Acta Tropica 151 (2015) 88–93

Contents lists available at ScienceDirect

Acta Tropica

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

Trypanosoma cruzi population dynamics in the Central Ecuadorian Coast

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ARTICLE INFO

Article history:
Received 26 May 2015
Received in revised form 13 July 2015
Accepted 15 July 2015
Available online 19 July 2015

Keywords:
Trypanosoma cruzi
Rhodnius ecuadoriensis
Chagas disease
microsatellite
Transmission cycle
DAPC
Ecuador

ABSTRACT

Chagas disease is the most important parasitic disease in Latin America. The causative agent, *Trypanosoma cruzi*, displays high genetic diversity and circulates in complex transmission cycles among domestic, peridomestic and sylvatic environments. In Ecuador, *Rhodnius ecuadoriensis* is known to be the major vector species implicated in *T. cruzi* transmission. However, across vast areas of Ecuador, little is known about *T. cruzi* genetic diversity in relation to different parasite transmission scenarios.

Fifty-eight *T. cruzi* stocks from the central Ecuadorian coast, most of them derived from *R. ecuadoriensis*, were included in the study. All of them were genotyped as *T. cruzi* discrete typing unit I (DTU TcI). Analysis of 23 polymorphic microsatellite loci through neighbor joining and discriminant analysis of principal components yielded broadly congruent results and indicate genetic subdivision between sylvatic and peridomestic transmission cycles. However, both analyses also suggest that any barriers are imperfect and significant gene flow between parasite subpopulations in different habitats exists. Also consistent with moderate partition and residual gene flow between subpopulations, the fixation index (F_{ST}) was significant, but of low magnitude. Finally, the lack of private alleles in the domestic/peridomestic transmission cycle suggests the sylvatic strains constitute the ancestral population.

The *T. cruzi* population in the central Ecuadorian coast shows moderate tendency to subdivision according to transmission cycle. However, connectivity between cycles exists and the sylvatic *T. cruzi* population harbored by *R. ecuadoriensis* vectors appears to constitute a source from which the parasite invades human domiciles and their surroundings in this region. We discuss the implications these findings have for the planning, implementation and evaluation of local Chagas disease control interventions.

1. Introduction

Chagas disease is the most important parasitic disease in Latin America [1], currently affecting an estimated 10 million people [2], ~40% of which will develop chronic complications such as cardiac and/or digestive alterations [3]. The causative agent, *Trypanosoma cruzi*, circulates in complex transmission cycles which occur in wild (sylvatic), peridomestic and domestic environments, and involve mammalian hosts (including human) and blood-feeding triatomine bugs [4]. Unraveling the dynamics of parasite transmission cycles at a local scale is pivotal for designing effective intervention strategies [5], among which, vectorial control is of paramount importance [6].

Chagas disease is endemic in Ecuador [7]. *Rhodnius ecuadoriensis* is the major *T. cruzi* vector [8,9]; including in Manabí province, Central Coast. This area constitutes an important target for the National Chagas disease control program. In contrast to entomological findings in southern Ecuador, *R. ecuadoriensis* rarely colonizes (i.e., establishes breeding triatomine populations) inside houses in Manabí [8]. However, this vector species is frequently associated with peridomestic habitats, especially if firewood and is accumulated near the house [10].

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http://dx.doi.org/10.1016/j.actatropica.2015.07.017

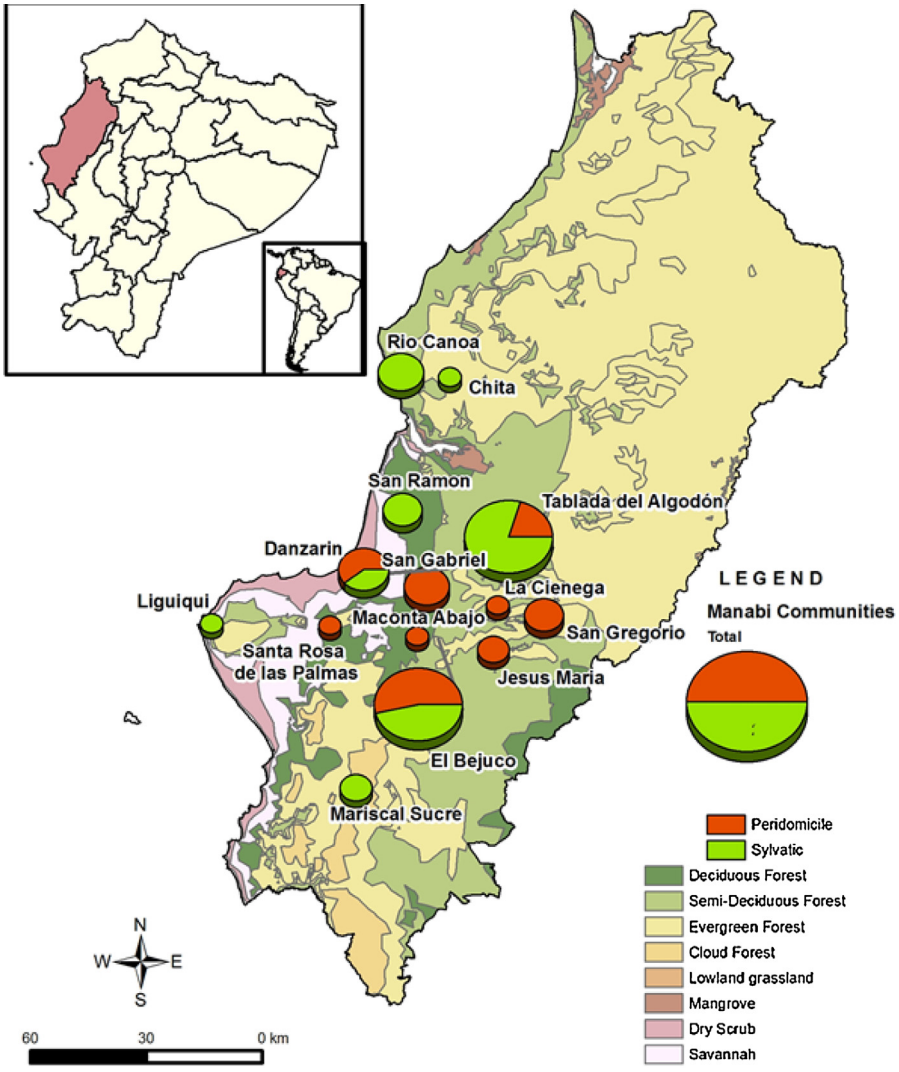

Fig. 1. Studied communities in Manabí. The locations of the studied communities are shown. The area of each circle corresponds to the number of *T. cruzi* stocks analyzed in a given locality. Colors indicate the proportion samples collected in sylvatic (green) vs. domestic/peridomestic (orange) habitats. Inserts show the location of Manabi province in Ecuador and of Ecuador in South America. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In genetic terms, *T. cruzi* is a highly heterogeneous species, divided into six major genetic lineages, referred to as discrete typing units (DTUs), or "near-clades" [11], named TcI through TcVI [12], may have been the result of long-term restrained genetic recombination with occasional bouts of genetic exchange and/or hybridization, i.e., predominant clonal evolution (PCE) [11,13] although there is controversy surrounding the applicability of this model to evolution to *T. cruzi* [14]. Each DTU displays different biological characteristics, as well as particular phylogeographic and eco-epidemiological traits, although these differences are not clear-cut [15]. Previous studies in Southern Ecuador, have detected the presence of the TcI DTU exclusively [5]. The DTUs present as well as *T. cruzi*'s population dynamics in Manabí province remain uncharacterized.

In this study, we genotyped the *T. cruzi* lineages circulating in the vector population of the central Ecuadorian coastal region through restriction fragment length polymorphism (PCR-RFLP) analysis. Additionally, we employed 27 previously described microsatellite polymorphic loci [16] to gain insight into the local parasite population dynamics in the region. Our findings, which point towards moderate population partitioning and the existence of genetic flow between peridomestic and sylvatic *T. cruzi* populations, are discussed in the context of the long-term Chagas disease control efforts in this geographic area, especially vector control interventions directed towards *R. ecuadoriensis,* the major vector species in Manabí and in Ecuador in general.

2. Materials and methods

2.1. Study area

Samples included in the study come from 14 different rural communities of Manabí province (Fig. 1), in the central Ecuadorian coast. The terrain is mostly flat, with hills of low altitude (0–350 m.a.s.l), covered mostly by subtropical dry and tropical humid forests [17]. Agriculture is the main economic activity and Manabí is one of the least affluent provinces in the country, despite being the third largest in terms of population size. In rural communities, housing in Manabí is typically built with cane walls and palm roofs, although cement/brick walls and zinc plate roofs are also common [10].

2.2. T. cruzi *sample panel*

A panel of 58 *T. cruzi* samples was assembled for the study (Additional file 1). All samples were isolated from triatomines col-

lected for previous studies performed by our group at the Center for Infectious and Chronic Disease Research, Pontificia Universidad Católica del Ecuador, in Quito, Ecuador. The panel included samples collected in Manabi province in domestic (within domiciles) or peridomestic habitats (near human dwellings or associated with human activities such as chicken coops, piles of construction material or wood, stones, bricks, piles of agricultural products, and other structures, as described in [18]). Additionally, samples came from triatomines which were collected in sylvatic habitats (at least 20 m away from human domiciles) by manual searches following procedures described in [19], or in quadrants as previously described in [20,21].

Collected triatomines were sacrificed/sanitized by immersion in Whités solution (0.25% $HgCl_2$, 0.65% NaCl, 0.125% v/v HCl, 25% ethanol) in a biohazard level II cabinet and their intestinal contents or feces were aseptically inoculated into biphasic NNN agar to isolate *T. cruzi* epimastigotes. To reduce the number of polyclonal cultures included in the analysis, plate-cloned cultures (as described in [22]) were included whenever available.

2.3. DNA extraction and quantification.

DNA was extracted from epimastigote cultures with either DNAzol or DNAeasy kits, following manufacturer's instructions. DNA was quantified in a Nanodrop 1000 spectrophotometer (Thermo Scientific,Wilmington, DE, USA).

2.4. T. cruzi genotyping

Assignment to major genetic lineages was performed using a previously described polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) based on the D7 domain of the 24Sα rDNA (LSU rDNA), glucose-6-phosphate isomerase (GPI) and heat shock protein 60 (HSP60) [23]. Parasite lineage was assigned by comparing the resulting restriction banding patterns with those obtained using DNA from standard strains for each *T. cruzi* DTU, as follows: TcI: SilvioX/10 cl1 strain; TcII: Esmeraldo cl3 strain; TcIII X109/2; TcIV: Can III cll; TcV: bug 2148 cl1; TcVI: CL Brener.

2.5. Microsatellite amplification and size determination.

PCR products for a panel of 27 previously described microsatellite loci were amplified under the conditions described in [16]. Amplification product sizes were measured in an automated capillary sequencer (ABI3730, Applied Biosystems, UK), employing fluorescently tagged standards. Sizes were determined "blind" (to prevent user bias) in GeneMapper Software®, and the accuracy of the calls was verified manually.

2.6. Analysis of population genetic structure

D_{AS} distances (1-proportion of shared alleles at all loci/n) were estimated employing MSAT2 software and used to build the dendrogram in Fig. 2. Multi-allelic loci were included in the analysis by performing one thousand random diploid re-samplings of each multi-allelic profile through a script written in Microsoft Visual Basic and individual-level genetic distances were calculated as the mean across multiple re-sampled datasets as in [5]. Additionally, population subdivision was explored through *K*-means clustering and discriminant analysis of principal components [24]. The fixation index (F_{ST}) and other population-level genetic parameters were calculated in Genalex software [25].

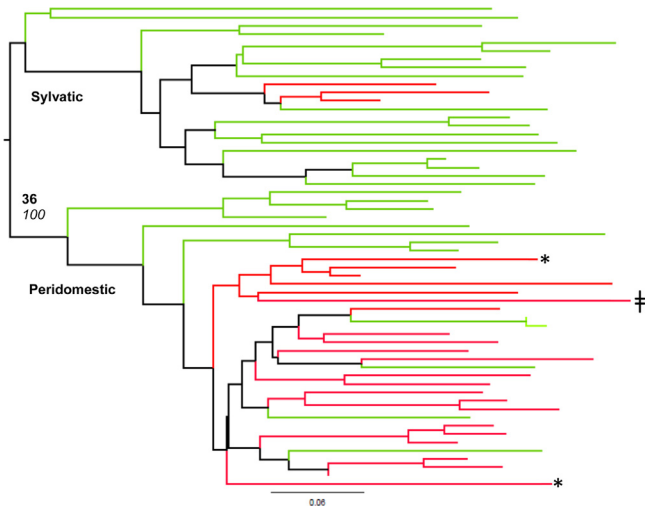

Fig. 2. Pairwise genetic distance-based neighbor-joining tree. The dendrogram was built based on D_{AS} values corresponding to the mean of thousand random re-samplings of the 23 microsatellite loci datasets. Branch colors correspond to the location of sample collection: red for domestic/peridomestic (sample labelled with‡ was the only intradomestic sample) and green for sylvatic habitats. All samples were originally isolated from *Rhodnius ecuadoriensis* vectors, except those labeled with an asterisk, which were isolated from *Panstrongylus howardi*. Allelic bootstrap values (percentage congruence over 1000 trees from 1000 random diploid datasets) are indicated in italics. Loci level bootstraps (percentage congruent tress over 1000 randomly sampled diploid datasets) are indicated in bold. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. T. cruzi lineages and microsatellite dataset

Fifty-eight *T. cruzi* isolates and clones were included in the analysis. All of them were determined to belong to DTU TcI. The microsatellite dataset comprised 2504 alleles across all studied loci (excluding missing data (Additional file 2). Multiple (≥3) alleles were found in 3.75% of studied loci.

3.2. T. cruzi population genetic structure

Two clusters are evident in the D_{AS} pairwise genetic distance-based neighbor-joining dendrogram, although bootstrap support for this subdivison was weak (36%, Fig. 2). The clusters correspond to the transmission cycles: one of them is predominantly composed of samples collected in the peridomestic environment (along with the single domestic sample available), while the other is predominantly composed of sylvatic samples. Discriminant analysis-based clustering roughly conforms to these subdivisions as well, although it shows population partition into three clusters: one composed primarily of peridomestic isolates (equivalent to that found by Neighbor Joining), while the other two comprise mainly sylvatic samples (Fig. 3). For both the Neighbor joining and DAPC analysis, some peridomestic isolates grouped within the sylvatic cluster and vice versa, showing that parasite flow between these habitats occurs (Figs. 2 and 3). The clusters found by DAPC are very similar to those found by Neighborgh joining: The peridomestic cluster is equivalent in both analysis, whereas the addition of the two sylvatic clusters identified by DAPC is equivalent to the sylvatic cluster found by Neighborgh Joining. The samples connecting the two cycles are the same in both analysis. Thus, overall, both analysis yield very similar results.

Table 1
Population genetic parameters for peridomestic and sylvatic *T. cruzi* populations in Manabí province, Ecuador.

Population	*G*/*N*	PL	PA	MA/*S*	Ar ± SE	H_O[a]	H_E[a]	F_{IS} ± SE	I_A (*p*-value)
Peridomestic	26/26	10	0.826	0.654	4.447 ± 2.552	0.354	0.448	0.214 ± 0.355	2.417 (<0.001)
Sylvatic	32/32	16	1.826	0.938	5.103 ± 2.955	0.371	0.564	0.346 ± 0.373	1.245 (0.001)

G = Number of multilocus genotypes per population.
N = Number of isolates in population.
PL = Number of polymorphic loci.
PA = Mean number of private alleles per locus.
MA/S = Mean number of multiple (+3) alleles per sample.
Ar ± SE = Allele richness as a mean over loci ± standard error, calculated in FSTAT.
F_{IS} ± SE = F_{IS} over loci and standard error, calculated in FSTAT.
I_A = Association index, calculated in Microsat.
[a] Mean observed and expected heterozygocity across all loci, calculated in Arlequin 3.5.1.2.

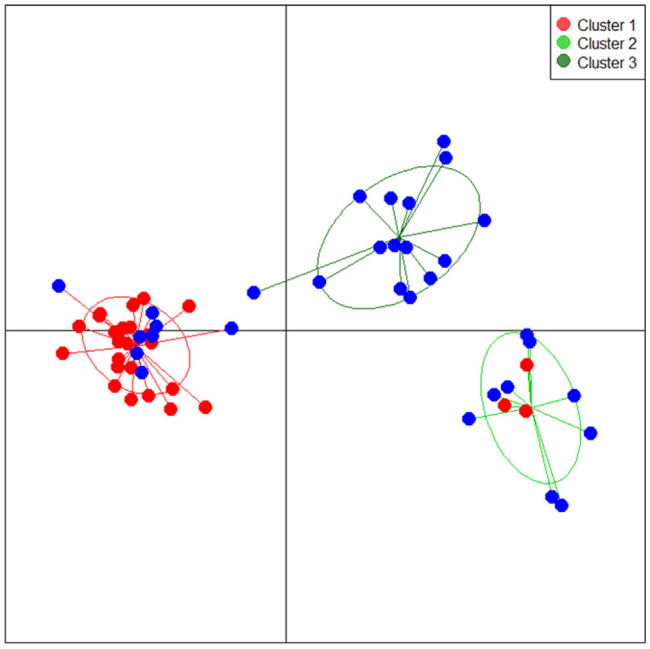

Fig. 3. *T. cruzi* genetic clustering by discriminant analysis of principal components. Eight principal components, explaining 80% of the total genetic diversity in the dataset, were retained in the analysis. The ellipses represent the optimal number of clusters among the dataset according to the minimum Bayesian information criterion. Red: samples collected in domestic/peridomestic habitats. Blue: Samples collected in sylvatic habitats. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Population genetic parameters

Population genetic parameters are shown in Table 1. Peridomestic and sylvatic populations were characterized by considerable genetic diversity, as evidenced by high measurements of allelic richness (A_r = 4.447 and 5.103, respectively); number of private alleles per locus were higher among sylvatic strains (0.826 *vs.* 1.826, respectively). Both populations were also deficit in heterozygosity (F_{IS} = 0.214 and 0.346, respectively). Moderate subdivision between habitats was supported by the fixation index, which was significant ($p < 0.0001$), but of low magnitude (F_{ST} = 0.12871). To compare the magnitude of the parasite flow between habitats in Manabi with that of other *T. cruzi* populations in the South of the country, F_{ST} was recalculated using 10 microsatellite markers for which the information for Loja province was available [5]. Recalculated F_{ST} for the Manabí population using only those ten markers was 0.131, $p < 0.0001$, compared to the reported F_{ST} value of 0.212 for the population in Loja. The index of association I_A (linkage disequilibrium, or nonrandom association of genotypes at different loci) was significant when calculated for the entire dataset (2.230, $p < 0.001$) as well as for the peridomestic and sylvatic subpopulations individually (Table 1), supporting preponderant clonality in these populations.

4. Discussion

We have used a PCR-RFLP-based genotyping scheme combined with high-resolution molecular markers to genetically characterize the *T. cruzi* population associated with triatomine vectors in the central Ecuadorian coastal region. Our samples, consisting primarily of isolates obtained from *R. ecuadoriensis*, allow us to make inferences regarding the *T. cruzi* population harbored by the most epidemiologically important and prevalent vector species in the area.

Similar to southern Ecuador [5], we find DTU TcI to be the only *T. cruzi* genetic lineage associated with triatomine vectors in the central Ecuadorian coast. Except for one isolated study [26], DTU TcI is the only genetic lineage reported in Ecuador to date. Our results are consistent with the notion of DTU TcI being predominant in the northern region of South America [27,28].

In Southern Ecuador (Loja) and the North of neighboring Peru, *R. ecuadoriensis* has been shown to colonize human dwellings [18,29] as well as being present in sylvatic environments [19–21]. Morphometric analysis suggest that two distinct *R.*ec *uadoriensis* populations exist in Southern Ecuador, one in sylvatic and another in domestic/peridomestic habitats [30]. In agreement with these findings, we have previously identified distinct (although not completely separated) sylvatic and domestic/peridomestic *T. cruzi* transmission cycles in Loja [5]. In contrast, in Manabí, morphometric analysis shows that *R. ecuadoriensis* specimens collected in sylvatic and peridomestic habitats are phenotypically similar, suggesting greater interconnection and exchange of individuals between these two habitats [30]. Furthermore, sylvatic populations of *R. ecuadoriensis* have been shown to re-infest domestic/peridomestic habitats after insecticide spraying campaigns [8]. Therefore, greater parasite flow between habitats in Manabí than in Loja would be expected.

In this study, our data show that the local *T. cruzi* sylvatic transmission cycle appears to constitute a source from which the parasite reaches human domiciles. It is likely that *R. ecuadoriensis* acts as a link between transmission cycles in Manabí. The low fixation index, coupled with entomological evidence [8,30] suggest that parasite flow between sylvatic and peridomestic environments in Manabí has greater intensity than in other areas of the country where the same vectors species predominates (i.e., Loja). Additionally, the lack of private alleles in the domestic/peridomestic population and the branching patterns in the neighbor-joining tree, suggests the sylvatic population constitutes the ancestral population from which it was derived. Even though domiciliated *R. ecuadoriensis* populations are uncommon in Manabí, the pre-

dominant housing materials (cane walls and palm roofs) are not sufficient to prevent home invasion/reinvasion by vectors, and integral control measures will require house improvement. These considerations are key for long-term control of Chagas disease in the area.

Several components of the *T. cruzi* transmission network remain to be studied in the central Ecuadorian coast. Despite the preponderant role played by *R. ecuadoriensis*, less prevalent vector species such as *Panstrongylus howardi* have been reported to occur in Manabí [17,20]. Only two isolates from this secondary vector species could be included in this study. Furthermore, the role of wild, synanthropic and domestic mammalian reservoirs in the transmission chain remains to be explored**.** Clarifying the role of these additional elements in the local *T. cruzi* transmission network will be crucial for the implementation of effective control measures, which will also require concerted efforts by researchers, public health agencies, and the local population.

Competing interests

The authors declare having no competing interests of any kind related to this article.

Authors' contributions

JAC participated in study design, analysed and interpreted results, drafted the manuscript and provided overall coordination of the study. MAJP performed PCRs for genotyping and microsatellite amplification, analysed results and helped draft the manuscript. MSL participated in study design and guided data analysis and interpretation. LAM participated in study design and determined microsatellite amplicon sizes. SOM conducted parasite isolation and supervised parasite cloning. AGV directed entomological collection and identified triatomine species. MT participated in data analysis and interpretation. MJG conceived the study, and helped draft the manuscript. All authors read an approved the final manuscript.

Acknowledgements

The authors wish to thank Sara Silva Pereira, Tapan Bhattacharyya, Amber MacDonald, Pedro Pineda, Alejandra Zurita and Andrés Merino for technical assistance. This study received the financial support of the Institut de recherche pour le développement (AIRD), Global Infectious Diseases Training Grant-Fogarty International Center-National Institutes of Health (D43TW008261), National Institutes of Health, USA, European Commission Framework Programme Project "Comparative epidemiology of genetic lineages of Trypanosoma cruzi" ChagasEpiNet, Contract No. 223034, Children's HeartLink USA and Pontificia Universidad Católica del Ecuador. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2015.07.017

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