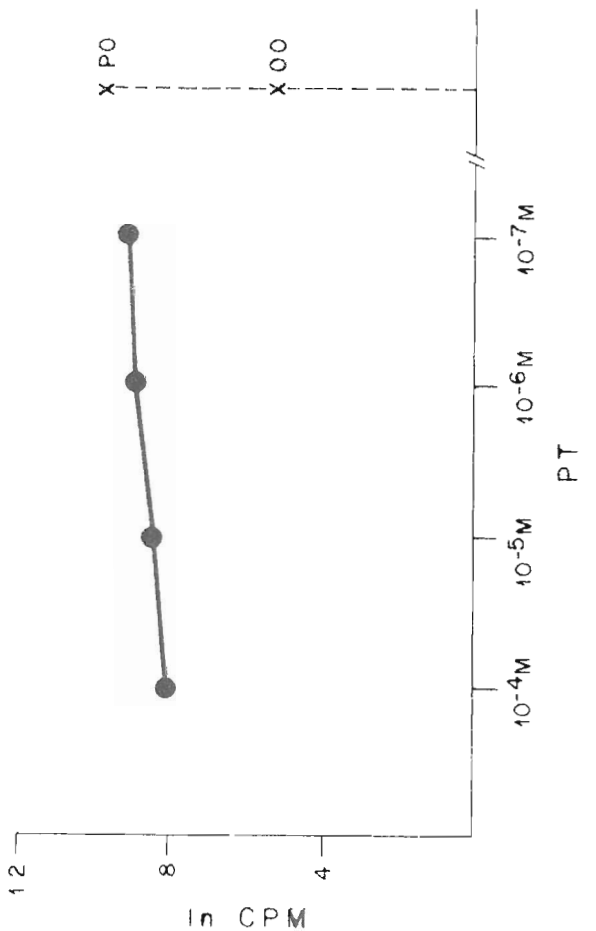

Figure 1. Incorporation of tritiated thymidine by testosterone-treated cultures (PT). PO indicates control PHA-stimulated cultures, and 00 control non-stimulated cultures. Each result (as natural logarithmic values of cpm) represents the mean of duplicate cultures. The cultures treated with testosterone concentrations lower than 10^{-5}M exhibited non significant differences in thymidine uptake when compared with PO values.

Table I - Effect of testosterone on mitotic activity of lymphocytes from two normal male donors.

Donors	Treatment	M	B	N	P
A	PO	41	747	212	< 0.01
	PT	24	669	307	
B	PO	48	718	234	0.01 - 0.05
	PT	30	698	272	

PO indicates control cultures and PT the testosterone-treated ones. The number of dividing (M), blast (B) and undifferentiated cells (N) are scored for each group of cultures.

Table II - Effect of testosterone on mitotic activity of lymphocytes from a TFS patient and control.

	Treatment	M	B	N	P
Control	PO	114	1428	458	< 0.01
	PT	58	1355	587	
Patient	PO	62	1028	910	< 0.01
	PT	29	805	1066	

PO indicates control cultures and PT the testosterone-treated ones. The number of dividing (M), blast (B) and undifferentiated cells (N) are scored for each group of cultures.

Table III - [^{3}H] TdR uptake (expressed in cpm) by normal male lymphocytes incubated for different periods of time.

Incubation period (hours)	00	PT	PO	P
24	388 ± 10	317 ± 6	436 ± 64	> 0.05
28	365 ± 10	489 ± 66	1 109 ± 51	< 0.01
32	367 ± 24	988 ± 48	3 856 ± 131	< 0.01
72	371 ± 35	4 708 ± 113	22 592 ± 4 480	< 0.01

The radioactive marker was added two hours prior to culture termination. Data refer to control non-stimulated cultures (00), control stimulated cultures (PO) and stimulated and testosterone-treated cultures (PT). Each result represents the mean and standard deviation of a duplicate culture. The differences between means were cheked using the SNK test, the solid lines indicating those which are non significant.

Table IV - [^{3}H] Td uptake (expressed in cpm) by TFS and normal male lymphocytes treated with testosterone.

	00	PO	PT	P
Control	1 221 ± 1 223	186 078 ± 10 544	79 172 ± 2 570	< 0.01
Patient	1 160 ± 175	146 777 ± 21 621	47 955 ± 2 789	< 0.01

Data refer to control non-stimulated cultures (00), control stimulated cultures (PO), and stimulated and testosterone-treated cultures (PT). Each results represents the mean and standard deviation of a triplicate culture. Comparisons were made between PO and PT.

Table V - [^{3}H] - isoleucine uptake (expressed in cpm) by normal male lymphocytes incubated for 24 hr.

00	PO	PT	P
2 945 ± 927	9 231 ± 906	8 388 ± 443	> 0.22

The radioactive marker was added 4 hr prior to culture termination (1 μCi/ml, act. sp. 105 Ci/m mole). Data refer to control non-stimulated cultures (00), control stimulated cultures (PO), and stimulated and testosterone-treated cultures (PT). Each result represents the mean and standard deviation of a triplicate culture. Comparisons were made between PO and PT.

marker was added only two hours before culture termination. Table IV shows the results of [^{3}H] TdR incorporation in 72 hours lymphocytes cultures from a normal donor and the TFS patient. All the testosterone-treated cultures exhibited a significantly depressed [^{3}H] TdR uptake.

DISCUSSION

Sex hormones exert widespread suppressive effects on mitogen--stimulated lymphocytes. PHA response of human lymphocytes is depressed by oestradiol (Ghosh and Athreya, 1976) and by serum of pregnant women, which exihibits high levels of oestrogen and progesterone (Walker et al., 1972). Calcium and parathyroid hormone-induced mitogenesis is inhibited by oestradiol (Morgan and Perris, 1974) and diethylstilbestrol reduces the PHA response of bovine lymphocytes (Chrisman and Lasley, 1975). It was also shown that testosterone causes depletion of thymus and bone marrow lymphocytes in rats (Frey-Wettestein and Craddock, 1970) and reduces imunological performance in mice (Eidinger and Garrett, 1972).

The present study reports an inhibitory effect of testosterone on PHA - induced mitogenesis both in normal and TFS human lymphocytes. This effect is probably independent of the testosterone receptor and carrier

proteins in the lymphocyte membrane and cytoplasm, since it occurs on TFS lymphocytes which are functionally defective with respect to such proteins (Ohno, 1976). As far as we can conclude, it is impossible to detect carriers for TFS gene based on the tolerance of part of their lymphocytes to high doses of testosterone, as proposed by Ohno (1971) since the hemizygous affected individuals do not differ in their lymphocyte response from the normal control.

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3:1 SEGREGATION IN A CARRIER OF A (6; 21) TRANSLOCATION[1]

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ABSTRACT

A patient with Down syndrome and a 47, XY, - 6, + der (6), + der (21), t (6; 21)(q21; q22) karyotype is reported. His mother and maternal uncle were found to be balanced carriers. The propositus' grandfather was, most probably, the balanced carrier in the previous generation. The segregation behavior of the translocated chromosomes and their normal homologues is discussed.

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CASE REPORT

The propositus, R.H.P., born in September 1970, is affected by Down syndrome. He is the only son of phenotypically normal and nonconsanguineous parents. His mother and father were aged 20 and 24 respectively, at his birth. The father presents seizures (epilepsy?). There is no history of abortions. No other cases of mongolism are known in the family.

The propositus was born after approximately 35 weeks of an uneventful pregnancy and a normal delivery; birth weight was 2,800g. On the second day of life he developed jaundice which regressed after three days of phototherapy. He held his head up at five months, sat at eight months and walked when he was 18 months old. At three years of age he can say some intelligible words, but is unable to make up a sentence.

At physical examination at five months of age, he showed generalized hypotonia. Weight was 6,500g, height, 60cm (below the third percentile) and head circumference was 39cm (below the 2nd percentile). He had brachicephaly and a flat occiput. His flattened facies was typical of mongolism. He had bilateral epicanthal folds, mongoloid slanting of palpebral fissures, flat nasal bridge, protruding tongue, narrowly arched palate and micrognathism. His ears appeared normally implanted and differentiated. The neck was short. Chest and abdomen were unremarkable. He had a small penis and descended testes. The hands were short; both showed clinodactily of the fifth digit; a simian crease was present on the right hand and a transitional crease on the left hand. The hallux and the second toe were widely separated bilaterally.

Dermatoglyphics are summarized in Table I. The Walker index (Walker, 1957) was + 2,61, supporting the diagnosis of mongolism.

Table I - *Dermatoglyphic findings*

	Digits					atd		Hallucal
	I	II	III	IV	V	angle	Palmar formula	areas
R	U	U	U	U	U	102º	9(10).X.5"(6).5' -t"-A^c/L^u. 0.0.0.0	A^t
L	U	U	U	U	U	97.5º	11(10).X.7(6).5' -t" -A^c/L^u. 0.0.0.V	A^t

Cytogenetic studies

Chromosome analyses were performed in peripheral blood lymphocytes. Chromosomes were identified through Q-banding (Caspersson *et al.*, 1971) and G-banding (Summer *et al.*, 1971). In routine Giemsa-stained preparations, the propositus showed 47 chromosomes including an extra chromosome in the D group; in addition, one of the shortest chromosomes in the C group was metacentric (Fig. 1). The mother and maternal uncle of the propositus, both with 46 chromosomes, had the same described abnormalities in the C and D groups, and a missing G chromosome (Fig. 2). The banding patterns of the chromosomes of the mother of the propositus (Figs. 3 and 4) showed that a chromosome 21 was missing and that there was only one chromosome with the typical pattern of chromosome 6. One arm of the metacentric C had the banding pattern of the short arm of chromosome 6, and the pattern in the other arm corresponded to that of the proximal half of the normal long arm of that chromosome. One of the large acrocentrics differed from the D's: the banding in the proximal one third of its long arm was similar to that in the proximal segment of the long arm of a chromosome 21 and the pattern in its distal two thirds was comparable to that of the distal one half of the long arm of a normal 6.

The father and the maternal grandmother of the propositus had normal karyotypes. No other relatives were available for examination.

DISCUSSION

We interpreted the abnormal chromosomes as resulting from a reciprocal translocation between the long arms of a chromosome 6 and 21. The banding studies allow the localization of the breakpoints approximately at the middle of the long arms of both chromosomes, namely 6q21 and 21q22. The exchange of segments led to the formation of a metacentric C (der 6) and an acrocentric similar to those in the D group (der 21). Since the propositus has both translocated chromosomes and two free chromosomes 21, he is a trisomic 21 with a 47, XY, -6, + der (6), + der (21), t (6;21) (q21; q22) karyotype. His mother and maternal uncle are balanced carriers with a 46, XX (or XY), t (6; 21) (6 pter → 6q21::21q22 → 21qter; 21pter → 21q22::6q21 → 6qter) karyotype. The translocation should therefore be

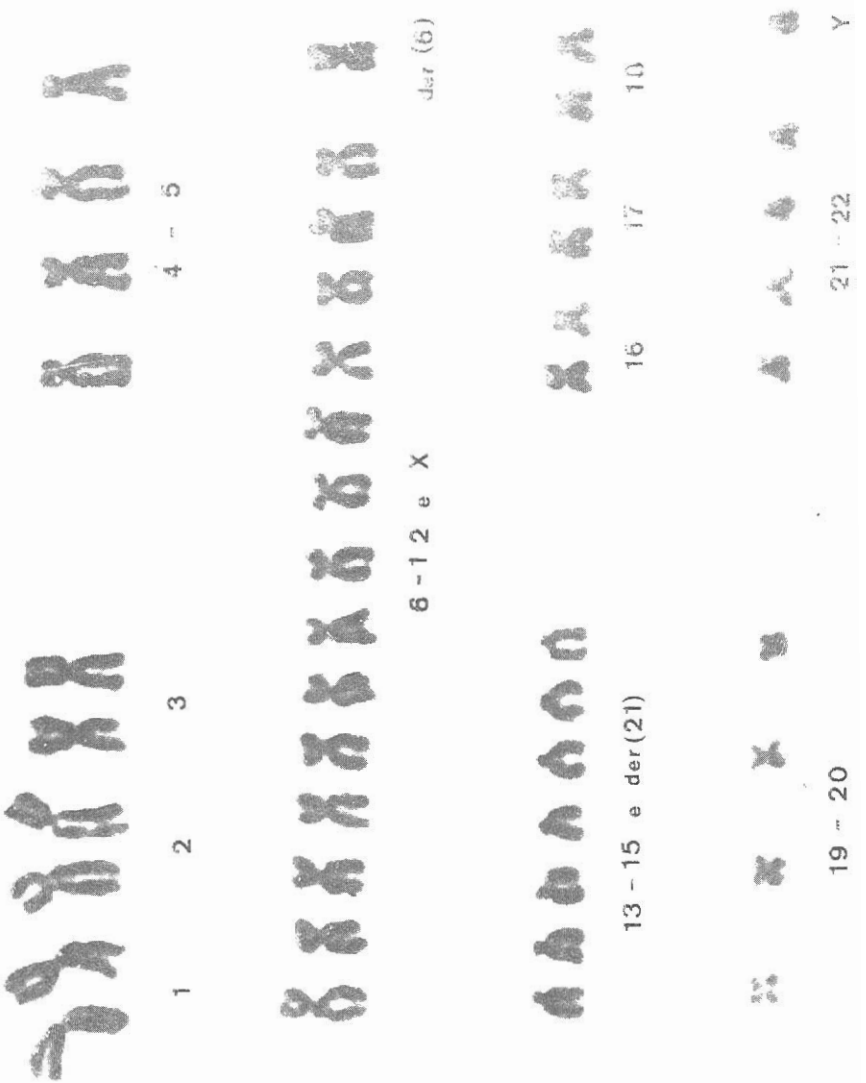

Figure 1. Karyotype 46, XY, -6, + der (6), + der (21), t (6;21) (q21; q22) of the propositus.

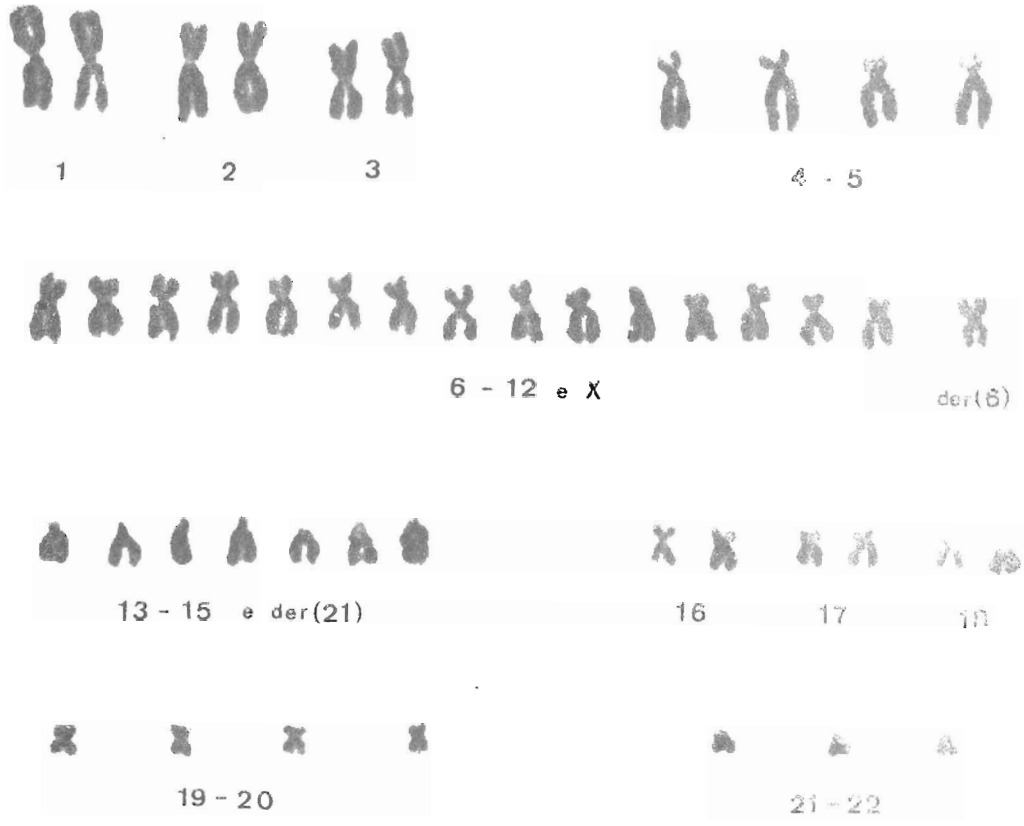

Figure 2. Karyotype 46, XX, t (6;21) (q21; q22) of the propositus' mother.

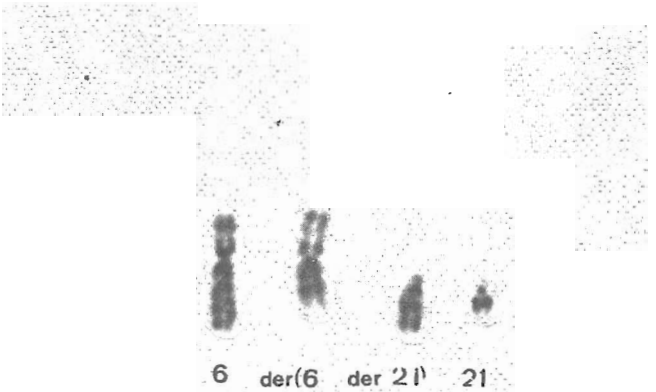

Figure 3. G-bands of the translocated chromosomes and their normal homologues from the propositus' mother.

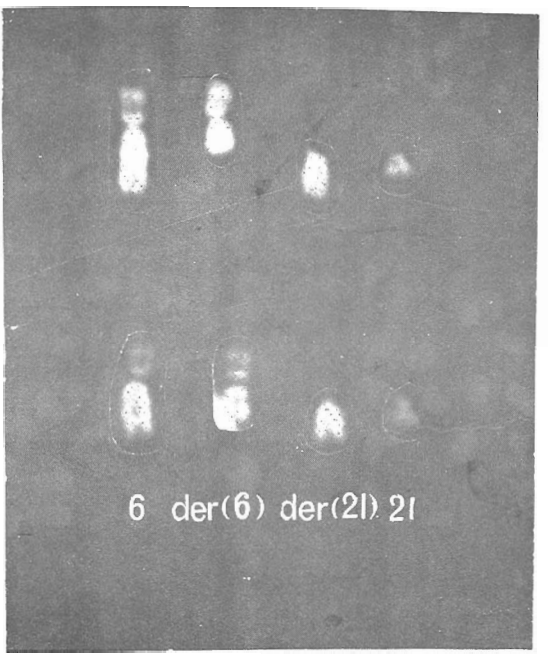

Figure 4. Q-bands of the translocated chromosomes and their normal homologues from two cells of the propositus' mother.

present in the previous generation, and since the maternal grandmother has a normal karyotype, the noninvestigated maternal grandfather is, most probably, a balanced carrier.

The abnormal karyotype of the propositus presumptively resulted from a 3:1 segregation of the translocation chromosomes and their homologues during maternal gametogenesis. Segregation of this type may result from two mechanisms: a) a discordant orientation of the quadrivalent (a chain IV); or b) the formation of a trivalent (chain III) and a univalent (I), the latter passing to either pole at random.

Hamerton (1971) studied the segregation of reciprocal translocations in 71 families. Eleven of these had unbalanced progeny with 47 or 45 chromosomes resulting from 3:1 segregation. He suggested that reciprocal translocations showing 3:1 disjunction are characterized by a) extreme asymmetry, b) involvement of an acrocentric and c) the presence of very short interstitial segments. Lindenbaum and Bobrow (1975) evaluated the factors that seemed to predispose to 3:1 disjunction, comparing a series of 37 translocations disjoining 3:1 with a control group of 23 translocations segregating 2:2. They concluded that the "extreme asymmetry" of Hamerton (1971) must refer to gross disparity in the length of the chromosomes involved, and not to the unequal length of the exchanged segments or to the asymmetry at pachytene. In addition, this disparity appeared to predispose to interchange trisomy, i.e., complements in which the two translocation chromosomes are present together with one of their normal homologues, as in the present case. Lindenbaum and Bobrow (1975) also observed that, among the acrocentrics, the G-group chromosomes were those more frequently involved in the 3:1 series. Different viability may explain this observation. Alternately, it may reflect the fact that the involvement of a G chromosome in a translocation assures the presence of short interstitial and pairing segments in which chiasmata are less likely to occur, thus predisposing to the formation of a chain III and a univalent. The present translocation fits the conditions for a 3:1 segregation, since a) the chromosomes involved differ markedly in length, b) one of them is a small acrocentric and c) there is one short interstitial segment and one short pairing segment.

Other familial translocations involving a C and a G chromosome and ascertained through unbalanced offspring with 47 chromosomes were described by Pfeiffer *et al.* (1967), Weiss and Wolf (1968), Mikkelsen (1969), Vianna (1970), Giraud et al. (1974), Lidenbaum and Bobrow (1975). In the case of Pfeiffer *et al.* (1967), the translocation chromosomes were identified

through autoradiography as a 6 and a 21, as in the present report. In the case of Giraud et al. (1974), the chromosomes involved were a 7 and a 21 and the probable breakpoints were localized at 7q22 and at the tip of the long arm of chromosome 21, according to the results of Q-and R-banding. The two translocations described by Lindenbaum and Bobrow (1975) were identified by Q- and G-banding, respectively, as t (7; 21) (q31; q22) and t (7; 21) (p22; q22).

As to the empirical risk for further unbalanced livebirths in families showing 3:1 disjunction, it appears to be similar to that estimated for carriers ascertained through 2:2 malsegregation (Lindenbaum and Bobrow, 1975), namely 10-20% for carrier females and 5-10% for carrier males.

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