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ARTHROPODS AND MEDICAL ENTOMOLOGY - ORIGINAL PAPER

The cost of being a killer's accomplice: *Trypanosoma cruzi* impairs the fitness of kissing bugs

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Abstract

Relatively little is known about the fitness effects and life history trade-offs in medically important parasites and their insect vectors. One such case is the triatomine bugs and the parasite *Trypanosoma cruzi*, the key actors in Chagas disease. Previous studies have revealed some costs but have not simultaneously examined traits related to development, reproduction, and survival or their possible trade-offs. In addition, these studies have not compared the effects of genetically different *T. cruzi* strains that differ in their weakening effects in their vertebrate hosts. We compared the body size of the bugs after infection, the number of eggs laid, hatching/non-hatching rate, hatching success, survival, and the resulting number of parasites in *Meccus* (*Triatoma*) *pallidipennis* bugs that were experimentally infected with two strains of *T. cruzi* (Chilpancingo [CH], the most debilitating in vertebrates; and Morelos [MO], the least debilitating) (both belonging to TcI group). Our results showed that infection affects size (MO < CH; MO and CH = control), number of eggs laid (MO and CH < control) hatching/non-hatching rate (MO < control < CH), hatching success (control < MO, CH = control = MO), and survival (Chilpancingo < Morelos < control). In addition, the CH strain produced more parasites than the MO strain. These results suggest that (a) infection costs depend on the parasite's origin, (b) the more debilitating effects of the CH strain are due to its increased proliferation in the host, and (c) differences in pathogenicity among *T. cruzi* strains can be maintained through their different effects on hosts' life history traits. Probably, the vectorial capacity mediated by a more aggressive strain could be reduced due to its costs on the triatomine, leading to a lower risk of vertebrate and invertebrate infection in natural populations.

Keywords Development · Fecundity · Survival · Chagas · Triatominae · *Meccus pallidipennis* · *Trypanosoma cruzi*

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Introduction

Parasitism has profound effects on host ecology and evolution (reviewed in Schmid-Hempel 2011). This involves an arms race to increase the potential for exploitation by the parasites and reduction of costs by the hosts (Kaltz and Shykoff 1998; Kawecki 1998; Thompson 2005). The correlated evolution of each of these parties can be viewed as a collection of adaptations and counter-adaptations (reviewed by Dolgova and Lao 2019).

One way to infer the costs paid by the host due to its interaction with a parasite is the response of the host's life history traits when it is infected (reviewed in Lambrechts et al. 2006; Poulin 2007; Schmid-Hempel 2011). It is assumed that a reduction in host development, reproduction, and/or survival is evidence of the costs the parasite. Another way of understanding these costs is the maximization of one trait at the expense of another (Sheldon and Verhulst 1996; Agnew et al. 2000). This trade-off between life history traits is often interpreted as

the adaptive prioritization of resource use by the host. For example, the host may increase its investment in fecundity when it is likely to die (e.g. Keymer 1980; Gwynn et al. 2005; Kalinda et al. 2017). Studies have documented a plethora of examples of negative effects of parasites on a single life history trait of its host or on trade-offs between life history traits (reviewed by Poulin 2007; Schmid-Hempel 2011).

While there is a large body evidence that parasites impose life history costs on their hosts, how this knowledge can be applied in parasites of medical importance is less clear (reviewed in Mahmud et al. 2018). An example of this limitation is the case of parasites transmitted by insect vectors. In these cases, most of the information on the costs of parasitism to their vectors comes from studies in mosquitoes (e.g. Schwartz and Koella 2001; Tripet et al. 2008; Vézilier et al. 2012). This information is essential in order to understand, for example, if the vectorial capacity is reduced (i.e. whether infection of the insect vector by the parasite makes the vector less effective at transmitting the parasite to its next host; Paaijmans et al. 2011; Kramer and Ciota 2015; Bara et al. 2015). One would suppose that a reduction in the survival of the vector once it is infected would lead to fewer opportunities for it to be in contact with people and therefore lower probability that humans will become infected. This has been corroborated in mosquitoes. For example, it was recently demonstrated that West Nile virus decreases survival but increases fecundity in infected mosquitoes, reducing the mosquito's vectorial capacity (Ciota et al. 2011).

One case in which knowledge of the effects of parasitism on vectors is still limited is that of the triatomine bugs (reviewed in Hurd 2003; De Fuentes-Vicente et al. 2018; de Oliveira et al. 2018). This subfamily of insects is responsible for carrying and transmitting the parasite *Trypanosoma cruzi*, which causes Chagas disease in humans and other mammals. Chagas disease is an illness endemic to Latin America; it often goes undetected but has an estimated 7 million people currently infected and annual mortality of 22,000 people in the Americas (De Fuentes-Vicente et al. 2018; Flores-Ferrer et al. 2018). The bugs ingest blood from birds and mammals, during which they transfer the parasite which then infects the vertebrate host. There are ideas that suggest a coevolutionary scenario based on the costs to the triatomine in terms of development (Botto-Mahan et al. 2006), reproduction (e.g. fecundity, Fellet et al. 2014), and survival (Elliot et al. 2015; Hinestroza et al. 2016; Peterson et al. 2016). However, these studies have not simultaneously evaluated the effects of carrying the parasite on all three of these life history traits (development, reproduction, and survival), which does not allow the recognition of possible trade-offs in the bug. Examining these traits simultaneously would allow us to deduce whether the bugs adaptively maximize one function relative to others when infected. This lack of studies explains why sometimes parameters measured independently show no apparent costs of the parasite (e.g. Zeledón et al. 1970; Schaub 1988; Elliot et al. 2015).

In this study, we show the results of the effects of infection by two different *T. cruzi* strains on the bug (*Meccus* (*Triatoma*) *pallidipennis*) Stål 1872, in terms of development (i.e. body size), reproduction (i.e. number of eggs laid and hatched), and survival. Currently, the different *T. cruzi* populations are broadly classified into seven discrete typing units (DTU), TcI to TcVI and Tcbat, which are known to differ in ecological and pathological associations (reviewed by Zingales 2018). Some studies have found that different variants of *T. cruzi* show genetic differences associated with the symptomatology they produce on their hosts (Macedo and Pena 1998: Macedo et al. 2002, 2004, reviewed in Jiménez et al. 2018) and that could lead to different coevolutionary outcomes in either the bugs' defense systems or those of their vertebrate hosts (Peterson et al. 2015; Flores-Ferrer et al. 2018). Here, we have used two strains that differ in the magnitude of their symptomatology in mammals and that could differ in their costs to triatomines as well. If this was the case, we expected to find differences in the costs to the bugs of being infected depending on the strain with which they are infected. In order to provide clues to these possible differences in virulence, we also compared the number of parasites of each strain after the bugs were infected. We have also added information in regard to the DTU group classification of our strains.

Material and methods

Insects

We kept fifth-stage nymphs (N5) of *M. pallidipennis* from a colony established 21 years ago in Oaxtepec, Morelos, under controlled temperature, humidity, and photoperiod (28 ± 2 °C, 60 ± 5 Rh, 12:12 h) in the Laboratory of Parasite Biology, Department of Microbiology and Parasitology, in the Faculty of Medicine at the Universidad Nacional Autónoma de México (UNAM). "New" individuals from the same site of origin have been added regularly into our colony.

Origin of the strains

We used two strains of *T. cruzi*, ITRI/MX/12/MOR (Morelos) and ITRI/MX/14/CHIL (Chilpancingo), named in accordance with World Health Organization nomenclature. The first strain was obtained from a *M. pallidipennis* male captured in the state of Morelos, Mexico. The second was obtained from a *M. pallidipennis* female, captured in Chilpancingo, Guerrero, Mexico. Both strains were obtained in 2014 and were characterized as TCI (all authors' unpublished data). Both have been maintained in the Laboratory of Parasite Biology in the Faculty of Medicine, UNAM by multiple cyclic passages in a murine model, CD-1 (Favila-Ruiz et al. 2018). Despite being kept in laboratory conditions, both strains are still pathogenic and, thus, representative of wild-caught strains.

DTU group determination of strains

Amplification of the non-transcribed spacer of the intergenic region of the mini-exon gene was achieved by a multiplex PCR assay using a pool of oligonucleotides: TcI:5′ GTGTCCGCCACCTCCTTCGGGCC (specific to TcII to TcVI groups), TcII: 5′GTGTCCGCCACCTCCTTCGGGCC (specific to the TcI groups), Tc: 5′CCCCCCTCCCAGGC CACACTG (common oligonucleotide of the TcI to TcVI strains). The thermal profile was 94 °C/ 1 min; 27 cycles of 94 °C/30 s, 55 °C/30 s, 72 °C/30 s; and 72 °C/10 min. Amplification products were analyzed in 2% agarose gel (Souto et al. 1996).

Infection of nymphs and feeding conditions

A total of 120 fifth-stage nymphs of *M. pallidipennis* (unfed for 15 days after molting) were infected by feeding on female CD-1 mice 20–25 g in mass that were previously inoculated with 20,000 blood trypomastigotes per milliliter. The triatomines were fed for 30 min in groups of 5 insects per mouse in the dark. Three experimental groups of 40 insects each were formed: nymphs infected with the ITRI/MX/12/ MOR strain (hereafter, Morelos), those infected with the ITRI/MX/14/CHIL strain (hereafter, Chilpancingo), and the control group nymphs which were fed on uninfected mice. In order to ensure that the nymphs carried out their molt to adulthood, they were fed again on uninfected mice with the same specifications as before: fed four times, with 15 days between feedings, in 30-min sessions, and with five insects per mouse in the dark. The temperature, humidity, and photoperiod conditions were the same as previously mentioned.

Confirmation of infection

Fifteen days after infection by feeding (the second feeding), a fecal sample was obtained from each insect from the infected groups. To do so, a drop of isotonic saline solution was placed on a microscope slide. The expulsion of feces or urine was provoked by rectal stimulation and gentle abdominal compression of the insect, and the sample was observed under a microscope at × 40 magnification (Olympus CH-2). This compression did not kill any animal. The whole sample was searched for the presence of parasites, considering insects positive for *T. cruzi* when they presented at least one parasite in the sample. The parasites were found in the form of blood trypomastigotes, epimastigotes, and transitional (spheromastigotes).

Changes in body size

The total length of the nymphs (from the end of the abdomen to the flexion point of the proboscis, in mm) was measured at two different times during the life of the animal: at the beginning of the experiment (stage N5) and when they molted to the adult stage. Each time the measurement was taken, it was repeated three times by the same person using a digital caliper (Hornady 050800 ®). The observer was blind to the treatment of each bug. The average of the three repetitions of the measurement was used for the total length analysis. Note that these were the same bugs that were used for the survival analysis described below. This means that independently of either when the bug died and treatment, the size change was recorded.

Survival

The survival of the bugs was recorded daily immediately after the infection. The nymphs of each group were checked daily, gently manipulating them with entomological tweezers when they did not show signs of life. Our criterion for survival/ mortality was that bugs that did not move for 5 min even after this manipulation were considered dead.

Egg production and hatching success

Ten adult females were randomly chosen (using the random number generator at https://www.random.org/) from each of the three experimental groups to be mated with uninfected virgin males (not from our control group). We used only uninfected males for crossings to avoid any chance that our experimental design would be compromised by horizontal parasite transmission by coprophagy (Schaub 1988). The males used for insemination had been fed ad libitum when they molted to the adult stage on female CD-1 mice weighing 20–25 g. At the time of copulation, the males were approximately three-weeks old as adults. Prior to copulation, the females were fed for two 30-min sessions on uninfected female CD-1 mice weighing 20–25 g in groups of five insects per mouse in the dark. The first feeding occurred 5 days after they molted to the adult stage and the second feeding 30 days later. Then, they were not fed for 20 days before mating. The copulation was considered successful based on two criteria: (a) the phallus was introduced into then extracted from the vagina and (b) the duration of the introduction was at least 5 min. These criteria are consistent in triatomines (Téllez–García et al. In press). The number of eggs laid (both fertile and infertile) was recorded to calculate the lifetime fecundity rate (fertile eggs/total eggs laid) per female. For fertile eggs, the time until hatching was recorded in days.

Quantification of parasites

For insects from all three treatments, insects that died in the adult stage were dissected shortly after death to expose the end of the intestine (rectum) and obtain as much of the rectal content as possible. The person who carried out the dissection

was blind to the animals' treatment group. The contents were placed in an Eppendorf tube with 20 μL isotonic saline solution and homogenized by vortexing (Thomas Scientific 945700, New Jersey, USA) for 1 min to carry out a 1:10 dilution. This dilution was placed in Neubauer counting chamber (Brand™) to count the number of parasites under a microscope at × 40 magnification (Olympus CH-2). The parasite count was carried out three times, and the average was used for the analysis.

Statistical analyses

The development data (size) was analyzed as a proportion of increase in length from the N5 nymph stage to the adult stage. In other words, we calculated the ratio of final length to initial length of individual bugs. The data were then standardized with a mean of 0 and standard deviation of 1. Survival was evaluated using the number of insects that died per day in each treatment (Chilpancingo, Morelos, and control), using generalized linear models (GLM) with a Poisson error distribution. We analyzed three aspects of fecundity: number of eggs laid, number of hatched eggs divided by the number of unhatched eggs, and hatching success rate over time. The number of eggs laid was evaluated as a function of treatment (Chilpancingo, Morelos, and control) using a GLM with a Poisson error distribution. For the second, we evaluated number of hatched eggs/unhatched eggs as a function of treatment (Chilpancingo, Morelos, and control) using a GLM with a binomial error distribution. Hatching success over time was evaluated as the proportion of hatched eggs per treatment (Chilpancingo, Morelos, and control) as a function of time (days) using a GLM with Poisson error distribution. Finally, to evaluate the number of parasites in the rectal content as a function of treatment (Chilpancingo, Morelos, and control), we used GLM with a binomial error distribution.

The statistical analyses of our data were carried out using the R programming language (https://www.r-project.org/).

Results

DTU determination

PCR amplification of the mini-exon gene of both the Morelos and Chilpancingo strains amplified a fragment of 350-bp that is characteristic of the TcI genetic group (see Suppl. Mat. Fig. 1).

Development: changes in body size

The increase in length differed significantly between Chilpancingo and Morelos ($F = 3.83$, df = 2, $p = 0.02$): Morelos grew less than Chilpancingo ($t = -2.670$, $p < 0.001$). The control group did not differ from either of the infected groups (Fig. 1).

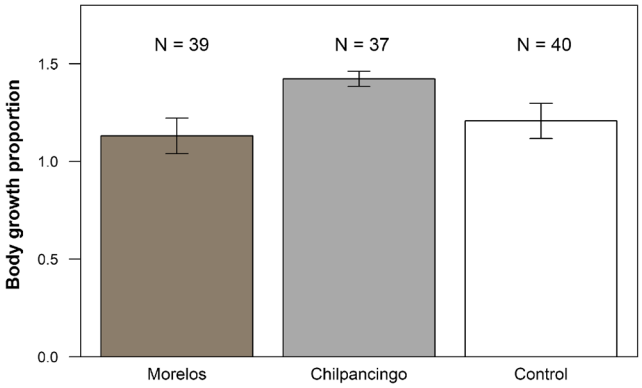

Fig. 1 Differences (mean and 95% confidence interval) in the proportional growth (final length/initial length) of *Meccus pallidipennis* bugs after exposure to two different *Trypanosoma cruzi* strains (Chilpancingo and Morelos) versus control group. Numbers above bars indicate sample sizes

Fecundity

Treatment affected the number of eggs laid ($\chi^2 = 11.29$, df = 2, $p < 0.001$; Fig. 2): Chilpancingo and Morelos laid fewer eggs than the control group ($z = 9.45$, $p < 0.001$; $z = 9.52$, $p < 0.001$). There was no difference between Morelos and Chilpancingo ($z = -1.22$, $p = 0.43$; Fig. 2).

Treatment affected the proportion of hatched to unhatched eggs ($\chi^2 = 38.086$, df = 2, $p < 0.001$): Chilpancingo had higher hatching rate than the control group ($z = 13.143$, $p < 0.001$), while Morelos group had lower values than the control group ($z = -8.449$, $p < 0.001$) and Chilpancingo ($z = -20.177$, $p < 0.001$).

Hatching rate differed significantly among groups over time ($\chi^2 = 12.7078$, df = 2, $p < 0.001$): Morelos hatched faster ($z = -2.31$, $p < 0.001$) than the control group, while Chilpancingo did not differ significantly from the control group ($z = 1.74$, $p = 0.08$). There was no difference between Morelos and Chilpancingo.

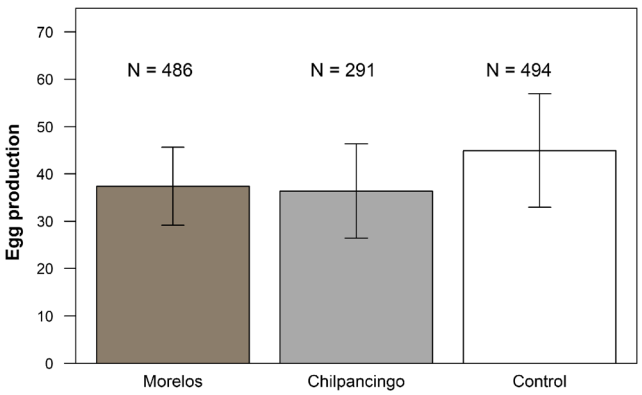

Fig. 2 Egg production (mean ± standard error of the number of eggs laid over the lifetime; by female *Meccus pallidipennis* according to experimental treatment (Morelos and Chilpancingo strains of *Trypanosoma cruzi* and control group). Numbers above bars indicate total number of eggs

Survival

The treatment had an effect on survival ($\chi^2 = 96.57$, df = 2, $p < 0.001$; Fig. 3). Morelos and Chilpancingo survived less than the control group ($z = -61.96$, $p < 0.001$; $z = -31.88$, $p < 0.001$, respectively), while Morelos had higher survival than Chilpancingo ($z = 30.43$, $p < 0.001$).

Parasitism rate

The number of parasites per treatment differed between Morelos and Chilpancingo ($\chi^2 = 826{,}220$, df = 1, $p < 0.001$; Figure 4): Morelos had fewer parasites per milliliter than Chilpancingo ($z = -2.571$, $p < 0.001$). The control group had no parasites.

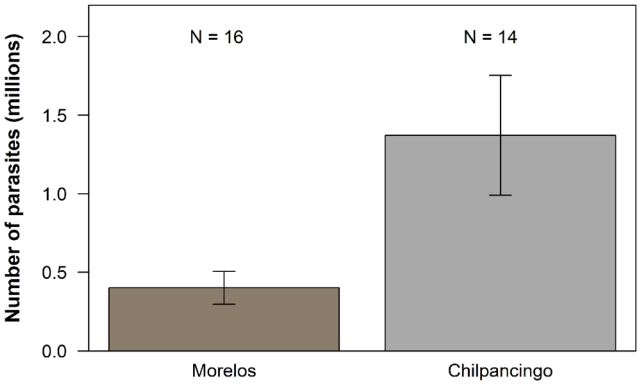

Fig. 4 Parasitemia (in millions of parasites; mean ± standard error) in male and female *Meccus pallidipennis* according to experimental treatment (exposed to two different *Trypanosoma cruzi* strains). Note that the control group was also checked, but they showed no parasites so that they were no added to this comparison. Numbers above bars indicate sample sizes

Discussion

Our results suggest costs of *T. cruzi* in most of the life history parameters we measured in the bug *M. pallidipennis*. These general results do not differ from other studies carried out in other species of triatomines. For example, negative effects of infection have been detected in terms of development (Botto-Mahan et al. 2006), fecundity (Fellet et al. 2014), and survival (Elliot et al. 2015; Hinestroza et al. 2016; Peterson et al. 2016), though there are also studies that have not found these effects (e.g. Zeledón et al. 1970; Schaub 1988; Elliot et al. 2015). Unlike these previous studies which each studied the effects of *T. cruzi* on a single life history parameter, we measured development, survival, and reproduction in the same individuals. This allowed us to associate the effects in all parameters and, from there, reveal possible trade-offs. In this sense and on one hand, even though the Chilpancingo strain drastically reduced the bugs' survival, they gained in size, laid more eggs, and had a higher hatching rate. These contrasts could be interpreted as an adaptive response by the bugs to increase their size and reproduce quickly via fecundity compensation once survival was compromised. On the other hand, these results contradict the delay in development as a response to stressful environmental factors as has been suggested for Triatominae (Menu et al. 2010). In other words, *T. cruzi* would be expected to delay the development of the bugs to increase the probability of passing to a vertebrate host before the bug host dies, which was not the case in our results. Our results, rather, suggest terminal investment (Williams 1966), where an increased likelihood of mortality drives the bugs to divert all of their resources into increasing their body size and producing eggs with higher hatching rates (in the Morelos strain) as a "last ditch" effort to reproduce before dying. This would maximize their fitness under a shortened life expectancy. Trade-offs between fecundity (decreased number of eggs) and longevity (reduced duration in each life stage) when exposed to a stress factor have been observed in bugs with different levels of resistance to the insecticide deltamethrin, but in the absence of infection (Germano and Picollo 2014). These results indicate that there is not only a high cost of infection to the bugs, but also that in general, trade-offs are a clear response of triatomines to different stress factors.

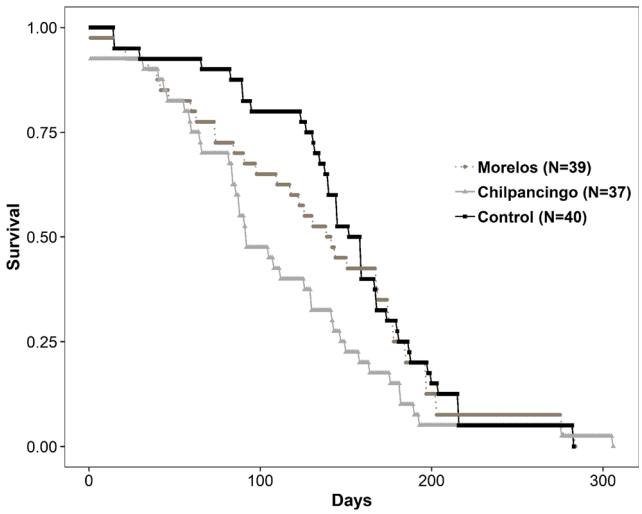

Fig. 3 Survival in days of male and female *Meccus pallidipennis* according to experimental treatment (two different *Trypanosoma cruzi* strains vs a control group)

Our work also clarifies that the cost of infection is not the same between *T. cruzi* strains. Even though both strains can be classified as TcI, the variation in their costs is likely explained by their different genetic backgrounds. We found that the number of parasites varies depending on the strain, with Chilpancingo having a larger number than Morelos. This elevated parasite load in Chilpancingo could be the explanation for the higher costs, assuming that parasite abundance is correlated with a more debilitating effect. However, this may not

be the case as, alternatively, it could be that the Chilpancingo strain is more virulent to the bug than the Morelos strain without requiring differences in parasite abundance. In this respect, little is known about the proximate consequences of infection by *T. cruzi* for the bug. While it is known that the infection does activate the immune response of the bug (e.g. Flores-Villegas et al. 2016, reviewed in de Fuentes-Vicente et al. 2018) which would lead to high (up to 80%) parasite mortality within the bug within a few hours of infection (Ferreira et al. 2016), we do not have a clear idea of the physiological changes in the bug due to the parasites that remain. Our knowledge is limited to the changes in intestinal structure (Gutiérrez-Cabrera et al. 2014) and their possible effects on parasite development (Kollien and Schaub 2000). One alternative explanation to the difference in virulence between the two strains is that the bug is more tolerant to the Chilpancingo strain than the Morelos strain. The idea that triatomines may evolve tolerance to *T. cruzi* has not yet been directly tested (as is the case for many animal systems; Råberg et al. 2008) even though there is evidence that the bugs do not kill all of the parasites soon after infection. Thus, given our results, triatomines could be expected to evolve means to reduce the harm caused by the parasite according to a tolerance scenario. In general, our results indicate that, similar to results with respect to genetic variance among strains and their pathogenic effects on vertebrate hosts (reviewed in Jimenez et al. 2018), bugs also experience different costs. This could also explain the variation in the costs of *T. cruzi* infection documented among studies. That would include apparent lack of costs (e.g. Zeledón et al. 1970; Schaub 1988) if the strain of *T. cruzi* that was used was not very virulent or if the bug used had mechanisms to counteract that virulence. For example, if we had only examined the effects in the Morelos group versus the control group, it is likely that we would not have detected costs or trade-offs, which are clear in the Chilpancingo group. Note that this variation in the costs depending on the strain could also be interpreted as different stages of the coevolution between bugs and *T. cruzi* (Peterson et al. 2015; Flores-Ferrer et al. 2018), where the different costs that have been documented so far by the bug show those moments. A situation like that in the Chilpancingo group would illustrate that the parasite is "winning," while the Morelos group shows the bug is in the lead.

What are the consequences of our study for the risk of infection with Chagas in vertebrates? A crucial factor is that we do not know if the vectorial capacity of the bug varies for different strains. While survival did decrease in the Chilpancingo strain, the bugs maximized their growth and hatching success, which could imply increased use and demand for energy reserves, which would lead to higher feeding rates. More frequent feeding could lead to more parasite transmission even if the bug dies sooner. We know that infection can double the bite rate of the bug as well as decrease defecation times (Botto-Mahan et al. 2006). However, we do not know if these values depend on the strain. One prediction is that the Chilpancingo strain will lead to lower vectorial capacity than the Morelos strain. However, the fecundity compensation experienced in the face of reduced survival is a crucial factor as there would be more eggs being laid and with a higher hatching success that are likely to increase bug infestation even when these eggs are parasite-free. Future experiments should compare whether the parasite is more successfully transmitted when it comes from a more aggressive strain.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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