Infection, Genetics and Evolution 27 (2014) 348–354

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Preponderant clonal evolution of *Trypanosoma cruzi I* from Argentinean Chaco revealed by Multilocus Sequence Typing (MLST) ☆

Nicolás Tomasini [a,b,*,1], Juan J. Lauthier [a,b,*,1], María M. Monje Rumi [a,b], Paula G. Ragone [a,b], Anahí M. Alberti D'Amato [a,b], Cecilia Pérez Brandán [a,b], Miguel A. Basombrío [b], Patricio Diosque [a,b]

[a] Unidad de Epidemiología Molecular (UEM), Instituto de Patología Experimental, Universidad Nacional de Salta-CONICET, Av. Bolivia 5150, CP4400 Salta, Argentina
[b] Instituto de Patología Experimental, Universidad Nacional de Salta-CONICET, Av. Bolivia 5150, CP4400 Salta, Argentina

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 1 August 2014
Accepted 2 August 2014
Available online 8 August 2014

Keywords:
Chagas disease
TcI
PCE
Genetic exchange
SL-IR
MLST

ABSTRACT

Trypanosoma cruzi has been historically classified as a species with preponderant clonal evolution (PCE). However, with the advent of highly polymorphic markers and studies at geographically reduced scales, the PCE in *T. cruzi* was challenged. In fact, some studies have suggested that recombination in *T. cruzi* lineage I (TcI) is much more frequent than previously believed. Further analyses of TcI populations from different geographical regions of Latin America are needed to examine this hypothesis. In the present study, we contribute to this topic by analyzing the population structure of TcI from a restricted geographical area in the Chaco region, Argentina. We analyzed TcI isolates from different hosts and vectors using a Multilocus Sequence Typing (MLST) approach. These isolates were previously characterized by sequencing the spliced leader intergenic region (SL-IR). Low levels of incongruence and well-supported clusters for MLST dataset were obtained from the analyses. Moreover, high linkage disequilibrium was found and five repeated and overrepresented genotypes were detected. In addition, a good correspondence between SL-IR and MLST was observed which is expected under PCE. However, recombination is not ruled out because five out of 28 pairs of loci were incompatible with strict clonality and one possible genetic exchange event was detected. Overall, our results represent evidence of PCE in TcI from the study area. Finally, considering our findings we discuss the scenario for the genetic structure of TcI.

1. Introduction

The clonal theory of parasitic protozoa proposed by Tibayrenc and colleagues (Tibayrenc and Ayala, 2012; Tibayrenc et al., 1990) gives a framework to study parasitic diversity for different diseases. The final goal is to identify "clones" of parasites that are associated with certain relevant epidemiological features that require more attention. In this regard, the parasitic diversity may have a role in different aspects such as drug susceptibility, clinical manifestations of the disease and certain epidemiological features. Several years ago, diversity of different parasite protozoa was commonly analyzed by methods like Multilocus Enzyme Electrophoresis (MLEE) or Random Amplified Polymorphic DNA (RAPD) where "clones", major groups or near-clades (Tibayrenc and Ayala, 2012) were identified for different species like *Trypanosoma cruzi* or *Leishmania* spp. (reviewed in Miles et al., 2009). Most recently, more sophisticated methods like sequencing of intra-specific variable genes, Multilocus Microsatellite Typing (MLMT) (Barnabe et al., 2011; Llewellyn et al., 2009a,b; Macedo et al., 2001; Messenger et al., 2012; Zumaya-Estrada et al., 2012) or Multilocus Sequence Typing (MLST) (Lauthier et al., 2012; Mauricio et al., 2006; Yeo et al., 2011; Zemanova et al., 2007) succeeded the previous ones. Additionally, a greater amount of isolates were available and diversity was observed within previously identified groups. This is the case for *Trypanosoma cruzi* the causative agent of Chagas Disease, which is considered a clonal organism with very rare events of genetic exchange (Gaunt et al., 2003; Tibayrenc and

Abbreviations: MLST, Multilocus Sequence Typing; PCE, preponderant clonal evolution; SL-IR, sliced leader intergenic region; MLEE, multilocus enzyme electrophoresis; RAPD, random amplified polymorphic DNA; MLMT, multilocus microsatellite typing; DTU, discrete typing units; DP, discriminatory power.

☆ GenBank, EMBL and DDBJ: KF264002–KF264049; KF268601–KF268680, JN129503–JN129510; JN129536–JN129543; JN129569–JN129576; JN129602–JN129609; JN129635–JN129642; JN129668–JN129675; JN129734–JN129741 and JN129767–JN129774.

* Corresponding authors at: Instituto de Patología Experimental, Universidad Nacional de Salta-CONICET, Av. Bolivia 5150, CP4400 Salta, Argentina. Tel.: +54 387 4255333.

E-mail addresses: nicotomasini@yahoo.com.ar (N. Tomasini), juanjoselauthier@yahoo.com.ar (J.J. Lauthier).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.meegid.2014.08.003
1567-1348/

Ayala, 1987, 2012; Tibayrenc et al., 1990; Tomazi et al., 2009; Westenberger et al., 2005). The *T. cruzi* species has been subdivided into six major Discrete Typing Units (DTUs, TcI–TcVI) (Zingales et al., 2009, 2012), and recently it was discovered a seventh group from bats called TcBat (Marcili et al., 2009; Pinto et al., 2012). However, considerable variability is observed within some of these DTUs (Llewellyn et al., 2009a, 2009b). The variability within *T. cruzi* DTU I (TcI) has been particularly studied (Cura et al., 2010; Guhl and Ramirez, 2011; Herrera et al., 2013; Llewellyn et al., 2009b; Tomasini et al., 2011). This DTU is widely distributed from the south of the USA to the north of Argentina and Chile and it is the main causative of Chagas Disease in countries at north of the Amazon basin. The internal diversity and the wide distribution of this DTU suggest that this group may have a particular genetic structure. Studies based on MLMT proposed a geographical-based structure more than a strong phylogenetic structure (Llewellyn et al., 2009b; Messenger et al., 2012; Zumaya-Estrada et al., 2012). However, some studies based on the sequence of the intergenic region of Spliced-Leader gene (Cura et al., 2010; Herrera et al., 2007, 2013) have proposed different subgroups and some of them showed broad distribution questioning the hypothesis of strict geographical structuring. However, SL-IR and MLMT had some disadvantages to analyze the structuring. For example, the SL-IR sequencing is a single-locus approach which could not correctly account for the phylogeny of TcI; while MLMT is an approach very susceptible to homoplasy. In this sense, MLST could be a useful method to overcome these disadvantages.

In addition, a possible cause of the vast diversity of genotypes is the existence of genetic exchange in natural populations. However, it is not clear if genetic exchange has or had an impact into the genetic structure. Recent works based on MLMT analyses and maxicircle genes (Messenger et al., 2012; Ramirez et al., 2012) proposes that recombination should be frequent. However, the widespread distribution of certain genotypes or groups (Cura et al., 2010; Tomasini et al., 2011) suggests that the model of Predominant Clonal Evolution (PCE) is applicable and genetic exchange is rare.

In a previous work, we characterized four groups of TcI in our study area by SL-IR sequencing. They were called Chaco-1 to Chaco-4 (Tomasini et al., 2011). Chaco-1 corresponded with the previously described TcIa/TcI_{DOM} (Zumaya-Estrada et al., 2012). Chaco-2 and Chaco-3 had a microsatellite motif in the SL-IR sequence identical to strains TcId from Colombia but phylogenetic analyses of entire sequence clustered them near to Chaco-4/TcIe. These results suggested that TcId is a paraphyletic group and the TcId microsatellite motif is an ancestral character (Tomasini et al., 2011).

Here, we characterized 24 parasite stocks by Multilocus Sequence Typing which were previously analyzed by SL-IR sequencing (Tomasini et al., 2011). This dataset is particularly interesting because different genotypes were identified in a restricted geographical area, allowing the analysis of existence and frequency of genetic exchange. In addition, correspondence between SL-IR and MLST markers was evaluated. Furthermore, we discuss about the genetic structure and the role of recombination in TcI.

2. Material and methods

2.1. Parasites

The analyzed stocks are listed in Table 1, and they are the same that were previously examined by SL-IR (Tomasini et al., 2011), except the stock LL040-P33.R1, which was not included in the present study because it was a mixed isolate of TcI and TcIII. Eight out of the 24 isolates were previously analyzed by MLST by Lauthier et al. (2012). In addition, M5631 (TcIII) and IVV (TcII) strains were used as outgroups. Maintenance, harvest and DNA extraction of the 24 TcI stocks isolated from the Chacabuco and 12 de Octubre counties, Chaco Province, Argentina, were previously described in Tomasini et al. (2011).

Table 1
Geographical and host origins of *T. cruzi* DTU I stocks (Chaco, Argentina) and the corresponding SL-IR group.

Stock	Host	Geographical origin	SL-IR group
798R1	*T. infestans*	TRES ESTACAS, Chacabuco	Chaco-1
TEV91cl5	*T. infestans*	TRES ESTACAS, Chacabuco	Chaco-1
Rata3 938-A	*R. rattus*	TRES ESTACAS, Chacabuco	Chaco-1
TEV55cl1	*T. infestans*	TRES ESTACAS, Chacabuco	Chaco-2
PAV00cl7	*T. infestans*	PAMPA AVILA, Chacabuco	Chaco-2
PalDa24	*D. albiventris*	EL PALMAR, 12 de Octubre	Chaco-2
PalDa3cl4	*D. albiventris*	EL PALMAR, 12 de Octubre	Chaco-2
PalDa1cl9	*D. albiventris*	EL PALMAR, 12 de Octubre	Chaco-3
Da28	*D. albiventris*	EL PALMAR, 12 de Octubre	Chaco-3
PalDa22cl7	*D. albiventris*	EL PALMAR, 12 de Octubre	Chaco-3
TEDa2cl4	*D. albiventris*	TRES ESTACAS, Chacabuco	Chaco-3
PalDa20cl3	*D. albiventris*	EL PALMAR, 12 de Octubre	Chaco-3
PalDa4cl8	*D. albiventris*	EL PALMAR, 12 de Octubre	Chaco-3
PalDa25	*D. albiventris*	EL PALMAR, 12 de Octubre	Chaco-3
LL051-P23R0	*C. familiaris*	LAS LEONAS, 12 de Octubre	Chaco-3
PalDa30-Po1 R0	*D. albiventris*	EL PALMAR, 12 de Octubre	Chaco-3
PalDa31-Po1 R0	*D. albiventris*	EL PALMAR, 12 de Octubre	Chaco-3
Ll022-1 R2	*T. infestans*	LAS LEONAS, 12 de Octubre	Chaco-3
802-R1	*T. infestans*	TRES ESTACAS, Chacabuco	Chaco-3
LL017 Po0 R0	*T. infestans*	LAS LEONAS, 12 de Octubre	Chaco-4
LL027-21R1	*T. infestans*	LAS LEONAS, 12 de Octubre	Chaco-4
LL027-21R2	*T. infestans*	LAS LEONAS, 12 de Octubre	Chaco-4
PalV1cl1	*T. infestans*	EL PALMAR, 12 de Octubre	Chaco-4
PalV2-2cl5	*T. infestans*	EL PALMAR, 12 de Octubre	Chaco-4

2.2. PCR amplification and sequencing

Gene fragments used for MLST analysis were: superoxide dismutase A (*SODA*), superoxide dismutase B (*SODB*), leucine aminopeptidase (*LAP*), glucose-6-phosphate isomerase (*GPI*), glutathione peroxidase (*GPX*), pyruvate dehydrogenase E1 component alpha subunit (*PDH*), 3-hydroxy-3-methylglutaryl-CoA reductase (*HMCOAR*) and small GTP-binding protein rab7 (*GTP*) as is described in Lauthier et al. (2012).PCRs were carried out in reaction volumes of 50 μl containing 100 ng of DNA; 0.2 μM of each primer, 1 U of goTaq DNA polymerase (Promega), 10 μl of 5X buffer (supplied with the goTaq polymerase) and a 50 μM concentration of each dNTP (Promega). Cycling conditions were as follow: 5 min at 94 °C followed by 35 cycles of 94 °C 1 min; 55 °C 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 5 min. Amplified fragments were precipitated with 70% ethanol and sequenced on both strands in an ABI PRISM_310 Genetic Analyzer (Applied Biosystems).

2.3. Data analysis

The obtained sequences were aligned using the ClustalW algorithm included in the Mega v. 5.2 package (Tamura et al., 2011). Diploid Sequence Types (DSTs) were determined using MLSTest 1.0 (http://ipe.unsa.edu.ar/software) (Tomasini et al., 2013). Discriminatory power (DP) was calculated according to Hunter (1990) and confidence intervals were calculated using the jackknife pseudo-values procedure as is proposed by Severiano et al. (2011) using the online tool available at http://darwin.phyloviz.net/ComparingPartitions/.

Loci concatenation and distance matrices were built using MLSTest 1.0 (Tomasini et al., 2013). Correspondence between MLST and SL-IR distance matrices was calculated by using Mantel test with