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TRYPANOSOMA CRUZI : MEASUREMENT OF DNA QUANTITY IN DIFFERENT ISOENZYMIC STRAINS. INFERENCES ON THE PLOIDY OF THESE STRAINS

by

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Summary — The DNA quantity in different isoenzymic strains of *Trypanosoma cruzi* appears to be the same among these strains. This seems to indicate that these strains have the same ploidy. Genetic distances may be used for the taxonomy of the isoenzymic strains of *T. cruzi*.

KEYWORDS : *Trypanosoma cruzi*; DNA quantity; Isoenzymic Strains; Ploidy; Genetic Distance.

Introduction

Based on the genetic analysis of *Trypanosoma cruzi* zymograms (Tibayrenc *et al.*, 1981a, 1981b) we proposed the hypothesis of diploidy for this parasite. Lanar *et al.* (1981) arrived at the same conclusion by a study of *Trypanosoma cruzi* DNA. Nevertheless, our observations were principally based on one of the main Bolivian isoenzymic strains, which showed numerous heterozygous loci (strain 2). The other main strain (strain 1) was almost totally homozygote. The quantity of DNA in both strains was compared in order to verify the constancy of ploidy within the *T. cruzi* complex.

Material and Methods

— Origin of the stocks :

Table 1 summarizes the origin of the stocks used. Two stocks of each Bolivan isoenzymic strain were studied; two laboratory reference stocks,

TABLE 1

Origin of the stocks examined

Stock	Geographical origin §	Host
C8	Chiwisivi	*T. infestans*
C37	Chiwisivi	*T. infestans*
Tehuentepec	Mexico (Brumpt)	Triatominae
C80	Chiwisivi	*T. infestans*
92-80	Santa Cruz	Human
Tulahuen	Chile	?

§ Chiwisivi : a river valley at 60 km S.E. of La Paz, altitude 2700 m.
Santa Cruz : 400 km S.E.E. of La Paz, altitude 400 m.

closely related to each strain, were added for comparison. Bolivian stocks C8, C37 and C50 were isolated from *Triatoma infestans* using a method described elsewhere (Tibayrenc *et al.*, 1982). 92-80 was a human stock. The laboratory reference stocks were Tehuentepec and Tulahuen. Isoenzymic classification (Tibayrenc *et al.*, 1983) : C8 and C37 are referred to Bolivian homozygous strain 1. C50 and 92-80 are referred to Bolivian heterozygous strain 2. The genetic distance (average number of codon differences per gene between two populations (Nei, 1972; Tibayrenc, 1980) between these two strains, measured on 12 isoenzymic loci, is very high : about 1.7. Tehuentepec is closely related to strain 1 and Tulahuen to strain 2 (only one allelic difference : genetic distance about 0.03) (see matrix).

Matrix of genetic distances

	Strain 1	Tehuentepec strain	Strain 2
Strain 1	0		
Tehuentepec	0.03	0	
Strain 2	1.7	1.7	0
Tulahuen	1.7	1.7	0.03

— Culture *in vitro* :

For each strain, 200 ml synchronous cultures were obtained on GLSH monophasic medium at 28 °C (Jadin & Wéry, 1963; Jadin & Le Ray, 1969). The parasites were centrifugated at 2.000 g for 15 min. The supernatants were discarded and the pellets were washed four times with a Hanks-Wallace solution (Hanks & Wallace, 1949) in order to discard the culture medium contaminants. Washing solutions were cooled to 4 °C in order to minimize the spontaneous lysis of the parasites. The culture forms were then resuspended in a 1 per cent NaCl solution at a ratio of 1 ml per gram (wet weight) of packed cells. The suspensions were then frozen and stored at − 70 °C until use.

— Nucleic acid and protein analyses :

A weighted quantity of organisms of each stock was first extracted three times with 0.2N perchloric acid at + 4 °C. It was agitated for 30 min. at + 4 °C, then centrifuged at 3.000 g for 15 min. The supernatant was discarded and the pellet was again twice extracted with 0.5 N perchloric acid at + 70 °C. It was agitated for 30 min. and then centrifuged at 3.000 g for 15 min. The supernatants were combined and total nucleic acid was estimated by absorption at 260 nm (Gales & Folkes, 1953), and DNA by the diphenylamine method (Burton, 1956). Purified calf thymus DNA was used as a standard. RNA content of organisms was taken to be the difference between the two values obtained. Protein of the pellet remaining after the hot perchloric acid extractions, was estimated by the Folin method (Lowry *et al.*, 1951). Crystalline bovine albumin (Sigma) was used as a standard. All determinations were made three times.

— Statistical analysis :

All paired results were compared by Student's t-test, with a Hewlett-Packard computer (Series n° 9825A). We verified that the variances were

comparable by means of F test ($P < 2$ per thousand) and supposed that the populations followed the Normal Law.

TABLE 2

Protein and nucleic acid contents of the stocks examined

Stock	Isoenzymic strain	Wet weight (mg)	Protein/mg of (μg) wet weight	RNA/mg of (μg) wet weight	DNA/mg of (μg) wet weight	DNA/mg of (μg) Prot.
C8	1	220	30.00 ± 0.86 (3)	5.45 ± 0.17 (3)	1.64 ± 0.09 (3)	54.5 ± 1.55 (3)
C37	1	225	28.44 ± 0.72 (3)	5.11 ± 0.27 (3)	1.67 ± 0.08 (3)	58.8 ± 1.21 (3)
Tehuentepec	1 (1)	303	29.10 ± 1.04 (3)	5.28 ± 0.19 (3)	1.58 ± 0.1 (3)	54.5 ± 1.24 (3)
Tulahuen	2 (2)	292	25.30 ± 0.88 (3)	3.94 ± 0.31 (3)	1.32 ± 0.07 (3)	51.9 ± 1.19 (3)
C50	2	209	29.66 ± 0.78 (3)	3.83 ± 0.25 (3)	1.61 ± 0.08 (3)	54.2 ± 1.31 (3)
92-80	2	239	26.40 ± 0.72 (3)	4.18 ± 0.18 (3)	1.46 ± 0.08 (3)	55.2 ± 1.58 (3)

(1) Closely related to strain 1.
(2) Closely related to strain 2.
(3) Mean, standard deviations and number of determinations.

TABLE 3

Comparison of all pair results by student's T-test

Isoenzymic strain	Protein/mg of (μg) wet weight		RNA/mg of (μg) wet weight		DNA/mg of (μg) wet weight		DNA/mg of (μg) protein	
	M (1)	P (2)	M	P	M	P	M	P
Isoenzymic strain 1	29.18		5.28		1.63		55.93	
		0.05		0.001		0.05		0.05
Isoenzymic strain 2	27.12		3.98		1.46		53.76	

(1) Arithmetic mean.
(2) Probability; comparison of two means by student's T-test.

Results and discussion

The results are summarized in tables 2 and 3; there are little differences between the average concentrations of DNA (per mg of protein and per mg of wet weight) of the two strains. Student's t-test shows that these differences are not significant. The constancy of DNA concentration among isoenzymic strains of *T. cruzi* suggests that the different strains have the same ploidy. The ratio of kinetoplastic DNA is indeed about 20 per cent of the total DNA. So, if a strain were haploid and the other were diploid, the variability in DNA quantity between them would be much higher (Ayala, 1982). One possible objection is that the cells of one strain could be much larger than the ones of the other strain. But all the isoenzymic strains of *T. cruzi* seem to have comparable cell size (Miles, personal communication; Tibayrenc, unpublished data). Other studies on *T. cruzi* DNA (see for example Dvorak *et al.*, 1982) concern the concentration of DNA in individual cells of *T. cruzi.* This kind of technique is very useful to detect slight differences of DNA concentration. But in our study, it is not at all indispensable as we looked for *important* differences of DNA amount. Thus an average concentration (DNA/mg of protein and DNA/mg of wet weight) was a sufficient determination. Nevertheless, our study is not a proof for diploidy in *T. cruzi.* It is only an evidence for the same ploidy among the different isoenzymic strains.

One may notice that protein quantity is also rather constant among the different strains. This is not the case for RNA concentration : there are significant differences between the stocks of strain 1 and the stocks of strain 2. We hare no explanation for this.

Conclusion

Measurement of DNA quantity for different isoenzymic strains of *Trypanosoma cruzi* indicates that these strains have the same ploidy : they are consequently probably all diploid. This fact confirms our former genetic interpretations (Tibayrenc *et al.*, 1981 a & b; Tibayrenc & Miles, 1983) and allows the use of genetic distances for a phylogenetic classification of the isoenzymic strains.

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Trypanosoma cruzi : **mesure de la quantité d'ADN chez des souches isoenzymatiques différentes. Inférences sur la ploïdie de ces souches.**

Résumé — La quantité d'ADN chez des souches isoenzymatiques différentes de *Trypanosoma cruzi* semble être la même. Cela paraît indiquer que ces souches ont la même ploïdie. Ce résultat autorise l'utilisation des distances génétiques pour la taxonomie des souches isoenzymatiques de *T. cruzi*.

Trypanosoma cruzi : meting van de hoeveelheid DNA in verschillende isoenzyme stammen. Betekenis voor de ploidie van deze stammen.

Samenvatting — De hoeveelheid DNA in verschillende isoenzyme stammen van *Trypanosoma cruzi* was gelijk. Dit wijst erop dat deze stammen dezelfde ploidie hebben. De genetische relatie kan gebruikt worden voor de taxonomie van isoenzyme stammen van *T. cruzi*.

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