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SEXUAL STAGES IN TRYPANOSOMES AND IMPLICATIONS

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SUMMARY

The basis for concluding that a system of genetic exchange exists in African trypanosomes of the *Trypanosoma brucei* group is outlined by the accumulated data from variation in natural parasite populations and from laboratory crossing experiments. The results indicate that these parasites can complete their life cycle either with or without genetic exchange. This sexual event involves meiosis and syngamy, but the order of these processes and the sexual stages involved are not yet known.

RÉSUMÉ : **Stades sexuels chez les trypanosomes et perspectives.**

Les bases pour conclure à un système d'échange génétique chez les trypanosomes africains du groupe *Trypanosoma brucei* sont fournies par l'accumulation des données recueillies sur les variations observées en milieu naturel chez les populations de parasites et par les expériences de croisement génétique en laboratoire. Les résultats indiquent que ces parasites peuvent compléter leur cycle de vie avec ou sans échange génétique. Cette mutation sexuelle implique méiose et syngamie, mais l'ordre de ces processus et les stades sexuels impliqués ne sont pas encore connus.

REPRODUCTION AND SEXUALITY IN AFRICAN TRYPANOSOMES

Trypanosomes of the subgenus *Trypanozoon* primarily replicate by binary fission and have a complex life cycle in the *Glossina* vector and in mammalian hosts. In both groups of hosts, they differentiate into several morphologically distinct forms. In the following, the name *Trypanosoma brucei* will be used in the broad sense, *i. e.* include all subgenus types *(T. brucei, T. rhodesiense* and *T. gambiense).*

Although much is known about the cyclical development of *T. brucei,* the important question of whether these organisms pass a sexual cycle has been debated since the original description of these parasites (Baker 1989, Tait 1983).

The existence of genetic exchange between *T. brucei* trypanosomes could account for the evolution of extensive molecular variation and polymorphism observed (Gibson *et al.* 1980). Direct observations of meiosis and mitosis are lacking as trypanosome chromosomes do not condense throughout the cell division cycle and thus cannot be visualized by classical cytological methods. However, indirect evidence of genetic exchange has been obtained from the analysis of enzyme electrophoretic variation between natural parasite populations from field isolations (Gibson *et al.* 1980, Tait 1980). Tait (1983) compared the observed and expected frequencies of iso-enzyme patterns of *T. brucei* populations and he found suggestive evidence for the existence of a gene exchange system.

Essential to the analysis of genetic exchange is the knowledge of the ploidy and DNA content of the organism under study. Three independent series of experiments have revealed that *T. brucei* organisms are diploid for most genes. This conclusion is based on the analysis of enzyme electrophoretic banding patterns (Gibson *et al.* 1980, Tait 1980), the kinetic complexity of the genome and the measured DNA content (Borst *et al.* 1982) as well as on the data by Gibson *et al.* (1985) on the analysis of restriction fragment length polymorphisms (RFLP's) of single copy genes.

GENETIC EXCHANGE IN *Trypanosoma brucei*

Direct evidence for genetic exchange between two cloned populations of *T. brucei* has recently been obtained by Jenni *et al.* (1986). The cloned parental populations had been selected from a number of recent field isolates after extensive characterization by iso-enzyme electrophoresis, southern blot analysis of genes encoding for variable surface antigens, analysis of fitness for life cycle development and vector specificity. Three cloned progeny were recovered which exhibited « hybrid » characters for different genotypic and phenotypic parameters including 2D-PAGE patterns of parental clones and hybrid progeny (Pearson and Jenni 1989). However, the analysis of several cloned progeny showed that genetic exchange is not obligatory for the successful cyclical development in the fly. Hybrid

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progeny could be recovered from clones directly initiated with single metacyclic forms, which indicates that genetic exchange has occured during cyclical development of the trypanosomes in *Glossina*. The analysis of cyclically retransmitted hybrid progeny showed that the individual clones were stable with regard to their genotype and phenotype.

Tait and Turner (1990) have recently summarized and reviewed the number of crosses which have been carried out so far (Gibson 1989, Jenni *et al.* 1986, Paindavoine *et al.* 1986, Sternberg *et al.* 1988, Sternberg *et al.* 1989, Wells *et al.* 1987). Six successful experiments from different laboratories have been listed which produced 61 hybrid clones in total. In one experiment (Gibson 1989), a completely new parental pair of trypanosomes was used while in the other crosses at least one of the « original » parental clones (STIB 247 or STIB 386) was used. In addition, using *T. brucei* clone STIB 247 and another clone from East Africa (STIB 777) for simultaneous transmission, 4 new hybrid clones could be isolated from one tsetse fly (Schweizer *et al.*, unpublished) which fall into two different groups after current preliminary iso-enzyme analyses.

So far, parental clones of *T. gambiense* were not involved in any of the successful cross experiments.

Frequency of genetic exchange

The genetic exchange observed under laboratory conditions may not be a regular feature of the life cycle of *T. brucei* in a natural field environment. Although the predicted genotype frequencies were generally in a good agreement with those data obtained from natural populations, significant deviations from Hardy-Weinberg equilibrium were observed in one population of *T. brucei* (Tait 1983). Cibulkis (1988) carried out an in depth statistical analysis and he concluded that if sample size is small, agreement cannot definitely be interpreted as indicating that genetic exchange is occuring.

The frequency and timing of genetic exchange between the two « original » parental clones STIB 247 and STIB 386 has been investigated (Schweizer *et al.* 1988). Series of subsequent bloodstream populations initiated in mice by the bite of 23 infective flies were characterized by iso-enzyme electrophoresis. Both parental clones were homozygous for iso-citrate dehydrogenase (ICD) and alkaline phosphatase (AP). Heterozygous patterns for both enzymes have been found in progeny from at least 9 of the 23 tsetse flies. This indicates that the frequency of genetic exchange can be high if compatible clones develop together in the same vector at optional conditions. There was further evidence that hybrid trypanosomes were not extruded continuously but could alter over time with the detection of either or both of the parental clones. This also indicates that mating is not obligatory for cyclical development in the tsetse fly, but sexual events seem to be co-existent with asexual development in the same individual vector.

Sexual stages

Although there still exists some controversy about the mechanisms of gene exchange in *T. brucei* with regard to a classical mendelian mechanism, the basic genetic rules of the sexual process in *T. brucei* have now been established and summarized by Tait and Turner (1990). The compiled data clearly indicate that meiosis and syngamy do occur in the tsetse fly but the order of these genetic events is still not known. There is no clear cytological observation of the corresponding sexual cell types and observed variations in DNA content of some progeny are difficult to interpret. Baker (1989) has reviewed the available data on observations of several workers on particular forms which were interpreted as being involved in sexual fusions. This important lack of information clearly needs a detailed analysis and further work.

Preliminary results from experiments with the objective to localize the sexual stages of *T. brucei* in *Glossina* indicate that hybrid trypanosomes may not be formed within the midgut parts of the vector (Schweizer *et al.* unpublished). If mating represents a rare event which may not occur continuously in the same vector, the detection of the origin of gene exchange and the corresponding sexual stages may be difficult but is however indispensable for the determination of the precise mechanisms of meiosis and syngamy.

Conclusions

Genetic exchange and recombination in *T. brucei* are of fundamental importance in the generation of diversity. Organisms which possess a mating system are capable of producing a greater range of novel genotypes than those which develop by asexual reproduction only. The ability for genetic exchange could allow this group of trypanosomes to generate progeny with novel specifications such as new sets of variable surface antigens, drug resistance and resistance against normal host factors. The latter would allow the establishment in new hosts. Genetic exchange could have some implications for the successful development of new methods of disease control in the future.

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