

Trypanosoma cruzi and *Trypanosoma rangeli* co-infection patterns in insect vectors vary across habitat types in a fragmented forest landscape

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SUMMARY

The transmission of parasites can be influenced by their co-occurrence with other parasites, in some cases increasing or reducing transmission. *Trypanosoma cruzi*, aetiologic agent of Chagas disease, often co-occurs with *Trypanosoma rangeli*, a parasite not pathogenic for mammal hosts. Both parasites can reduce the fitness of their insect vectors (the triatomine bugs; Hemiptera: Reduviidae), with *T. rangeli* being more pathogenic for some species. Here, we study the prevalence of *T. cruzi* and *T. rangeli* in the triatomine *Rhodnius pallescens* across a heterogeneously transformed landscape in Panamá. We found that single *T. rangeli* infections were more common in contiguously forested habitats, while single *T. cruzi* infections predominated in anthropogenically disturbed habitats. *Trypanosoma cruzi*–*T. rangeli* co-infections were more common in contiguous forests and in peridomestic areas. Furthermore, adult insects were more likely to be co-infected than nymphs. Our results suggest that human-mediated landscape transformation might have increased the predominance of single infections with *T. cruzi* within vectors. An important mechanism driving changes in trypanosome infection patterns in triatomines at a landscape scale includes alterations in host species composition that may vary with different degrees of deforestation. Trypanosome co-infection may also confer a survival advantage for *R. pallescens* to and/or throughout adulthood.

Key words: deforestation, Panamá, *Trypanosoma cruzi*, *Trypanosoma rangeli*, kissing bugs, *Rhodnius pallescens*, Triatominae.

INTRODUCTION

Animals are co-infected by a wide range of parasite species (Graham, 2008; Telfer *et al.* 2008; Johnson *et al.* 2015) whose interactions can affect both parasites and hosts across spatial and temporal scales (Rohani *et al.* 2003; Seabloom *et al.* 2009). Co-infecting parasite species may interact directly or indirectly within the host via direct competition, immune-mediated competition, or facilitation, which can enhance susceptibility to a co-infecting parasite, or result in a change in parasite virulence and/or within-host pathogenicity of one or more parasites (Petney and Andrews, 1998; Chaves *et al.* 2009; Eswarappa *et al.* 2012). At a population level, co-occurring infections may also impact the dynamics and persistence of infectious diseases

through a variety of mechanisms that drive the emergence of human and/or animal diseases (Dobson, 1985; Pedersen and Fenton, 2007; Jolles *et al.* 2008; Diuk-Wasser *et al.* 2015). One mechanism is ecological interference, where changes in the susceptibility to one parasite due to population-level phenomena can impact the transmission dynamics in a host population, as in the case of measles outbreaks indirectly changing *Bordetella* dynamics (Rohani *et al.* 2003); a phenomenon previously described as heterologous immunity in places where different malaria species co-occur (Cohen, 1973; Richie, 1988); or cross-immunity in places where different strains also co-exist (Bruce *et al.* 2000).

Experimental studies have shown that within-community parasite species richness is negatively associated with within-host parasite persistence (Hoverman *et al.* 2012; Johnson and Hoverman, 2012). Similarly, a host's environment (e.g. resources, climate) may influence co-infection and in turn, influence infection outcomes at the

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population and community level. For instance, in drought years, *Babesia* and canine distemper virus co-infections are frequent, and host mortality is increased; this is likely due to interactions between immunosuppression caused by canine distemper virus, and increased *Babesia* co-infections from increased numbers of questing ticks after drought-induced ungulate die-offs (Munson *et al.* 2008). Habitat type and land use may also influence population-level patterns of parasite co-infection, as has been observed in spirochete co-infection in ticks (Sytykiewicz *et al.* 2015). Studying patterns of vector-co infection at habitat and landscape scales [as reviewed in (Diuk-Wasser *et al.* 2015)] is a critical component of predicting and preventing zoonotic disease transmission risk.

In Panamá, the triatomine species *Rhodnius pallescens* (Hemiptera: Reduviidae) is an important vector of the parasite *Trypanosoma cruzi*. *Trypanosoma cruzi* infects a wide range of domestic and wild mammal hosts, and is the causative agent of Chagas disease in humans. Prior studies in Panama have shown that *T. cruzi* infection in *R. pallescens* increases in response to anthropogenic disturbance (Gottdenker *et al.* 2012). Furthermore, *R. pallescens* is frequently co-infected by *T. cruzi* congeneric *T. rangeli*, a trypanosome species that is entomopathogenic in experimentally infected *Rhodnius prolixus*, but is not believed to cause disease in mammals (Groot and Hernandez Mora, 1947; Groot *et al.* 1951; Herbig-Sandreuter, 1957; Añez, 1981; Añez *et al.* 1985; Nieves and Añez, 1992; Peterson and Graham, 2016). *Trypanosoma rangeli* is epidemiologically important, as it shares 60% of its antigens with *T. cruzi*, and the two parasites can cross-react in serological tests (Guhl and Marinkelle, 1982; Saldana & Sousa, 1996a, b; Guhl and Vallejo, 2003).

Recent studies have also shown that, like *T. rangeli*, *T. cruzi* infection can have negative impacts on vector fitness (Añez *et al.* 1992; Fellet *et al.* 2014; Peterson *et al.* 2015). Additionally, triatomines (*R. prolixus*) experimentally co-infected with *T. cruzi* and *T. rangeli* have higher survival than insects infected with just one of the parasites (Peterson *et al.* 2016). This evidence that infection by one or both of the parasites can affect vector fitness suggests that these infections could influence vector population dynamics, the distribution and frequency of *T. cruzi*-infected *Rhodnius* spp. and, in turn, *T. cruzi* transmission to humans.

In this study, we evaluate how *T. cruzi*–*T. rangeli* co-infection in *R. pallescens* varies in response to anthropogenic landscape transformation. We propose two competing hypotheses to explain what is driving observed patterns of co-infection. First, we propose that a lower frequency of co-infected vectors than expected due to chance would suggest that individual or population-level interference

between *T. cruzi* and *T. rangeli* (within their insect vectors or mammal hosts) structures co-infection patterns found in *R. pallescens*. In this scenario, co-infection patterns would be independent of landscape factors, and thus would not differ between landscape classes, nor be explained by spatial segregation of the parasites. We may also see a higher frequency of co-infected vectors, independent of habitat type, if infection by one parasite facilitates infection by another. Alternatively, a higher frequency of co-infection than expected by chance would suggest that habitats, acting as ‘templates’ of host community structure (Southwood, 1977), more strongly shape parasite transmission patterns. In this scenario, we would expect to see environmental or habitat-based differences in co-infection patterns across different landscape types (due to associated differences in the species composition of mammal hosts between habitats), and thus co-infection patterns would differ between landscapes.

METHODS

Rhodnius pallescens ($N = 643$) were captured from *Attalea butyracea* palm crowns during the wet season of 2007 (May–December) in a total of 45 study sites located to the east (Chilibre) and west (La Chorrera) of the Panamá Canal within the Panamá Canal Watershed. Sites ($N = 45$) were located in (a) contiguous late secondary tropical moist forests in Soberania National Park and (b) in four different habitats in anthropogenically transformed landscapes: mid-secondary forest remnants (seven sites), early secondary forest patches (eight sites), cattle pasture (eight sites) and peridomiliary areas within 100 m of a human dwelling (seven sites). Five randomly selected palms were sampled from each site. Study site locations are shown in Fig. 1. Detailed descriptions of the moist tropical forest habitat types have been reported previously (Gottdenker *et al.* 2011, 2012). Contiguous forests are fully protected sites within Soberania National Park, mid-secondary forest patches are remnants of original contiguous forest, generally around riparian areas, and early secondary forest patches refer to forest patches that were previously deforested, and in the early secondary forest stage of ecological succession. For all study sites, permission of the landowners and/or Soberania National Park management was obtained. Permits for triatomine collection were obtained through the Autoridad Nacional de Ministerio de Ambiente (ANAM) of Panamá.

To collect triatomines, Noireau traps were used in combination with direct searching. Three Noireau traps (Abad-Franch *et al.* 2000, 2010; Noireau *et al.* 2002) were placed within the crown of each *A. butyracea* palm and checked the following day for *R. pallescens* (Gottdenker *et al.* 2011, 2012).

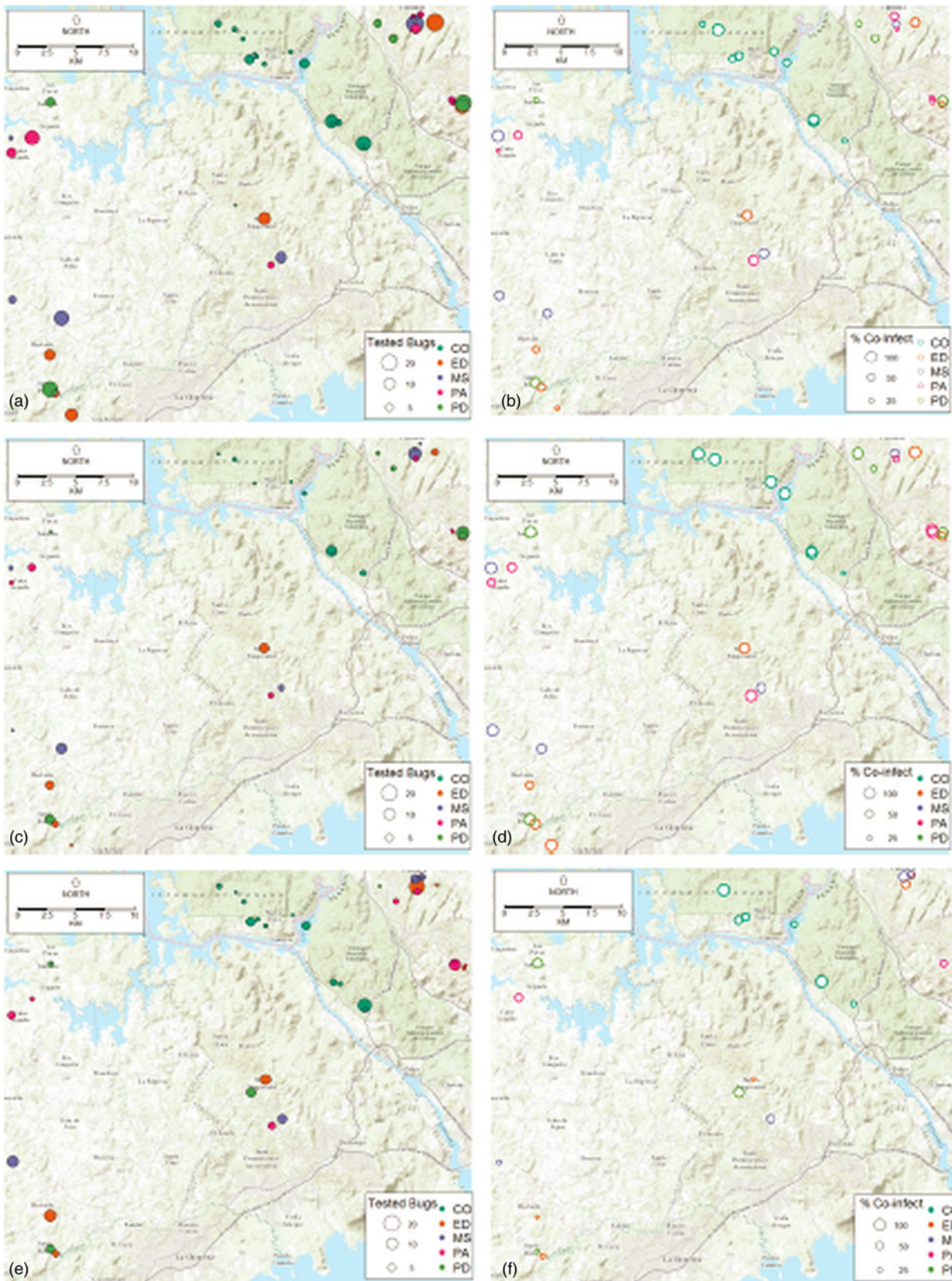


Fig. 1. Maps of infection prevalence with single *Trypanosoma cruzi* infection, single *Trypanosoma rangeli* infection, and *T. cruzi*–*T. rangeli* co-infection at study sites. CO, contiguous forest; ED, early secondary forest; MS, mid-secondary forest remnant; PA, cattle pasture; PD, peridomestic. (a) Total number of *Rhodnius pallescens* tested, (b) per cent of *R. pallescens* co-infected, (c) total number of adult bugs tested, (d) per cent of adult bugs co-infected. (e) Total number of nymphal bugs tested, (f) per cent of nymphal bugs co-infected.

Traps were approved by the Gorgas Memorial Institute Animal Care and Use Committee in accordance with Panamá's regulations for animal use. After recovering the baited traps, palm crowns were manually examined for triatomines by a skilled individual for 10 min. Palm crowns were accessed with a 20 feet tall ladder or by climbing the palm tree with a rope and harness climbing technique modified for palm trees.

After capture, triatomines were identified to the species level, following the key by Lent and Wygodzinsky (1979) and classified by developmental stage. To diagnose trypanosome infection legs and wings of adults were removed, triatomine bodies were macerated (Calzada *et al.* 2006; Pineda *et al.* 2008; Gottdenker *et al.* 2012) and DNA was extracted with a commercial kit (Promega, Madison, WI) following manufacturer instructions. *Trypanosoma cruzi* and *T. rangeli* DNA was amplified in a duplex PCR using the primer set Tc189Fw (5'-CCAACGC TCCGGGAAAAC-3') and Tc189Rv3 (5'-GCGTC TTCTCAGTATGGACTT-3') for *T. cruzi* and TrF3 (5'-CCCCATACAAAACACC CTT-3') and TrR8 for *T. rangeli* (5'-TGGAATGACGGTG CGGCGAC-3') (Chiurillo *et al.* 2003). PCR products mixed with loading dye were run on a 1.5% agarose gel and *T. cruzi* (100 bp) and *T. rangeli* (170 bp) specific bands were observed. Negative controls of extraction and pcr as well as positive controls for pcr were run with known standards of *T. cruzi* and *T. rangeli* from cultured isolates from *R. palllescens* collected near areas of study as positive controls (single reactions) and *T. cruzi* and *T. rangeli* in the same tube as well as co-infected and singly infected samples from known-infected bugs.

Statistical analyses

Descriptive statistics were performed on singly infected and co-infected bugs for each habitat type. Confidence intervals for the proportion of bugs infected were calculated using a 1-sample proportions test with continuity correction. Chi-squared (χ^2) analysis was used to evaluate univariate associations between triatomine stage (N3, N4, N5 and adult) and infection status (co-infected with *T. cruzi*, singly infected with *T. cruzi* or *T. rangeli*, or uninfected) as well as habitat and infection status. Association plots were used to visualize independence in two-way contingency tables for single/co-infection statuses among all bugs in each habitat, as well as bug stage and single/co-infection status using the vcd package in R (Meyer *et al.* 2015). Maps were made of the overall number of *R. palllescens* bugs sampled and found infected. Generalized Estimating Equation models (GEEM) (Venables and Ripley, 2002) were used to evaluate the probability of vector infection in each habitat type using the Yags package (Carey, 2004) in the R

statistical computing environment (R Development Core Team, 2015). Logistic GEEM models with binomial errors were used because triatomines captured within each site were not independent from one another and we assumed that the correlation structure in the models was independent. An advantage to the logistic GEEM models is that empirical sandwich estimators can be used that result in valid confidence intervals for fixed effects, even if there is an incorrect or uncertain correlation structure (Venables and Ripley, 2002). For each binomial response variable (*T. cruzi* single infection, *T. rangeli* single infection, *T. cruzi*-*T. rangeli* co-infection), we began the analysis building a full model for each type of infection that included the following independent variables: habitat type, insect stage and covariates of *T. cruzi* or *T. rangeli* infection status (e.g. in the case where *T. cruzi* single infection was a response variable, *T. rangeli* was included as a covariate in the full model and vice versa in the case of *T. rangeli* single infection as a response variable). A total of 632 bugs were available for this GEE analysis, less than the 643 tested for trypanosomes because 11 bugs were not identified to stage. The Quasilikelihood information criterion (QIC), a model selection metric analogous to the Akaike Information Criterion (AIC), was used to identify the best fitting model among the set of candidate models for each infection type (Pan, 2001).

RESULTS

Figure 1 shows a map of the locations of the numbers of bugs tested (Fig. 1a), as well as the percentage of co-infected nymphs and adults (Fig. 1b), the number of adult bugs (Fig. 1c) and nymphs tested (Fig. 1e) and their co-infection (Figs 1d and 1f, respectively). In general, co-infections were more common in adult bugs (Fig. 1d), yet most samples were nymphs (Fig. 1e). Figure 2 shows patterns of the mean proportion of co-infected and singly infected bugs within and between each habitat type (Supplementary table A also shows the number of infected and co-infected bugs in each habitat type). Overall, across all sites, there was a significant association between habitat type and trypanosome infection in *R. palllescens* ($\chi^2 = 77.74$, $df = 12$, P -value = 1.11×10^{-11}). The proportion of bugs singly infected with *T. cruzi* increased in disturbed habitats, while the proportion of bugs singly infected with *T. rangeli* markedly increased in contiguous forest as compared to anthropogenically disturbed habitats (Fig. 2). Association plots (Fig. 3) demonstrating habitat-related differences in infection status show that the frequencies of co-infected bugs were significantly lower than expected due to chance in cattle pasture. *Trypanosoma rangeli* single infections were significantly more frequent than expected due to chance in contiguous forests, and less frequent than

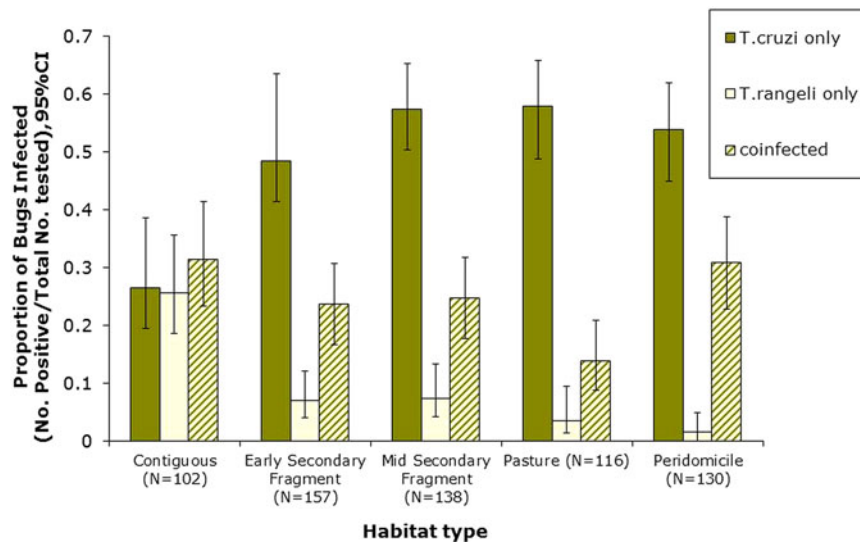


Fig. 2. Single and co-infection of *Rhodnius pallescens* with *Trypanosoma cruzi* and *Trypanosoma rangeli* in different habitat types (Contig, contiguous forest; ES, early secondary forest patch; MS, mid-secondary forest remnant; Past, cattle pasture; PD, peridomicile).

expected in peridomiciliary habitats, and *T. cruzi* single infections were less than expected in contiguous forests (Fig. 3). Generalized estimating equations evaluating relationships between infection (co-infection, single infection with *T. cruzi* and *T. rangeli*, respectively), habitat and insect stage show that the odds of infection differed across habitats depending on the infection type (Table 1). Although overall triatomine infection status (single and co-infections) was more evenly distributed in contiguous forests, the odds ratio of single infections with *T. cruzi* in *R. pallescens* was greater in deforested habitats (mid-secondary forest remnants, early secondary forest patches, cattle pasture) compared with contiguous forests (Table 1). The odds of vector co-infection contiguous forests and peridomiciliary sites were significantly higher than in cattle pasture (Table 1). The odds of single infections with *T. rangeli* were significantly higher in contiguous forests relative to cattle pasture (Table 1). All other variables mentioned in the methods were left out by the process of model selection, or because models containing them did not converge numerically because of collinearity issues, i.e. some of the covariates had a nearly perfect correlation.

General stage-specific patterns of trypanosome infections are shown in Table 2. Overall, there was a significant association between triatomine stage and infection status (non-infected, co-infected, singly infected with *T. cruzi*, singly infected with *T. rangeli*) ($\chi^2 = 63.46$, $df = 7$, P -value = 2.877×10^{-10}). Generalized estimating equations showed that stage was only significantly associated with infections in the case of co-infections (adults were more likely to be co-infected than nymphs) (Table 1).

DISCUSSION

Our results show that patterns of single and co-infection of *R. pallescens* with *T. cruzi* and *T. rangeli* differ as a function of habitat type. *Trypanosoma cruzi* single infection frequency was markedly higher in anthropogenically disturbed habitats, and this was supported by its greater association with mid-secondary forest fragments, cattle pasture, and peridomiciliary sites and less frequent than expected occurrence in contiguous forests (Fig. 2, Table 1). On the other hand, *T. rangeli* single infection frequency was significantly greater than expected in contiguous forests. *Trypanosoma cruzi* and *T. rangeli* co-infection seemed to take on occurrence patterns of both infections, as co-infection was significantly more likely to occur in contiguous forests and peridomiciliary sites, and least likely in pasture habitats (Table 1).

One potential explanation for the habitat-related difference in patterns of trypanosome infections in triatomines is that anthropogenic disturbance can lead to differences in host community structure. This in turn can lead to changes in the principal mammalian reservoir of *T. cruzi* in each habitat type, and the hosts that are coming into contact with the triatomine vectors. In theory (Dobson, 2004; Chaves *et al.* 2007; Roche *et al.* 2013) and in practice, host community structure and/or variation in contact with hosts of varying competence can influence vector infection prevalence in a wide variety of vector-borne disease systems, including West Nile Virus (Loss *et al.* 2009; Hamer *et al.* 2012), Chagas disease (Kjos *et al.* 2009; Gottdenker *et al.* 2012; Gurtler *et al.* 2014; Gurtler and Cardinal, 2015) and a variety of mosquito-borne

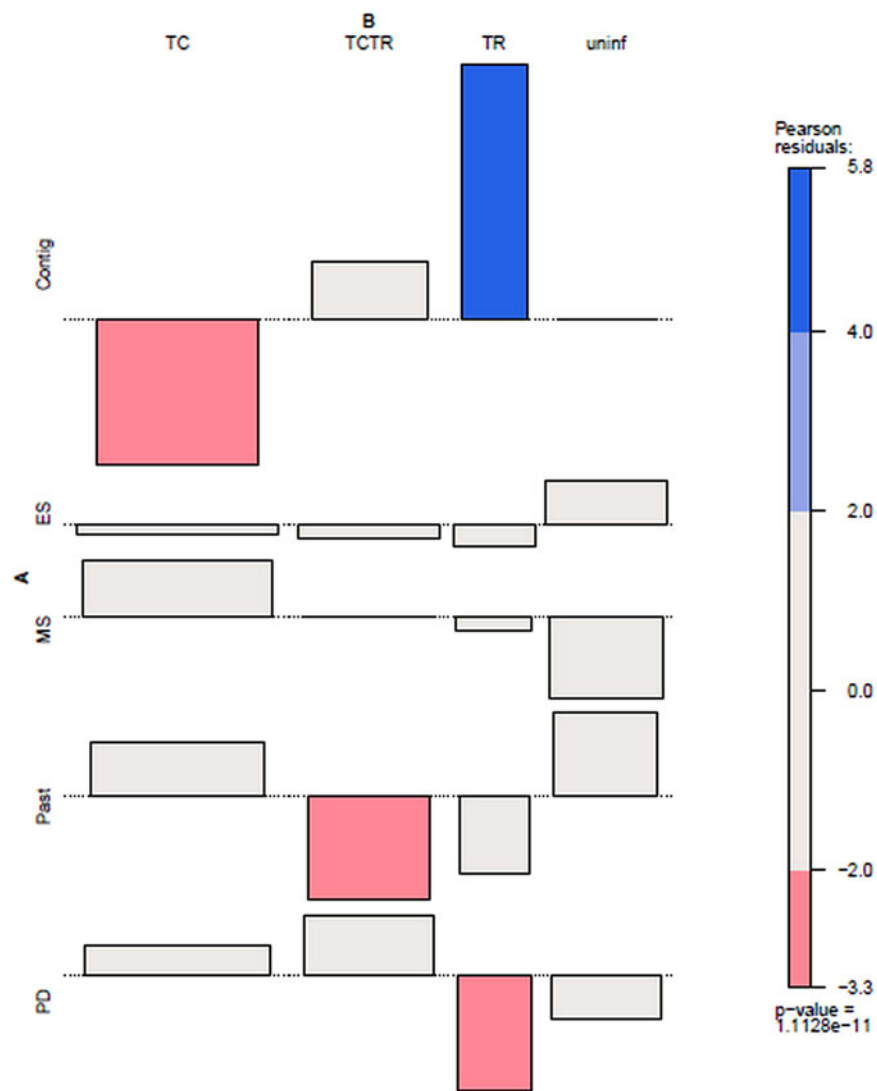


Fig. 3. Association plot of habitat and trypanosome co-infection patterns in *Rhodnius pallescens*. Contig, contiguous forest; ES, early secondary forest patch; MS, mid-secondary forest remnant; Past, cattle pasture; PD, peridomicile; TC, single infection with *Trypanosoma cruzi*; TCTR, co-infection with *T. cruzi* and *Trypanosoma rangeli*; TR, single infection with *T. rangeli*; uninf, uninfected. Pearson residual values of χ^2 associations are shown to the right. Bar width corresponds to the square root of the expected counts, and the height of the bars are proportional to the Pearson Residual values.

pathogens (Chaves *et al.* 2010). There is evidence of differential mammalian host competence for *T. cruzi* across taxa, but less is known about mammal host competence for *T. rangeli*. *Trypanosoma rangeli* infection has been frequently found in Pilosa (sloths and tamanduas) (Zeledon *et al.* 1979; Miles *et al.* 1983; Dereure *et al.* 2001; Dias *et al.* 2010; De Araujo *et al.* 2013) and their vectors. Blood meal analysis of *R. pallescens* vectors (Gottdenker *et al.* 2012) showed that Pilosa (sloth and tamandua) blood meals are present across all habitats, tamandua, sloth and primate blood meals dominate in *R. pallescens* captured in primary forests, and sloth blood meals are relatively common in all habitats. This suggests that sloths may be commonly infected with *T. rangeli* across landscape types, and is in accordance with previous observations in Panamá, where sloths were frequently infected with *T. rangeli* (Herrer and

Christensen, 1980). Although *T. rangeli* has been isolated from a wide range of vertebrate taxa (Miles *et al.* 1983), including sloths, non-human primates, bats, rats, and humans, the role different mammal species play in *T. rangeli* transmission is not well known, and one report states that there are no particular *T. rangeli*-specific vertebrate host associations (Maia Da Silva *et al.* 2007).

Our finding of significantly increased infection prevalence with *T. rangeli* in contiguous forests and increased odds of infection in forest fragments, i.e. mid-secondary and early secondary (Table 1) suggests that there may be some similarities in the host community composition or the identity of species coming into contact with vectors between these habitat types. Single infections with *T. cruzi* were more likely in triatomines captured in anthropogenically disturbed sites such as in cattle

Table 1. Parameter estimates for the logistic generalized estimating equation models explaining infection status for *Rhodnius pallescens* singly infected with *Trypanosoma cruzi* only (a), co-infected with *T. cruzi* and *Trypanosoma rangeli* (b), singly infected with *T. rangeli* (c). Sites were the clustering factor in the analysis.

Parameter	Estimate	±Sandwich S.E.	Z*
(a) <i>T. cruzi</i> single infection			
Intercept (Pasture + N3)	0.19	0.34	0.61
Contiguous	-1.32	0.41	-3.21
Early secondary Forest	-0.37	0.31	-1.20
Mid-secondary forest	-0.04	0.40	-0.11
Peridomiciliary	-0.16	0.35	-0.45
Stage A	0.004	0.230	0.019
Stage N4	0.38	0.34	1.10
Stage N5	0.31	0.32	0.97
(b) <i>T. cruzi</i> - <i>T. rangeli</i> co-infection			
Intercept (Pasture + N3)	-2.59	0.37	-7.05
Contiguous	1.17	0.40	2.96
Early secondary	0.65	0.35	1.84
Mid-secondary	0.68	0.36	1.86
Peridomiciliary	1.04	0.38	2.44
Stage A	1.34	0.28	4.82
Stage N4	0.21	0.38	0.57
Stage N5	0.61	0.35	1.74
(c) <i>T. rangeli</i> – single infection			
Intercept (Pasture + N3)	-3.43	0.57	-5.98
Contiguous	2.24	0.61	3.68
Early secondary	0.72	0.62	1.16
Mid-secondary	0.80	0.83	0.96
Peridomiciliary	-0.80	0.78	-1.02
Stage A	-0.21	0.51	-0.40
Stage N4	0.08	0.55	0.15
Stage N5	0.69	0.64	1.08

* Significant ($P < 0.05$) when $|“Z”| > 1.96$, Significant parameters are in bold.

Table 2. Stage-specific patterns of *Rhodnius pallescens* single and co-infections with *Trypanosoma cruzi* and *Trypanosoma rangeli* across all habitat types.

Stage (N = 632) ^a	<i>T. cruzi</i> (95% CI)	<i>T. cruzi</i> - <i>T. rangeli</i> co-infection (95% CI)	<i>T. rangeli</i> (95% CI)
Adults (N = 239)	46.9% (40.4, 53.3)	37.2% (31.1, 43.7)	6.3% (3.7, 10.3)
N5 (N = 110)	55.5% (45.7, 64.8)	21.8% (14.7, 30.9)	11.8% (6.7, 19.7)
N4 (N = 134)	55.2% (46.3, 63.7)	17.2% (11.4, 24.8)	8.2% (4.4, 14.6)
N3 (N = 149)	45.6% (37.5, 54.0)	13.4% (8.9, 20.2)	8.1% (4.4, 13.9)

^a 632 of 643 total bugs examined by PCR were identified to stage.

pasture, mid-secondary forest remnants and peridomiciliary sites, where triatomines are also more likely to come in contact with ‘anthropic’ species that are highly competent for *T. cruzi*, such as opossums (*Didelphis marsupialis* and *Metachirus nudicaudatus*). Previous studies show a positive association with *T. cruzi* infection in *R. pallescens* and opossum blood meal frequency (Gottdenker *et al.* 2012).

Trypanosome co-infection patterns within triatomine vectors could be related to potential interactions between trypanosome species within vectors and/or hosts. Laboratory studies have shown that infection with one species of trypanosome in the

vertebrate host may lead to lower infection rates with the second congeneric parasite due to an immunoprophylactic effect of infection of the first parasite. For instance, laboratory mice injected with epimastigotes of *T. rangeli* developed a lower parasitaemia upon challenge with a virulent *T. cruzi* strain, which led to decreased severity of disease outcomes, and 100% survival of all mice (Palau *et al.* 2003; Basso *et al.* 2008). Domestic dogs vaccinated with *T. rangeli* and subsequently challenged with *T. cruzi* had less parasitaemia and lower rate of infection to bugs (Basso *et al.* 2007). However, we did not find significantly lower than expected levels of

T. cruzi–*T. rangeli* co-infection in triatomines at a landscape or habitat level with the exception of cattle pastures (Fig. 1), lending little support to our initial hypothesis that ecological interference (competition, indirect interactions) or immunological interference (cross-immunity) between *T. cruzi* and *T. rangeli*, drive single and co-infection patterns. Regardless, immunologically mediated interactions between *T. cruzi* and *T. rangeli* may occur within vectors, such as a previous infection with *T. rangeli* that may facilitate *T. cruzi* infection and/or replication in the vector. Further studies of trypanosome co-infection in wild vectors and hosts would help understand other potential mechanisms driving vector infection patterns.

In situ, mammal co-infection with *T. cruzi* and *T. rangeli* is relatively common in Neotropical wildlife where both parasites circulate between vectors and mammalian hosts (Christensen and de Vasquez, 1981; Miles *et al.* 1983; Yeo *et al.* 2005; Da Silva *et al.* 2008). It is possible that lower co-infection rates in cattle pastures may be due to a lower availability of mammal hosts of *T. rangeli* or co-infected vertebrates, or fewer contacts between these mammals and the bugs. Further research into the relative competence in different wild and domestic mammal species found across deforestation gradients for *T. cruzi* and *T. rangeli* is important to fully understand vector co-infection. Xenodiagnosics, a common means to assess reservoir competence, may be difficult in rare, evasive and/or cryptic species. In this case, indirect methods, such as molecular blood meal analysis in combination with extensive host sampling, may be important in inferring relative host competence and its role in vector infection prevalence.

In terms of potential parasite interactions within the vector, we found that co-infections with *T. cruzi* and *T. rangeli* increased with stage (Tables 1 and 2), with adults being the stage with highest predicted odds of co-infection (Table 2). In *R. prolixus*, co-infection with *T. cruzi* and *T. rangeli* results in increased vector fitness (Peterson, 2015). If *R. pallescens* responded similarly to co-infection, then the positive association of adult bugs and co-infection may potentially be the result of co-infection conferring a survival advantage to and/or throughout adulthood in these bugs.

Another potential explanation for this result is that co-infection allows for more virulent *T. rangeli* strains to persist in vector populations. *Trypanosoma rangeli* has been observed to negatively affect the feeding behaviour and life history of some species of experimentally infected *Rhodnius* (Watkins, 1971a, b; Añez, 1984; Añez and East, 1984; Añez *et al.* 1992; Kollien *et al.* 1998; Eichler & Schaub, 2002; Schaub, 2006; Maia Da Silva *et al.* 2007; Vallejo *et al.* 2009). Thus, it is possible that higher single *T. rangeli* infection rates in contiguous forests act to regulate vector populations. A

moderate decline of *T. rangeli* infection in deforested landscapes may cause ecological release, allowing for increased reproductive success of *R. pallescens* in deforested landscapes, as previous studies have shown higher vector abundance in deforested habitats (Gottdenker *et al.* 2011). Currently, *R. pallescens* occupancy rates in *A. butyracea* palms in Panamá are the highest recorded among triatomines infesting palms throughout Latin America (Abad-Franch *et al.* 2015). Studies of the relative *T. cruzi* and *T. rangeli* parasite loads in single and co-infected *R. pallescens*, and of the impacts of trypanosome infection on *R. pallescens* fitness and pathogenicity at individual and population levels are necessary in order to better understand potential impacts of trypanosome infection patterns on *R. pallescens* population dynamics.

To summarize, we show evidence that anthropogenic land use and habitat type is related to patterns of single and co-infection with *T. cruzi* and *T. rangeli* in the triatomine bug species *R. pallescens*. These findings have implications for our understanding of Chagas disease infection dynamics and the role that co-infections may play on vector and host population dynamics at a population and landscape level.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at <http://dx.doi.org/10.1017/pao.2016.9>.

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