

# Thermal Tolerance Plasticity in Chagas Disease Vectors *Rhodnius prolixus* (Hemiptera: Reduviidae) and *Triatoma infestans*

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## Abstract

Temperature is recognized as the most influential abiotic factor on the distribution and dispersion of most insect species including *Rhodnius prolixus* (Stål, 1859) and *Triatoma infestans* (Klug, 1834), the two most important Chagas disease vectors. Although, these species thermotolerance range is well known their plasticity has never been addressed in these or any other triatomines. Herein, we investigate the effects of acclimation on thermotolerance range and resistance to stressful low temperatures by assessing thermal critical limits and ‘chill-coma recovery time’ (CCRT), respectively. We found positive effects of acclimation on thermotolerance range, especially on the thermal critical minimum of both species. In contrast, CCRT did not respond to acclimation in either. Our results reveal the plasticity of these Triatomines thermal tolerance in response to a wide range of acclimation temperatures. This presumably represents a physiological adaptation to daily or seasonal temperature variation with concomitant improvement in dispersion potential.

**Key words:** acclimation, chill-coma recovery time, CTmax, CTmin, triatomines

Insects of the subfamily Triatominae (Hemiptera: Reduviidae) are obligate hematophagous bugs with more than 100 species distributed in the Americas (from the United States to Argentina and Chile) and several of them being vectors of Chagas disease. *Triatoma infestans* (Klug, 1834) is the most important Chagas disease vector in southern South America, while *Rhodnius prolixus* (Stål, 1859) is in Central and northern South America (Schofield 1994), and it remains a classical model in insect physiology (Schilman 2017). *Triatoma infestans* is distributed over Venezuela, Colombia, and Central America, especially Guatemala, Honduras, and El Salvador. Whereas in Venezuela and Colombia, *R. prolixus* inhabits mainly wild environments, such as palm trees, and has a wide range of hosts including birds, rodents, marsupials, sloths, and reptiles. In Central America, the species is adapted to domestic environments (Lent and Wygodzinsky 1979, Schofield 1994). *Triatoma infestans* is widespread in the Southern Cone countries of South America: Bolivia, Argentina, Uruguay, Paraguay, Chile, Ecuador, Brazil, and Peru. In all these countries *T. infestans* was initially considered to be almost exclusively adapted to the domestic environment, except

for small wild foci primarily found in the valleys of Cochabamba in Bolivia. However, the discovery of wild populations in other ecoregions such as Bolivian Chaco, Argentina, Paraguay, and Chile indicates that wild populations of *T. infestans* are more widely distributed than previously assumed (Buitrago et al. 2016 and references therein).

Across Latin America, it is estimated that 13% of the population is at risk for Chagas disease infection (WHO 2015); contact with feces of triatomines infected with *Trypanosoma cruzi* is the major exposure route of this disease. *Rhodnius prolixus* and *T. infestans* dispersion and infestation show a seasonal variation (Vazquez-Prokopec et al. 2006, Abraham et al. 2011), due to environmental temperature fluctuation. In addition, seasonal variation also occurs in other vectors of *T. cruzi* such as *Mepraia spinolai* (Porter, 1933). The population density and home range variation indicate that *T. cruzi* would have a higher probability of transmission during warmer months (Botto-Mahan et al. 2005). As vector dispersion also occurs in warmer months, there is a direct relationship between environmental temperature increase (i.e., during spring and summer),

and increasing frequency of new cases of Chagas disease (Ibáñez-Cervantes et al. 2018).

Given the low thermal inertia of small ectotherms, their survival depends largely on the ambient temperature. In this sense, among environmental factors defining the distribution of triatomines and Chagas disease transmission, temperature is one of the most influential factors affecting vector's infective capacity (Tamayo 2018) and multiple physiological processes. For instance, life cycle duration (Pippin 1970, da Silva 1990), molting success (Okasha 1968, Guarneri et al. 2003), developmental rate (Rabinovich et al. 2006), hatching success (Guarneri et al. 2003), flight initiation (Gurevitz et al. 2006), water loss, and nutrient conversion rate (Schilman and Lazzari 2004, Rolandi et al. 2014) are among some of the life history traits known to be affected by temperature in Triatomines. Therefore, it is expected that the physiological thermal limits of Triatomines can be good predictors of their distribution (de la Vega et al. 2015, de la Vega and Schilman 2018).

A common method for assessing physiological thermal limits of insect species is the estimation of critical thermal maxima (CTmax) and minima (CTmin), which jointly define the thermal tolerance range. Beyond this range, insects lose neuromuscular control (Lutterschmidt and Hutchison 1997, Chown and Nicolson 2004, Chown and Terblanche 2007). Probably, then, CTmax and CTmin constitute physiological bounds out of which insects cannot perform vital activities for survival and reproduction. Thus, given the ecological value of critical thermal limits, it is not surprising that insects realize their niches in places where ambient temperature remains within their thermal tolerance range (Calosi et al. 2008, Diamond et al. 2017, de la Vega and Schilman 2018).

However, climatic conditions are not static: they naturally vary at temporal and spatial scales. In the face of thermal variation, staying within thermotolerance range may be a permanent challenge for insects (Pörtner et al. 2006). Therefore, ambient temperature variation may impose detrimental effects that depend on an insect's thermal sensitivity and capacity to buffer thermal variation (Helmuth et al. 2005, Rolandi and Schilman 2018). For instance, thermal acclimatization (or acclimation, if thermal variation is experimentally controlled in laboratory studies) can help organisms to keep physiological processes constant within a range of environmental thermal variation (Willmer et al. 2009). By means of biochemical responses, acclimatization has long been known as a physiological strategy of insects to confront daily or seasonal climatic variation, and deal with thermal stress (Hu and Appel 2004, Terblanche et al. 2006, Overgaard and Sørensen 2008, Sheldon and Tewksbury 2014).

In the last years, given impending global warming (IPCC 2014), insect acclimation response has received more attention (Sgro et al. 2016). Studying insect plasticity may help not only to understand, but to anticipate insect population's responses to novel environmental changes. Therefore, incorporating plasticity of thermal tolerance range into species distribution models may improve the overall prediction power (Kearney and Porter 2009).

Just beyond lower or upper thermal tolerance limits, there is a thermal range in which insects can survive for a certain amount of time, despite having no neuromuscular activity (MacMillan and Sinclair 2011, Overgaard and MacMillan 2017). Thus, an insect's capacity to resist and to recover from temperatures away from their thermal tolerance range is another way to confront thermal stress and extend (spatial or temporarily) their fundamental niche. In line with this, acclimatization may also modulate insect's capacity to recover from extremes temperatures. An overused measure to evaluate resistance to stressing low temperatures in insects is 'chill-coma recovery time' (CCRT). CCRT is determined by exposing

individuals to low temperatures (below CTmin), and then measuring the time they needed to recover neuromuscular activity (David et al. 1998, Gibert et al. 2001, Overgaard and MacMillan 2017).

In this study, acclimation effects on thermal tolerance and resistance in two of the most important Chagas disease vectors, *T. infestans* and *R. prolixus*, was investigated in order to achieve a better understanding of the fundamental niche exhibited by both species. This may provide helpful information for modeling Triatomines' future distribution and dispersion in a global climate change scenario which may ultimately contribute to more effective control programs of these vectors.

## Materials and Methods

### Experimental Design

To explore the effect of acclimation on the thermal tolerance of *R. prolixus* and *T. infestans* four thermal treatments or acclimations were produced. Each treatment consisted of exposing individuals to a certain environmental temperature (14, 21, 28, or 35°C) for a week. These temperatures were chosen to be 7°C above, and 7 and 14°C below the rearing temperature (i.e., 28°C). On the seventh day, three standard thermotolerance tests were performed, i.e., critical thermal maxima (CTmax), critical thermal minimum (CTmin) or CCRT, and they were determined as response variables. Since these species are limited by cold weather (de la Vega et al. 2015, de la Vega and Schilman 2018), two cold tolerance tests were performed, CCRT and CTmin, whereas CTmax measurements allowed the calculation of the thermotolerance range and polygons. For each temperature and response variable, 14 to 25 seven-day-old fifth-instar nymphs were measured. The use of fifth instar nymphs allowed us to compare the results with previous studies (de la Vega et al. 2015; de la Vega and Schilman 2018). Furthermore, this nymph stage is the largest stage, therefore it is easier to handle than a smaller stage, more precise to measure and without any physiological variations, e.g., due to reproduction. The total number of insects used for this study was 222 for *T. infestans* and 245 for *R. prolixus*. To this end, insects were placed in a chamber (PTC-1 Peltier Effect Cabinet; Sable System International (SSI), Las Vegas, Nevada, United States, connected to a temperature controller (Pelt-5 (SSI) under a 12:12 photoperiod (light on 9:00 am). All insects came from a lab population reared at 28°C for over 10 generations.

### Critical Thermal Maxima

To determine CTmax, defined as the temperature at which insects lose activity after multiple muscle spasms, we adapted the protocol implemented by de la Vega et al. (2015) and de la Vega and Schilman (2018), which was originally developed by Lighton and Turner (2004). According to them, CTmax is equally determined by loss of spiracular or locomotor activity. Thus, locomotor activity was measured by placing unrestrained insects individually in a glass chamber (RC-M chamber; SSI) connected to an AD-2 activity detector (SSI). The chamber was located inside a SSI's PTC-1 Peltier Effect cabinet controlled to  $\pm 0.2^\circ\text{C}$  by a SSI's Pelt-5 temperature controller. Clean dry air was drawn through the RC-M chamber at a flow rate of ca. 100 ml min<sup>-1</sup> STP by a SS4 sub sampler (SSI). In order to equilibrate the temperature of the RC-M chamber with that inside cabinet, the air flow passed through a copper coiled tube (ca. 6.5 meters long) placed inside the cabinet. The temperature was measured by a thermocouple attached to a SSI TC-2000 thermocouple meter (accuracy 0.2 and resolution 0.01°C). The Pelt-5 temperature controller generates a rising temperature ramp of 0.25°C min<sup>-1</sup> for 100 min after a baseline of 15 min at 35°C. The analog outputs from the analyzers measuring insect's activity,

temperature of the chamber and air flow rate were connected to an A/D converter (SSI UI-2, 16 bits basic accuracy = 0.05%) and stored in a computer by ExpeData data acquisition software (SSI). For each specimen, activity output was plotted as the cumulative sum of the absolute differences between adjacent data points across assay time, and the inflection point of the curve was identified as the point at which short-term variability in the activity data declines abruptly (for more details of the set up and procedure see supplementary material from de la Vega et al. 2015).

### Critical Thermal Minimum

After 1 wk acclimation, insects were placed individually on a temperature plate (Pelt-plate (SSI)) within a cylindrical acrylic container (5 cm diameter) with plastic mesh on top. The temperature plate was connected to the temperature controller (Pelt-5; SSI), where a descending temperature ramp was set up at a rate of  $-0.25^{\circ}\text{C min}^{-1}$  for 100 min. Before starting the ramp, insects were 15 min at  $20^{\circ}\text{C}$ .

To determine CTmin, defined as the temperature at which insects lose coordinated muscular function (Terblanche et al. 2006, MacMillan and Sinclair 2011), the insects were mechanically stimulated through the mesh with a thin metal filament and motor response was observed. CTmin was recorded as the temperature at which insects did not respond to the mechanical stimulus (de la Vega and Schilman 2018).

### Thermal Tolerance Range

Thermal tolerance polygons give more information than thermal tolerance endpoints alone. Overall, polygon area provides a convenient and useful comparative index of eurythermicity between species. Polygons were created using a modification of Eme and Bennett (2009) by connecting CTmin and CTmax regressions by its lowest and highest acclimation temperature to produce a quadrilateral figure expressed quantitatively using the areal units,  $^{\circ}\text{C}^2$ . Polygons were divided into an intrinsic tolerance zone (i.e., thermal tolerance independent of previous thermal acclimation) and acquired tolerance zones (i.e., thermal tolerance gained through acclimation) by dividing polygons with horizontal lines from extrapolated the lowest CTmin and CTmax values (see Fig. 1 and Eme and Bennett 2009).

### Chill-Coma Recovery Time

The insect specimens were placed in an adiabatic container with liquid water and ice (ca.  $0^{\circ}\text{C}$ ) for 1 h. Then, each specimen was placed individually ventral side up on filter paper in a Petri dish. The Petri dish was placed inside a temperature controlled cabinet at  $28^{\circ}\text{C}$  (rearing temperature), and the time elapsed until insects recover its normal position (ventral side down) was recorded within 1 h. Insects that did not recover after 1 h were designated as 'non-recovered'. All insects were discarded after the assay.

### Statistical Analyses

Lineal regression was applied to model CTmin, CTmax and CCRT as functions of acclimation temperature and species in R (R Core Team 2013) using lm function (stats package). Significance of each model term ( $P < 0.05$ ) was evaluated by a sequential analysis of variance using anova.lm function (stats package).

## Results

### Critical Thermal Maximum

Analysis of variance showed that the effect of acclimation temperature on CTmax was marginally significant ( $F = 23.72$ ;

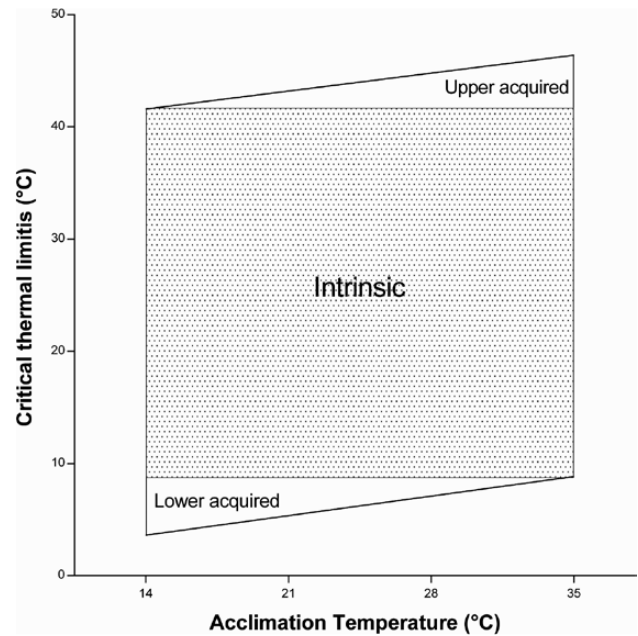


Fig. 1. Thermal range polygon. Intrinsic tolerance zone (i.e., thermal tolerance independent of previous thermal acclimation) and acquired tolerance zones (i.e., thermal tolerance gained through acclimation) were indicated.

$df = 1, 120$ ;  $P = 0.056$ ), and species dependent ( $F = 5.43$ ;  $df = 1, 120$ ;  $P = 0.021$ ). Therefore, a separated regression analysis was performed for each species. For *R. prolixus* we found a significant slope of 0.049 ( $F = 8.93$ ;  $df = 1, 63$ ;  $P = 4.0e-3$ ). In contrast, CTmax did not seem to depend on acclimation temperature for *T. infestans* ( $F = 0.14$ ;  $df = 1, 57$ ;  $P = 0.710$ ) (Fig. 2 and Table 1).

### Critical Thermal Minimum

Acclimation temperature affected positively CTmin for both species ( $F = 174.3$ ;  $df = 1, 187$ ;  $P < 2.2e-16$ ). Its effect, however, was dependent on the species ( $F = 23.4$ ;  $df = 1, 187$ ;  $P = 2.69e-6$ ), being more pronounced for *R. prolixus* (slope = 0.20; 95% CL = 0.19 – 0.21) than for *T. infestans* (slope = 0.08; 95% CL = 0.06 – 0.10) (Fig. 3 and Table 1). All insects survived 24 h after the assay.

### Thermal Tolerance Range

The total thermal tolerance range, estimated as the area of the polygon formed between CTmin and CTmax curves, was 18% larger for *T. infestans* ( $1004.22^{\circ}\text{C}^2$ ) than for *R. prolixus* ( $819.72^{\circ}\text{C}^2$ ), while the intrinsic thermal tolerance, i.e., thermal tolerance independent of previous thermal acclimation, was 23.6% larger for *T. infestans* ( $985.89^{\circ}\text{C}^2$ ) than for *R. prolixus* ( $753.48^{\circ}\text{C}^2$ ; Table 2). *R. prolixus* acquired greater amount of upper (1.7%) and lower (7%) thermotolerance through acclimation than *T. infestans* (0 and 1.8% upper and lower tolerance respectively; see Table 2).

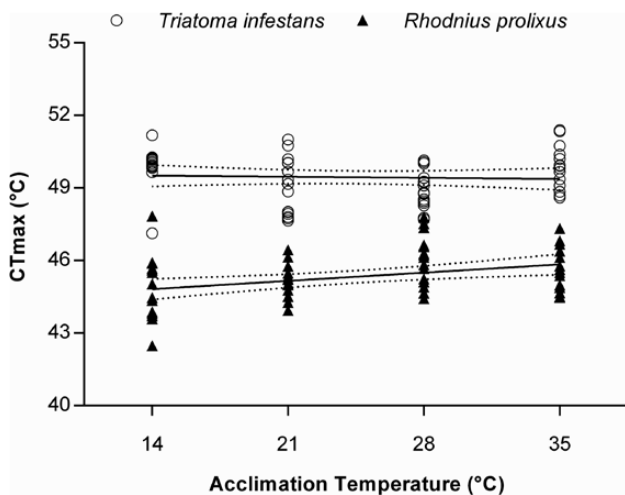
### Chill-Coma Recovery Time

The effect of acclimation temperature on CCRT could not be evaluated for *R. prolixus* because most of the insects did not recover during the hour that the assay lasted. All *T. infestans* tested recovered from chill coma during the assay. However, no relationship between CCRT and acclimation temperature was found ( $F = 0.315$ ;  $df = 1, 79$ ;  $P = 0.576$ ) (Fig. 4 and Table 1).

## Discussion

Thermal tolerance of *R. prolixus* and *T. infestans* in fifth-instar nymphs was shown to be plastic in response to a wide range of acclimation temperatures (i.e., 14, 21, 28 and 35°C) at which they are exposed for a week. As observed in other insect species, this plasticity is likely an adaptation associated to physiological responses to daily or seasonal temperature changes (Hu and Appel 2004, Terblanche et al. 2006, Overgaard and Sørensen 2008, Sheldon and Tewksbury 2014). Our results are in accordance to the widely reported ectotherms ability to alter their thermotolerance range when facing environmental changes (Terblanche et al. 2005; Jumbam et al. 2008a,b; Allen et al. 2012).

*Triatoma infestans* exhibited a higher thermotolerance range (CTmax-CTmin) than *R. prolixus* for all acclimation temperatures assessed. Even the narrowest thermotolerance range for *T. infestans* (46.9°C) was higher than the widest range for *R. prolixus* (40.4°C). Stillman (2003) proposed that species which have developed wider thermotolerance ranges have necessarily lost acclimation capacity. Supporting this hypothesis, we found that *T. infestans*, with a wider thermotolerance range, has a lower acclimation capacity



**Fig. 2.** Effect of acclimation on CTmax. Each point represents an individual insect. Full lines were fitted by simple linear regression and dotted lines show the 95% CIs. Acclimation effect was marginally significant for *R. prolixus*, but not significant for *T. infestans*.

than *R. prolixus*. Since *T. infestans*' geographical distribution is more austral than that of *R. prolixus*, our results also support the 'climatic variability hypothesis', which argues that species inhabiting more variable environments (e.g., high latitudes or altitudes) exhibit wider thermotolerance ranges (Addo-Bediako et al. 2000, Sunday et al. 2011). Given that, *T. infestans*' geographical distribution range includes areas with colder winters than *R. prolixus* range, it would be not surprising that the former is better adapted to deal with extremely cold temperatures. Thus, we can expect that chilled-coma treatment affects more severely *R. prolixus* than *T. infestans*, which was what we observed. While all *T. infestans* insects did recover during the assay, the vast majority of *R. prolixus* insects did not. Therefore, acclimation effect on CCRT could only be explored in *T. infestans*. For this species, there isn't any significant differences in CCRT across nymph stages (unpublished results). However, in contrast to the plasticity we found for the thermotolerance range, there was not any effect of acclimation on CCRT. One possible explanation for this is that the mechanisms involved in overcoming chilled-coma differs from those involved in preventing it (see MacMillan and Sinclair 2011 to a major understanding of the mechanisms underlying chill-coma). In fact, Macdonald (2004), and Ransberry and collaborators (2011) found evidence that CTmin and CCRT are mechanistically decoupled. Another possible explanation for the lack of acclimation effects on CCRT is that more drastic values for chilled-coma temperature or duration should be used to see acclimation effects on CCRT in *T. infestans*. In *R. prolixus*, that seems more susceptible to cold temperatures, a less drastic chilled-coma may allow acclimation flexibility. This pattern was previously observed for the same species by de la Vega and collaborators (2015), although thermal plasticity was not considered. To our knowledge, this is the first study evaluating thermotolerance range amplitude in triatomines, taking into account their plasticity to acclimation temperature, and furthermore it may help to gain a better understanding of their physiology, fundamental niche, and dispersion potential, as well as to predict more accurately how global climatic change is affecting their distribution.

Although our observations reveal a plastic thermotolerance range, the upper and lower limits of this range (CTmax and CTmin, respectively) did not equally respond to acclimation. Plasticity was notably higher for the lower than for the upper limit in both species, being the latter non-plastic at all for *T. infestans* (Figs. 2 and 3 and Table 2). This result suggests a decoupling response for the lower and upper thermal limits to acclimation that may be associated

**Table 1.** Summary of CTmax, CTmin, and CCRT obtain from *R. prolixus* and *T. infestans* acclimated for 1 wk at 14, 21, 28, or 35°C

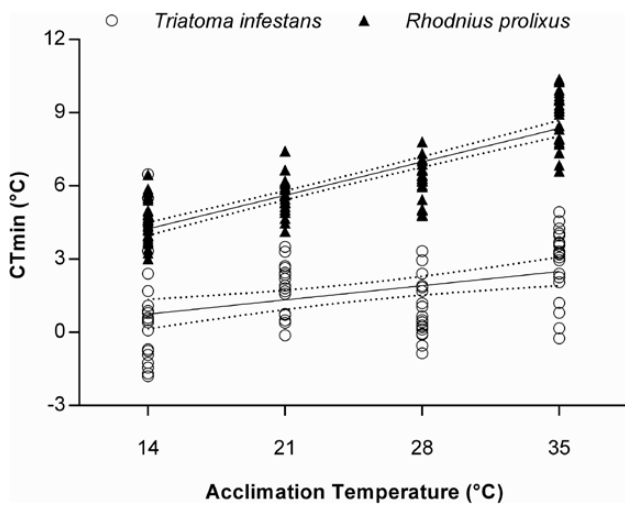
	Acclimation temperature (°C)	<i>Rhodnius prolixus</i>	ANOVA	<i>Triatoma infestans</i>	ANOVA
CTmax (°C)	14	44.71 ± 0.24 (17)	$F_{(1,63)} = 8.93$ $P = 0.004$	49.93 ± 0.24 (15)	$F_{(1,57)} = 0.14$ $P = 0.71$
	21	45.09 ± 0.26 (15)		49.08 ± 0.24 (15)	
	28	45.96 ± 0.24 (17)		48.93 ± 0.24 (15)	
	35	45.53 ± 0.25 (16)		49.86 ± 0.25 (14)	
CTmin (°C)	14	4.66 ± 0.18 (24)	$F_{(1,88)} = 201.91$ $P < 0.0001$	0.73 ± 0.34 (20)	$F_{(1,80)} = 13.40$ $P < 0.001$
	21	5.57 ± 0.20 (20)		1.85 ± 0.35 (19)	
	28	6.16 ± 0.19 (21)		1.03 ± 0.34 (21)	
	35	8.83 ± 0.18 (25)		2.90 ± 0.33 (22)	
CCRT (min)	14	>60 (25) <sup>a</sup>	N/A	14.77 ± 2.64 (20)	$F_{(1,79)} = 0.31$ $P = 0.58$
	21	>60 (23)		13.26 ± 1.26 (20)	
	28	>60 (21) <sup>b</sup>		12.12 ± 1.13(20)	
	35	>60 (21)		16.63 ± 2.01(21)	

Data are reported as mean ± SE. Number between brackets indicate sample size (*n*). N/A = data not available.

<sup>a</sup>Except four individuals.

<sup>b</sup>Except two individuals.





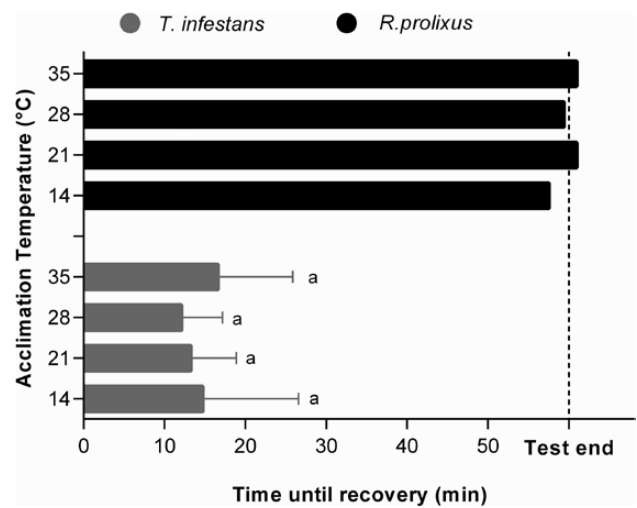
**Fig. 3.** Effect of acclimation on CTmin. Each point represents an individual insect. Full lines were fitted by simple linear regression and dotted lines show the 95% CIs. Acclimation effect was significant for both *R. prolixus* and *T. infestans*.

**Table 2.** Areal values (°C<sup>2</sup>) of thermal range polygons for two triatomines species

Thermal range polygons (°C <sup>2</sup> )	<i>R. prolixus</i>	<i>T. infestans</i>
Total tolerance	819.72	1004.20
Intrinsic tolerance	753.48	985.89
Upper acquired tolerance	13.09	—
Lower acquired tolerance	53.15	18.31

with the nocturnal habits of these species. *Rhodnius prolixus* and *T. infestans*, as well as other Triatomine species spend most of the daytime inside crevices or other refuges (Lazzari et al. 2013), where they benefit from being exposed to less thermal variation (Lorenzo and Lazzari 1999, Heger et al. 2006). However, two times a day, both species show activity peaks: one occurs right after dusk, and the other right before dawn. Between these activity peaks (i.e., at night), individuals remain out of their shelter, exposed to climate variation (Barrozo et al. 2004). Given their nocturnal habits, these species exhibit higher levels of activity during the coldest hours of the day. Therefore, the high plasticity of CTmin may reflect an adaptation to deal with cold nights.

Previous studies show that environmental temperatures have significant effects on the distribution of these triatomines species (Gorla 2002, de la Vega et al. 2015, de la Vega and Schilman 2018). For other species, e.g., *T. vitticeps* (Stål, 1859), precipitation has also a great effect on its distribution (de Souza et al. 2010, de la Vega and Schilman 2018). However, that was not the case for *R. prolixus* and *T. infestans*, which showed high tolerance to desiccation at high relative values of water vapor pressure deficit (e.g., hot and dry; de la Vega and Schilman 2017). This high tolerance to desiccation can be explained, at least in part for *R. prolixus*, by its low cuticular permeability (Rolandi et al. 2014). In addition, active dispersion capability of this species is strongly affected by temperature (Vazquez-Prokopec et al. 2006, Gurevitz et al. 2006). Therefore, the high plasticity of the lower limit of thermotolerance range here reported has most likely enhanced the ability of these species to disperse and conquer new habitats. However, dispersion through challenging environments also requires behavioral strategies useful to avoid pervasive temperatures (May 1979), especially



**Fig. 4.** Acclimation effect on CCRT. Each bar shows the average CCRT and SD for each acclimation temperature. Except for four individual nymphs acclimated at 14°C and two acclimated at 28°C, *R. prolixus* did not recover within 1 h (test duration). Only individual nymphs of *T. infestans* were statistically compared. Different letters indicate significant differences ( $P < 0.05$ ).

for Triatomines that are usually found inside climate controlled domestic dwellings. For instance, microhabitat selection (Heger et al. 2006) and modification of temporal activity cycles (Barrozo et al. 2004) may act as thermoregulatory behaviors that, jointly with the physiological responses we found, influence on the distribution of these vectors.

Regardless of the acclimation temperature, interspecific differences were also higher for lower (CTmin) than for upper (CTmax) thermotolerance. One explanation for this reduced CTmax variation is that for these species (and likely for most ectotherms) the CTmax is close to the physiological thermal ceiling or upper lethal temperature (ULT). In fact, the poor plasticity of thermotolerance upper limits (both critical and lethal temperatures), as well as its low variability across species or populations, is a generalized pattern in ectotherms (Araújo et al. 2013), including insects (Jumbam et al. 2008a, Allen et al. 2012), though there are few counterexamples (e.g., Jumbam et al. 2008b).

In addition to the benefits that follow plasticity, costs are also implied. For instance, depending on the thermal stress timescale to which insects are subjected, costs may overcome potential benefits, and thus plasticity would not be expected to evolve. The thermal plasticity involves protein expression, changes in cell membrane, and chaperones action (Chown and Nicolson 2004). Since these activities are energy demanding, and sustained over time, they can reduce investments to other physiological processes such as the ones required for reproduction. Indeed, a recent study by Rolandi and Schilman found lower fecundity of mated females in a fluctuating thermal environment than at constant temperature (Rolandi and Schilman 2018). For a better understanding of the scope of our results, new studies are needed to evaluate how thermal plasticity can influence different stages and on multiples traits directly or indirectly involved with fitness and survival in Triatomines.

Currently, medical treatments to cure Chagas disease are restricted to infection early stages. Despite the treatment's high effectiveness, many people at risk do not have access to a timely treatment. Furthermore, since there are no vaccines for this infection, vector control is the most effective measure aimed at preventing this disease (WHO 2018). In this context, changes in

the Triatomines distribution areas could represent a risk to health, especially for socioeconomically vulnerable populations. The plastic thermotolerance ranges for these Chagas disease vectors may have very important implications to distribution models, control programs, and climate change projections. At least two different studies predict the impact of global climate change on the future scenario of diseases. Both of them, suggest an important reduction on the distribution, and in some cases local extinctions of disease vectors of Chagas (Medone et al. 2015), or some of the most important diseases (Escobar et al. 2016). Our results indicate that this conclusion is, at least, an overestimation in the reduction of these species distribution area, and there is a high risk that policy makers might take the wrong decisions with detrimental consequences in health issues.

## Data Availability Statement

Data from this study are available on figshare (<https://figshare.com/s/2cc6f5821f2f96ae36a3>).

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