Land Cover and Life Stage Associations with Trypanosome Infections in the Kissing Bug *Rhodnius pallescens*

Bryna Wilson[1], Juliana Hoyos[2], Vanessa Pineda[5], Kadir Gonzalez[5], Daniel Mendieta Moreno[6], Azael Saldaña[5,7,8], Christina Varian[3,4], Kaylee Arnold[2,4], Nicole Gottdenker[3,4]

[1]Biology Department, Grove City College [2]Odum School of Ecology, University of Georgia [3]Department of Pathology, College of Veterinary Medicine, University of Georgia [4]Center for the Ecology of Infectious Diseases, University of Georgia
[5]Instituto Conmemorativo Gorgas de Estudios de la Salud (ICGES) [6]Facultad de Ciencias Naturales Exactas y Tecnología, Universidad de Panamá [7]Centro de Investigación y Diagnóstico de Enfermedades Parasitarias (CIDEP) [8]Facultad de Medicina, Universidad de Panamá

Background Information

- Chagas disease is a major concern throughout Central and South America because it can result in severe cardiac, gastrointestinal, and neurological complications. It is caused by *Trypanosoma cruzi*, a protozoan parasite spread by kissing bugs.
- Kissing bugs become infected with *T. cruzi* and a related species, *T. rangeli*, by feeding on the blood of infected mammals (1).
- *T. cruzi* is transmitted when infected kissing bugs feed on mammal blood and defecate near the bite site or when wild mammals ingest infected bugs. The trypanosomes from the insects' guts enter the host through broken skin or mucous membranes.

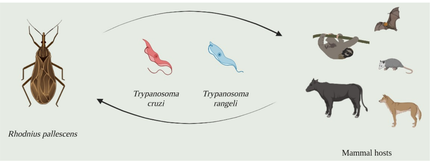


Figure 1. Cycle of trypanosome transmission.

- Changes in land use and forest cover can influence vector-borne pathogen transmission by interfering with the vector's habitat.
- Previous studies have demonstrated that habitat type can affect both the number of kissing bugs present in a landscape (2,3) and their likelihood of being infected with *T. cruzi* (4).
- This project used DNA extracted from 366 *Rhodnius pallescens* kissing bugs collected from *Attalea* palm trees in Panama in 2017 and 2023.

Research Questions

1. Does habitat type influence the prevalence of *Trypanosoma cruzi* and its relative *T. rangeli* in kissing bug vectors?

2. How do the proportions of infected kissing bugs vary relative to the insects' life stages?

Hypotheses

1. We expect forest habitat to have the lowest prevalence of *T. cruzi* infections and the highest prevalence of *T. rangeli* infections. We expect coinfections to be lowest in pasture habitat. (1)

2. We expect to see positive correlations between life stages and infection rates because older insects have ingested more blood meals.

Methods

1. **Initial screening of 70 samples from 2023**: SYBR Green qPCR was used to test the extracted DNA for the presence of *T. cruzi* DTU1 (the most prevalent genetic variant in Panama).

2. **Confirmation of 70 SYBR results and testing of 296 samples from 2017 with duplex qPCR**: TaqMan qPCR was used to test for *T. cruzi* and *T. rangeli* by amplifying a region of the 24Sα subunit of ribosomal DNA.

3. **Determination of habitat types at sampling sites**: Google Earth Engine was used to define the dimensions of each patch of palms, and a 200-meter buffer was added around the polygon. The land cover within the patch and buffer zone was determined using forest cover and land use data from 2021 published by Panama's Ministry of the Environment.

Results and Data Analysis

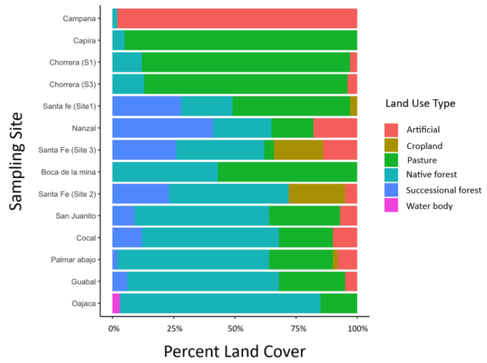


Figure 2. Land composition of sampling sites.

Table 1. Summary of qPCR results.

Stage	*T. cruzi* (95% CI)	*T. rangeli* (95% CI)	Coinfection (95% CI)
N1	6.41% (3.58, 9.24)	7.69% (6.23, 9.15)	17.95% (15.68, 20.22)
N2	16.42% (13.12, 19.72)	7.46% (5.76, 9.16)	20.90% (18.25, 23.54)
N3	31.58% (28.67, 34.49)	9.21% (7.71, 10.71)	28.95% (26.62, 31.28)
N4	37.04% (28.85, 45.22)	0% (-4.22, 4.22)	33.33% (26.78, 39.89)
N5	51.72% (44.10, 59.34)	3.45% (-0.48, 7.38)	20.69% (14.59, 26.79)
Adult	40.45% (37.97, 42.93)	4.49% (3.21, 5.78)	33.71% (31.72, 35.70)

The samples display a mixture of infection types. There is a **lower overall prevalence** of *T. rangeli* (Fig. 3b) compared to *T. cruzi* (Fig. 3a).

The habitat at sampling sites consists of varying proportions of six land use types (Fig. 2).

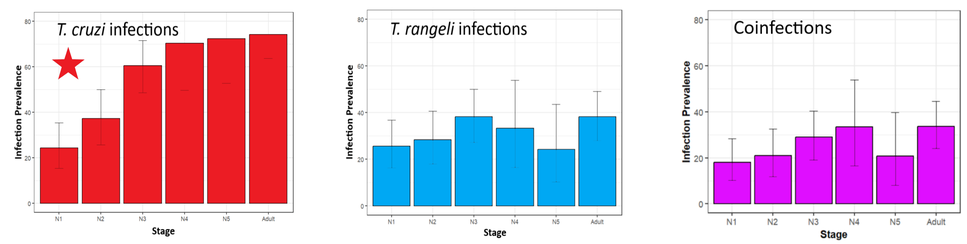
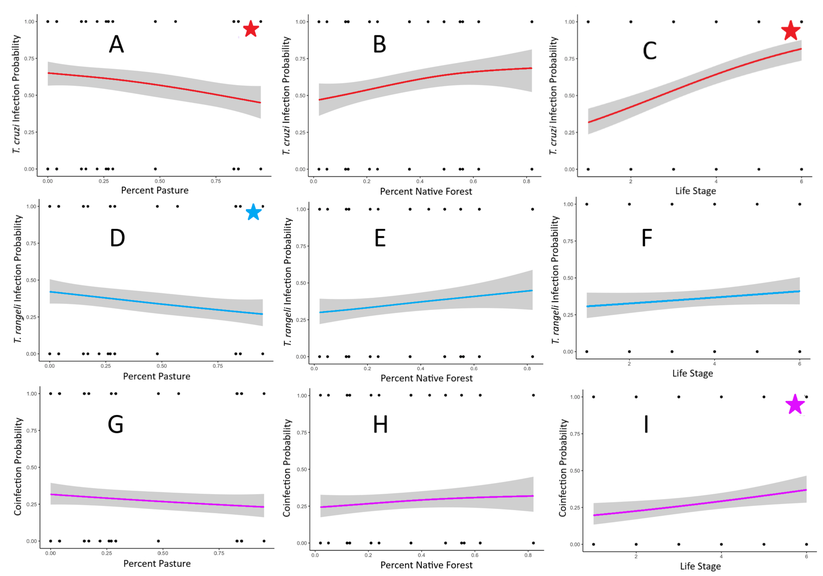


Figure 3. Percent of insects in each life stage that were (3a) infected with *T. cruzi* (χ = 38.484, df = 5, p = 3.016e-7), (3b) infected with *T. rangeli* (χ = 6.133, df = 5, p = 0.294), and (3c) coinfected (χ = 9.296, df = 5, p = 0.098). Stars denote a significant association between life stage and infection.

There are **significant positive associations** between insect life stage and probability of *T. cruzi* infections (Fig. 4c) and coinfections (Fig. 4i). There is **no significant association** between life stage and probability of *T. rangeli* infections (Fig. 4f).

There appear to be positive associations between the percent of native forest and the prevalence of each infection type (Figs. 4b, 4e, 4h), but these findings are **not significant**.



There are **significant negative associations** between the percent of pasture habitat and the prevalence of *T. cruzi* infections (Fig. 4a) and *T. rangeli* infections (Fig. 4d). There is **no significant association** between pasture and coinfections (Fig. 4g).

Figure 4. Generalized Additive Models (GAMs) depicting the relationships between infection type, habitat, and life stage. Significant associations are marked with stars.

Discussion

- **The lower prevalence of *T. rangeli* and the lack of association between *T. rangeli* infections and life stage may be due to the parasite's pathogenic effect on kissing bugs**. Previous studies have noted a decrease in fitness of *T. rangeli*-monoinfected kissing bugs in laboratory settings (5). This phenomenon may cause decreased reproduction and higher mortality rate in the wild, reducing the number of *T. rangeli*-infected bugs available for capture.
- **The increase in coinfections across life stages may result from a beneficial effect of coinfections on host fitness**. One study demonstrated that *Rhodnius prolixus* kissing bugs coinfected with *T. cruzi* and *T. rangeli* had better fitness and greater parasite tolerance than monoinfected insects (6).
- **The differences in infection prevalence between habitats may stem from the number and species identity of animals in contact with kissing bugs in these habitats.** Kissing bugs can feed on mammals, birds, or reptiles; however, only mammals can serve as reservoirs for *T. cruzi* and *T. rangeli*. Host competence for one or both parasites may also vary among mammal species (1). The decline in infections observed in pasture habitat may stem from a higher prevalence of birds, while the increase in infections observed in forest habitat may be due to a greater number and diversity of mammals.

Future Directions

- Determine the mechanisms underlying the differences in infection proportions between habitat types. Examine the mammal species composition of each habitat and evaluate which mammal species have greater host competence for *T. cruzi* or *T. rangeli*.
- Sample changing landscapes consistently over time to determine how the proportions of infected bugs change due to human activities such as deforestation.
- Study *R. pallescens* in the lab to determine how *T. rangeli* infections and coinfections affect species fitness across life stages.

References

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