Acta Tropica 160 (2016) 9–14

Contents lists available at ScienceDirect

Acta Tropica

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

Fluctuations in *Trypanosoma cruzi* discrete typing unit composition in two naturally infected triatomines: *Mepraia gajardoi* and *M. spinolai* after laboratory feeding

Camila Egaña[b], Raquel Pinto[b], Fernanda Vergara[b], Sylvia Ortiz[b], Ricardo Campos[a], Aldo Solari.[b,*]

[a] *Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile*
[b] *Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile*

ARTICLE INFO

Article history:
Received 10 August 2015
Received in revised form 22 March 2016
Accepted 19 April 2016
Available online 21 April 2016

Keywords:
Trypanosoma cruzi
Single-mixed natural infections
Mepraia
Prospective study
Triatome feeding

ABSTRACT

Mepraia species are hematophagous insects and the most important wild vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease in southeastern South America. Because the domestic *Triatoma infestans* is already controlled, the transmission of different *T. cruzi* discrete typing units (DTUs) by *Mepraia* species deserves attention. Our aim is to gather information on the diversity of *T. cruzi* DTUs circulating in natural insect populations.

Two groups of naturally infected bugs 21 *Mepraia gajardoi* and 26 *Mepraia spinolai* were followed-up after two or more laboratory feedings by means of minicircle-PCR assays to evaluate the composition of four *T. cruzi* DTUs by hybridization tests. Fluctuations from positive *T. cruzi* detection to negative and the converse, as well as single to mixed infections with different *T. cruzi* DTUs and the opposite were frequent observations after laboratory feeding in both *Mepraia* species. Single and mixed infections with more than two *T. cruzi* DTUs were detected after the first feeding; however mainly mixed infections prevailed after the second feeding. Laboratory feeding on three or more occasions resulted in a decreasing trend of the parasite burden. In a comparison with 28 infected and fed *M. gajardoi* collected one year before from the same vector colony *T. cruzi* DTUs composition changed, indicating that temporal variations occur in *T. cruzi*.

Natural populations of *Mepraia* species can transmit complex mixtures *T. cruzi* DTUs which fluctuate over time after feeding, with a tendency to eliminate the parasitism after prolonged feeding.

1. Introduction

Chagas disease is one of the main tropical diseases of humans in Central and South America, affecting approximately 8 million people (WHO, 2012). Triatomine vectors acquire and transmit *Trypanosoma cruzi*, the etiological agent of the disease, after a blood feeding on a mammalian reservoir. The taxon *T. cruzi* consists of discrete typing units (DTUs), named TcI-TcVI, including Tcbat (Zingales et al., 2012). *T. cruzi* is transmitted by contamination of mammal skin lesions or oral ingestion of triatomine feces containing parasites, contaminated blood and congenitally. *T. cruzi* colonizes the midgut and hindgut, mainly in the latter of triatomines without infecting cells. During or after the triatomine blood meal diuretic hormones are released, moving water and ions quickly from the stomach and hemolymph into the Malpighian tubules and later into the rectum. This way triatomines can release metacyclic trypomastigotes via urine/feces with a rapid to delayed behavior, as occurs in insects with good or poor vectorial capacity, respectively. (Kollien and Schaub, 1997). In pioneer studies of nymph survival of starved *T. cruzi*-infected *Triatoma infestans*, infected bugs reduced the life span by 14–17% (Schaub and Lösch, 1989). This result may be explained by competition between insect, enterobacteria and parasites for nutrients, making the infected bugs hungry more rapidly as described (Botto-Mahan et al., 2006). In nature triatomines most probably are starved, therefore an infection with *T. cruzi* can induce an earlier feeding response than in a non-infected triatomine. Triatominae subfamily is composed of 146 species distributed in 18 genera and grouped into six tribes. In Chile, the wild vectors of Chagas disease are *Mepraia* species (Hemiptera,

* Corresponding author at: Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile, 8380453 Santiago, Chile.
E-mail address: asolari@med.uchile.cl (A. Solari.).

http://dx.doi.org/10.1016/j.actatropica.2016.04.008
0001-706X/

Triatominae), which have diurnal habits and include three species: *Mepraia gajardoi*, *Mepraia parapatrica* and *Mepraia spinolai* (Frías and Atria, 1998; Frías-Lasserre, 2010; Lent et al., 1994). Natural populations of *M. gajardoi* and *M. spinolai* their infection rate increased substantially after feeding (Egaña et al., 2014). In nymphs of *M. spinolai T. cruzi* retarded development time, reduced body weight and reduced survival, and also induced earlier defecation compared to uninfected bugs (Botto-Mahan et al., 2006; Botto-Mahan, 2009). These *Mepraia* species are frequently associated with stony areas of arid or semiarid zones, and rarely within human dwellings. Infection rates in *M. gajardoi* right after collection range from 11% to 35%, and in *M. spinolai* from 0 to 54% (Toledo et al., 2013; Botto-Mahan et al., 2005). These numbers after feeding in the laboratory varied in a survey from 20% to a cumulative figure of 87% in *M. gajardoi* and from 0% to 59–62% in *M. spinolai* (Egaña et al., 2014). All species of Triatominae, a subfamily of Reduviidae are potential vectors of the Chagas disease parasite *T. cruzi.* Information on *T. cruzi* DTU composition in *M. gajardoi* and *M. spinolai* from several endemic localities is available (Toledo et al., 2013; Coronado et al., 2009). Triatomines may harbor *T. cruzi* populations on very high genetic heterogeneity, but little is known about how this diversity changes over time. The purpose of this study was to determine *T. cruzi* DTU composition in naturally infected *Mepraia* spp. after being fed on laboratory mice several times to address the following questions: 1. Do naturally infected *Mepraia* species transmit the same *T. cruzi* DTU composition after different laboratory feeding events? 2. Does *T. cruzi* DTU composition change from single to mixed infections in naturally infected *Mepraia* species after several laboratory feeding events? 3. Does *T. cruzi* burden decrease in naturally infected triatomines after several laboratory feedings? None of these questions have been previously addressed.

2. Material and method

2.1. Populations under study

M. gajardoi (nymph stages III-V) were captured during the austral summer of 2012 and (nymph stages III-V and adults) 2013 from Caleta Vitor Arica (18°45′45″S, 70°20′34″W). *M. spinolai* (nymph stages III-V) were captured from Las Chinchillas National Reserve, Illapel, Chile (31°30′28″S, 71°06′19″W). Individuals were collected by two trained people using humans as bait on 3 consecutive days for 2 h within the time of maximum insect activity (11 am-2 pm). Captured insects were classified by nymph stage and kept individually to avoid cross-contamination. In the laboratory, all insects (nymphs III- adults) were maintained under optimal conditions (27 °C; 75% relative humidity). Feces/urine samples were obtained after feeding on uninfected *Mus musculus* as described for nymphs (Egaña et al., 2014). Insects were maintained for up to three or four consecutive feedings (approximately 8–12 weeks). This study was conducted after approval of the Ethics Committee of the Faculty of Medicine, University of Chile, with code Protocol CBA# 0443 FMUCH dated August 16, 2011. We state clearly that no specific permissions were required for these locations to collect triatomines. We confirm that the field studies did not involve endangered or protected species.

2.2. Triatomine laboratory feeding conditions

Triatomines fed on laboratory *M. musculus* 1 or 2 days after arrival from the field and the first drop feces/urine sample were obtained from each specimen. This first feeding allowed us to determine the infective status right after collection. Second and third samples were obtained after molting and feeding 4 weeks later to assess changes in infective status and the *T. cruzi* DTU composition.

2.3. Triatomine samples

The urine/feces samples were diluted with 150 ul distilled water, boiled for 10 min and individually frozen at −20 °C. DNA was extracted with the Blood DNA EZNA kit and re-suspended in 100 μl of elution buffer (Omega Bio-tech, Georgia,USA). One to five μl DNA template were used for PCR, since the method sensitivity is 0.01 parasite DNA equivalents/assay (Schijman et al., 2003).

2.4. PCR assays

PCR was performed as previously reported using primers 121 (5′AAA TAA TGT ACG G (T/G) GAG ATG CAT GA-3′) and 122 (5′GGG TTC GAT TGG GGT TGG TGT-3′) to amplify the variable region of minicircle DNA (Wincker et al., 1994). Each experiment included positive and negative controls. Samples were tested several times using different DNA concentrations as template; a reaction was considered positive when at least one of the several assays showed a positive result. False negatives were evaluated by incubation of the DNA sample on a positive control. The PCR products were analyzed by electrophoresis on 2% agarose gels and visualized by staining with ethidium bromide. A 330-base pair product indicated a positive result.

2.5. DNA blots

These analyses were performed using 10 μl of each positive PCR assay. The PCR products were electrophoresed, transferred onto Hybond N+ nylon membranes (Amersham, Little Chalfont, UK) and cross-linked by UV light for DNA fixation. After transferring PCR products, membranes were pre-hybridized for at least 2 h at 55 °C, and finally hybridized separately with the four kDNA random priming labelled with ^{32}P (1×10^6 cpm/membrane) as probes. After hybridization, membranes were washed three times for 30 min each with 2 × SSC, 0.1% sodium dodecyl sulphate (SDS) at 55 °C and then exposed in a Molecular Imager FX (Bio-Rad Laboratories, Hercules, California,USA).

2.6. Trypanosoma cruzi genotyping

For *T. cruzi* genotyping, four different *T. cruzi* clones isolated in Chile (sp104 cl1, CBB cl3, NR cl3 and v195 c11), corresponding to TcI, TcII, TcV, and TcVI, respectively, were used to generate specific probes. Construction of minicircle probes and validation of this method to genotype with the old clonets and present *T. cruzi* DTUs was performed as previously described (Veas et al., 1991).

2.7. Statistical analysis

Analysis of the infection status after each laboratory feeding event between populations was performed using $\varkappa^2$ and a regression test (Fox, 2005)

3. Results

In the first group of bugs from Vitor 21 out of 35 insects were *T. cruzi* infected (14 nymph stages III-IV and 7 adults) (Table 1A). In a second group the 14 insects were uninfected or died during the follow-up. The results of repeated PCR assays of the 21 infected insects performed during this follow-up are shown in Table 1A. After the first feeding the ratio of positive results/total assays performed was 0.51, however after the second, third, and fourth feeding, this proportion changed to 0.23, 0.23 and 0.17, respectively. The linear regression of feeding on proportion of positives studied up to the third feeding according to data on Table 1A were F-statistic: β = −1.8748; R-squared = 0.165; P: 0.0019. At the

Table 1A
Trypanosoma cruzi in *Mepraia gajardoi* after successive feedings (F) in the laboratory. x/y: number of kDNA PCR assays +/number of total assays. D: dead insect.

Insect N°/death before x Feeding	1st F x/y	2nd F x/y	3rd F x/y	4th F x/y
4/-	3/9	3/15	2/12	0/9
5/5F	4/9	3/15	4/10	0/9
6/2F	9/13	D	D	D
8/5F	6/13	6/13	4/10	0/9
9/3F	9/16	2/15	D	D
13/5F	2/9	4/9	4/12	0/9
15/5F	1/11	2/12	10/12	0/9
16/5F	3/9	1/22	3/16	0/9
175F	11/14	5/13	2/11	0/9
21/3F	5/11	4/13	D	D
22/5F	6/15	2/20	0/9	0/9
23/5F	12/25	4/9	5/13	4/9
25/5F	7/9	8/22	4/9	9/9
26/2F	9/14	D	D	D
27/-	9/23	2/17	3/15	4/9
28/-3F	10/16	3/9	D	D
30/-4F	12/22	1/22	0/15	D
31/-3F	6/10	0/11	D	D
33/-3F	9/9	7/9	D	D
34/-4F	4/14	6/18	4/9	D
35/-4F	8/12	1/9	1/9	D
Average	0.51	0.23	0.23	0.17
frequency	145/283	64/273	37/162	17/99

Table 1B
Temporal changes of the *Trypanosoma cruzi* DTUs composition in the urine/feces of *M. gajardoi* after successive feedings (F) in the laboratory. D: dead insect.

Insect N°/death before x Feeding	Tc I 1F 2F3F 4F5F	Tc II 1F 2F3F 4F5F	Tc V 1F 2F3F 4F5F	Tc VI 1F 2F3F 4F5F	FIS
4 N/-	+ + – – –	– – – – –	– – + – –	– – – – –	M
5 N/5F	+ + – – D	– – – – D	– + – – D	– – – – D	M
6 N/2F	+ D	– D	– D	– D	S
8 N/-	+ + – – –	– – – – –	– – – – –	– – – – –	S
9 N/3F	+ + D	– – D	– – D	+ – D	M
13 N/5F	– + – – D	– + – – D	– – – – D	– + – – D	M
15 N/5F	– – – – D	– + – – D	– – – – D	– + – – D	M
16 N5F	– – – – D	– – – – D	– – – – D	– – – – D	ND
17 N/5F	+ – – – D	+ – – – D	+ – – – D	– + – – D	M
21 N/3F	+ – D	– – D	+ + D	– – D	M
22 A/5F	+ – – – D	– + – – D	– + – – D	– – – – D	M
23 N/5F	– – – – D	– + – – D	+ – – – D	+ + – – D	M
25 N/5F	– – – – D	+ + + + D	– – – – D	– – – – D	S
26 N/2F	+ D	– D	– D	+ D	M
27 N/5F	+ – – – D	+ – – – D	– + – – D	+ + – – D	M
28 A/3F	+ + D	– – D	– – D	+ – D	M
30 A/4F	– – – D	– – – D	+ – – D	+ + – D	M
31 A/3F	– – D	+ – D	– – D	– – D	S
33 A/3F	+ + D	+ + D	+ – D	– – D	M
34 A/4F	– – – D	– – + D	– – – D	– – – D	S
35 A/4F	+ – – D	– – – D	– – – D	– – + D	M
Total	13 7 0 0	5 6 2 1 0	5 4 1 0	6 1 0	25%S 75%M

1F-5F: first feeding-fifth feeding; FIS: final infection status; S: single infection; M: mixed infection; ND: not determined; A: adult; N: nymph; D: death.

fourth feeding only three out of eleven insects that survived gave positive PCR assays (insect N° 23, 25 and 27). The linear regression results were $\beta = -1.5193$; R-squared = 0.109; P: 0.0286. These results suggest prolonged feeding reduce parasite burden in the vector. Eleven out of twenty one survived up to the fourth feeding: one bug contained very low numbers of parasites, not allowing a genotyping (insect N°16). Another one maintained a high infection level (insect N° 25) and insect N° 8 lost the infection. Both single and mixed infections with more than one *T. cruzi* DTU exist. The final infection status reached 75% mixed infections (see Table 1B); all the *T. cruzi* DTUs were detected at similar levels (TcI = 20; TcII = 14; TcV = 10; TcVI = 13) in this follow-up with several feedings. Infections with *T. cruzi* DTUs changed from positive to negative and viceversa except in insect N° 25, which was positive and single infected with TcII in all samples (Table 1B). The *T. cruzi* DTU composition in each insect was studied. No specific combinations of *T. cruzi* DTU mixtures were detected. Another group of 70 *M. gajardoi* from Vitor collected one year earlier was analyzed twice. Right after collection they were fed and sampled; 14 were infected with single infections of TcII; 50 of them, mainly nymphs in the III-V stages, survived and were not infected. These were studied after a second feeding: then, 28 insects were infected and the parasites genotyped (Table 2). The 14 previously infected with TcII conserved that status but infections with TcI, and TcV were observed. No TcVI were detected. Another 16 were not infected, 7 died during the period and 5 gave PCR DNA bands insufficient to genotype. An infection rate of 67% was obtained from the insects that resulted negative after the first feeding, compared to 20% (14 out of 70) analyzed right after collection. Single and mixed infections with two DTUs (7 insects) and three DTUs (4 insects) were obtained; there were 48% single and 52% mixed infection. The *T. cruzi* DTUs most frequently found were TcI = 14, TcII = 14 and TcV = 17. Regarding the

Table 2
Trypanosoma cruzi DTU composition in *Mepraia gajardoi* nymphs III-V after a feeding event.

Sample N°	Insect N°	TcI	TcII	TcV	TcVI	IS
1	1	+	–	–	–	S
2	2	+	–	–	–	S
3	3	–	+	+	–	M
4	5	–	–	–	–	ND
5	6	+	+	+	–	M
6	11	+	–	–	–	S
7	14	+	+	–	–	M
8	15	–	+	+	–	M
9	17	+	+	+	–	M
10	18	+	–	+	–	M
11	21	+	+	+	–	M
12	22	+	+	–	–	M
13	23	+	+	–	–	M
14	25	–	+	–	–	S
15	26	–	+	+	–	M
16	27	–	+	+	–	M
17	28	+	–	+	–	M
18	29	+	+	–	–	M
19	34	–	–	+	–	S
20	37	+	+	+	–	M
21	38	–	–	+	–	S
22	42	–	–	+	–	S
23	46	–	–	+	–	S
24	47	–	–	+	–	S
25	48	–	–	+	–	S
26	49	–	–	+	–	S
27	50	+	–	–	–	S
28	51	–	+	–	–	S
Total		14	14	17	0	48% S 52% M

IS: infection status; S = single infection; M = mixed infection; ND = not determined.

other triatomine species, in a similar follow-up study with three feedings of 32 *M. spinolai*, no infection was detected after only the first feeding (Table 3), containing less mixed infections (50%) after the second feeding and 77–80% after the third one ($\varkappa^2 = 4.24$; df:1; p = 0.039). The level of mixed infections reached an average of 77%. The combination of TcI and TcV was the most represented (11 times out of 15 cases with double infections). The most represented *T. cruzi* DTUs after the second feeding were TcV, TcI, and much less TcVI. However *T. cruzi* DTU TcII was detected only after the third feeding (9 insects). This *T. cruzi* DTU was compared in the second and third feeding ($\varkappa^2 = 10.88$; d.f. = 1; p = 0.001). Fluctuations from positive to negative and the opposite were observed during this follow-up. Similarly fluctuations from single to mixed infections or the opposite were frequent observations. Only one case of a single infection with TcV was maintained (insect N° 17). Negative cases were repeated up to 4 times using extracted and 10× concentrated DNA samples. We looked for the presence of DNA polymerase inhibitors but none was detected in these cases (not shown). Fig. 1 shows specific patterns of hybridization of the four probes tested. Each one cross reacts only with its homologue. The probes against *T. cruzi* DTUs TcI and TcII only cross reacted with the homologous parasite but not against *T. cruzi* DTUs of other countries (Bolivia and Brazil), suggesting high heterogeneity in these two *T. cruzi* DTUs.The probes against *T.cruzi* DTUs TcV and TcVI showed to be highly sensitive to react against the tested *T.cruzi* clones.

4. Discussion

In a previous study we reported higher infection rates of naturally infected *M. gajardoi* and *M. spinolai* nymphs after feeding in the laboratory (Egaña et al., 2014). The first feeding performed right after collection allowed nourishing the insects, prolonging their lives and further feces/urine samples. After additional feedings of bugs maintained isolated every 4–5 weeks, new feces/urine samples were taken to determine the infective status of the insect and the infection course without *T. cruzi* re-infection. One group of *M. gajardoi* collected in the summer of 2012 was followed-up only after two feedings. Another group of the same species collected one year later from the same colony was followed-up with pro-

Table 3
Trypanosoma cruzi DTU composition in Mepraia spinolai insects after successive feeding events.

	Second feeding					Third feeding					
Insect N°	TcI	TcII	TcV	TcVI	IS	TcI	TcII	TcV	TcVI	IS	FIS
1	+	–	+	+	**M**	+	–	+	–	**M**	**M**
2	+	–	+	–	**M**	+	+	+	–	**M**	**M**
3	–	–	+	–	**S**	+	+	+	+	**M**	**M**
4	–	–	+	–	**S**	+	–	+	–	**M**	**M**
5	+	–	+	–	**M**	+	–	+	–	**M**	**M**
6	–	–	–	–	**ND**	–	+	+	–	**M**	**M**
7	+	–	+	–	**M**	–	–	–	–	**ND**	**M**
8	–	–	+	–	**S**	–	–	–	–	**ND**	**S**
9	+	–	+	–	**M**	+	+	+	+	**M**	**M**
10	–	–	+	+	**M**	+	–	+	–	**M**	**M**
11	–	–	+	–	**S**	+	–	–	–	**S**	**M**
12	+	–	+	–	**M**	+	–	–	–	**S**	**M**
13	+	–	+	–	**M**	+	–	+	–	**M**	**M**
14	–	–	+	–	**S**	–	+	+	–	**M**	**M**
15	–	–	+	–	**S**	–	–	–	–	**ND**	**S**
16	+	–	+	–	**M**	+	–	+	–	**M**	**M**
17	–	–	+	–	**S**	–	–	+	–	**S**	**S**
18	–	–	+	–	**S**	–	+	+	–	**M**	**M**
19	–	–	+	–	**S**	–	–	–	–	**ND**	**S**
20	–	–	–	–	**ND**	–	+	+	–	**M**	**M**
21	+	–	–	–	**S**	–	–	–	–	**ND**	**S**
22	+	–	–	–	**S**	+	–	+	–	**M**	**M**
23	+	–	–	–	**S**	–	–	–	–	**ND**	**S**
24	+	–	+	–	**M**	–	+	+	–	**M**	**M**
25	+	–	+	–	**M**	–	–	+	–	**S**	**M**
26	+	–	+	–	**M**	–	+	+	–	**M**	**M**
Total	**14**	**0**	**21**	**2**	**50%S** **50%M**	**12**	**9**	**18**	**2**	**20%S** **80%M**	**23%S** **77%M**

IS: infection status. FIS: final infection status; ND: not determined; S: single infection; M: mixed infection.

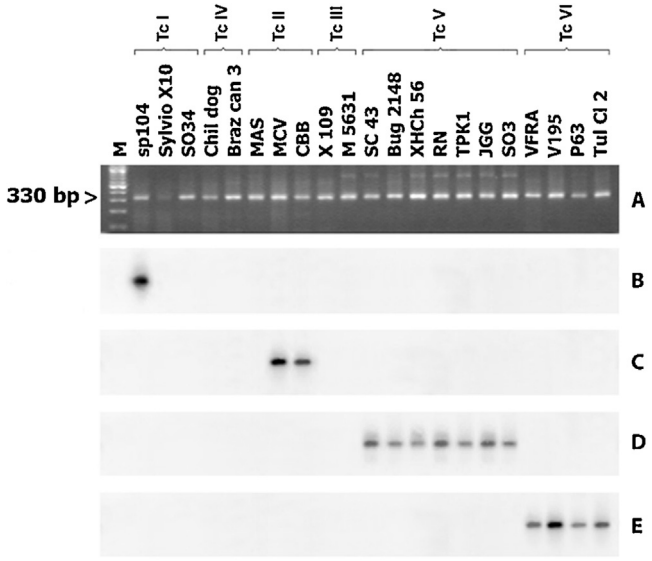

Fig. 1. Specificity of DNA probes. Hybridization patterns of different *Trypanosoma cruzi* stocks belonging to different DTUs. A. minicircle PCR amplicons stained with ethidium bromide; B. Hybridization with probe TcI; C. Hybridization with probe TcII; D. Hybridization with probe TcV; E. Hybridization with probe TcVI. Lane M, 100-base pair (bp) DNA ladder. Sylvio X10 (TcI) and MAS (TcII) are from Brazil, and SO34 (TcI) is from Bolivia.

longed feeding (5 consecutive events and during 4 months). The *T. cruzi* DTU composition changed after successive feedings from a pattern of single infection to a mixed one with up to three *T. cruzi* DTUs of the four tested. The three most represented *T. cruzi* DTUs were TcI, TcII and TcV. However this tendency was different in another group of insects collected from the same locality the following year. In this group TcVI was detected as frequently as the others. The most probable explanation for changes in *T. cruzi* DTUs composition in *M. gajardoi* of the same colony after one year is that reservoirs where this insect species feeds may change over time. These reservoirs are wild rodents; however other possible blood sources are marine birds and lagomorphs, even though the former are refractory to infection (Teixeira et al., 2006). During the present follow-up study fluctuations in *T. cruzi* DTU composition were detected. Different *T. cruzi* DTUs are transmitted over time, except in one case of *M. gajardoi* (insect N° 8) infected with TcI only. Previous results in insects from Vitor analyzed right after collection detected mostly TcI, TcII and TcV in single infection (Toledo et al., 2013). With insects of the same locality fed in the laboratory we found more mixed than single infections. Regarding *T. cruzi* burden in this prolonged follow-up study with *M. gajardoi* this tended to decrease after three consecutive feedings (8–15 weeks, equivalent to one nymph stage period); suggesting that triatomines need to be re-infected to transmit *T. cruzi* and behave as a competent vector. Infected insects can survive with high parasite burden as revealed in 3 out 21 insects which died after the fourth feeding, including insect N° 25 which maintained a high parasite burden. The analysis of *T. cruzi* DTU composition in *M. spinolai* was performed. Very similar results of fluctuations in *T. cruzi* DTU composition were obtained with the *M. spinolai* sample analyzed after three feedings. Mixed infections were evident after two or three feedings, suggesting that the nutritional status of both bug species allows them to cope with complex *T. cruzi* compositions of two, three and up to four *T. cruzi* DTUs. Single infections almost disappeared in *M. spinolai* under this feeding condition except in one case (insect N° 17). Prolonged laboratory feeding on up to three occasions allowed the amplification of residual *T. cruzi* DTUs, as is the case with TcII, while other *T. cruzi* DTUs were detected at the same rates. Further studies with more naturally infected *M. spinolai* are necessary to confirm this preliminary observation. Previous results of *T. cruzi* DTU composition in the same locality in *M. spinolai* analyzed right after collection mainly detected TcI, much less TcII, very few cases of TcV, TcVI and few mixed infections (Coronado et al., 2009). The results in *M. spinolai*, similar to those obtained in the *M. gajardoi* sample, suggest that complex mixtures of *T. cruzi* can be hosted in properly fed *Mepraia* species even though vectors may contain a complex aggressive microbiota composition and/or can have innate immune responses that may attenuate the parasitism (Eichler and Schaub, 2002; Azambuja et al., 2004; Whitten et al., 2007; Gourbiere et al., 2012). Even though *M. gajardoi* and *M. spinolai* are two different species of the genus *Triatoma* they allow to amplify and develop the four tested *T. cruzi* DTUs (TcI, TcII, TcV and TcVI), in contrast to triatomine species of different genera such as *Rhodnius*, *Triatoma*, and *Panstrongylus*. Experimental infections of several triatomine species of Colombia showed that TcII only developed in *Panstrongylus* and *Triatoma* species but not in any *Rhodnius* species. According to other investigations, TcI developed in all the triatomine species tested including *Rhodnius* (Mejia-Jaramillo et al., 2009; Araujo et al., 2014). Therefore it is considered vectors are biological filters transmitting different *T. cruzi* DTUs (Vallejo et al., 2009). In detailed studies performed with experimental infections in *T. infestans* with well-established *T. cruzi* DTUs belonging to the zymodemes 1 and 2 bol which correspond to TcI and TcV, respectively. TcI rather than TcV-infected hosts reached higher *T. cruzi* densities between 2 and 12 weeks post infection in the midgut and rectum with permanent feeding between molts (Schaub, 1989). The feeding protocol used in our study represents a permanent feeding able to cope with the development of each *T. cruzi* DTU present in natural triatomine infections. Some of the DTUs need only one feeding but others, probably less represented as is the case of TcII in *M. spinolai*, need more than one feeding to amplify and reach the minimum to be detected by the minicircle PCR assay. In experimentally TcI-infected *T. infestans* fed after long periods of starvation, parasite density increased in the rectum by the 10th day after feeding, with important changes in the different *T. cruzi* forms (Kollien and Schaub, 1998). These studies resembled ours in which natural populations of *Mepraia* were collected and probably are starved. After experimental infection of *T. infestans* with two different *T. cruzi* DTUs no particular *T. cruzi* DTU of the mixture proliferated preferentially in the host (Pinto et al., 1998; Da Silveira et al., 2000). Our results do not allow quantifying exactly the *T. cruzi* DTU burden; although semi-quantitatively it is possible to estimate the parasite burden by conventional PCR and the most abundant *T. cruzi* DTUs in *Mepraia* species mixed infections by means of the hybridization signal sizes. No specific *T. cruzi* DTU compositions are present in *M. gajardoi* and *M. spinolai* mixed infections. Complex mixtures of *T. cruzi* may coexist within *Mepraia* species provided feeding is prolonged in time. *T. cruzi* DTU detection undergoes fluctuations through this process. These observations are important epidemiologically since infecting *Mepraia* species makes them hungrier, searching for food more frequently and improving their vector capacity to any vertebrate that is a competent reservoir. Some *T. cruzi* DTUs require prolonged feeding to be detected; they are probably less represented as observed with DTU TcII in *M. spinolai*. However *Mepraia* seem to require re-infections to maintain their infectious state, otherwise the triatomine decreases its parasite burden after successive feedings without re-infections of *T. cruzi*.

5. Conclusions

After having followed up in the laboratory about what is *T. cruzi* DTUs composition in naturally infected vector we can conclude the following: 1. naturally infected *Mepraia* species transmit different

T. cruzi DTU composition after different laboratory feeding events 2. *T. cruzi* DTUs composition change from single to mixed infections and viceversa in naturally infected *Mepraia* species after several laboratory feeding events 3. Prolonged laboratory feedings with non-infected hosts on two and three occasions produced a decrease in *T. cruzi* burden in naturally infected triatomines.

Acknowledgement

This work was supported by FONDECYT-Chile 1120122 to A. Solari and 3150289 to R. Campos.

References

Araujo, C.A., Waniek, P.J., Jansen, A.M., 2014. TcI/TcII co-infection can enhance *Trypanosoma cruzi* growth in *Rodnius prolixus*. Parasite Vector 7, 94.
Azambuja, P., Feder, D., García, E., 2004. Isolation of *Serratia marcescens* in the midgut of *Rhodnius prolixus*: impact on the establishment of the parasite *Trypanosoma cruzi* in the vector. Exp. Parasitol. 107, 89–96.
Botto-Mahan, C., Ortiz, S., Rozas, M., Cattan, P.E., Solari, A., 2005. DNA evidence of *Trypanosoma cruzi* in the Chilean wild vector *Mepraia spinolai* (Hemiptera: reduviidae). Mem. I. Oswaldo Cruz 100, 237–239.
Botto-Mahan, C., Cattan, P.E., Medel, R., 2006. Chagas disease parasite induces behavioural changes in the kissing bug *Mepraia spinolai*. Acta Trop. 98, 219–223.
Botto-Mahan, C., 2009. *Trypanosoma cruzi* induces life-history trait changes in the wild kissing bug *Mepraia spinolai*: implications for parasite transmission. Vector-Borne Zoonotic Dis. 9, 505–510.
Coronado, X., Rozas, M., Botto-Mahan, C., Ortíz, S., Cattan, P.E., Solari, A., 2009. Molecular epidemiology of Chagas disease in the wild transmission cycle: the evaluation in the sylvatic vector *Mepraia spinolai* from an endemic area of Chile. Am. J. Trop. Med. Hyg. 81, 656–659.
Da Silveira, P.A., de Lana, M., Britto, C., Bastrenta, B., Tibayrenc, M., 2000. Experimental *Trypanosoma cruzi* biclonal infection in *Triatoma infestans*: detection of distict clonal genotypes using kinetoplast DNA probes. Int. J. Parasitol. 30, 843–848.
Egaña, C., Vergara, F., Campos, R., Ortiz, S., Botto-Mahan, C., Solari, A., 2014. Trypanosoma cruzi infection in *Mepraia gajardoi* and *Mepraia spinolai*: the effect of feeding nymphs from the field. Am. J. Trop. Med. Hyg. 91, 534–536.
Eichler, S., Schaub, G.A., 2002. Development of symbionts in triatomine bugs and the effects of infections with trypanosomatids. Exp. Parasitol. 100, 17–27.
Fox, J., 2005. The R commander: a basic statistics graphical user interface to R. J. Stat. Softw. 14, 1–42.
Frías, D., Atria, J., 1998. Chromosomal variation, macroevolution and possible parapatric speciation in *Mepraia spinolai* (Porter) (Hemiptera:Reduviidae). Genet. Mol. Biol. 21, 179–184.
Frías-Lasserre, D., 2010. A new species and karyotype variation in the bordering distribution of *Mepraia spinolai* (Porter) and *Mepraia gajardoi* Frias, et al. (Hemiptera; Reduviidae; Triatominae) in Chile and its parapatric model of speciation. Neotrop. Entomol. 39, 572–583.
Gourbiere, S., Dorn, P., Tripet, F., Dumonteil, E., 2012. Genetics and evolution of triatomines: from phylogeny to vector control. Heredity 108, 190–202.
Kollien, A.H., Schaub, G.A., 1997. Trypanosoma cruzi in the rectum of the bug *Triatoma infestans*: effects of blood ingestion of the vector and artificial diuresis. Parasitol. Res. 83, 781–788.
Kollien, A.H., Schaub, G.A., 1998. Development of *Trypanosoma cruzi* after starvation and feeding of the vector—a review. Tokai J. Exp. Clin. Med. 23, 335–340.
Lent, H., Jurberg, J., Galvo, C., 1994. Revalidacao de genero *Mepraia* mazza, gajardo and jorg, 1940 (Hemiptera, reduviidae triatominae). Mem. I. Oswaldo Cruz. 89, 347–352.
Mejia-Jaramillo, A.M., Peña, V.H., Triana-Chavez, O., 2009. *Trypanosoma cruzi* Biological characterization of lineages I and II supports the predominance of lineage I in Colombia. Exp. Parasitol. 121, 83–91.
Pinto, A.S., de Lana, M., Bastrenta, B., Barnabé, C., Quesney, V., Noël, S., Tibayrenc, M., 1998. Compared vectorial transmissibility of pure and mixed clonal genotypes of *Trypanosoma cruzi* in *Triatoma infestans*. Parasitol. Res. 84, 348–353.
Schaub, G.A., Lösch, P., 1989. Parasite/host—interrelationships of the trypanosomatids *Trypanosoma cruzi* and *Blastocrithidia triatomae* and the reduviid bug *Triatoma infestans*: influence of starvations of the bug. Ann. Trop. Med. Parasitol. 83, 215–223.
Schaub, G.A., 1989. Does *Trypanosoma cruzi* stress its vectors? Parasitol. Today 5, 185–188.
Schijman, A.G., Altcheh, J., Burgos, J.M., Biancardi, M., Bisio, M., Levin, M.J., Freilij, H., 2003. Aetiological treatment of congenital Chagas' disease diagnosed and monitored by the polymerase chain reaction. J. Antimicrob. Chemother. 52, 441–449.
Teixeira, A.R.L., Nascimento, R.J., Sturn, N.R., 2006. Evolution and pathology in Chagasídisease—a review. Mem. I. Oswaldo Cruz. 101, 463–491.
Toledo, A., Vergara, F., Campos, R., Botto-Mahan, C., Ortiz, S., Coronado, X., Solari, A., 2013. Trypanosoma cruzi genotypes in *Mepraia gajardoi* from wild ecotopes in Northern Chile. Am. J. Trop. Med. Hyg. 88, 285–288.
Vallejo, G.A., Guhl, G., Schaub, G.A., 2009. Triatominae—*Trypanosoma cruzi/T: rangeli*: vector-parasite interactions. Acta Trop. 110, 137–147.
Veas, F., Breniere, S.F., Cuny, G., Brangues, C., Solari, A., Tibayrenc, M., 1991. General procedures to construct highly specific kDNA probes for clones of *Trypanosoma cruzi* for sensitive detection by polymerase chain reaction. Cel. Mol. Biol. 37, 73–84.
WHO, Research priorities for Chagas disease, human African trypanosomiasis and leishmaniasis Technical report series no. 975, (2012).
Whitten, M., Sun, F., Tew, I., Schaub, G., Soukou, C., Nappi, A., 2007. Differential modulation of *Rhodnius prolixus* nitric oxide activities following challenge with *Trypanosoma rangeli*, *Trypanosoma cruzi* and bacterial cell wall components. Insect. Biochem. Mol. Biol. 37, 440–452.
Wincker, P., Britto, C., Pereira, J.B., Cardoso, M.A., Oeleman, O., Morel, C.M., 1994. Use of a simplified polymerase chain reaction procedure to detect *Trypanosoma cruzi* in blood samples from chronic chagasic patients in a rural endemic area. Am. J. Trop. Med. Hyg. 51, 771–777.
Zingales, B., Miles, M.A., Campbell, D.A., Tibayrenc, M., Macedo, A.M., Teixeira, M.M.G., Schijman, A., Llewellyn, M.S., Lages-Silva, E., Machado, C.R., Andrade, S.G., Sturn, N.R., 2012. The revised *Trypanosoma cruzi* subspecific nomenclature: rationale, epidemiological relevance and research applications. Infect.Genet. Evol. 12, 240–253.