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Population genetic structure of Central African *Trypanosoma brucei gambiense* isolates using microsatellite DNA markers

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ABSTRACT

Genetic variation of microsatellite loci is a widely used method for the analysis of population genetic structure of microorganisms. Seven microsatellite markers were used here to characterize *Trypanosoma brucei gambiense* isolates from Central Africa sub-region in order to improve knowledge on the population genetic structure of this subspecies. These markers confirmed the low genetic polymorphism within Central African *T. b. gambiense* isolates from the same focus and strong differentiation between different foci. The presence of many multilocus genotypes of *T. b. gambiense* and the excess of heterozygotes found in this study play in favour of a clonal reproduction of this parasite. But some data may be indicative of a unique recombination event in one subsample. The high F_{ST} value indicates low migration rates between *T. b. gambiense* subpopulations (foci). Very negative F_{IS} suggests fairly small clonal population sizes of this pathogen in the different human trypanosomosis foci of Central Africa.

1. Introduction

The causative agent of Human African trypanosomiasis (HAT) or sleeping sickness is a trypanosome belonging to *Trypanosoma brucei* (*T. brucei*) species which is classically divided into three subspecies: *T. brucei brucei* (*T. b. brucei*), responsible for "nagana" in animals; *Trypanosoma brucei rhodesiense*, the agent of the acute form of HAT in East Africa; and *T. b. gambiense*, responsible for the chronic form of HAT in West and Central Africa. In West Africa, two groups of *T. b. gambiense* have been identified (Gibson, 1986). One referred to as group 1, represents more than 90% of all West African isolates, possessing the classical attributes of chronic disease in patients and low virulence in experimental rodents (Gibson, 1986). *T. b. gambiense* group 2 has greater virulence in rodents and can further be differentiated from group 1 by biochemical and molecular markers (Mehlitz et al., 1982; Godfrey et al., 1990; Stevens et al., 1992; Biteau et al., 2000).

T. b. gambiense group 1 populations were initially considered genetically very similar. However, several biochemical and molecular biology techniques such as isoenzyme electrophoresis (Godfrey and Kilgour, 1976; Gibson et al., 1980), Random amplified polymorphic DNA (RAPD) (Stevens and Tibayrenc, 1995; Kanmogne et al., 1996a), restriction fragment length polymorphism (RFLP) (Hide et al., 1990; Kanmogne et al., 1996b), mini satellite and microsatellite DNA amplification (MacLeod et al., 2000; Biteau et al., 2000; Koffi et al., 2007), mobile genetic element PCR (MGE-PCR) (Simo et al., 2005) and amplified fragment length polymorphism (AFLP) (Simo et al., 2008) revealed substantial genetic polymorphism within *T. b. gambiense* group 1 populations. Furthermore, the identification of distinct classes of zymodemes of *T. b. gambiense* group 1 in the same patients in West Africa demonstrates clearly that different clones of *T. b. gambiense* group 1 coexist in the same HAT foci and sometimes in the same patient (Truc et al., 2002; Jamonneau et al., 2002). Despite these important advances in the understanding of the epidemiology of HAT, the taxonomical implications of the genetic variability of *T. b. gambiense* are not yet well elucidated. Indeed, considerable controversy about the existence of genetic exchange among different *T. brucei* strains within each subspecies in the field has been reported (Tibayrenc, 1995; Gibson and Stevens, 1999; MacLeod et al., 2000, 2001a,b).

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Unequivocal evidence for genetic exchange was reported when two distinct genotypes of *T. b. brucei* or *T. b. brucei* and *T. b. gambiemse* group 2 were used to co-infect tsetse flies in the laboratory (Jenni et al., 1986; Sternberg et al., 1989; Gibson, 1989; Turner et al., 1990). All *T. brucei* subspecies, with the exception of group 1 *T. b. gambiense*, have been shown to be capable of genetic exchange when experimentally co-transmitted by tsetse flies in the laboratory (Jenni et al., 1986; Gibson, 1989; Turner et al., 1990; Gibson and Stevens, 1999). For *T. b. gambiense* group 1, Gibson (2007) mentioned that this trypanosome subspecies may also be capable of genetic exchange with other *T. brucei* sub-groups. Until now, this has not been experimentally demonstrated since *T. b. gambiense* is not easily transmitted by the commonly used laboratory tsetse flies species such as *Glossina morsitans*. Nevertheless, investigations on the population genetic structure of Central African *T. b. gambiense* group 1 isolates could provide data that may enable to confirm the hypothesis of genetic exchanges between genetically different *T. b. gambiense* clones (coexisting in the same patients or in the same HAT focus), and also to understand how *T. b. gambiense* populations evolve (in space and time). For such investigations, the classical genetic analysis based on genetic tools like microsatellite markers appears as the best approach that may be used to study the population genetic structure of Central African *T. b. gambiense* isolates.

Microsatellite DNA sequences or simple sequence repeats (SSRs) are hyper-variable, ubiquitous and co-dominant (Macedo et al., 2001; Schwenkenbecher et al., 2004). They occur randomly and abundantly in eukaryotic genomes (Hamada et al., 1982) and they are widely used in genetics and phylogenetic studies (Oliveira et al., 1998; Macedo et al., 2004; Schwenkenbecher et al., 2004, 2006). Variability of the microsatellites is thought to occur through slipped-strand mispairing, which is caused by strand mismatching of the neighbouring repeats during the DNA replication (Van Belkum et al., 1998). This type of mutation leads to a higher degree of observed genetic variability with microsatellite as compared to other genetic markers such as isoenzyme and RFLP markers. The high variability in microsatellites makes them suitable for identifying genetic variability in closely related species. During this decade, a panel of SSR specific for subgenus *Trypanozoon* has been developed for the characterization of trypanosome isolates and for the assessment of the population structure and reproductive mode of *T. b. gambiense* group 1 (Biteau et al., 2000; Koffi et al., 2007, 2009; Morrison et al., 2008) and also for the construction of genetic map of these parasites (MacLeod et al., 2005; Cooper et al., 2008). These markers have been used here to evaluate the genetic structure of Central African *T. b. gambiense* isolates in order to improve knowledge on the population genetic structure of this trypanosome subspecies.

2. Materials and methods

2.1. Trypanosome isolates

Except EATRO 1125, A005 and JUA, the remaining trypanosome isolates used in this study were obtained using the 'Kit for *In Vitro* Isolation of trypanosomes' (Aerts et al., 1992). The 72 *T. brucei* s.l. isolates analyzed here were collected from a range of hosts (human, pig and zebu) and geographical origins (Fig. 1), including one *T. b. gambiense* group 2 (TH1), 4 *T. b. brucei* and 67 *T. b. gambiense* group 1 isolated in 5 countries (Cameroon, Equatorial Guinea, Central African Republic, Uganda and Republic of Congo) of the Central African sub-region (Table 1). These trypanosome isolates have been previously characterized by isoenzyme electrophoresis, MGE-PCR and AFLP (Nkinin et al., 2002; Njiokou et al., 2004; Simo et al., 2005, 2008). Genomic DNA from each

Table 1
Trypanosoma brucei s.l. isolates used, size of alleles at each microsatellite locus and multilocus genotypes.

Isolates	Year of isolation	Hosts	Country	HAT foci	Species	MLGs	Microsatellite markers						
							Micbg1	Micbg5	Micbg6	MinsatG9	Minsatg4	M6C8	MT3033
133	1998	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
959	1998	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
2903	1998	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
2434	1998	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
2700	1998	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
3126	1998	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
3205	1998	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
3392	1998	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
3578	1998	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
3738	1998	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
4282	1998	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
3769	1998	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
SOMABc	1999	Man	CM	Campo	Tbg1[a]	17	162/194	170/226	182/266	190/198	115/145	085/157	154/170
Bat7	1999	Man	CAR	Batangafo	Tbg1[a]	15	162/194	176/230	182/266	128/190	111/145	085/157	154/170
Bat21	1999	Man	CAR	Batangafo	Tbg1[a]	15	162/194	176/230	182/266	128/190	111/145	085/157	154/170
Bat28	1999	Man	CAR	Batangafo	Tbg1[a]	15	162/194	176/230	182/266	128/190	111/145	085/157	154/170
Bat26	1999	Man	CAR	Batangafo	Tbg1[a]	15	162/194	176/230	182/266	128/190	111/145	085/157	154/170
Bat47	1999	Man	CAR	Batangafo	Tbg1[a]	15	162/194	176/230	182/266	128/190	111/145	085/157	154/170
Bat35	1999	Man	CAR	Batangafo	Tbg1[a]	15	162/194	176/230	182/266	128/190	111/145	085/157	154/170
Bat3	1999	Man	CAR	Batangafo	Tbg1[a]	15	162/194	176/230	182/266	128/190	111/145	085/157	154/170
Bat42	1999	Man	CAR	Batangafo	Tbg1[a]	15	162/194	176/230	182/266	128/190	111/145	085/157	154/170
Bat24	1999	Man	CAR	Batangafo	Tbg1[a]	15	162/194	176/230	182/266	128/190	111/145	085/157	154/170
Bat44	1999	Man	CAR	Batangafo	Tbg1[a]	15	162/194	176/230	182/266	128/190	111/145	085/157	154/170
Bat45	1999	Man	CAR	Batangafo	Tbg1[a]	15	162/194	176/230	182/266	128/190	111/145	085/157	154/170
Bat46	1999	Man	CAR	Batangafo	Tbg1[a]	15	162/194	176/230	182/266	128/190	111/145	085/157	154/170
Bip39	1999	Man	CM	Bipindi	Tbg1[a]	7	198/248	172/180	180/256	128/190	111/145	085/085	154/170
Bip40	1999	Man	CM	Bipindi	Tbg1[a]	7	198/248	172/180	180/256	128/190	111/145	085/085	154/170
Bip38	1999	Man	CM	Bipindi	Tbg1[a]	7	198/248	172/180	180/256	128/190	111/145	085/085	154/170
Bip35	1999	Man	CM	Bipindi	Tbg1[a]	7	198/248	172/180	180/256	128/190	111/145	085/085	154/170
Bip33	1999	Man	CM	Bipindi	Tbg1[a]	7	198/248	172/180	180/256	128/190	111/145	085/085	154/170
Bip31	1999	Man	CM	Bipindi	Tbg1[a]	7	198/248	172/180	180/256	128/190	111/145	085/085	154/170
Bip29	1999	Man	CM	Bipindi	Tbg1[a]	7	198/248	172/180	180/256	128/190	111/145	085/085	154/170
Bip28	1999	Man	CM	Bipindi	Tbg1[a]	7	198/248	172/180	180/256	128/190	111/145	085/085	154/170