Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 45 (1988)
Heft: 1

Artikel: Nuclear DNA content of "Trypanosoma congolense"
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DOI: https://doi.org/10.5169/seals-314055

Acta Tropica 45, 27–31 (1988)

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Nuclear DNA content of *Trypanosoma congolense*

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Summary

We have measured the nuclear DNA content of the major life cycle stages of *Trypanosoma congolense,* in two clones of geographically distant origin. We find that nuclear DNA content in epimastigote, mammalian blood-stream and metacyclic forms is constant and that the nuclear DNA contents of the two clones were 0.1 pg and 0.09 pg, respectively.

Key words: *Trypanosoma congolense;* nuclear DNA content; microdensitometry.

Introduction

The parasitic protozoan, *Trypanosoma congolense,* causes a major cattle disease problem in tropical Africa. Following the demonstration of a sexual cycle in the related trypanosomatid parasite, *T. brucei* (Jenni et al., 1986), we have been interested in the possible occurrence of similar processes in *T. congolense.* As mixed infections of tsetse flies with *T. congolense, T. brucei* and *T. vivax* have been reported (Moloo et al., 1982), it is likely that mixed *T. congolense* infections occur, and if so a genetic exchange system would have important implications for the epidemiology of the disease, in terms of the spread of surface antigen variants, virulence and drug resistance. In the absence of chromosome condensation in trypanosomes, three methodologies are available to resolve the question of whether a sexual cycle exists. Firstly, the analysis of polymorphic markers such as isoenzymes or DNA probes as has been undertaken with *T. brucei* (Tait, 1983; Gibson et al., 1985); secondly the demonstration of the formation of hybrids by mixing different genetically marked stocks as has been shown with *T. brucei* (Jenni et al., 1986); and thirdly the demon-

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stration of a "gamete" like stage either morphologically or by the observation of a reduction of nuclear DNA content by a factor of two. This latter approach has also been undertaken with *T. brucei* and Zampetti-Bosseler et al. (1986) showed that the metacyclic stage of *T. brucei* had a 50% lower nuclear DNA content than mammalian bloodstream forms or tsetse fly proventricular forms. It was argued that the metacyclic trypanosomes were products of meiosis in the fly salivary glands and that the fusion of the haploid metacyclics in the mammalian host restored the DNA content to the normal value and completed the sexual cycle. The possibility of a similar process occurring during metacyclogenesis in *T. congolense,* and the fact that each life cycle stage of this parasite can be cultured and cyclically differentiated in vitro led us to undertake measurements of nuclear DNA content in metacyclic, epimastigote and mammalian bloodstream stages.

Materials and Methods

Source of trypanosome clones

TREU 1676 was a cloned derivative of the Tanzanian isolate Serengeti/66/LUMP/12 and was cited as TREU 1468 by Luckins and Gray (1983). TREU 1627 was a cloned derivative of the Gambian isolate Kantong Kunda/77/LUMP/1794 (Ross et al., 1985).

Trypanosome culture

Cultures of *T. congolense* insect forms were maintained at 28°C in Eagles Minimal Essential Medium supplemented with 20% fetal calf serum (FCS) and 4 mM glutamine, using the methods of Gray et al. (1981, 1984). Epimastigote forms were obtained from the supernatants of 4–6-day-old cultures when virtually all the trypanosomes present are in this life cycle stage. Supernatants of cultures older than 10 days also contain the metacyclic forms, and these were separated from the other trypanosomes on DE-52 anion exchange columns (Gray et al., 1984).

Cultures of bloodstream forms were initiated by introducing column separated metacyclic trypanosomes to bovine aorta endothelial cell monolayers (Gray et al., 1984). Bloodstream forms were harvested for DNA analysis at least 8 days after growth with feeder cells.

DNA content measurements

Trypanosomes of the different life cycle stages were suspended in PBSG (60 mM phosphate, 45 mM NaCl, 1% glucose pH 8.0) and mixed with appropriately diluted fresh chicken blood to provide chicken erythrocyte nuclei as standards on all slides (2.5 pg DNA/nucleus; Rasch et al., 1971). The mixture was allowed to settle on a microscope slide. After 10 min the slides were immersed in 50% (v/v) EtOH for 30 sec and then 90% (v/v) EtOH for 5 min, all at room temperature, and air dried. The slides were then feulgen stained as described by Borst et al. (1982). Briefly, the slides were subjected to hydrolysis in 5N HCl for 60 min at room temperature and washed extensively in distilled water. The slides were then immersed for 60 min in feulgen reagent preapared as follows. 1 g of pararosaniline (Sigma) was dissolved in 85 ml H_2O. To this 1.8 g of sodium metabisulphite and 15 ml 1N HCl was added and the mixture left for 3 days with intermittent shaking at room temperature. The resulting reagent was decolourised with activated charcoal and filtered.

After staining, the slides were extensively washed in water, and then air dried under DPX mountant (Gurr). Integrated optical density measurements were made of the trypanosome nuclei using a Vickers M86 scanning microdensitometer. The scanning spot size was 0.5 μm, with a wavelength of 560 nm. A measuring window was chosen which allowed the optical density measurements of the nuclei to be free from interference from the kinetoplast. In all measurements, local background corrections were made.

Results

The quantitative reaction of the feulgen reagent with DNA was used to determine the quantity of DNA per trypanosome nucleus. Using a scanning microdensitometer, the integrated optical density of each nucleus could be measured, and the reference standard chicken erythrocyte nuclei included in all slides allowed the determination of absolute DNA content per trypanosome nucleus. The trypanosomes were grown under the different culture conditions described in Materials and Methods to provide homogeneous epimastigote, blood-stream form or metacyclic trypanosomes. Microscopic examination of the various populations showed no contamination with different life cycle stages.

Fig. 1 shows histograms representing the frequency of measured nuclear DNA content in the different life cycle stages of the two cloned stocks examined, TREU 1676 and TREU 1627. Metacyclic trypanosomes are considered

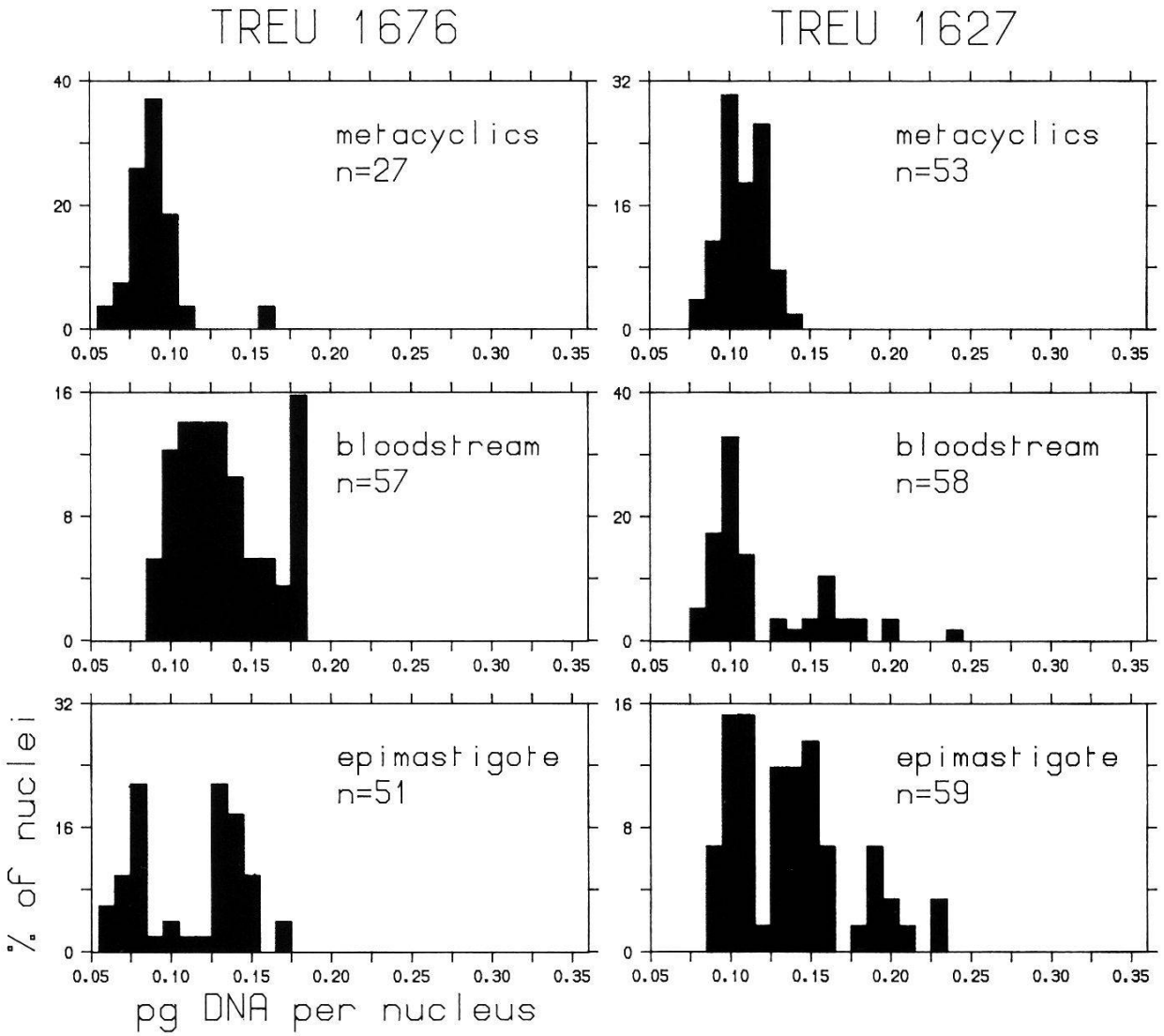

Fig. 1. Histograms showing the nuclear DNA content of metacyclic, epimastigote and blood-stream forms of TREU 1676 and TREU 1627.

to be non-dividing forms (Shapiro et al., 1984), and examination of the nuclear DNA content distribution for both stocks analysed in the metacyclic form shows a modal distribution, with a peak at 0.09 pg/nucleus for TREU 1676 and 0.1 pg/nucleus for TREU 1627. In both cases the standard deviation is 0.015 pg and this is to be expected from considerations of the inherent "noise" in the cytophotometry system.

The distributions of nuclear DNA content for the blood-stream and epimastigote forms are more complex. These trypanosomes are actively dividing forms, however the proportions of cells in the G_1, S and G_2 phases of the cell cycle are influenced by conditions in the culture from which they were isolated. Although the shape of the distribution is therefore variable, a range of nuclear DNA contents from a G_0/G_1 value to a value double this for G_2 nuclei is expected. Taking the TREU 1676 trypanosomes first, the blood-stream forms show a range of nuclear DNA content of 0.09–0.18 pg. This confirms the DNA content derived from the metacyclic trypanosomes of 0.09 pg DNA/nucleus for G_0/G_1 cells. The epimastigote population shows a range of nuclear DNA content from 0.06–0.17 pg/nucleus. This distribution is apparently shifted to values around 0.02 pg lower than would have been expected for a nuclear DNA content of 0.09 pg/nucleus. As there is no evidence for such an effect in the TREU 1627 epimastigotes (see below) this shift may be due to a sampling error. This might be expected to happen if the culture from which the trypanosomes were derived was largely stationary.

In the case of the TREU 1627 trypanosomes, the blood-stream forms show a range of nuclear DNA content from 0.08–0.24 pg. The first peak value is 0.1 pg/nucleus which is in accordance with the metacyclic derived value for G_0/G_1 nuclei, and this suggests that the trypanosomes at 0.2 pg DNA/nucleus are G_2 cells. The isolated peak at 0.24 pg/nucleus probably represents cells reentering S phase before the completion of nuclear division at telophase. The epimastigote population of TREU 1627 shows a range of nuclear DNA content from 0.09–0.23 pg/nucleus. Again, the value for the first peak is in accordance with a G_1 nuclear DNA content of 0.1 pg/nucleus and the smaller peak around 0.2 pg/nucleus represents the G_2 nuclear DNA content. As discussed above, the trypanosomes at 0.23 pg/nucleus are probably derived from a new round of DNA synthesis before the completion of nuclear division.

From these measurements, we have shown that clones of two *T. congolense* isolates from widely separated regions of Africa (Tanzania and the Gambia) have a similar nuclear DNA content. Also, throughout the life cycle stages observed, no evidence for a change in nuclear DNA content was found, except for a possible light fall in TREU 1676 epimastigotes. It has been postulated by Zampetti-Bosseler et al. (1986) that in *T. brucei,* the metacyclic form is haploid and represents a "gamete" stage in a sexual cycle, although the only direct evidence for genetic exchange, based on the isolation of metacyclic clones (Jenni et al., 1986) shows that hybrid formation occurs prior to metacyclogene-

sis. Our data shows that in *T. congolense,* metacyclic trypanosomes are not haploid and are therefore unlikely to be a sexually differentiated stage.

The question of the existence of a sexual cycle in *T. congolense* remains open and requires further studies of both allele frequencies and allelic combinations in natural populations as has been undertaken in *T. brucei* (Tait, 1983) in addition to the direct demonstration of mating.

Acknowledgments

This work was supported by grants from the Wellcome Trust (J. S. and A. T.) and the U.K. Overseas Development Agency (C. R.).

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