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Characterization of di-, tri-, and tetranucleotide microsatellite markers with perfect repeats for *Trypanosoma brucei* and related species

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Abstract

Trypanosoma brucei, unicellular parasites causing human sleeping sickness and animal nagana, have a great impact on the socio-economic environment of sub-Saharan Africa. The dynamics of the parasite are still poorly understood. We have characterized 14 polymorphic di-, tri-, and tetranucleotide microsatellite loci with perfect repeats (only one motif) exhibiting between 5 and 16 alleles in *T. brucei* isolates from all over Africa and from all described subspecies. The microsatellites will be useful in addressing population genetic questions in *T. brucei* to better understand the population structure and spread of this important parasite.

Keywords

Trypanosoma; microsatellites; population genetics; co-infection; cross-amplification; Kinetoplastid

Trypanosoma brucei, a protozoan parasite transmitted by the bite of tsetse flies (*Glossina* spp.), causes human sleeping sickness and animal Nagana in Africa. After near-elimination in the 1960's, sleeping sickness has resurged to record heights (Van Nieuwenhove et al., 2001) due to declining control efforts caused by neglect and civil unrest.

Microsatellite loci for *T. brucei* have been published previously: five microsatellites by Biteau et al. (2000), one by Truc et al. (2002) and a further 180 by MacLeod et al. (in press), which were used to generate a genetic map of the *T. brucei* genome strain (TREU927/4). Many of these markers, however, do not have perfect repeats (only motif included) and so may not follow a step-wise mutation model, limiting their use for population genetic analyses. And, in the case of the markers used for the genetic map, their variability is unknown. We therefore characterized a set of microsatellite loci with perfect repeats. Loci were identified by extracting all clean di-, tri-, and tetranucleotide repeats in the entire genome (TIGR *Trypanosoma brucei* genome database, <http://www.tigr.org/tdb/mdb/tbdb/>, as of Oct. 9, 2004) using Tandem Repeat Finder 3.21 (Benson, 1999). Primers were constructed with Primer3 (Rozen, Skaletsky, 2000). Where loci identified matched those of MacLeod et al. (in press), the same locus names and primers were used (Table 1). Microsatellite loci were amplified using the following PCR profile: 4 min 94°, followed by 35 cycles of: 45 sec 94°, 30 sec T_A (Table 1), 45 sec 72°, followed by 7 min 72°. Reactions contained 1× PCR buffer II with MgCl₂ (Applied Biosystems), 0.8 mM of each dNTP

(Promega), 0.2 μ M of each primer, 0.25 U AmpliTaq polymerase (Applied Biosystems). Allele sizes were determined using an ABI3100 Genetic Analyzer (Applied Biosystems) and GeneMapper 3.5 software (Applied Biosystems, 2002). GENEPOP 3.3 (Raymond, Rousset, 1995) was used to calculate observed and expected heterozygosities. Linkage disequilibrium between loci was not assessed because the loci are distributed over 9 of 11 chromosomes and thus in most combinations not physically linked. Furthermore, samples from all over Africa and from all sub-species were assessed to maximize detected allele diversity. Due to the geographical structure thus introduced, linkage disequilibrium is expected without physical linkage.

We screened between 21 and 106 *T. brucei* isolates at 41 loci to determine variability. Fourteen loci were highly variable and reliably produced allele patterns consistent with the repeat motif (Table 1). We assessed the potential of our markers to study related species by analyzing one isolate each of *T. evansi*, *T. equiperdum*, *T. vivax*, *T. congolense*, *T. lewisi*, *T. simiae*, *T. rangeli*, *Leishmania donovani*, and *L. major*. Most markers amplified *T. evansi* and *T. equiperdum* (the closest relatives of *T. brucei*) well, and the *Leishmania* species very weakly. *Trypanosoma simiae* was amplified by eight markers, the remaining *Trypanosoma* species by one to four markers (Table 1). Cross-amplification could potentially pose problems in those five markers that amplify *T. vivax* or *T. congolense* as these species have geographic and host ranges overlapping with *T. brucei*. The other species do not occur sympatrically or have very restricted host ranges (Mulligan, Potts, 1970). Genotyping of cultured parasites is unproblematic with all markers because all species survive under different culture conditions. Also, all species are distinguishable morphologically or with species-specific primers (Desquesnes, Davila, 2002) if necessary. The markers did not amplify tsetse flies (*Glossina spp.*), or a vertebrate host, wildebeest (*Connochaetes taurinus*).

The presented markers will help reveal the population structure and spread of this important parasite and greatly facilitate the determination of different strains directly in field samples.

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References

- Applied Biosystems. GeneMapper Software Version 3.0. Applied Biosystems; 2002.
- Benson G. Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Research*. 1999; 27:573–580. [PubMed: 9862982]
- Biteau N, Bringaud F, Gibson W, Truc P, Baltz T. Characterization of Trypanozoon isolates using a repeated coding sequence and microsatellite markers. *Molecular and Biochemical Parasitology*. 2000; 105:187–202. [PubMed: 10745102]
- Desquesnes M, Davila AMR. Applications of PCR-based tools for detection and identification of animal trypanosomes: a review and perspectives. *Veterinary Parasitology*. 2002; 109:213–231. [PubMed: 12423934]
- MacLeod A, Tweedie A, McLellan S, et al. The genetic map and comparative analysis with the physical map of *Trypanosoma brucei*. *Nucleic Acids Research*. in press.
- Mulligan, HW.; Potts, WH. The African trypanosomiasis. New York: Wiley-Interscience; 1970.
- Raymond M, Rousset F. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*. 1995; 86:248–249.

- Rozen, S.; Skaletsky, HJ. Primer3 on WWW for general users and for biologist programmers. In: Krawetz, S.; Misener, S., editors. Bioinformatics methods and protocols: methods in molecular biology. Totowa, NJ: Humana Press; 2000. p. 365-386.
- Truc P, Ravel S, Jamonneau V, N'Guessan P, Cuny G. Genetic variability within *Trypanosoma brucei gambiense*: evidence for the circulation of different genotypes in human African trypanosomiasis patients in Cote d'Ivoire. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2002; 96:52–55. [PubMed: 11925992]
- Van Nieuwenhove S, Betu-Ku-Mesu VK, Diabakana PM, Declercq J, Bilenge CM. Sleeping sickness resurgence in the DRC: the past decade. Tropical Medicine and International Health. 2001; 6:335–341. [PubMed: 11348528]

Characteristics of *Trypanosoma brucei* microsatellites

Table 1

Locus	Chromosome	Primer sequence (5'-3')	Repeat	T _A	n	N _A	Range (bp)	H ₀	H _E	Cross amplification
TB1/8	1	[FAM]-AGGTTTAAAGTGCATGTCGGA CCTGTTGTACGGAGGTCA	(CA)	53	103	11	97-117	0.38	0.69	Teq, Tev, Tv, Ts, Tr, Lm, Ld
TB2/19	2	[HEX]-CTGGTGCCTGTAACCTGTG GAAAGTGAGGACATGCACG	(AT)	53	24	11	84-104	0.71	0.86	Teq, Tev, Ld, Lm
TB2/21	2	[HEX]-CTGTGTGTGCTTGTTCATA AGTTTAAACAGCACTTCCATTT	(AT)	53	21	12	80-102	0.67	0.89	Teq, Tev, Ld, Lm
TB5/2	5	[HEX]-CAACCCGAAAGTAAGGGGAAC TCTGCCCTTCTTTGCC	(AT)	60	23	9	83-107	0.57	0.77	Teq, Tev, Tc, Ts, Ld, Lm
TB6/7	6	[HEX]-AAGCTGACAGGTGTTGA GAACATGCGTGCCTGTG	(AT)	53	24	12	104-136	0.50	0.84	Teq, Tev, Ts, Lm
TB8/11	8	[FAM]-TGTCAGTCGTACGCAC CACCCAAACGATGTAAGC	(AT)	53	22	13	97-127	0.86	0.90	Teq, Tev, Tc, Ld, Lm
TB9/6	9	[HEX]-TGATTCATTGGTTAAGACAGG AATGATAACTCGGATTACAC	(AC)	53	24	11	124-158	0.67	0.86	Teq, Tev, Lm
TB9/4	9	[HEX]-CAACTGTCACTCGTTACTT TTCAAATTGCCTATCGTTGTG	(AT)	53	24	9	101-119	0.50	0.82	Tev, Ts, Ld, Lm
TB10/5	10	[FAM]-AAAGCGATATGTTATTATGA ATTGGGTATACTGTCCCTCA	(TA)	60	24	13	79-115	0.75	0.86	Tev
TB11/12	11	[FAM]-CCTTCACCTCTTAAAGTGAAG GTAGCCATTCTGCGTCC	(AT)	53	23	12	83-109	0.48	0.85	Tev, Ts, Ld, Lm
TB11/13	11	[FAM]-CAAGAACTCTGCATTGAGC ATCTGTTGGCGATGGTGA	(AT)	53	91	16	125-161	0.78	0.88	Teq, Tev
Tr301-1	1	[HEX]-CTCCCTCCTCCTCCTCAG GTTTGACATGACAAATAACACAGG	(TTA)	60	25	10	69-108	0.56	0.85	Tev, Tc, Tl, Ts, Ld, Lm
Tr401-1	1	[HEX]-GTGAAAAAGGAAAGGCAACG TGAGTTCAACAATCTTTATTC	(GTGA)	53	106	6	126-150	0.28	0.58	Teq, Tev, Ts, Ld, Lm
Tr407-1	7	[HEX]-AACAAATATCTGACAAATGAGGATGG GTTTAGATGGGTGAAAGGGTAGG	(AAAC)	53	76	5	157-173	0.16	0.53	Teq, Tev, Tc, Tl, Ts, Ld, Lm

Locus, TB1-TB11 as in MacLeod et al. (in press); T_A, annealing temperature; n, number of isolates assessed; N_A, number of alleles detected in *T. brucei*; H₀, observed heterozygosity; H_E, expected heterozygosity; Cross amplification, further species that amplified: Teq, *T. equiperdum*; Tev, *T. evansi*; Tc, *T. congolense*; Tl, *T. lewisi*; Ts, *T. simiae*; Tr, *T. rangeli*; Tv, *T. vivax*; Ld, *Leishmania donovani*; Lm, *L. major*.