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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 *T.brucei* has been analysed by generating large numbers of independent progeny clones from two crosses, one between two cloned isolates of *T.b.brucei* and one between cloned isolates of *T.b.brucei* and *T.b.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.

Key words: genetics; *Trypanosoma brucei*; allelic segregation; meiosis

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.gambien*se 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-like stages 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* x *T.b.brucei*, *T.b.rhodesiense* x *T.b.brucei* and *T.b.gambiense* (Type 2) x *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* x *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 undertaken in *Plasmodium falciparum* [17,18], *Plasmodium chabaudi chabaudi* [19,20,21], *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* x *T.b.brucei* and *T.b.brucei* x *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 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 (F 9/45, 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 [8,9,10], 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 (250mg/kg body weight of cyclophosphamide). The stabilates used were: F974/78, F532/72, F532/63, F124/28 (STIB 247 x TREU 927) and F9/45, F492/50, F9/41, F19/31, F28/46, F29/46 (STIB 247 x 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 Cunninghams culture medium with 15% heat-inactivated (56°C) foetal calf serum. The clones derived from the different life cycle stages are designate by m (metacyclic stage), bs (bloodstream stage) or p (procyclic stage) 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 a series of microsatellite markers identified from the genome sequence of TREU 927 [26,27, MacLeod et al., submitted] and by five new microsatellite markers identified by 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 seconds, 50°C for 50 seconds and 65°C for 50 seconds for 30 cycles, using primer concentrations and the PCR buffer described elsewhere [25]. The products were separated by electrophoresis on 3% Nusieve (Flowgen) agarose gels and visualized under UV. Minisatellite markers, *MS42*, *292* and *CRAM*, were analysed as described previously [25].

3. Results

3.1. Identification and characterization of unique progeny clones

To investigated whether the genetic system in *T. brucei* is Mendelian, the previously obtained progeny clones [8,9,10] and a large number of clones generated by further cloning of the cryopreserved uncloned progeny from crosses between STIB 247 and STIB 386 or TREU 927 were screened with five previously described markers (*MS42, 292, CRAM*, JS2 and PLC [24,25]). As STIB 386 and TREU 927 are heterozygous for all five markers and STIB 247 is heterozygous for one marker (JS2), a total of 64 different F1 genotypes would be predicted in a Mendelian system for each cross. Independent progeny clones were defined as those hybrids with unique genotypes or those derived from different flies. Any bloodstream stage hybrids from the same fly with the same genotype (using the five markers) were treated as one sample, as they were potentially the vegetative progeny of one initial product of mating. In conjunction with the

previously isolated progeny clones, 39 and 40 independent progeny from the STIB 247 x STIB 386 and the STIB 247 x TREU 927 crosses, respectively, were generated for analysis (Table 1).

3.2 Marker Identification and selection

From the available sequence data [26,27,29] of stock TREU 927, microsatellite loci were identified using the program, Tandem Repeat Finder [28] and a small selection, which consisted of more than 12 repeat units and were distributed across each of the 11 megabase chromosomes (excluding the subtelomeric/telomeric regions), were used to analyse polymorphism in the parental stocks. PCR primers were designed to the sequence flanking each locus and used to test the parental stocks for allele size differences. For stocks TREU 927 and STIB 386, one heterozygous marker from each of the housekeeping chromosomes was picked for segregation analysis (see Tables 2 and 3). Of the 189 markers that were heterozygous in TREU 927 (MacLeod et al, submitted), only a few were heterozygous in STIB 247, precluding analysis of markers on all chromosomes in this stock. However, four markers distributed on Chromosomes II, III, V and IX were heterozygous (Table 4) and were used to genotype progeny clones. An example of a marker (1J15/1) amplified by PCR for the parental stocks, STIB 386 and 247 and a selection of progeny clones from the cross between these stocks is illustrated in Fig 1. Both parental stocks are heterozygous and it can be seen that one allele from each parent segregates in the progeny. Based on this initial screen for heterozygous markers, 11 were analysed for both TREU 927 and STIB 386 and 4 for STIB 247 in both crosses.

3.3. *Segregational Analysis*

All progeny from the STIB 247 x TREU 927 cross were genotyped for 11 markers that were heterozygous in TREU 927. For every marker, it was clear that each progeny clone inherited one of each pair of alleles from TREU 927 thus showing allele segregation. Under a Mendelian system, one allele at each locus would be inherited by 50% of the progeny clones. A comparison of the observed frequency to that expected assuming Mendelian inheritance, was made for each pair of alleles on each of the 11 megabase chromosomes, and tested for agreement by χ^2 (Table 2). The results indicate no significant deviation from Mendelian ratios for any of the markers. Analysis of the segregation of the 11 markers that were heterozygous in STIB 386 in the progeny of the cross between STIB 386 and STIB 247 was undertaken and the results for each locus are presented in Table 3 together with the values of χ^2 for deviation between expected and observed. There was no significant deviation from a 1:1 ratio for the markers on chromosomes II – XI, but a significant deviation from the predicted ratio was observed for the *MS42* marker on chromosome I. Two potential explanations can be offered for this result, which are not mutually exclusive. Firstly, that it is a type 1 error that has arisen because we have made multiple comparisons. Second, that segregation distortion has occurred. To analyse this second possibility further, 6 markers [MacLeod et al, submitted] spanning the length of this chromosome were used to genotype the progeny and determine the allele segregation. The results are presented in Fig 2 and show that the distortion is limited to the left end of the chromosome with markers in right hand end segregating in the predicted 1:1 ratio. This is likely to be due to selection on the progeny clones at a locus on the left end of the chromosome (see discussion). An equivalent

analysis was undertaken for the 4 informative markers identified in STIB 247 and the results, for both crosses, are presented in Table 4, showing no significant deviation from expectation. Overall these results show that in all three stocks Mendelian segregation occurs with all but one of the loci tested.

Alleles at loci located on different chromosomes should be inherited independently in a Mendelian system, as each homologue will assort independently. In order to test for independent assortment, allele segregation for the 11 heterozygous markers on TREU 927, each located on a different chromosome, was compared, for all pair wise combinations using the χ^2 test of independence (Table 5). The results establish independent assortment of unlinked loci, thus establishing Mendel's second law. A similar analysis was undertaken with the 11 markers located on different chromosomes in STIB 386 and the 4 markers on STIB 247 (Table 6 and data not shown). For this analysis another marker on chromosome I, ChI/15B, was substituted for *MS42* to avoid any complications segregation distortion at this locus might generate. No association between the unlinked loci was observed.

4. Discussion

The genotypes of the progeny demonstrate that the genetic system in *T. brucei* follows Mendel's laws of allele segregation and independent assortment, in the ratios predicted for a Mendelian system. Previous analysis with a limited number of markers on chromosomes I and II has provided evidence that recombination and crossing over occur between physically linked markers [24,26,27]. Taken together these findings provide evidence for meiosis and a standard diploid Mendelian system in contrast to the fusion

model proposed in the related kinetoplastid, *T. cruz*i [30]. These results mean that *T. brucei* is amenable to genetic mapping and linkage analysis and, based on this, a genetic map of TREU 927 has been constructed (MacLeod et al., submitted). The data presented formally prove that *T.b.brucei* (TREU 927 and STIB 247) and Type 2 *T.b.gambiense* (STIB 386) are diploid and thus the genetic analysis shows all the features of a classical Mendelian system of genetic exchange. There is one exception to this pattern of inheritance, where there is a predominance of one allele at several loci on the left end of chromosome I in the progeny of the cross between STIB 386 and STIB 247. As alleles at loci on all the other chromosomes and the left region of chromosome I segregate with the predicted ratio in these progeny, this cannot be explained at the level of meiosis. All the progeny require growth in mice before marker analysis can be undertaken, and so the most likely explanation for this segregation distortion is selection for alleles at loci on one end of one of the homologues of chromosome I. This could either be due to the creation of a deleterious effect of the new combination of alleles at these loci or just that this combination leads to a phenotype with a lower growth rate in rodents. An interesting feature of the marker analysis of the parental stocks is the high level of homozygosity of STIB 247. It is, of course, uncertain how this could have arisen although the most likely explanation would be that this isolate has undergone a high level of inbreeding in the field. In this context, previous results analysing progeny clones from a cross with STIB 386, showed that STIB 247 underwent self-fertilisation [31], which, if it occurred regularly in the field would lead to most loci becoming homozygous.

Previous analysis of progeny from these crosses [8,9,10] has been consistent with the conclusions presented here but the small numbers of progeny had precluded testing

Mendelism in a statistically robust manner. In addition, two crosses have been undertaken between other *T.b.brucei* stocks [13,32,33] from Uganda, Zambia and Cote d'Ivoire but as relatively few progeny clones were isolated, it is difficult to conclude more than that the results were consistent with a diploid Mendelian system. Two further sets of crosses have been undertaken between 2 stocks of *T.b.brucei* from Cote d'Ivoire and *T.b.rhodesiense* from Zambia [11,13]. Analysis of the progeny has shown that a significant proportion had a raised DNA content, raising the question of how this arises and whether it suggests that the system is non-Mendelian. Karyotype analysis by pulse field gel electrophoresis has shown that these progeny with raised DNA content are trisomic and possibly triploid and this was confirmed using a range of markers. While in one cross, it appears that the *T.b.rhodesiense* stock contributes two homologues to the progeny [34] in the other it is the *T.b.brucei* stock that is the source of the additional homologue [35]. Analysis of the progeny clones of crosses between STIB 386 and either STIB 247 or TREU 927 have also identified progeny with raised DNA content and karyotype analysis has suggested that these are triploid with the human infective stock contributing the additional chromosome homologues. In this context meiosis is not perfect as, in humans, a high proportion of aborted fetuses are triploid or trisomic as a result of non-disjunction of the chromosomes at meiosis [36]. At the present time there are insufficient genome wide data to distinguish between triploidy and trisomy, although the markers described here would provide the means to do this. This would be necessary to distinguish between the hypotheses that the raised DNA contents arise as a result of the non-disjunction of the parental chromosomes (triploidy) or the detrimental effects of allelic combinations on specific chromosomes for the growth of the progeny (trisomy). It

is also possible, given the occurrence of such aberrant progeny in crosses between *T.b.rhodesiense* and *T.b.brucei*, that the human infective sub-species has begun to loose the ability to undergo meiosis due to their clonal population structure [3,4]. There are no extensive data on the role of genetic exchange in *T.b.gambiense* Type 2 field populations as relatively few isolates have been analysed [37], however in the laboratory, they clearly have the ability to undergo meiosis as shown here. The extensive data on *T.b.gambiense*, Type 1 populations show that this sub-species has expanded clonally [3] and so may have lost the ability to undergo meiosis.

Previous analyses of *T.brucei* crosses have led to several different models of how mating takes place [12,13]. The data presented here clearly do not support the parental fusion and chromosome loss model originally proposed to explain the increased DNA content of some progeny [14,15]. Essentially two other models have been proposed: (1) a conventional system where parental cells undergo meiosis to produce haploid gametes that then fuse to generate diploid recombinant progeny and (2) a 'ciliate/flagellate' model in which fusion of diploid cells occurs, followed by meiosis in a heterokaryon. This would produce 8 haploid nuclei so that loss of all but two of these and fusion of the remaining two would be required to yield diploid progeny [13]. In principal, the data presented here fit both models. However, we favour the conventional model as there is no necessity to generate the ciliate model to explain the data and secondly, unless there is some selective fusion of haploid nuclei from the two parents, this model would produce equal numbers of self-fertilisation products from both parents. While self-fertilisation for one parent has been reported [31], the major products of mating are the F1 progeny and our data on clones from several crosses does not support the occurrence of self-

fertilisation at the same frequency as cross-fertilisation (unpublished observations). It seems, from the analysis of this large collection of progeny, that only one round of mating occurs as only F1 progeny are detected and none of the clones have a genotype consistent with the products of either a backcross to one of the parents or an F2 produced by mating between F1 progeny. In conclusion our data demonstrate that the system of genetic exchange in trypanosomes is a classical Mendelian one and that the mechanism would most likely involve the production and fusion of haploid gametes. The exceptions to the classical system are largely due to crossing different sub-species where either non-disjunction occurs or there is allelic incompatibility at certain loci.

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References

[1] Hoare, CA. *The trypanosomes of mammals*. Oxford: Blackwell Scientific Publications. 1972

[2] Gibson WC. Will the real *Trypanosoma b. gambiense* please stand up. Parasitol Today 1986; 2: 255-57.

[3] Tibayrenc M. Population genetics of parasitic protozoa and other micro-organisms. Adv Parasitol 1995; 36: 48-112.

[4] MacLeod A., Tweedie A., Welburn SC, Maudlin I, Turner CMR, Tait A. Minisatellite marker analysis of *Trypanosoma brucei*: reconciliation of clonal, panmictic, and epidemic population genetic structures. Proc Natl Acad Sci USA 2000; 97:13442-47.

[5] MacLeod A, Tait A, Turner CMR. The population genetics of *Trypanosoma brucei* and the origin of human infectivity. Phil Trans Roy Soc B 2001;356:1-10.

[6] MacLeod A, Welburn SC, Maudlin I, Turner CMR, Tait A. Evidence for multiple origins of human infectivity in *T.brucei* revealed by minisatellite variant repeat mapping. J Mol Evol 2001; 52: 290-301.

[7] Gibson WC, Stevens J. Genetic exchange in the Trypanosomatidae. Adv Parasitol 1999; 43:1-45.

[8] Turner CMR, Sternberg J, Buchanan N, Smith E, Hide G, Tait A. Evidence that the mechanism of gene exchange in *Trypanosoma brucei* involves meiosis and syngamy Parasitol 1990;101:377-86.

[9] Jenni L, Marti S, Schweizer J, Betschart B, LePage RWF, Wells JM, et al. Hybrid formation between African trypanosomes during cyclical transmission. Nature 1986; 322:173-75.

[10] Sternberg J, Turner CMR, Wells JM, Ranford-Cartwright LC, Le Page RFW, Tait A. Gene exchange in African trypanosomes: frequency and allelic segregation. Mol Bioch Parasitol 1989; 34: 269-80.

[11] Gibson WC. Analysis of a genetic cross between *Trypanosoma brucei rhodesiense* and *T. b. brucei*. Parasitol 1989; 99: 91-402.

[12] Tait A, Turner CMR. Genetic exchange in *Trypanosoma brucei*. Parasitol Today 1990; 6: 70-5.

[13] Gibson W. The significance of genetic exchange in trypanosomes. Parasitol Today 1995; 11:465-68.

[14] Le Page RWF, Wells J, Prospero TD Sternberg J. In: Turner M J, Arnott D, editors, Current communications in molecular biology: molecular genetics of parasitic protozoa, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1988; 65-71. .

[15] Paindovoine, P. et al Trypanosome hybrids generated in tsetse flies by nuclear fusion. EMBO J 1986;5:3631-36.

[16] Hope M, MacLeod A, Melville S, Tait A, Turner CMR. Maintenance of diploidy (in megabase chromosomes) in *Trypanosoma brucei* after genetic exchange. Mol Biochem Parasitol 1999;104:1-9.

[17] Su X.-Z, Ferdig MT, Huang Y, Huynh CQ, Lui A, You J, et al. A genetic map and recombination parameters of the human malaria parasite *Plasmodium falciparum*. Science 1999;286:1351-53.

[18] Su X, Kirkman LA, Fujioka H Wellems TE. Complex polymorphisms in an approximately 330 kDa protein are linked to chloroquine-resistant *P. falciparum* in Southeast Asia and Africa. Cell 1997; 91:593-603.

[19] Martinelli A, Hunt P, Cheesman SJ, Carter R. The application of Amplified Fragment Polymorphism to the genetic analysis of selectable phenotypes in malaria parasites. Mol Biochem Parasitol 2004;136:117-22.

[20] Culleton R, Martinelli A, Hunt P, Carter R. Linkage group selection: rapid gene discovery in malaria parasites. Genome Res 2005;15:92-7.

[21] Martinelli A, Cheesman S, Hunt P, Culleton R, Raza A, Mackinnon M, et al. A genetic approach to the de novo identification of targets of strain specific immunity in malaria parasites. Proc Natl Acad Sci USA 2005;102:814-19.

[22] Su C, Howe DK, Dubey JP, Ajioka JW, Sibley LD. Identification of quantitative trait loci controlling acute virulence in *Toxoplasma gondii*. Proc Natl Acad Sci USA 2002; 99:10753-56.

[23] Shirley MW, Harvey DA. A genetic linkage map of the apicomplexan protozoan parasite *Eimeria tenella*. Genome Res 2000; 10:1587-1593.

[24] Tait, A., Masiga, D., Ouma, J., MacLeod, A., Sasse, J., Melville, S. et al. (2002) Genetic analysis of phenotype in *Trypanosoma brucei*: a classical approach to potentially complex traits *Philosophical Transactions of the Royal Society London B*, **357,** 89-99.

[25] MacLeod, A., Turner, C.M.R. and Tait, A. (1999) A high level of mixed *Trypanosoma brucei* infections in tsetse flies detected by three hypervariable minisatellites *Molecular and Biochemical Parasitology*, **102,** 237-248.

[26] El-Sayed NMA, Ghedin E, Song J, MacLeod A, Bringaud F, Larkin C, et al. The sequence and analysis of *Trypanosoma brucei* chromosome II. Nucl Acids Res 2003;31: 4856-63.

[27] Hall N, Berriman M, Lennard NJ, Harris BR, Bart-Delabesse EN, Hertz-Fowler C, et al. The DNA sequence of chromosome I of an African trypanosome: gene content, chromosome organisation, recombination and polymorphism. Nuc Acids Res 2003; 31:4864-73.

[28] Benson G. Tandem repeats finder: a program to analyze DNA sequences. Nucl Acids Res 1999; 27:573-80.

[29] http://www.genedb.org/genedb/tryp/index.jsp

[30] Gaunt MW, Yeo M, Frame IA, Stothard JR, Carrasco H.J, Taylor MC, et al. Mechanism of genetic exchange in American trypanosomes. Nature 2003; 421: 936-39.

[31] Tait A, Buchanan N, Hide G, Turner CMR. Self-fertilisation in *Trypanosoma brucei*. Mol Bioch Parasitol 1996;76: 31-42.

[32] Gibson WC, Garside LH. Genetic exchange in *T.b.brucei*: variable chromosomal location of housekeeping genes in different trypanosome stocks. Mol Biochem Parasitol 1991;45:77-90

[33] Schweizer J, Pospichal H, Hide G, Buchanan N, Tait A, Jenni L. Analysis of a new genetic cross between two East African *Trypanosoma brucei* clones. Parasitol 1994; 109:83-93.

[34] Gibson W, Bailey M. Genetic exchange in T.brucei: evidence for meiosis from analysis of a cross between drug-resistant transformants. Mol Biochem Parasitol 1994;64:241-52.

[35] Gibson W, Garside L, Bailey M. Trisomy and chromosome size changes in hybrid trypanosomes from a genetic cross between T.b.rhodesiense and T.b.brucei. Mol Biochem Parasitol 1992;52:189-200.

[36] Hassold TJ. Chromosome abnormalities in human reproductive wastage. Trends in Genet 1986;2:105-10.

[37] Hide G, Cattand P, Le Ray D, Barry JD, Tait A. The identification of *T.brucei* subspecies using repetitive DNA sequences. Mol Bioch Parasitol 1990;39:213-26.

FIGURE 1.

PCR amplification of the microsatellite, 1J15/1, from progeny clones, using primers 1J15/1-A and B. The products were separated on a 3% Nusieve agarose gel, stained with ethidium bromide and visualised by UV illumination. Lane 1, STIB 247; lane 2, STIB 386; lanes 3-12, progeny clones from the STIB 386 x STIB 247 cross.

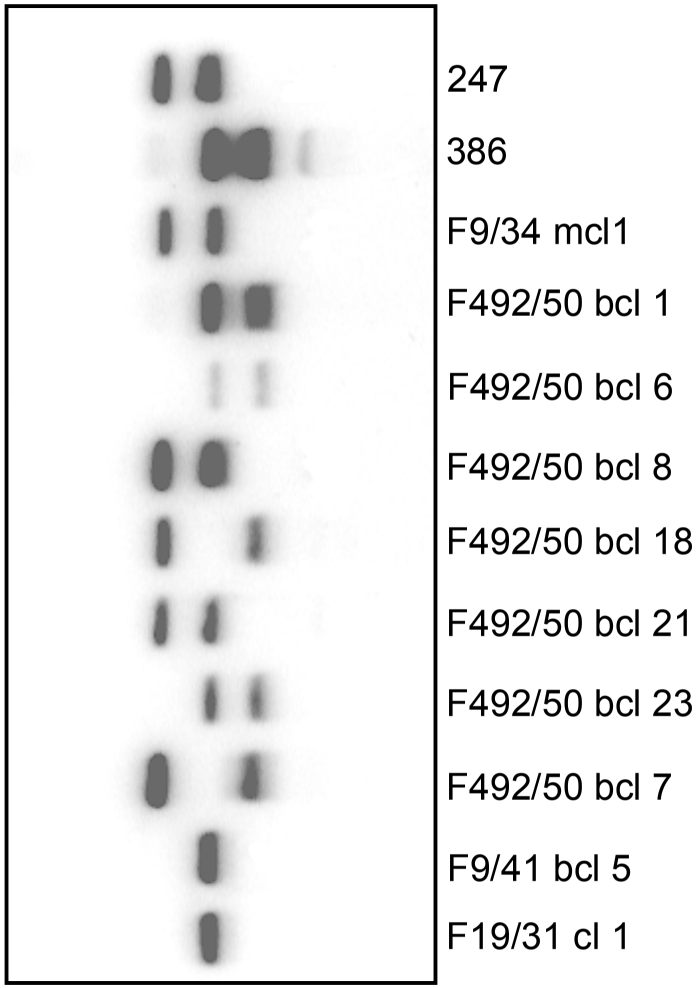
247
386
F9/34 mcl1
F492/50 bcl 1
F492/50 bcl 6
F492/50 bcl 8
F492/50 bcl 18
F492/50 bcl 21
F492/50 bcl 23
F492/50 bcl 7
F9/41 bcl 5
F19/31 cl 1

FIGURE 2.

Genotype segregation proportions for markers on chromosome I. Dashed horizontal lines delimit the approximate 95% probability range for equal segregation of alleles.

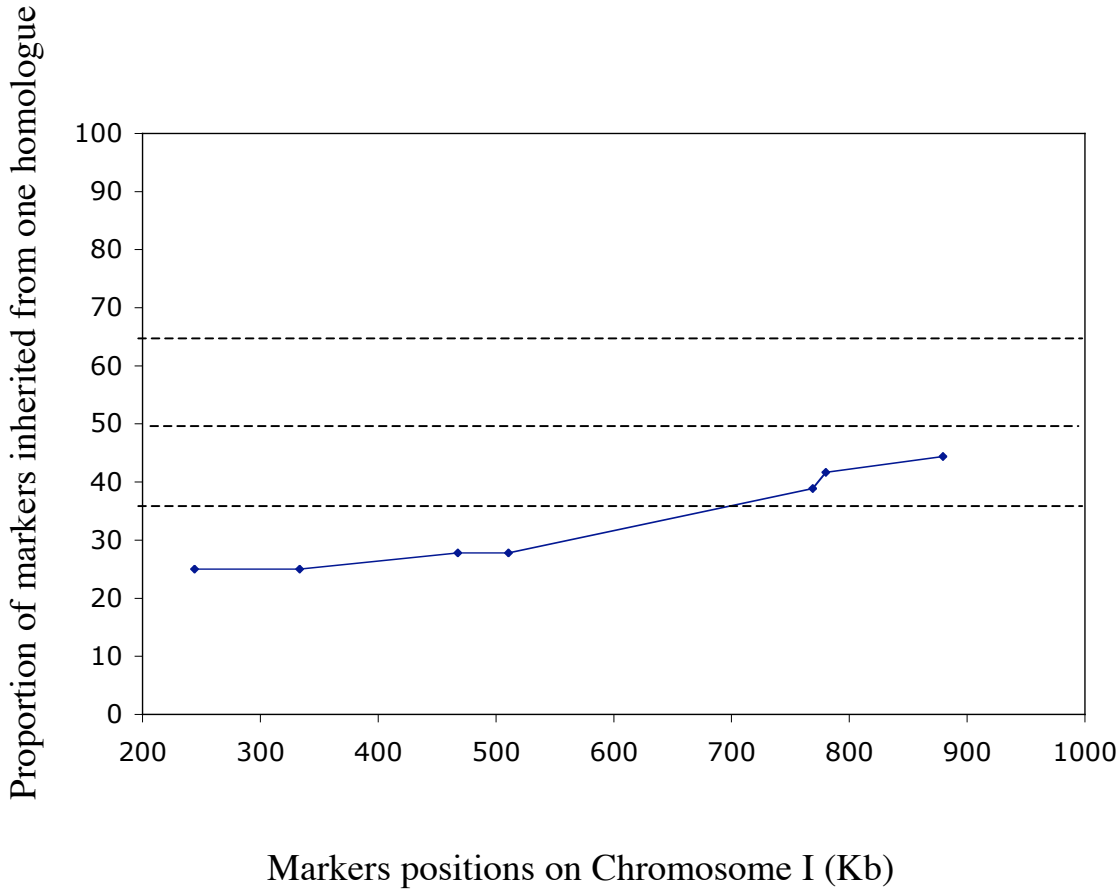
Proportion of markers inherited from one homologue
100
90
80
70
60
50
40
30
20
10
0
200
300
400
500
600
700
800
900
1000
Markers positions on Chromosome I (Kb)

TABLE 1
List of all unique hybrids from both crosses.

From STIB 247 x TREU 927	From STIB 247 x STIB 386
F124/28 bscl A1	F9/45 mcl 2
F124/28 bscl C5	F9/45 mcl 10
F124/28 bscl B3	F9/45 mcl 11
F974/70 mcl 4	F9/45 mcl 12
F532/63 bscl 3	F9/34 mcl 1
F532/63 bscl 7	B80 cl 2
F532/63 bscl 2	F492/50 bscl 1
F532/63 bscl 5	F492/50 bscl 6
F532/72 mcl 5	F492/50 bscl 8
F532/72 mcl 1	F492/50 bscl 9
F532/72 mcl 2	F492/50 bscl 12
F532/72 mcl 3	F492/50 bscl 14
F532/72 mcl 6	F492/50 bscl 18
F532/72 mcl 7	F492/50 bscl 21
F532/72 mcl 8	F492/50 bscl 23
F532/53 mcl 1	F9/41 bscl 5
F532/72 pcl 1	F9/41 bscl 7
F532/63 cl 16	F9/41 bscl 9
F532/72 mcl 9	F29/46 bscl 3
F532/63 cl A11	F29/46 bscl 4
F532/63bsclA14/1	F19/31clone 1
F532/72 bscl 1	F 19/31 bscl 11
F532/72 bscl 2	F28/46 bscl 6
F532/72 pcl 5 (P2D4)	F28/46 bscl 11
F124/28 bscl 1	F29/46 bscl 2
F532/72 pcl 7(P1E2)	F28/46 bscl 1
F124/28 bscl 9	F28/46 bscl 4
F124/28 bscl 12	F28/46 bscl 7
F124/28 bscl 13	F28/46 bscl 8
F124/28 bscl 15	F29/46 bscl 1
F124/28 bscl 20	F9/41 bscl 1
F124/28 bscl 3	F9/41 bscl 2
F124/28 bscl 5	F9/41 bscl 8
F974/78 bscl 3	F9/41 bscl 11
F974/78 bscl 6	F19/31 bscl 5
F974/78 bscl 7	F19/31 bscl 10
F124/28 bscl 22	F19/31 bscl 8
F532/72 pcl 8 (P2B3)	F492/50 bscl 7
F124/28 bscl 14	F19/31 bscl 2
F532 Bcl 15 clone 5B	

TABLE 2
Frequency of inheritance of TREU 927 alleles by F1 hybrid progeny of the cross TREU 927 x STIB 247, and test for departure from Mendelian expectations. The number of hybrids screened varied between markers. The p values shown have not been corrected for multiple comparisons.

Marker	**TREU 927 alleles inherited**	**n**	**observed**	χ^2	**p**
ChI-*MS42*	3	39	19	0.0256	0.95-0.9
	4		20		
ChII-*PLC*	3	39	21	0.2308	0.7-0.6
	4		18		
ChIII-*292*	3	40	23	0.9	0.4-0.3
	4		17		
ChIV-17F12	3	35	16	0.2571	0.7-0.6
	4		19		
ChV-JS2	3	40	16	1.6	0.3-0.2
	4		24		
ChVI-4F7	3	40	23	0.9	0.4-0.3
	4		17		
ChVII-8P12	3	34	14	1.0588	0.4-0.3
	4		20		
ChVIII-26A17	3	29	18	1.6897	0.2-0.1
	4		11		
ChIX-68	3	39	19	0.0256	0.9-0.8
	4		20		
ChX-*CRAM*	3	37	15	1.3243	0.3-0.2
	4		22		
ChXI-35	3	40	16	1.6	0.3-0.2
	4		24		

TABLE 3
Frequency of inheritance of STIB 386 alleles by F1 hybrid progeny of the cross TREU 927 x STIB 247, and test for departure from Mendelian expectations. The number of hybrids screened varied between markers. The p values shown have not been corrected for multiple comparisons.

Marker	**TREU 386 alleles**	**n**	**observed**	χ^2	**P**
ChI-*MS42*	1 2	35	26 9	4.387	0.05-0.025
ChII-*PLC*	1 2	38	13 25	1.893	0.2-0.1
ChIII-*292*	1 2	38	22 16	0.477	0.5-0.4
ChIV-2A13	1 2	32	21 11	1.602	0.3-0.2
ChV-JS2	1 2	38	18 20	0.053	0.9-0.8
ChVI-4F7/6	1 2	36	21 15	0.503	0.5-0.4
ChVII-29K4/A2	1 2	35	13 22	1.177	0.3-0.2
ChVIII-1J15/2	1 2	36	16 20	0.223	0.7-0.6
ChIX-1	1 2	35	17 18	0.014	0.8-0.7
ChX-*CRAM*	1 2	39	20 19	0.0256	0.9-0.8
ChXI-53	1 2	35	16 19	0.129	0.8-0.7

TABLE 4

Frequency of inheritance of STIB 247 alleles by F1 hybrid progeny of crosses TREU 927 x STIB 247 and STIB 386 x STIB 247, and test for departure from Mendelian expectations. The number of hybrids screened varied between markers. The p values shown have not been corrected for multiple comparisons.

Marker	**STIB 247 alleles**	**n**	**observed**	χ^2	**P**
STIB 386 x STIB 247 cross					
ChII-A4	5	39	27	3.164	0.1-0.05
	6		12		
ChIII-IJ15/1	2	36	18	0	1
	3		18		
ChV-JS2	5	38	19	0	1
	6		19		
ChIX/4	5	27	17	0.923	0.4-0.3
	6		10		
TREU 927 x STIB 247 cross					
ChII-A4	5	39	27	2.995	0.1-0.05
	6		12		
ChIII-IJ15/1	5	40	22	0.201	0.7-0.6
	6		18		
ChV-JS2	5	37	17	0.243	0.7-0.6
	6		20		
ChIX/4	5	36	19	0.056	0.9-0.8
	6		17		

TABLE 5

Test for independent assortment of alleles at unlinked loci in the TREU 927 x STIB 247 cross for 11 markers located on different chromosomes, using χ^2. $\chi^2 < 7.82$ for all pairwise comparisons; d.f. = 3, $p > 0.05$ in each case.

chromosome		II	III	IV	V	VI	VII	VIII	IX	X	XI
	marker	PLC	292	17F12	JS2	4F7	8P12	26A17	68	CRAM	35
I	MS42	0.487	1.308	0.588	2.538	0.692	1.788	2.571	6.842	3.359	1.308
II	PLC		0.526	1.060	1.158	0.947	0.818	3.414	0.526	2.842	1.923
III	292			0.543	2.600	2.200	1.294	1.999	1.923	2.600	7.400
IV	17F12				4.143	1.607	3.333	1.385	1.000	5.043	3.443
V	JS2					3.400	7.177	2.862	1.923	3.600	3.600
VI	4F7						3.176	2.309	3.767	2.600	2.600
VII	8P12							1.000	1.059	5.059	4.823
VIII	26A17								2.310	3.689	2.310
IX	68									2.128	1.513
X	CRAM										3.200

TABLE 6

Test for independent assortment of alleles at unlinked loci in the STIB 386 x STIB 247 cross for 11 markers located on different chromosomes, using χ^2. $\chi^2 < 7.82$ for all pairwise comparisons; d.f. = 3, $p > 0.05$ in each case.

chromosome		II	III	IV	V	VI	VII	VIII	IX	X	XI
	marker	PLC	292	2A13	JS2	4F7/6	29K4/A2	1J15/2	1	CRAM	53
I	15B	3.339	4.298	3.816	1.682	4.786	2.205	1.291	1.018	0.705	2.590
II	PLC		3.96	3.438	4.856	4.446	4.409	4.038	3.725	2.867	3.077
III	292			3.132	1.038	1.624	2.289	3.731	0.900	0.902	1.569
IV	2A13				1.386	3.915	2.561	2.660	2.286	2.096	2.286
V	JS2					0.901	1.018	1.137	1.299	0.495	0.793
VI	4F7/6						2.703	0.559	1.059	0.488	0.296
VII	29K4/A2							7.340	1.649	1.365	2.407
VIII	1J15/1								3.077	2.955	0.188
IX	1									2.917	0.059
X	CRAM										0.278