Trypanosoma cruzi–Trypanosoma rangeli co-infection ameliorates negative effects of single trypanosome infections in experimentally infected *Rhodnius prolixus*

JENNIFER K. PETERSON[1]*, ANDREA L. GRAHAM[1], RYAN J. ELLIOTT[1], ANDREW P. DOBSON[1] *and* OMAR TRIANA CHÁVEZ[2]

[1] *Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey 08544, USA*
[2] *Grupo BCEI, Universidad de Antioquia, Calle 70 No. 52-21, Medellín, Colombia*

(*Received 12 February 2016; revised 10 March 2016; accepted 15 March 2016; first published online 13 May 2016*)

SUMMARY

Trypanosoma cruzi, causative agent of Chagas disease, co-infects its triatomine vector with its sister species *Trypanosoma rangeli*, which shares 60% of its antigens with *T. cruzi*. Additionally, *T. rangeli* has been observed to be pathogenic in some of its vector species. Although *T. cruzi–T. rangeli* co-infections are common, their effect on the vector has rarely been investigated. Therefore, we measured the fitness (survival and reproduction) of triatomine species *Rhodnius prolixus* infected with just *T. cruzi*, just *T. rangeli*, or both *T. cruzi* and *T. rangeli*. We found that survival (as estimated by survival probability and hazard ratios) was significantly different between treatments, with the *T. cruzi* treatment group having lower survival than the co-infected treatment. Reproduction and total fitness estimates in the *T. cruzi* and *T. rangeli* treatments were significantly lower than in the co-infected and control groups. The *T. cruzi* and *T. rangeli* treatment group fitness estimates were not significantly different from each other. Additionally, co-infected insects appeared to tolerate higher doses of parasites than insects with single-species infections. Our results suggest that *T. cruzi–T. rangeli* co-infection could ameliorate negative effects of single infections of either parasite on *R. prolixus* and potentially help it to tolerate higher parasite doses.

Key words: *Trypanosoma cruzi*, *Trypanosoma rangeli*, *Rhodnius prolixus*, *T. cruzi–T. rangeli* co-infection, Chagas disease, infected-vector fitness.

INTRODUCTION

Upon infection of its insect vector, the parasite *Trypanosoma cruzi*, aetiological agent of Chagas disease, joins a diverse microbial community (Eichler and Schaub, 2002; Espino *et al*. 2009; Vallejo *et al*. 2009), consisting of up to eight species of bacteria (Vallejo *et al*. 2009), six genera of fungi (De Moraes *et al*. 2001, 2004; Luz *et al*. 2004), four other trypanosomatid species (Schaub, 1992) and at least one virus (Marti *et al*. 2015). These taxa can interact indirectly via resource competition, immune modulation, competition for immune-free space (Dobson, 1985; Cox, 2001; Pedersen and Fenton, 2007) and even sometimes directly through physical attack (Azambuja *et al*. 2004; Castro *et al*. 2007), all of which have potential consequences for the survival and reproduction of the insect.

One species of particular interest is *Trypanosoma rangeli*, a *T. cruzi* congeneric that infects several of the same mammal and triatomine species as *T. cruzi*. *Trypanosoma rangeli* is of interest in the study of Chagas disease because it shares at least 60% of its antigens with *T. cruzi* (Guhl and Marinkelle, 1982; Saldaña and Sousa, 1996; Guhl and Vallejo, 2003). These antigenic similarities can lead to cross-reactions in immunogenic diagnostic tests, which can result in erroneous Chagas disease diagnoses (Guhl *et al*. 1987) and in turn interfere with the ability to predict and describe Chagas disease distribution in Chagas-endemic regions. *Trypanosoma cruzi* and *T. rangeli* are often found co-infecting together in field-caught triatomine bugs of the genus *Rhodnius* (Fig. 1), some of which are considered key vectors of *T. cruzi* to humans (Gorla and Noireau, 2010).

Although not pathogenic in mammals (Herbig-Sandreuter, 1957), in triatomine bugs, *T. rangeli* has been observed to negatively affect the survival and development of the triatomine species *Rhodnius prolixus* when experimentally infected with the parasite (Grewal, 1957; Tobie, 1965; Gómez, 1967; Watkins, 1971; Añez, 1984; Añez *et al*. 1987). Until recently, *T. cruzi* was not believed to have negative consequences for its invertebrate hosts (Schaub, 1989*a*, 1992, 1994), although this has now been shown to be variable (Elliot *et al*. 2015; Peterson *et al*. 2015). Little is known about the consequences of *T. cruzi–T. rangeli* co-infection for the triatomine bug, and to our knowledge, has been investigated just once (Añez *et al*. 1992); that study reported delayed nymphal development and

* Corresponding author: Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey 08544, USA. E-mail: jenni.peterson@gmail.com

Parasitology (2016), **143**, 1157–1167.
doi:10.1017/S0031182016000615

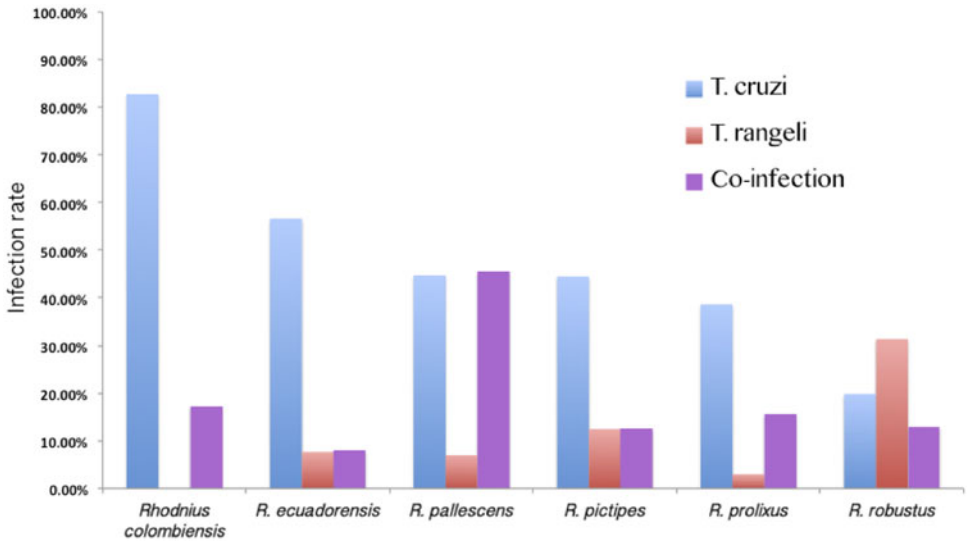

Fig. 1. Reported co-infection prevalence in field-caught *Rhodnius* triatomines (Carcavallo *et al*. 1975; Vallejo *et al*. 1988; Pavia *et al*. 2007; Pineda *et al*. 2008; Grijalva *et al*. 2012).

increased mortality in *R. prolixus* co-infected with *T. cruzi* and *T. rangeli* compared with singly-infected insects. However, the sustained effects of such co-infection on triatomines (e.g. on their reproduction or overall fitness) have never been investigated. In mammals, it was found that *T. rangeli* exposure in vertebrates prior to *T. cruzi* infection modulated the host immune response to *T. cruzi*, resulting in reduced disease severity in both acute and chronic *T. cruzi* infections (Basso *et al*. 1991, 2007, 2008, 2014; Marini *et al*. 2011; Basso, 2013). These studies suggest that *T. cruzi–T. rangeli* co-infection could affect triatomine fitness differently than single-species infections.

Here, we compared the fitness of triatomine bugs (*R. prolixus*) experimentally co-infected with *T. cruzi* and *T. rangeli* with the fitness of bugs with single-species infections of *T. cruzi* or *T. rangeli*. We defined fitness as the net contribution to future generations of each insect. We aimed to determine if there is a difference in fitness between bugs with different infection types, as we propose that the extent to which *T. cruzi–T. rangeli* co-infection alters the impact of each infection on individual vector fitness may in turn alter the transmission potential of the parasites. This, in turn, could have implications for vector control and Chagas disease prevention strategies.

MATERIALS AND METHODS

Experimental design

We infected 100 *R. prolixus* fifth instar females with just *T. cruzi*, just *T. rangeli*, or *T. cruzi* and *T. rangeli* (Table 1). A total of 33 additional uninfected insects were used as controls, for a total of 133 insects used in the experiment. After moulting into the adult stage, each female was mated with an uninfected male, and survival and reproduction were measured for up to 96–140 days. All experiments were carried out in the laboratory of the Grupo de la Biología y Control de Enfermedades Infecciosas [Biology and Control of Infectious Diseases Group (BCEI)], University of Antioquia, Medellín, Colombia.

Table 1. Treatment groups

Treatment group	
T. cruzi (Gal61 strain)	24
T. rangeli (Choachí strain)	33
T. cruzi–T. rangeli co-infection	43
Control	33

Triatomines

All *R. prolixus* used in the experiment were from laboratory colonies reared in the BCEI insectary, where triatomine colonies are kept under semi-controlled climate conditions (~27 ± 1 °C and 65 ± 15% RH) and a 12 h photoperiod, and given the opportunity to feed twice weekly on hens according to the animal ethics committee regulations of the Sede de Investigación Universitaria [University Investigation Headquarters (SIU)] of the University of Antioquia. Insects used in the experiment were fed on hens once per oviposition cycle, described below. Colonies were founded by *R. prolixus* eggs collected in Colombia between 2000 and 2009. All insects used in the experiment were 5th instar nymphs at the time of infection. Nymphs were collected manually from the colonies, and sex was subsequently determined (prior to infection)

https://doi.org/10.1017/S0031182016000615 Published online by Cambridge University Press

by examining the two concentric terminal segments around the anus on the insect's ventral side under a dissecting microscope, as described in Chiang *et al*. (2013) and Gillet (1935).

Parasites

We used the parasite strains 'Gal61' (*T. cruzi*) and 'Choachí' (*T. rangeli*). Gal61 was originally isolated from a mouse in Galeras, Colombia, and belongs to the *T. cruzi* discrete typing unit (DTU) group I (Rojas *et al*. 2007; Falla *et al*. 2009). Choachí was originally isolated from an *R. prolixus* individual collected in Cundinamarca, Colombia (Grisard *et al*. 1999; Vargas *et al*. 2000; Urrea *et al*. 2011), and belongs to the KP1(+) kDNA (kinetoplastid deoxyribonucelic acid) group (Vallejo *et al*. 2002), which is associated with the Prolixus complex of *Rhodnius* (Urrea *et al*. 2005).

Trypanosoma cruzi parasites were cultured and maintained as described in Peterson *et al*. (2015). Briefly, epimastigotes were cultured at 28 °C in a RPMI-1640 liquid medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS). Epimastigotes of the *T. rangeli* Choachí strain were supplied by Professor Gustavo Vallejo of the University of Tolima, where they were cultured at 28 °C in NNN medium and supplemented with 10% FBS. Infectivity was maintained by cyclic *R. prolixus*–mouse passages every 3 months.

Insect infection

We prepared the parasites (epimastigote stage) and infected the insects as described in Peterson *et al*. (2015). Briefly, parasites were counted in a Neubauer chamber, washed through centrifugation and resuspended in 1 mL of sterile phosphate-buffered saline. Insects were starved for about 2 weeks before the infection, upon which each bug was marked with a small dot of non-toxic water-based paint at the top of the pronotum, and then weighed before and after feeding to estimate the number of parasites ingested. Only females were fed infected blood, while the females from the control group and all males were fed uninfected blood. A total of 5–10 insects were grouped in small jars, which were then placed under a membrane feeder containing defibrinated, de-complemented human blood (heated to 37·5 °C) supplemented with inactivated FBS with an estimated concentration of $3{\cdot}3–3{\cdot}5 \times 10^6$ parasites/mL. This concentration falls within (a) the range of peak parasitaemias observed in mice and guinea pigs experimentally infected with *T. cruzi* (Bice and Zeledon, 1970; Urdaneta-Morales and Rueda, 1977; Perlowagora-Szumlewicz and Muller, 1982; Schaub and Losch, 1989*a*; Schaub *et al*. 1989; Kollien *et al*. 1998) and *T. rangeli* (Urdaneta-Morales and Tejero, 1986; Zuñiga *et al*. 1997*a*, *b*), and oral infectious doses used in prior published studies of *T. cruzi* and *T. rangeli* infection in triatomines (Garcia *et al*. 1994, 2004; Mello *et al*. 1996; Ratcliffe *et al*. 1996; Whitten *et al*. 2001; Borges *et al*. 2006; Araújo *et al*. 2007, 2014; Nogueira *et al*. 2007; Mejía-Jaramillo *et al*. 2009; Ferreira *et al*. 2010; Castro *et al*. 2012, 2014; Fellet *et al*. 2014). *Trypanosoma cruzi–T. rangeli* co-infections were carried out at a similar total parasite concentration, consisting of equal concentrations of each species (i.e. $1{\cdot}65 \times 10^6$ of each parasite species/mL of blood, for a total of $3{\cdot}8 \times 10^6$ parasites/mL of blood).

Insect reproduction

After moulting into the adult stage, we paired each female with a recently fed adult male (Buxton, 1930; Davey, 1965). Males were paired with females of just one treatment group throughout the experiment to avoid cross-contamination. Copulation was determined 1 day after insects were paired from the presence of the spermatophore casing in the jar, ejected by the female (Ruegg and Davey, 1979). If we did not find the spermatophore casing after the first night, then 2–3 additional males were placed in the jar with the female, and left for another night (G. Chiang, personal Communication, 2013). If copulation did not occur after three nights with several males, then we recorded the female as unmated for that oviposition cycle. Unmated individuals from the first oviposition cycle were given a second opportunity to mate for the second oviposition cycle. After mating, females were fed on hens (males were fed 3–4 days prior to copulation for sperm production). We marked each female with a small coloured dot of non-toxic, water-based paint on the pronotum (Mac Cord *et al*. 1983; Henriques *et al*. 2012), weighing it before and after feeding to calculate the volume of blood ingested. We recorded oviposition and eclosion 3–4 times per week until the second oviposition cycle, 31–38 days later.

We measured reproduction as fecundity (egg production) and the percentage of oviposited eggs that hatched. Fecundity in *R. prolixus* is correlated with the quantity of blood ingested and weight before feeding (Friend *et al*. 1965), and the standard index used when comparing fecundity in *R. prolixus* is the *E* value (Ruegg and Davey, 1979). The *E* value is calculated as the total number of eggs produced by a given individual divided by the product of the blood meal volume multiplied by its pre-feeding weight. This represents the efficiency with which the insect converts nutrition (blood) into food, while normalizing for blood and insect mass, allowing for comparison across feedings. The *E* value is independent of the timing of the oviposition cycle in an insect's lifetime. In analysing the *E* values,

https://doi.org/10.1017/S0031182016000615 Published online by Cambridge University Press

we did not include insects that died before an oviposition cycle began (i.e. resulting in an E value of 0), in order to compare E value independent of mortality rate. In addition to these measurements, time-dependent reproductive values were also generated for each individual in our fitness analyses, described below.

Infection confirmation

After insect death, we extracted total DNA from each insect using Qiagen DNeasy blood and tissue kit. Additionally, we extracted DNA from pooled males and pooled offspring to check for horizontal and vertical transfer of parasites. We amplified DNA in an RT–PCR (StepOnePlus Real-Time PCR System, Applied Biosystems), with the *T. cruzi* primer pair [TcZ1/2 (Cummings and Tarleton, 2003)] and *R. prolixus* reference gene primer (RP18S, Paim *et al*. 2012). To obtain a *T. rangeli*-specific primer of the optimal size [<150 base pairs (bp)] that did not cross-amplify *T. cruzi*, we designed a primer denoted as 'PEEL5' −F (5′-TGCTTTCGTAGTTGGCACTG-3′) and −R (5′-ACGCACCTCCTCCTCTCTCT-3′), which amplifies a 93 bp fragment of *T. rangeli* telomeric DNA. We designed this primer from the *T. rangeli* clone TrTel 10 telomeric sequence (GenBank ID: AF426020·1), using the Primer3 plus software (Untergasser *et al*. 2007).

Statistical analyses

We carried out all statistical analyses using the R statistical computing environment software version 3.03 (R Core Team, 2014) using non-parametric tests to avoid normality assumptions. We tested for differences between treatments in the amount of parasites or blood ingested per unit of insect mass using the Kruskal–Wallis rank sum tests. We tested for differences in the amount of parasites or blood ingested per unit of body weight using Wilcoxon Rank Sum tests. We applied the 'kruskalmc' function from the 'pgirmess' package (Giraudoux, 2013) to carry out multiple comparisons and control for family wise error when a difference was found in Kruskal–Wallis tests. This function implements comparisons between treatments, and one- and two-tailed comparisons *vs* control. We accepted P-values under 0·05 as statistically significant.

We analysed survival function for each treatment group using the Kaplan–Meier (K–M) method in the R 'survival' package (Therneau and Grambsch, 2000; Therneau, 2015). We compared survival function (the probability of total time until failure) between treatment groups using the 'survdiff ' function in the 'survival' package, a two-tailed test for censored data that implements the G–ρ family of tests (Harrington and Fleming, 1982), where deaths at various times are weighted by a factor of $S(t)^\rho$ (S = K–M estimate; t = time), and ρ is a scalar parameter that determines the type of test used. When set at 0, all deaths are weighted equally across time and a log-rank test is used. When set at 1, deaths at the beginning of the time period are more heavily weighted, and the Peto and Peto test (Peto and Peto, 1972) is employed. We set ρ at 1, to offset insect death events related to senescence. We carried out pairwise comparisons between K–M survival curves with Chi-squared (χ^2) distribution tests and adjusted P-values to control for the familywise error rate using the Holm–Bonferroni correction method (Holm, 1979).

We used Cox proportional hazards (PH) models (Cox, 1972) to examine the main effects and two-way interactions of parasite treatment, parasite dose and blood ingested on treatment hazard rates (the instantaneous rate of failure at any given time, given that the individual has survived up until that time). The PH assumption, (i.e. hazards were proportional over time) was tested with the Coxph function in the 'survival' package. We selected model covariates using Akaike's Information Criterion (AIC) with the stepAIC function in the 'MASS' package (Venables and Ripley, 2002), and manual one-variable-at-a-time reduction.

We log2 transformed parasite dose data, and centred them on the log2 transformation of $5{\cdot}0 \times 10^5$ parasites, the round number closest to the mean. We used the Predict function from the 'rms' package (Harrell, 2014) to estimate log relative hazards and their 95% confidence intervals based on 1000 simulations of the model.

We ran the Cox model with three variations. In the first variation, we investigated the interaction between treatment and blood:weight ratio, and compared the parasite treatment group hazards with the control hazard. In the second and third variations, we included only parasite treatment groups to investigate relative hazard. To control for a possible effect of absolute number of parasites *vs* relative number of each parasite species in the mixed parasite species dose, we ran the model with data for the absolute number of parasites ingested by the mixed group in the second variation. In the third variation, we ran data for the mixed group as the relative number of each parasite species ingested. This does not change the power of the model or the summary statistics; the change was reflected only in effect size. Cox PH model outputs are in Tables S1–S3 in the Supplementary Materials.

Fitness estimates

We used individual survival and reproduction data to construct an age-classified population projection matrix for each insect (McGraw and Caswell, 1996;

https://doi.org/10.1017/S0031182016000615 Published online by Cambridge University Press

Twombly *et al*. 1998). Each matrix was 3 × 3, with age-specific survival (P_i) on the sub-diagonal [always 0 or 1 in individual matrices (McGraw and Caswell, 1996)], and age-specific realized reproductive output (F_i) in the first row. All other matrix elements were zeros. Each time step (t_i) in the matrix represented one month (with t_0 being the day of insect infection). The model for each individual A was constructed as:

$$A = \begin{matrix} 0 & F_2 & F_3 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \end{matrix}$$

The dominant eigenvalue (λ) of each matrix is a maximum-likelihood estimate of individual fitness, with values above one indicating population growth, and values below one indicating population shrinkage. The dominant left eigenvector of each matrix is an estimate of individual reproductive value v_i for each time step. We calculated dominant eigenvalues (λ) using the eigenfunction in the R base package, and reproduction values were calculated by hand based on these values, as in McGraw and Caswell (McGraw and Caswell, 1996; based on Fisher, 1930). The reproductive value for t_1 (v_1) is scaled to one, and other values are given relative to v_1. In an individual population projection model where F_1 is equal to 0, v_2 is equal to lambda. The model assumes a closed population with unlimited resources, no genetic structure, and does not account for effects of population density.

RESULTS

Parasites ingested

Insects ingested between 30·1 and 337·9 mg of blood (mean 214·8 mg), and an estimated 62 000–1 079 000 total parasites (mean 708 000). The ratio of the volume of blood ingested to insect pre-feeding weight ranged from 0·99 to 14·25 (mean 8·23), and the ratio of the estimated number of parasites ingested per mg of insect biomass ranged from 2000 to 48 000 parasites (mean 28 000). There were no differences between treatments in the absolute parasites dose, nor were there any linear relationships between the parasite dose and death day, E value, reproductive value or estimate of total fitness. There was a significant difference between treatments in the ratio of the volume of blood ingested per mg of insect biomass (Kruskal–Wallis, blood: $P = 1{\cdot}67 \times 10^{-4}$; parasites: $P = 0{\cdot}01$), with the mixed group ingesting significantly more blood than the *T. cruzi* or control groups (Fig. 2; KruskalMC, $P < 0{\cdot}05$ for comparisons).

Reproduction

87·8–97·6% of insects in each group laid eggs, and there was no significant difference between treatment groups in this respect. The E values were significantly different between treatments in both the first and second oviposition cycles (Kruskal–Wallis; cycle 1: $P = 8{\cdot}98 \times 10^{-8}$; cycle 2: $P = 3{\cdot}24 \times 10^{-4}$, Fig. 3A and B). In both cycles, E values for the *T. cruzi* or *T. rangeli* treatments were significantly lower than the co-infected treatment E values (Kruskalmc, $P < 0{\cdot}05$). The *T. cruzi* treatment had a significantly lower E value than the control group in cycle 1 only (Kruskalmc, $P < 0{\cdot}05$). The mean percentage of oviposited eggs that hatched ranged between 79·4 and 84·3% for cycle 1; 62·4–81·8% for cycle 2; and 77·6–83·7% overall. The percentage of eggs that hatched was not significantly different between treatments. Additionally, there was no association between E value and per cent of eggs hatched.

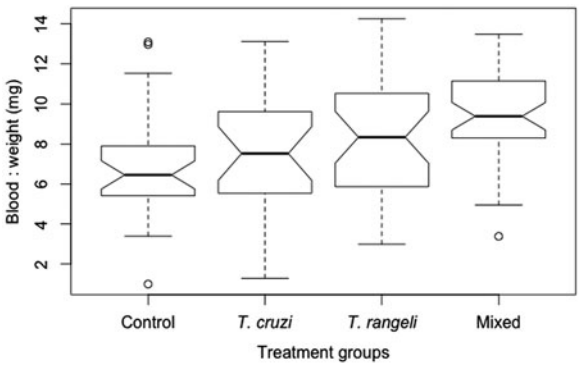

Fig. 2. The distribution of the ratio of the volume of blood consumed in the infective blood meal to mg of insect biomass, across treatments. The mixed group blood: weight ratio was significantly higher than that of the *T. cruzi* and control groups.

Survival function

K–M survival curves (representing survival function, i.e. the probability of total time until failure), were significantly different from each other ($\chi^2 = 8{\cdot}4$, 3 df, $P = 0{\cdot}03$, Fig. 4). The *T. cruzi* treatment group had a significantly shorter time to failure than the mixed treatment group (χ^2 distribution comparisons, $P < 0{\cdot}05$).

Hazards analysis

The Cox model variation investigating the interaction of treatment with blood:weight ratio was significant (Likelihood ratio test, 24·67, 7 df, $P = 8{\cdot}67 \times 10^{-4}$; Supplementary Materials Table S1), suggesting hazard (i.e. instantaneous risk of death) was not the same between treatment groups even when blood meal and body size were taken into account. Investigating the blood:weight ratio allowed us to control for differences in insect size by measuring the effect of the quantity of blood (and also therefore,

https://doi.org/10.1017/S0031182016000615 Published online by Cambridge University Press

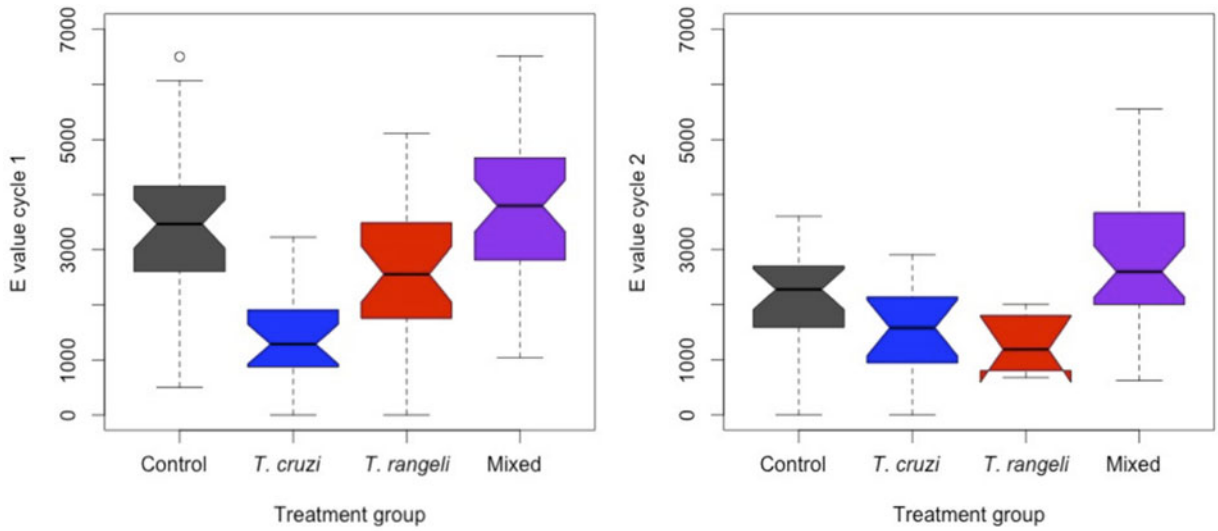

Fig. 3. E value distributions in each treatment group for oviposition cycle 1 (left) and oviposition cycle 2 (right). In both cycles, the mixed group *E* values were significantly higher than the *T. cruzi* and *T. rangeli* treatment group *E* values. The control group *E* values were significantly higher than the *T. cruzi* and *T. rangeli* treatments in cycle 1. In cycle 2, the control group is higher than just the *T. rangeli* treatment.

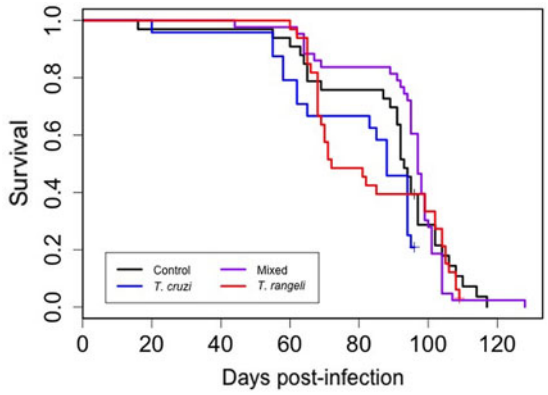

Fig. 4. K–M survival curves for each treatment group. The *T. cruzi* treatment survival function was significantly different than that of the mixed group.

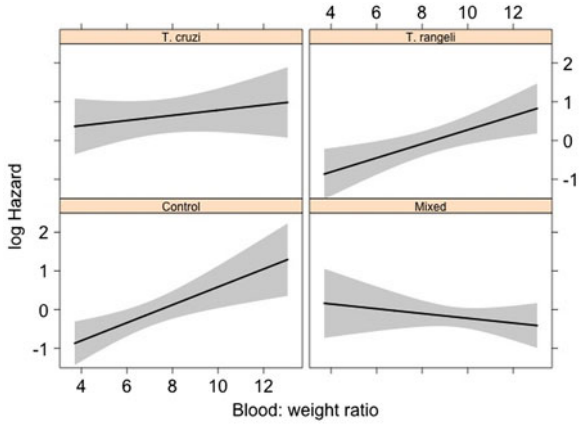

Fig. 5. Interaction of treatment with the blood:weight ratio of the infective blood meal. Hazards were predicted after 1000 simulations of the model. Figures are centred on the mean ratio, 8·23. Grey shading indicates 95% confidence intervals. Just the interactions in the bottom row (the control and mixed treatment groups) were significant.

number of parasites for infected groups) per unit of body mass. Quantity of blood was used in the calculation rather than parasite dose to be able to include the control group. The main effects of *T. cruzi* treatment were significant, with a hazard 2·17 times that of the control group ($e^{\beta} = 2{\cdot}17$, $P = 4{\cdot}33$–04). The control and mixed treatments interacted significantly with the blood:weight ratio, but in opposite directions; the control group hazard increased as the blood: weight ratio increased, while the mixed group hazard decreased with increases in the blood:weight ratio (control: $e^{\beta} = 1{\cdot}26$, $P = 1{\cdot}55 \times 10^{-3}$; mixed: $e^{\beta} = 0{\cdot}74$, $P = 5{\cdot}64$–03, Fig. 5).

The Cox model investigating the main and interaction effects of parasite dose was also significant (Likelihood ratio test, 29·63, 5 df, $P = 1{\cdot}74 \times 10^{-5}$). The patterns and significant effects were the same in both variants of the model (examining the effect of absolute *vs* relative parasite dose), with effects being slightly larger in the model investigating absolute parasite dose. In both model variations there were no differences in the main effects of treatment on hazard. Main effects of parasite dose were significant for *T. rangeli* and marginally significant for *T. cruzi*, with a 3-fold increase in hazard at a dose of 1 million parasites from the hazard at 500 000 parasites (*T. rangeli:* $e^{\beta} = 3{\cdot}27$, $P = 4{\cdot}33$–04; *T. cruzi:* $e^{\beta} = 3{\cdot}07$, $P = 6{\cdot}5 \times 10^{-2}$). Effects of the interaction between treatment and parasite dose were significant for the mixed group in both model variations (absolute and relative parasite doses of the mixed group). At 250 000 parasites, the mixed group hazard was

https://doi.org/10.1017/S0031182016000615 Published online by Cambridge University Press

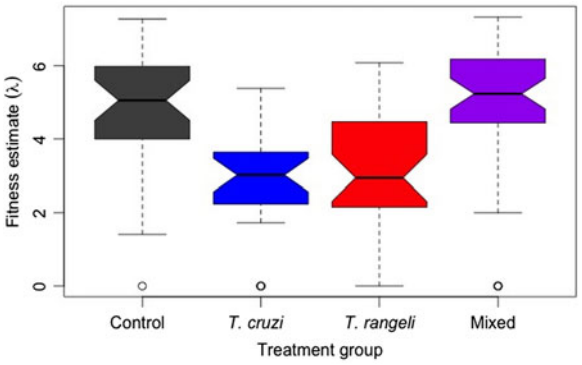

Fig. 6. Distribution of fitness estimates in each treatment group. The control and mixed groups had significantly higher fitness estimates than the *T. cruzi* and *T. rangeli* groups.

significantly higher than either single-species infection treatment, while at 1 million parasites the mixed group hazard was significantly lower (mixed *vs T. cruzi:* $P = 0{\cdot}025$; Mixed *vs T. rangeli,* $P = 0{\cdot}00006$; full summary in Supplementary Materials, Tables S2 and S3). Interaction effects were not significant when comparing the *T. cruzi* treatment with the *T. rangeli* treatment, suggesting their hazards were not significantly different from each other at any parasite dose.

Fitness

Fitness estimates (λ) and reproductive values v2 and v3 (corresponding to 60 and 90 days) were significantly different between treatments (Kruskal–Wallace; λ and v_2: $P = 1{\cdot}69 \times 10^{-7}$; v_3: $P = 1{\cdot}42 \times 10^{-2}$), with *T. cruzi* and *T. rangeli* treatment groups having significantly lower λ and v_2 values than the mixed and control groups (KruskalMC, $P < 0{\cdot}01$, Fig. 6). The reproductive value at 90 days (v_3) was significantly different between the *T. cruzi* and mixed group, with *T. cruzi* being lower (KruskalMC, $P < 0{\cdot}05$). The *T cruzi* and *T. rangeli* treatment group fitness estimates and reproductive values were not significantly different from each other at any time point.

Infection status at death

The difference between treatment groups in the proportion of samples that amplified in the qPCR was marginally non-significant (Fisher's Exact Test for Count Data, $P = 0{\cdot}09$), although there were no significant differences after performing individual comparisons between each treatment and adjusting the *P*-values for multiple comparisons. 90% of *T. cruzi* treatment group samples amplified; 76·92% of *T. rangeli* samples amplified; 61·53% of samples from the mixed treatment group amplified *T. cruzi*; and 84·61% amplified *T. rangeli*. There was no parasite DNA amplification for the pooled male and offspring groups.

DISCUSSION

Co-infection: advantageous for the host and parasite?

We observed that insects co-infected with *T. cruzi* and *T. rangeli* had higher survival, reproduction and overall fitness, suggesting that *T. Cruzi–T. rangeli* co-infection could reduce negative life history consequences of a single infection with *T. cruzi* or *T. rangeli* for *R. prolixus*. This could in turn, lead to increases in the transmission potential of *T. cruzi* and/or *T. rangeli*. Additionally, this could be a way that virulent strains persist, especially *T. rangeli*, which, as mentioned, is known to be pathogenic to *R. prolixus*. Reported prevalences of *T. cruzi–T. rangeli* co-infection in field-caught triatomines have been found to be higher than single infections of *T. rangeli* in *R. prolixus* (Groot, 1951; Vallejo *et al.* 1988), *R. pallescens* (Pineda *et al.* 2008; Calzada *et al.* 2010; Gottdenker *et al.* 2016); and *R. colombiensis* (Pavia *et al.* 2007), which would support the idea of a co-infection advantage for *T. rangeli* (Fig. 1). However, more data on fitness in trypanosome-infected field-caught triatomines are needed to support this result.

Additionally, we found a threshold parasite dose below which insects infected with a single species infection had a lower instantaneous hazard rate (i.e. risk of death) and above which co-infected insects had a lower risk. This might increase the transmission potential of the parasites if the parasite dose in the blood meal were associated with higher numbers of parasites transmitted by the bugs. However, *T. cruzi* infective dose does not correlate with the number of parasites excreted (Wood, 1954; Urdaneta-Morales and Rueda, 1977; Chowdury and Fistein, 1986; Azambuja *et al.* 2004, 2005), and the total trypanosome population size and composition (proportion of each form present) within a triatomine will fluctuate with feeding status; significant decreases in parasite numbers can occur within 4 h after feeding by as much as 50% in some parts of the bug (Schaub and Lösch, 1988; Schaub, 1989*b*; Kollien and Schaub, 1998*a*). Thus, it seems unlikely that the higher infective doses tolerated by co-infected insects increase the parasites' transmission potential, aside from increasing the transmission probability by keeping the insect alive longer.

Insect reproduction: quality vs quantity

While the efficiency of egg production seemed to be affected by parasite treatment, the per cent of oviposited eggs that hatched was not. It is known that the processes of egg growth and oviposition are

https://doi.org/10.1017/S0031182016000615 Published online by Cambridge University Press

controlled separately in *R. prolixus* (Mundall, 1978). Oviposition of badly formed eggs, which has been observed in *Cimex* species, is rare, even in cases of insect malnutrition (Buxton, 1930). This investment in egg quality over quantity could be a mechanism of insecticide resistance, which has been observed in *T. infestans* eggs (Toloza *et al*. 2008), and could be one factor that explains residual populations in human homes after insecticidal spraying.

T. cruzi *vs* T. rangeli *virulence*

As mentioned, *T. rangeli* is considered to be pathogenic to triatomines of the genus *Rhodnius*, while *T. cruzi* has been described in several publications as 'subpathogenic' (Schaub, 1989*a*, 1990, 1992; Schaub and Losch, 1989*a*), i.e. pathogenic only in the presence of external stress. In this light, it is surprising that the fitness of the treatment group infected with *T. cruzi* was not significantly higher than the fitness of the *T. rangeli* treatment group. However, the majority of studies supporting the subpathogenic theory of *T. cruzi* in triatomines have been carried out in the species *T. infestans* (Schaub, 1988*a*, *b*; Schaub and Lösch, 1988; Schaub and Losch, 1989*a*, *b*; Kollien and Schaub, 1998*a*, *b*; Kollien *et al*. 1998). Most studies investigating effect of *T. cruzi* on *R. prolixus* life history have found a mild effect (D'Alessandro and Mandel, 1969; Neves and Peres, 1975; Fellet *et al*. 2014), and effects have also been observed in *Panstrongylus megistus* (Lima *et al*. 1992) and *Mepraia spinolai* (Botto-Mahan, 2009). Additionally, Añez *et al*. (1992) also found no significant difference in development or mortality between insects infected with *T. cruzi* and insects infected with *T. rangeli*. Moreover, recent studies have found that *T. cruzi* can negatively affect *R. prolixus* life history outcomes, depending on temperature (Fellet *et al*. 2014; Elliot *et al*. 2015) and parasite strain (Peterson *et al*. 2015). This could be due to increased parasite replication rates at higher temperatures (Wood, 1954; Asin and Catalá, 1995). However, the insects in this study were reared under climate conditions similar to those found in *R. prolixus*-endemic areas of Colombia (Hoyos *et al*. 2007; Gutierrez *et al*. 2013), thus, if temperature were an underlying factor in *T. cruzi* virulence, it would suggest that *T. cruzi* may also be virulent to free-living Colombian *R. prolixus*.

Our survival results are not in agreement with the other published study of *R. prolixus* survival when co-infected with *T. cruzi* and *T. rangeli*, which found that insects with mixed infections had higher mortality (Añez *et al*. 1992). This could be due to differences in temperature between the studies (ours was carried out at higher temperatures), insect stage and/or parasite strains. Considering the high degree of polymorphism found within both the *T. cruzi* and *T. rangeli* species, it seems possible that the outcome of triatomine infection with either or both trypanosomes could lie in a wide range of outcomes from mildly virulent to positive. In our work, we have observed a wide range of survival in insects infected with different *T. cruzi* DTU I strains (Peterson *et al*. 2015).

Concluding remarks

Due to the inherent limitations of laboratory experiments, the extrapolation of effects observed in the laboratory to their meaning in the natural system must be carried out cautiously. That said, our findings suggest that some *T. Rangeli–T. cruzi* co-infections could ameliorate the negative effects of single-species infections, allowing more virulent strains to persist and potentially increasing the transmission potential of both parasites. Further research into *T. cruzi–T. rangeli* co-infections in other triatomine systems and in field-caught bugs will provide more insight into this topic.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at http://dx.doi.org/10.1017/S0031182016000615.

ACKNOWLEDGEMENTS

We would like to extend our gratitude to Germán Rodriguez from the Office of Population Research at Princeton University for help with the survival analyses, and Professor Gustavo Vallejo from the University of Tolima for providing the *T. rangeli* culture used in this work.

FINANCIAL SUPPORT

This work was supported by funding from the Universidad de Antioquia (to O.T.C.), the Princeton Program in Latin American Studies (to J.K.P.) and the Princeton Institute for International and Regional Studies (to J.K.P.).

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

J.K.P. conceived and designed the study, carried out all assays, performed the statistical analysis, and drafted the manuscript. A.L.G. participated in the study design, helped with statistical analysis and critically revised the manuscript. R.J.E. helped with insect and molecular assays and helped with the analyses of the results; A.P.D. participated in

the study design and revised the manuscript. O.T.C. participated in the design of the study, helped to coordinate the experiments, and critically revised the manuscript.

REFERENCES

Añez, N. (1984). Studies on Trypanosoma rangeli Tejera 1920. VII – Its effect on the survival of infected triatomine bugs. *Memorias do Instituto Oswaldo Cruz Inst Oswaldo Cruz* **79**, 249–255.

Añez, N., Nieves, E. and Cazorla, D. (1987). Studies on Trypanosoma rangeli Tejera, 1920. IX. Course of infection in different stages of Rhodnius prolixus. *Memorias do Instituto Oswaldo Cruz* **82**, 1–6.

Añez, N., Molero, M., Valderrama, E., Nieves, D., Cazorla, M. and Márquez, V. (1992). Studies on *Trypanosoma rangeli* Tejera, 1920 X- Its comparison with *Trypanosoma cruzi* Chagas, 1909. Infection in different stages of *Rhodnius prolixus* Stal, 1859. *KASMERA* **20**, 35–51.

Araújo, C. A. C., Cabello, P. H. and Jansen, A. M. (2007). Growth behaviour of two *Trypanosoma cruzi* strains in single and mixed infections: *in vitro* and in the intestinal tract of the blood-sucking bug, *Triatoma brasiliensis*. *Acta tropica* **101**, 225–231.

Araújo, C. a C., Waniek, P. J. and Jansen, A. M. (2014). TcI/TcII co-infection can enhance Trypanosoma cruzi growth in Rhodnius prolixus. *Parasites & Vectors* **7**, 94.

Asin, S. and Catalá, S. (1995). Development of *Trypanosoma cruzi* in *Triatoma infestans*: influence of temperature and blood consumption. *Journal of Parasitology* **81**, 1–7.

Azambuja, P., Feder, D. and Garcia, E. S. (2004). Isolation of *Serratia marcescens* in the midgut of *Rhodnius prolixus*: impact on the establishment of the parasite *Trypanosoma cruzi* in the vector. *Experimental Parasitology* **107**, 89–96.

Azambuja, P., Garcia, E. S. and Ratcliffe, N. a. (2005). Gut microbiota and parasite transmission by insect vectors. *Trends in Parasitology* **21**, 568–572.

Basso, B. (2013). Modulation of immune response in experimental Chagas disease. *World Journal of Experimental Medicine* **3**, 1–10.

Basso, B., Moretti, E. and Votrero-cima, E. (1991). Immune response and *Trypanosoma cruzi* infection in *Trypanosoma rangeli*-immunized mice. *American Journal of Tropical Medicine and Hygiene* **44**, 413–419.

Basso, B., Castro, I., Introini, V., Gil, P., Truyens, C. and Moretti, E. (2007). Vaccination with *Trypanosoma rangeli* reduces the infectiousness of dogs experimentally infected with *Trypanosoma cruzi*. *Vaccine* **25**, 3855–3858.

Basso, B., Moretti, E. and Fretes, R. (2008). Vaccination with epimastigotes of different strains of *Trypanosoma rangeli* protects mice against *Trypanosoma cruzi* infection. *Memórias do Instituto Oswaldo Cruz* **103**, 370–374.

Basso, B., Moretti, E. and Fretes, R. (2014). Vaccination with *Trypanosoma rangeli* induces resistance of guinea pigs to virulent *Trypanosoma cruzi*. *Veterinary Immunology and Immunopathology* **157**, 119–123.

Bice, D. E. and Zeledon, R. (1970). Comparison of Infectivity of Strains of *Trypanosoma cruzi* (Chagas, 1909). *The Journal of Parasitology* **56**, 663–670.

Borges, E. C., Machado, E. M. M., Garcia, E. S. and Azambuja, P. (2006). *Trypanosoma cruzi*: effects of infection on cathepsin D activity in the midgut of *Rhodnius prolixus*. *Experimental Parasitology* **112**, 130–133.

Botto-Mahan, C. (2009). *Trypanosoma cruzi* induces life-history trait changes in the wild kissing bug *Mepraia spinolai*: implications for parasite transmission. *Vector Borne and Zoonotic Diseases* (*Larchmont, N.Y.*) **9**, 505–510.

Buxton, P. (1930). The biology of a blood-sucking bug, *Rhodnius prolixus*. *The Transactions of the Entomological Society of London* **78**, 227–256.

Calzada, J. E., Pineda, V., Garisto, J. D., Samudio, F., Santamaria, A. M. and Saldaña, A. (2010). Human trypanosomiasis in the eastern region of the Panama Province: new endemic areas for Chagas disease. *The American Journal of Tropical Medicine and Hygiene* **82**, 580–582.

Carcavallo, R. U., Martinez Silva, R., Otero, A.M.A. and Tonn, R. J. (1975). Infeccion natural de *Rhodnius robustus* Larrouse y *Rhodnius pictipes* Stal por *T. cruzi* y *T. rangeli* en Venezuela. *Boletin de la Direcion de Malaiologia y Saneamiento Ambiental* **15**, 117–120.

Castro, D. P., Moraes, C. S., Garcia, E. S. and Azambuja, P. (2007). Inhibitory effects of d-mannose on trypanosomatid lysis induced by *Serratia marcescens*. *Experimental Parasitology* **115**, 200–204.

Castro, D. P., Moraes, C. S., Gonzalez, M. S., Ratcliffe, N. a., Azambuja, P. and Garcia, E. S. (2012). *Trypanosoma cruzi* immune response modulation decreases microbiota in *Rhodnius prolixus* gut and is crucial for parasite survival and development. *PLoS ONE* **7**, e36591.

Castro, L. A., Peterson, J. K., Saldaña, A., Perea, M. Y., Calzada, J. E., Pineda, V., Dobson, A. P. and Gottdenker, N. L. (2014). Flight behavior and performance of *Rhodnius pallescens* (Hemiptera: Reduviidae) on a tethered flight mill. *Journal of Medical Entomology* **51**, 1010–1018.

Chiang, R. G., Chiang, J. A., Hoogendoorn, H. and Lima, M. M. (2013). Exploring the role of rhodtestolin, a cardio-inhibitor from the testes of *Rhodnius prolixus*, in relation to the structure and function of reproductive organs in insect vectors of Chagas disease. *Insects* **4**, 593–608.

Chowdury, M. and Fistein, B. (1986). Excretion of *Trypanosoma cruzi* by various stages of *Rhodnius prolixus*. *International Journal for Parasitology* **16**, 353–359.

Cox, D. R. (1972). Regression models and life-tables. *Journal of the Royal Statistical Society B*, **34**, 187–220.

Cox, F. E. (2001). Concomitant infections, parasites and immune responses. *Parasitology* **122** (Suppl.), S23–S38.

Cummings, K. L. and Tarleton, R. L. (2003). Rapid quantitation of *Trypanosoma cruzi* in host tissue by real-time PCR. *Molecular and Biochemical Parasitology* **129**, 53–59.

D'Alessandro, A. and Mandel, S. (1969). Natural infections and behavior of *Trypanosoma rangeli* and *Trypanosoma cruzi* in the vector *Rhodnius prolixus* in Colombia. *Journal of Parasitology* **55**, 846–852.

Davey, K. (1965). Copulation and egg-production in *Rhodnius prolixus*: the role of the spermathecae. *Journal of Experimental Biology* **42**, 373–378.

De Moraes, A. M. L., Reis-de-Figueiredo, A., Vieira-Junqueira, a. C., Lara-da-Costa, G., Aguiar, R. K. and Cunha-de-Oliveira, P. (2001). Fungal flora of the digestive tract of *Panstrongylus megistus* (Reduviidae) used for experimental xenodiagnosis of *Trypanosoma* (*Schizotripanum*) *cruzi* Chagas, 1909. *Revista iberoamericana de micología* **18**, 79–82.

De Moraes, A. M. L., Junqueira, A. C. V., Celano, V., Da Costa, G. L. and Coura, J. R. (2004). Fungal flora of the digestive tract of *Rhodnius prolixus*, *Rhodnius neglectus*, *Diptelanogaster maximus* and *Panstrongylus megistus*, vectors of *Trypanosoma cruzi*, Chagas, 1909. *Brazilian Journal of Microbiology* **35**, 288–291.

Dobson, A. (1985). The population dynamics of competition between parasites. *Parasitology* **91**, 317–347.

Eichler, S. and Schaub, G. A. (2002). Development of symbionts in triatomine bugs and the effects of infections with trypanosomatids. *Experimental Parasitology* **100**, 17–27.

Elliot, S. L., Rodrigues, J. D. O., Lorenzo, M. G., Martins-Filho, O. a. and Guarneri, A. a. (2015). *Trypanosoma cruzi*, Etiological agent of chagas disease, is virulent to its Triatomine vector *Rhodnius prolixus* in a temperature-dependent manner. *PLoS Neglected Tropical Diseases* **9**, e0003646.

Espino, C. I., Gómez, T., González, G., do Santos, M. F. B., Solano, J., Sousa, O., Moreno, N., Windsor, D., Ying, A., Vilchez, S. and Osuna, A. (2009). Detection of Wolbachia bacteria in multiple organs and feces of the triatomine insect *Rhodnius pallescens* (Hemiptera, Reduviidae). *Applied and Environmental Microbiology* **75**, 547–550.

Falla, A., Herrera, C., Fajardo, A., Montilla, M., Vallejo, G. A. and Guhl, F. (2009). Haplotype identification within *Trypanosoma cruzi* I in Colombian isolates from several reservoirs, vectors and humans. *Acta Tropica* **110**, 15–21.

Fellet, M. R., Lorenzo, M. G., Elliot, S. L., Carrasco, D. and Guarneri, A. A. (2014). Effects of infection by *Trypanosoma cruzi* and *Trypanosoma rangeli* on the reproductive performance of the vector *Rhodnius prolixus*. *PLoS ONE* **9**, e105255.

Ferreira, L. L., Lorenzo, M. G., Elliot, S. L. and Guarneri, A. a. (2010). A standardizable protocol for infection of *Rhodnius prolixus* with *Trypanosoma rangeli*, which mimics natural infections and reveals physiological effects of infection upon the insect. *Journal of Invertebrate Pathology* **105**, 91–97.

Fisher, R. (1930). *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.

Friend, W. G., Choy, C. and Cartwright, E. (1965). The effect of nutrient intake on the development and the egg production of *Rhodnius prolixus* Stahl (Hemiptera: Reduviidae). *Canadian Journal of Zoology* **43**, 891–904.

Garcia, E. S., Mello, C. B., Azambuja, P. and Ribeiro, J. M. C. (1994). *Rhodnius prolixus*: salivary antihemostatic components decrease with *Trypanosoma rangeli* infection. *Experimental Parasitology* **78**, 287–293.

Garcia, E. S., Machado, E. M. M. and Azambuja, P. (2004). Inhibition of hemocyte microaggregation reactions in *Rhodnius prolixus* larvae orally infected with *Trypanosoma rangeli*. *Experimental Parasitology* **107**, 31–38.

Gillet, J. (1935). The genital sterna of the imature stages of *Rhodnius prolixus* (Hemiptera). *Transactions of the Royal Entomological Society of London* **83**, 1–5.

Giraudoux, P. (2013). pgirmess: Data analysis in ecology. R package version 1.5.8. http://CRAN.R-project.org/package=pgirmess

https://doi.org/10.1017/S0031182016000615 Published online by Cambridge University Press

Gómez, I. (1967). Nuevas observaciones acerca de la acción patógena del *Trypanosoma rangeli* Tejera, 1920 sobre *Rhodnius prolixus* Stal, 1859. *Revista do Instituto de Medicina Tropical de São Paulo* **9**, 5–10.

Gorla, D. E. and Noireau, F. (2010). Geographic distribution of Triatomine vectors in America. In *American Trypanosomiasis Chagas Disease* (ed. Telleria, J. and Tibayrenc, M.), pp. 209–231. Elsevier, Amsterdam.

Gottdenker, N. L., Chaves, L. F., Calzada, J. E., Peterson, J. K., Santamaría, A. M., Pineda, V. and Saldaña, A. (2016). *Trypanosoma cruzi* and Trypanosoma rangeli Co-infection patterns in insect vectors vary across habitat types in a fragmented forest landscape. *Parasitology Open* Submitted.

Grewal, M. S. (1957). Pathogenicity of *Trypanosoma rangeli* in the Invertebrate Host. *Experimental Parasitology* **6**, 123–130.

Grijalva, M. J., Suarez-Davalos, V., Villacis, A. G., Ocana-Mayorga, S. and Dangles, O. (2012). Ecological factors related to the widespread distribution of sylvatic *Rhodnius ecuadoriensis* populations in southern Ecuador. *Parasites & Vectors* **5**, 17.

Grisard, E. C., Campbell, D. a. and Romanha, a. J. (1999). Mini-exon gene sequence polymorphism among *Trypanosoma rangeli* strains isolated from distinct geographical regions. *Parasitology* **118** (Pt 4), 375–382.

Groot, H. (1951). Nuevo foco de trypanosomiasis humana en Colombia. *Anales de la Sociedad de Biologia de Bogotá* **4**, 220–221.

Guhl, F. and Marinkelle, C. J. (1982). Antibodies against *Trypansoma cruzi* in mice infected with *T. rangeli*. *Annals of Tropical Medicine and Parasitology* **76**, 361.

Guhl, F. and Vallejo, G. A. (2003). *Trypanosoma* (*Herpetosoma*) *rangeli* Tejera, 1920: an updated review. *Memórias do Instituto Oswaldo Cruz* **98**, 435–442.

Guhl, F., Hudson, L., Marinkelle, C. J., Jaramillo, C. a. and Bridge, D. (1987). Clinical *Trypanosoma rangeli* infection as a complication of Chagas' disease. *Parasitology* **94** (Pt 3), 475–484.

Gutierrez, F. R. S., Trujillo Güiza, M. L. and Escobar Martínez, M. D. C. (2013). Prevalence of *Trypanosoma cruzi* infection among people aged 15 to 89 years inhabiting the department of Casanare (Colombia). *PLoS Neglected Tropical Diseases* **7**, e2113.

Harrell, F. E., Jr (2014). rms: Regression Modeling Strategies. R package version 4.2-1. http://CRAN.R-project.org/package=rms

Harrington, D. P. and Fleming, T. R. (1982). A class of rank test procedures for censored survival data. *Biometrika* **69**, 553–566.

Henriques, C., Castro, D. P., Gomes, L. H. F., Garcia, E. S. and De Souza, W. (2012). Bioluminescent imaging of *Trypanosoma cruzi* infection in *Rhodnius prolixus*. *Parasites & Vectors* **5**, 1–15.

Herbig-Sandreuter, A. (1957). Further studies on *Trypanosoma rangeli* Tejera 1920. *Acta Tropica* **14**, 193–207.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* **6**, 65–70.

Hoyos, R., Pacheco, L., Agudelo, L. A., Zafra, G., Blanco, P. and Triana, O. (2007). Seroprevalencia de la enfermedad de Chagas y factores de riesgo asociados en una población de Morroa, Sucre. *Biomédica* **27**, 130–136.

Kollien, A. and Schaub, G. (1998*a*). *Trypanosoma cruzi* in the rectum of the bug *Triatoma infestans*: effects of blood ingestion by the starved vector. *American Journal of Tropical Medicine and Hygiene* **59**, 166–170.

Kollien, A. and Schaub, G. (1998*b*). The Development of *Trypanosoma cruzi* (Trypanosomatidae) in the Reduviid bug *Triatoma infestans* (Insecta): influence of starvation. *Journal of Eukaryotic Microbiology* **45**, 59–63.

Kollien, A. H., Schmidt, J. and Schaub, G. A. (1998). Modes of association of *Trypanosoma cruzi* with the intestinal tract of the vector *Triatoma infestans*. *Acta Tropica* **70**, 127–141.

Lima, M. M., Borges-Pereira, J., Albuquerque Dos Santos, J. A., Teixeira Pinto, Z. and Vianna Braga, M. (1992). Development and reproduction of *Panstrongylus megistus* (Hemiptera: Reduviidae) infected with *Trypanosoma cruzi*, under laboratory conditions. *Annals of the Entomological Society of America* **85**, 458–461.

Luz, C., Rocha, L. F. N. and Nery, G. V. (2004). Detection of entomopathogenic fungi in peridomestic triatomine-infested areas in central Brazil and fungal activity against *Triatoma infestans* (Klug) (Hemiptera: Reduviidae). *Neotropical Entomology* **33**, 783–791.

Mac Cord, J. R., Jurberg, P. and Lima, M. M. (1983). Marcacao individual de tratomineos para estudos comportamentais e ecologicos. *Memórias do Instituto Oswaldo Cruz* **78**, 473–476.

Marini, V., Moretti, E., Bermejo, D. and Basso, B. (2011). Vaccination with *Trypanosoma rangeli* modulates the profiles of immunoglobulins and IL-6 at local and systemic levels in the early phase of *Trypanosoma cruzi* experimental infection. *Memórias do Instituto Oswaldo Cruz* **106**, 32–37.

Marti, G. A., Balsalobre, A., Susevich, M. L., Rabinovich, J. E. and Echeverría, M. G. (2015). Detection of triatomine infection by Triatoma virus and horizontal transmission: protecting insectaries and prospects for biological control. *Journal of Invertebrate Pathology* **124**, 57–60.

McGraw, J. B. and Caswell, H. (1996). Estimation of individual fitness from life-history data. *The American Naturalist* **147**, 47–64.

Mejía-Jaramillo, A. M., Peña, V. H. and Triana-Chávez, O. (2009). *Trypanosoma cruzi*: biological characterization of lineages I and II supports the predominance of lineage I in Colombia. *Experimental Parasitology* **121**, 83–91.

Mello, C. B., Azambuja, P., Garcia, E. S. and Ratcliffe, N. a. (1996). Differential *in vitro* and *in vivo* behavior of three strains of *Trypanosoma cruzi* in the gut and hemolymph of *Rhodnius prolixus*. *Experimental Parasitology* **82**, 112–121.

Mundall, E. (1978). Oviposition in *Triatoma protracta*: role of mating and relationship to egg growth. *Journal of Insect Physiology* **24**, 321–323.

Neves, D. and Peres, R. (1975). Aspectos da biologia do *Rhodnius prolixus* quando alimentado em animais sadios ou infectados com o *Trypanosoma cruzi*. *Revista Brasileira de Biologia* **35**, 317–320.

Nogueira, N. F. S., Gonzalez, M. S., Gomes, J. E., de Souza, W., Garcia, E., Azambuja, P., Nohara, L. L., Almeida, I. C., Zingales, B. and Colli, W. (2007). *Trypanosoma cruzi*: involvement of glycoinositolphospholipids in the attachment to the luminal midgut surface of *Rhodnius prolixus*. *Experimental Parasitology* **116**, 120–128.

Paim, R. M., Pereira, M. H., Di Ponzio, R., Rodrigues, J. O., Guarneri, A. a, Gontijo, N. F. and Araújo, R. N. (2012). Validation of reference genes for expression analysis in the salivary gland and the intestine of *Rhodnius prolixus* (Hemiptera, Reduviidae) under different experimental conditions by quantitative real-time PCR. *BMC Research Notes* **5**, 128.

Pavia, P. X., Vallejo, G. A., Montilla, M., Nicholls, R. S. and Puerta, C. J. (2007). Detection of *Trypanosoma cruzi* and *Trypanosoma rangeli* infection in triatomine vectors by amplification of the histone H2A/SIRE and the sno-RNA-C11 genes. *Revista do Instituto de Medicina Tropical de São Paulo* **49**, 23–30.

Pedersen, A. B. and Fenton, A. (2007). Emphasizing the ecology in parasite community ecology. *Trends in Ecology & Evolution* (*Personal edition*) **22**, 133–139.

Perlowagora-Szumlewicz, A. and Muller, C. A. (1982). Studies in search of a suitable experimental insect model for xenodiagnosis of hosts with Chagas disease. 1-Comparative xenodiagnosis with nine triatomine species of animals with acute infections by *Trypanosoma cruzi*. *Memorias do Instituto Oswaldo Cruz* **77**, 37–53.

Peterson, J. K., Graham, A. L., Dobson, A. P. and Chavez, O. T. (2015). *Rhodnius prolixus* life history outcomes differ when infected with different *Trypanosoma cruzi* I strains. *American Journal of Tropical Medicine and Hygiene* **93**, 564–572.

Peto, R. and Peto, J. (1972). Asymptotically efficient rank invariant test procedures. *Journal of the Royal Statistical Society A* **135**, 185–207.

Pineda, V., Montalvo, E., Alvarez, D., Santamaría, A. M., Calzada, J. E. and Saldaña, A. (2008). Feeding sources and trypanosome infection index of *Rhodnius pallescens* in a Chagas disease endemic area of Amador County, Panama. *Revista do Instituto de Medicina Tropical de São Paulo* **50**, 113–116.

R Core Team (2014). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ratcliffe, N. A., Nigam, Y. N., Mello, C. B., Garcia, E. S. and Azambuja, P. (1996). *Trypanosoma cruzi* and Erythrocyte Agglutinins: a comparative study of occurrence and properties in the Gut and Hemolymph of *Rhodnius prolixus*. *Experimental Parasitology* **83**, 83–93.

Rojas, W., Caro, M. A., Lopera, J. G., Triana, O., Dib, J. C. and Bedoya, G. (2007). Análisis de polimorfismos en los genes tripanotión reductasa y cruzipaína en cepas colombianas de *Trypanosoma cruzi*. *Biomédica* **27**, 50–63.

Ruegg, R. P. and Davey, K. G. (1979). The effect of C18 juvenile hormone and Altosid on the efficiency of egg production in *Rhodnius prolixus*. *International Journal of Invertebrate Reproduction* **1**, 3–8.

Saldaña, A. and Sousa, O. (1996). *Trypanosoma rangeli*: Epimastigote Immunogenicity and Cross-reaction with *Trypanosoma cruzi*. *Journal of Parasitology* **82**, 363–366.

Schaub, G. A. (1988*a*). Development of isolated and group-reared first instars of *Triatoma infestans* infected with *Trypanosoma cruzi*. *Parasitology Research* **74**, 593–594.

Schaub, G. A. (1988*b*). Developmental time and mortality of larvae of *Triatoma infestans* infected with *Trypanosoma cruzi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 94–96.

Schaub, G. A. (1989*a*). Does Trypanosoma cruzi stress its vectors? *Parasitology Today* **5**, 185–188.

https://doi.org/10.1017/S0031182016000615 Published online by Cambridge University Press

Schaub, G. A. (1989*b*). *Trypanosoma cruzi*: quantitative studies of development of two strains in small intestine and rectum of the vector *Triatoma infestans*. *Experimental Parasitology* **68**, 260–273.
Schaub, G. A. (1990). Membrane feeding for infection of the reduviid bug *Triatoma infestans* with *Blastocrithidia triatomae* (Trypanosomatidae) and pathogenic effects of the flagellate. *Parasitology* **76**, 306–310.
Schaub, G. A. (1992). The effects of trypanosomatids on insects. *Advances in Parasitology* **31**, 255–319.
Schaub, G. A. (1994). Pathogenicity of trypanosomatids on insects. *Parasitology Today* **10**, 463–468.
Schaub, G. A. and Losch, P. (1989*a*). Parasite/host-interrelationships of the trypanosomatids *Trypanosoma cruzi* and *Blastocrithidia triatomae* and the reduviid bug *Triatoma infestans*: influence of starvation of the bug. *Annals of Tropical Medicine and Parasitology* **83**, 215–223.
Schaub, G. A. and Losch, P. (1989*b*). Parasite/host-interrelationships of the trypanosomatids *Trypanosoma cruzi* and *Blastocrithidia triatomae* and the reduviid bug *Triatoma infestans*: influence of starvation on the bug. *Annals of Tropical Medicine and Parasitology* **83**, 215–223.
Schaub, G. a. and Lösch, P. (1988). *Trypanosoma cruzi*: origin of metacyclic trypomastigotes in the urine of the vector *Triatoma infestans*. *Experimental Parasitology* **65**, 174–186.
Schaub, G. A., Grünfelder, C. G., Zimmermann, D. and Peters, W. (1989). Binding of lectin-gold conjugates by two *Trypanosoma cruzi* strains in ampullae and rectum of *Triatoma infestans*. *Acta Tropica* **46**, 291–301.
Therneau, T. (2015). A Package for Survival Analysis in S. version 2.38. http://CRAN.R-project.org/package=survival
Therneau, T. and Grambsch, P. (2000). *Modeling Survival Data: Extending the Cox Model*. Springer, New York.
Tobie, E. J. (1965). Biological factors influencing transmission of *Trypanosoma rangeli* by *Rhodnius prolixus*. *Journal of Parasitology* **51**, 837–841.
Toloza, A. C., Germano, M., Cueto, G. M., Vassena, C., Zerba, E. and Picollo, M. I. (2008). Differential patterns of insecticide resistance in eggs and first instars of *Triatoma infestans* (Hemiptera: Reduviidae) from Argentina and Bolivia. *Journal of Medical Entomology* **45**, 421–426.
Twombly, S., Clancy, N. and Burns, C. W. (1998). Life history consequences of food quality in the freshwater copepod *Boeckella triarticulata*. *Ecology* **79**, 1711–1724.
Untergasser, A., Nijveen, H., Rao, X., Bisseling, T., Geurts, R. and Leunissen, J. A. (2007). Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Research* **35**, W71–W74.
Urdaneta-Morales, S. and Rueda, I. G. (1977). A comparative study of the behavior of Venezuelan and Brazilian strains of *Trypanosoma* (*Schizotrypanum*) *cruzi* in the Venezuelan invertebrate host (*Rhodnius prolixus*). *Revista do Instituto de Medicina Tropical de São Paulo* **19**, 241–250.
Urdaneta-Morales, S. and Tejero, F. (1986). *Trypanosoma* (*herpetosoma*) *rangeli* Tejera, 1920. Intracellular amastigote stages of reproduction in white mice. *Revista do Instituto de Medicina Tropical de São Paulo* **28**, 166–169.
Urrea, D., Carranza, J. C., Cuba Cuba, C., Gurgel-Gonçalves, R., Guhl, F., Schofield, C. J., Triana, O. and Vallejo, G. a. (2005). Molecular characterisation of *Trypanosoma rangeli* strains isolated from *Rhodnius ecuadoriensis* in Peru, *R. colombiensis* in Colombia and *R. pallescens* in Panama, supports a co-evolutionary association between parasites and vectors. *Infection, Genetics and Evolution* **5**, 123–129.
Urrea, D. A., Guhl, F., Herrera, C. P., Falla, A., Carranza, J. C., Cuba-Cuba, C., Triana-Chávez, O., Grisard, E. C. and Vallejo, G. A. (2011). Sequence analysis of the spliced-leader intergenic region (SL-IR) and random amplified polymorphic DNA (RAPD) of *Trypanosoma rangeli* strains isolated from *Rhodnius ecuadoriensis, R. colombiensis, R. pallescens* and *R. prolixu*. *Acta Tropica* **120**, 59–66.
Vallejo, G. A., Marinkelle, C., Guhl, F. and de Sanchez, N. (1988). Comportamiento de la infección y diferenciación morfolóica entre *Trypanosoma cruzi* y *Trypanosoma rangeli* en el intestino del vector *Rhodnius prolixus*. *Revista Brasileira de Biologia* **48**, 577–587.
Vallejo, G. A., Guhl, F., Carranza, J. C., Lozano, L. E., Sánchez, J. L., Jamarillo, J. C., Gualtero, D., Castañeda, N., Silva, J. C. and Steindel, M. (2002). kDNA markers define two major *Trypanosoma rangeli* lineages in Latin-America. *Acta Tropica* **81**, 77–82.
Vallejo, G. A., Guhl, F. and Schaub, G. A. (2009). Triatominae-*Trypanosoma cruzi*/*T. rangeli*: vector-parasite interactions. *Acta Tropica* **110**, 137–147.
Vargas, N., Souto, R. P., Carranza, J. C., Vallejo, G. a. and Zingales, B. (2000). Amplification of a specific repetitive DNA sequence for *Trypanosoma rangeli* identification and its potential application in epidemiological investigations. *Experimental Parasitology* **96**, 147–159.
Venables, W. N. and Ripley, B. D. (2002). *Modern Applied Statistics with S*, 4th Edn. Springer, New York.
Watkins, R. (1971). *Trypanosoma rangeli*: effect on excretion in *Rhodnius prolixus*. *Journal of Invertebrate Pathology* **17**, 67–71.
Whitten, M. M., Mello, C. B., Gomes, S. a., Nigam, Y., Azambuja, P., Garcia, E. S. and Ratcliffe, N. a. (2001). Role of superoxide and reactive nitrogen intermediates in *Rhodnius prolixus* (Reduviidae)/*Trypanosoma rangeli* interactions. *Experimental Parasitology* **98**, 44–57.
Wood, S. F. (1954). Environmental temperature as a factor in development of *Trypanosoma cruzi* in *Triatoma protracta*. *Experimental Parasitology* **3**, 227–233.
Zuñiga, C., Penin, P., Gamallo, C. and de Diego, J. (1997*a*). Characterization of a *Trypanosoma rangeli* Strain of Colombian Origin. *Memórias do Instituto Oswaldo Cruz* **92**, 523–530.
Zuñiga, C., Paláu, M. T., Penin, P., Gamallo, C. and de Diego, J. A. (1997*b*). *Trypanosoma rangeli*: increase in virulence with inocula of different origins in the experimental infection in mice. *Parasitology Research* **83**, 797–800.