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## POPULATION STRUCTURE OF *TRYPANOSOMA BRUCEI* S. L. IN CÔTE D'IVOIRE ASSAYED BY MULTILOCUS ENZYME ELECTROPHORESIS: EPIDEMIOLOGICAL AND TAXONOMICAL CONSIDERATIONS

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**ABSTRACT:** Fifty-two *Trypanosoma brucei* stocks isolated in Côte d'Ivoire from sympatric locations were analyzed by cellulose acetate electrophoresis of isoenzymes. Of 13 genetic loci surveyed, 5 appeared as variable, which made it possible to delimit 12 different zymodemes. The most abundant zymodeme involved stocks isolated from both humans and pigs, which is consistent with the hypothesis that pig is a reservoir of human African trypanosomiasis in Côte d'Ivoire, as already proposed by other authors. Population genetic analysis of the isozyme data indicated a strong linkage disequilibrium, which suggests that genetic recombination is severely restricted in this sample and favors the hypothesis that the trypanosome populations surveyed are basically clonal. Nevertheless, additional studies are required to better estimate the long-term stability of these clones and the possible interference of gene exchange at an evolutionary scale. The results corroborate the hypothesis that a majority of human *T. brucei* stocks from West Africa correspond to a fairly homogeneous cluster of genotypes (*T. brucei gambiense* Group I, Gibson, 1986).

Since the pioneering work by Godfrey and Kilgour (1976), isozyme typing has been widely used for strain identification of *Trypanosoma brucei*, the agent of human African trypanosomiasis (HAT) (for recent review see Godfrey et al. [1990]). This made it possible to improve our knowledge on the epidemiology of HAT and on the basic biology of its causative agent.

*Trypanosoma brucei* mating system and population structure are still subjects of debate. The question of whether this parasite is sexual or not is epidemiologically relevant. If *T. brucei* populations undergo regular mating, this parasite's genotypes are ephemeral individual variants that cannot be used as markers for tracking epidemic outbreaks. The hypothesis that *T. brucei* is a sexual organism was first proposed by Tait (1980). Jenni et al. (1986) then successfully obtained recombinants in laboratory experiments. However, Cibulskis (1988) first proposed that gene exchange is restricted in *T. brucei*, which leads to the separate evolution of distinct parasitic lines. Tibayrenc et al. (1990) and Tibayrenc et al. (1991) then postulated that *T. brucei* is basically a clonal organism, similar to several other major parasitic protozoan species. Cibulskis (1992) later suggested

### MATERIALS AND METHODS

Table I gives the geographic origin, host, and date of isolation of the stocks studied. Two main regions were surveyed, the Daloa region close to the center of the country, and the Aboisso region, at the southeast corner of the country. The Tagoura locality is very close to the town of Daloa, while the Zoupkangou locality is located about 30 km west of Daloa. The Songan locality is located about 120 km north of the town of Aboisso. Primary isolates were intraperitoneally inoculated to either mice or *Mastomys natalensis*. Depending on the stocks inoculated, some of the experimental hosts were submitted to an immunosuppressive treatment through a single injection of 80 mg/kg cyclophosphamide (Endoxan®). Stabilates so obtained were reinoculated into Wistar rats, controlled until a parasitemia of  $10^8$ – $10^9$  parasites/ml was obtained. Trypanosomes were separated from blood on a DEAE-cellulose column (Lanham and Godfrey, 1970), harvested by centrifugation, and lysed by addition of an equal volume of hypotonic enzyme stabilizer (Godfrey and Kilgour, 1976). After centrifugation at 13,500 g, the supernatant was recovered and stored at  $-70$  °C until used for isozyme electrophoresis.

Cellulose acetate electrophoreses (Helena laboratories, Beaumont, Texas) were carried out according to Truc et al. (1991). The 11 enzyme systems used are listed in Table II.

Specific population genetic tests (Tibayrenc et al., 1990; Tibayrenc et al., 1991) were performed with special Turbo Pascal programs in order to explore the population structure of the sample. These tests

TABLE I. List of the 52 stock studied, with geographical origin, host, zymodeme determination (see Table IV), and date of isolation.

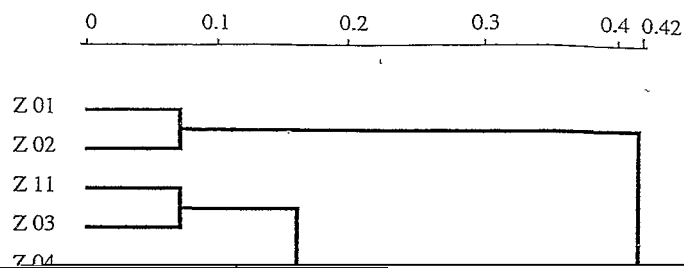
Stock	Host	Region	Zymo- deme	Date
HABS.I 1006	Human	SONGAN	1	7/11/86
HABS.I 1020	Human	SONGAN	2	7/11/86
HABS.II 0740	Human	ABOISSO	2	27/01/87
HPRCT 0494	Human	DALOA	1	?
HPRCT 1065	Human	DALOA	1	?
HPRCT 1072	Human	DALOA	2	?
HTAG 107/1	Human	TAGOURA	3	10/01/86
HTAG 128/6	Human	TAGOURA	3	11/01/85
HTAG 015/5	Human	TAGOURA	3	8/03/85
HZOUK 51/5	Human	ZOUKPANGBEU	2	28/11/84
PTAG 007	Pig	TAGOURA	4	6/01/85

TABLE II. Enzyme systems studied.

Enzyme system	Enzyme	E.C. number
Glyceraldehyde-phosphate dehydrogenase	GAPDH*	E.C.1.2.1.12
Glutamate oxaloacetate transaminase	GOT*	E.C.2.6.1.1
Glucose phosphate isomerase	GPI*	E.C.5.3.1.9
Isocitrate dehydrogenase	IDH*	E.C.1.1.1.42
Malate dehydrogenase	MDH	E.C.1.1.1.37
Malic enzyme	ME*†	E.C.1.1.1.40
Nucleoside hydrolase	NHi*†	E.C.3.2.2
Peptidase 2 (Leu-Ala)	PEP2	E.C.3.4.11 or 13
Phosphoglucomutase	PGM*	E.C.2.7.5.1
Threonine dehydrogenase	TDH*	E.C.1.1.1.10
6 Phosphogluconate dehydrogenase	6PGD*	E.C.1.1.1.44

TABLE IV. Isozyme profiles of the 12 zymodemes recorded for the 5 variable enzyme systems, with the corresponding number of stocks (last column).\*

Zd	Me 2	Pep 2	Got	Nh 2	Pgm	Size
1	1/3	II	1/1	1/1	1/2	3
2	1/3	II	1/1	1/1	2/2	4
3	1/1	I	1/1	2/2	2/2	21
4	1/1	I	2/2	2/2	2/2	9



heterogeneous *T. brucei gambiense* 'Group 2' (Gibson, 1986). In cluster 2, it is worth noting that the dominant genotype (zymodeme 3) has been recorded both from humans and pigs in the same locality, namely Tagoura (Fig. 1). This is consistent with the hypothesis that the pig is a reservoir for HAT in Côte d'Ivoire and, hence, confirms previous observations (Gibson et al., 1978, 1980; Mehlitz et al., 1982). Regarding the possible role of the pig as a reservoir, it is worth noting that apart from

ants within the subgenus *Trypanozoon*. *Advances in Parasitology* **29**: 1-74.

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