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Allelic segregation and independent assortment in *T. brucei* crosses: Proof that the genetic system is Mendelian and involves meiosis

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Abstract

The genetic system on *Trypanosoma brucei* has been analysed by generating large numbers of independent progeny clones from two crosses, one between two cloned isolates of *Trypanosoma brucei brucei* and one between cloned isolates of *T. b. brucei* and *Trypanosoma brucei gambiense*, Type 2. Micro and minisatellite markers (located on each of the 11 megabase housekeeping chromosomes) were identified, that are heterozygous in one or more of the parental strains and the segregation of alleles at each locus was then determined in each of the progeny clones. The results unequivocally show that alleles segregate in the predicted ratios and that alleles at loci on different chromosomes segregate independently. These data provide statistically robust proof that the genetic system is Mendelian and that meiosis occurs. Segregation distortion is observed with the minisatellite locus located on chromosome I of *T. b. gambiense* Type 2 and neighboring markers, but analysis of markers further along this chromosome did not show distortion leading to the conclusion that this is due to selection acting on one part of this chromosome. The results obtained are discussed in relation to previously proposed models of mating and support the occurrence of meiosis to form haploid gametes that then fuse to form the diploid progeny in a single round of mating.

Keywords: Genetics; *Trypanosoma brucei*; Allelic segregation; Meiosis

1. Introduction

Trypanosoma brucei is a zoonotic protozoan parasite species complex transmitted by tsetse flies and comprises three subspecies. *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* cause sleeping sickness in humans whereas the third subspecies, *Trypanosoma brucei brucei*, causes cattle disease but is not infective to humans [1]. Analysis of *T. b. gambiens*e isolates using a range of different markers has lead to the definition of two discrete groups, termed Type 1 and 2 [2]. While there is considerable controversy about the existence of genetic exchange between different strains within each subspecies in the field [3–7], there is unequivocal evidence for genetic exchange when two stocks of the parasite are used to infect the tsetse fly vector in the laboratory [8–11]. As no chromosome condensation has been observed in any life cycle stage and no gamete stages have been identified [12], the main approach to determining whether this parasite has a sexual cycle and undergoes meiosis has been to undertake classical genetic analysis.

Infection of tsetse flies with two genetically different lines of trypanosomes, followed by marker analysis of the metacyclic stage derived parasites has shown that these comprise a mixture of the original two parental lines together with parasites of novel, non-parental genotypes, which are the equivalent of F1 progeny [8–11]. To date crosses have been made between 10 pairs of different stocks including *T. b. brucei* × *T. b. brucei*, *T. b. rhodesiense* × *T. b. brucei*

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and *T. b. gambiense* (Type 2) × *T. b. brucei* (reviewed in [13]). The DNA contents of the progeny from the first cross [14,15] were shown to be elevated relative to the parental lines and this has also been observed in a high proportion (average 59%, $n = 24$) of progeny from crosses between *T. b. brucei* and *T. b. rhodesiense* where marker analysis suggests that these products of mating are trisomic or triploid [13]. In contrast, crosses between either *T. b. brucei* stocks or *T. b. brucei*/*T. b. gambiense* (Type 2) rarely (none in *T. b. brucei*, $n = 14$; 14% in *T. b. brucei* × *T. b. gambiense*, $n = 22$) lead to progeny with elevated DNA content [16]. These results have led to several models of genetic exchange being proposed [12,13], one of which is a conventional Mendelian system [12] involving meiosis. However, given the small number of available progeny clones generated from each cross, it has not been possible to prove Mendelian inheritance. The importance of determining the mechanism of genetic exchange in *T. brucei* lies in understanding this fundamental biological process of the parasite, providing a framework for the analysis of the population genetics and opening up the possibility of using genetic analysis as a tool for gene discovery, as has been undertaken in *Plasmodium falciparum* [17,18], *Plasmodium chabaudi chabaudi* [19–21], *Toxoplasma gondii* [22] and *Eimeria tenella* [23]. In contrast to these haploid apicomplexan parasites, *T. brucei* is diploid and so the progeny of a cross would be expected to be heterozygous for markers that are homozygous and different between the parents but would inherit only one allele from each locus that is heterozygous in the parents. In a Mendelian system, the two alleles at each heterozygous locus would be inherited in a 1:1 ratio and those on different chromosomes would be inherited independently of each other.

In this paper, we report the isolation of a large set of independent progeny clones from two crosses (*T. b. brucei* × *T. b. brucei* and *T. b. brucei* × *T. b. gambiense*, Type 2) and the analysis of the inheritance of micro and minisatellite markers located on different housekeeping, megabase chromosomes. The results allow a statistical analysis of allele segregation and independent assortment in crosses of *T. brucei*, involving three different stocks and thus provide unequivocal evidence for the mechanism of genetic exchange.

2. Materials and methods

2.1. Crosses and the isolation of progeny

Material from two previous crosses between STIB 386/STIB 247 and TREU 927/STIB 247 was used as a source of further progeny clones. The procedures for crossing and the origins of the stocks used have been described previously [8,9,24]. Briefly, the trypanosome stocks were grown up in MF1, ICR or TO Swiss mice and the bloodstream stage trypanosomes of two stocks were mixed, fed to teneral tsetse flies and, after completion of the life cycle stages in the fly, trypanosomes were sampled by allowing each infected tsetse to feed on a mouse. The resulting parasites were purified from the mice, lysed and the genotypes present, inferred from analysis with iso-enzyme markers [9,10]. The occurrence of mating between such populations in each infected tsetse fly was detected by identification of hybrid iso-enzyme patterns in the purified trypanosomes [9,10] The populations of trypanosomes containing hybrids from each fly were cryopreserved in liquid nitrogen from the first peak parasitaemias using standard methods [9,10]. The stabilates were designated by the fly number and the day (post-fly infection) on which the trypanosomes were sampled (F9/45, etc.). In several cases, the same fly was sampled more than once (F9/45, F9/56, etc.). Several previously identified progeny, derived either directly from metacyclic stage trypanosomes or from the resulting bloodstream stage [8–10], were used together with a panel of new progeny clones (derived as described above) from the same crosses. New clones were isolated from cryopreserved uncloned populations of bloodstream stage trypanosomes of the two crosses by the identification of single bloodstream trypanosomes optically and subsequent growth in immunosuppressed mice (250 mg/kg body weight of cyclophosphamide). The stabilates used were: F974/78, F532/72, F532/63, F124/28 (STIB 247 × TREU 927) and F9/45, F492/50, F9/41, F19/31, F28/46, F29/46 (STIB 247 × STIB386). In addition, the uncloned products of mating (F532/72) were transformed in vitro to the procyclic stage and clones established from these cultures by limiting dilution in Cunningham's culture medium with 15% heat-inactivated (56 °C) foetal calf serum. The clones derived from the different life cycle stages are designate by metacyclic stage (m), bloodstream stage (bs) or procyclic stage (p) and are listed in Table 1.

2.2. DNA preparation, markers and genotyping

The parental stocks and clones derived from the two crosses were amplified in mice or by procyclic culture, lysates of partially purified trypanosomes prepared (as described previously) and used as templates for PCR amplification [24]. These preparations were genotyped by PCR amplification of the minisatellite markers, *MS42*, *292* and *CRAM* [25], the two microsatellite markers JS2 and PLC [24] as well as five new microsatellite markers identified from the genome sequence of TREU 927 ([26,27] with the programme repeat finder [28], using previously described criteria. The primer sequences corresponding to the unique sequence flanking each of the new microsatellite markers are: CHVII/29K4/A2-A 5′-aggtctaagcaatatctatgc, CHVII/29K4/A2-B 5′-gggagagatcgtttgattcc, ChIII/1J15/2-A 5′-ggtggaatggaagatcagtt, ChIII/1J15/2-B 5′-gttggaattgttgttgctgt, ChIX/1-B 5′-gatgagcaatttgtagtgcc, ChIX/2-A 5′-cttgcttactgtatgtccg, CHXI/53-A 5′-cgtgtgtcttgtatatcttct, CHXI/53-B 5′-tgaataaacaaaacatgaaacgac, ChII/A41-A 5′-caaggtctaaggaaggtcag, ChII/A41-B 5′-tcaccgccattgcatct. The microsatellite markers were amplified from genomic DNA, under the following conditions: 95 °C for 50 s, 50 °C for 50 s