

Differences in competitive ability for the occupancy of shelters in triatomines

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Abstract. Triatomines (Hemiptera: Reduviidae: Triatominae) are nocturnal blood-sucking insects. During daylight hours they remain in an akinetic state inside their shelters, whereas at dusk they become active and move outside. When they are outside their shelters during the photophase, triatomines are vulnerable to diurnal predators and the period just before dawn is critical to their survival. This work analyses the existence of competitive interactions involved in the occupancy of shelters by triatomines. Behavioural assays were performed in which nymphs of different stages, nutritional status or species were released in an experimental arena containing a space-limited artificial shelter. The proportions of individuals occupying the shelter during the photophase were quantified to estimate the competitive abilities of each stage and species. Intraspecific comparisons showed higher levels of shelter occupancy for fourth over fifth instars and fed over unfed nymphs of *Triatoma infestans*. Interspecific comparisons showed higher rates of shelter occupancy for *Triatoma sordida* in comparison with *T. infestans*, and for *T. infestans* over *Rhodnius prolixus*. Arrival order was also relevant to determining shelter occupancy levels: early arrival was advantageous in comparison with later arrival. The study of intra- and interspecific competitive interactions for shelter occupancy provides relevant information about colonization and recolonization processes in the natural environments of triatomines.

Key words. *Rhodnius prolixus*, *Triatoma infestans*, *Triatoma sordida*, Chagas' disease, colonization, competition, shelter occupancy, triatomines.

Introduction

Triatomines are haematophagous insects and vectors of Chagas' disease in the Americas. Between six and seven million people are estimated to be infected worldwide, mostly in Latin America, with the parasite *Trypanosoma cruzi* (Trypanosomatida: Trypanosomatidae), the aetiological agent of this illness (World Health Organization, 2016). Most known triatomine species feed on the blood of vertebrate hosts and often live in close contact with their hosts, mostly inside or near host sleeping places.

During the day, triatomines usually remain inside their shelters, or refuges, which are often narrow, deep and dark. During

the night, they are active and present a bimodal pattern of locomotion activity which peaks first at dusk, when they exit their shelters and begin to search for food, and again at dawn, when the insects return to the shelters in which they remain during daylight hours (Lazzari, 1992; Lorenzo & Lazzari, 1998).

As well as time of day, nutritional status has been shown to modulate temporal patterns of shelter occupancy in these insects: longer periods of starvation promote higher frequencies of walking in and out of shelters (Lorenzo & Lazzari, 1998). Thus, searching behaviour and motivation to occupy a refuge can be modulated by the physiological state of an insect (Takano-Lee & Edman, 2001). For example, triatomines

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normally ingest large volumes of blood each time they feed, which increases their volume by several times and results in a transitory decrease in mobility. Recently fed *Triatoma infestans* (Klug 1834) (Hemiptera: Reduviidae) usually remain outside the refuge performing diuresis until dawn and then engage in the search for adequate shelter (Lorenzo & Lazzari, 1998). As staying outside the shelter makes triatomines vulnerable to predators, the ability to occupy a shelter at the end of the night is crucial to their survival.

The geographical distribution of most triatomine species is well described (Carcavallo *et al.*, 1999; Galvão, 2014). Moreover, at a local space scale, different species of triatomines are known to be found mainly in certain types of shelter, which indicates that some of them present clear sylvatic, peridomestic or domiciliary habits (Schofield, 1994). *Triatoma infestans* is considered the most important vector of Chagas' disease in the Southern Cone of South America (Dias, 2007), mainly as a result of its domiciliary habits. Fifth instar nymphs and adults of *T. infestans* have been found mostly around different materials near roofs, whereas less developed nymphal stages occupy shelters around the beds and belongings of human residents (Gürtler *et al.*, 1992). Inside dwellings in which roofs were made of thatch, tile or other materials that offer less adequate spaces for triatomines, all developmental instars of *T. infestans* were found mainly inside wall cracks (Dias & Zeledon, 1955), demonstrating that all five nymphal stages and adults can be found inside the same restricted area and may share the same shelter. Furthermore, this species also shows preferences for wall shelters located near the floor, even in experimental environments, which indicates the importance of proximity to the food source (Bar *et al.*, 1994). Domestic populations of *Rhodnius prolixus* (Stål 1859) (Hemiptera: Reduviidae), the most important vector in northern South America, have been described as preferring shelters near their hosts, in bedrooms, rather than in other parts of the house (Gómez-Núñez, 1965). Inside an experimental dwelling without refuges, *R. prolixus* nymphs tended to move upwards in search of suitable shelters and were found mainly in the upper wall and ceiling (Takano-Lee & Edman, 2001).

The occurrence of competitive processes in triatomines has been studied through the analysis of interactions between different species in laboratory conditions (Mota & Lorenzo, 2012), semi-natural environments (Bar *et al.*, 1994; Pereira *et al.*, 1995; Oscherov *et al.*, 2001, 2004) and in the context of natural colonization and recolonization processes in domestic and peridomestic environments (Cecere *et al.*, 1997; Pereira *et al.*, 2006). Most of these reports analysed changes in the density of a controlled population of a particular species in the presence of a second species and suggested that individuals presenting higher feeding rates would demonstrate adaptive advantages over those that need more time to attain full alimentation when food is limited.

In natural environments different species of triatomines can occupy different but close ecotopes. Species segregation patterns may be the consequence of differences in innate preferences for shelters, but also of an interspecific competitive displacement of one species that forces the worst competitor to make use of secondary habitats. For example, the temporary elimination of *T. infestans* as a result of vector control strategies in dwellings in Brazil provided empty spaces for other triatomine species such

as the mostly peridomestic *Triatoma sordida* (Stål 1859) (Dias, 1988; Diotaiuti *et al.*, 1995; Rossi *et al.*, 2015), which suggests that the former species was interfering with *T. sordida* spatial distribution. In addition, the destruction of natural ecotopes and profound demographic changes may cause sylvatic triatomines to start searching for more suitable habitats, which can include human dwellings (Diotaiuti *et al.*, 1995; Silveira & Dias, 2011). Simultaneous invasions of different species may also occur from extra-domestic ecotopes, leading to interspecific competition for a desirable but limited habitat. Observations of natural colonies formed by *T. infestans* and *T. sordida* in peridomestic ecotopes showed that the former increased in population numbers, whereas the latter decreased until extinction (Forattini *et al.*, 1983). Bar *et al.* (1994) reported that when similar populations of *T. infestans* and *T. sordida* were released simultaneously in controlled artificial environments, the *T. infestans* population remained stable but *T. sordida* became extinct after 14 months of coexistence. These results support the hypothesis that *T. infestans* is a more successful competitor than *T. sordida* and is capable of excluding the latter species to extinction, probably as a result of its shorter lifecycle. In this respect, Oscherov *et al.* (2001) proposed that if *T. sordida* is unable to find an alternative biotope, it may become extinct according to the competitive exclusion principle.

In experimental comparisons between *T. infestans* and *Panstrongylus megistus* (Burmeister 1835) (Hemiptera: Reduviidae), the former demonstrated a shorter lifecycle and requirements for smaller amounts of blood, which provides it with a competitive advantage over the latter. During the past century, *T. infestans* seems to have progressively displaced *P. megistus*, which was formerly the most abundant species in human dwellings in central and eastern Brazil (Pereira *et al.*, 2006). There have been reports of the coexistence of *T. infestans* and *P. megistus* in Brazil (Dias, 1955; Dias & Zeledon, 1955), but in all cases *T. infestans* presented higher densities. As *T. infestans* continued its spread to northern Brazil, it managed to displace other triatomine species from human dwellings, such as *T. sordida* (Schofield, 1980), *Triatoma brasiliensis* (Neiva 1911) and *Triatoma pseudomaculata* (Correa & Espínola 1964) (Silveira *et al.*, 1984).

One of the most effective strategies to interrupt the vector transmission of *T. cruzi* is the elimination of triatomine bugs from near or inside human bedrooms. In South America, *T. infestans* has been the main species targeted in field control programmes during the last decades. The resultant space liberated after the elimination of this species may favour the domiciliation of sylvatic or peridomestic triatomines that are not the usual targets of vector control strategies (Dias, 1988; Diotaiuti *et al.*, 1995). The study of triatomine vectors with high tendencies towards the invasion and colonization of human dwellings and their relationships with other triatomine species that may be present is therefore relevant.

To date, little is known about how different species of triatomines or even different individuals from the same species make use of available shelters. The aim of the present study is to investigate, under laboratory conditions, the intra- and interspecific competitive interactions involved in the use of shelters by triatomine nymphs.

Materials and methods

Insects

Triatoma infestans, *T. sordida* and *R. prolixus* were reared in an insectary at 28 ± 1 °C, $40 \pm 10\%$ relative humidity (RH) and under an LD 12 : 12 h photoperiod. Each week, newly emerged fourth and fifth instar *T. infestans*, fourth instar *T. sordida* and fifth instar *R. prolixus* were collected from the rearing chamber and maintained unfed for 10–15 days prior to their use in experiments. One fed group of fourth instar *T. infestans* was fed once on hens for 30 min at 4 or 5 days before experiments. Interspecific experiments were performed using different instars in order to minimize size differences: fourth instar *T. infestans* was compared with fourth instar *T. sordida* or fifth instar *R. prolixus*.

The insectary is frequently provided with new insects by the Servicio Nacional de Chagas (Santa María de Punilla, Córdoba, Argentina) in order to minimize any potential effects of inbreeding. All animals were handled according to the biosafety rules of the Servicio de Higiene y Seguridad of the University of Buenos Aires. Insects were used only once and then discarded.

Experimental device

To analyse the competitive abilities of different groups of triatomines in gaining access to a space-limited shelter, an artificial device was designed to allow the continual monitoring of the positions of insects able to freely enter or exit an artificial shelter. The experimental device consisted of a cylindrical Teflon[®] arena (14.5 cm in diameter, 8.0 cm in height) with a glass substrate, containing at its centre an artificial plastic shelter (3.0 × 2.0 × 2.0 cm) with opaque black walls and a transparent roof (Fig. 1). Two rectangular openings (1.5 cm wide, 0.5 cm high) at floor level on opposite walls enabled free access of walking nymphs to the shelter. Inside, a thin white cardboard substrate covered the base of the shelter (paper substrate is largely preferred over plastic or glass by triatomines). The whole device was illuminated by lamps located on each side of the shelter, which generated a homogeneous illuminated environment outside the shelter (70 lux; digital luxometer TES-1330; Tes Electrical Electronic Corp., Taipei, Taiwan), but less illumination inside (7 lux). A video camera (Camera JCC-300; Videoman, Seoul, South Korea) located 30 cm above the arena and connected to a time-lapse video cassette recorder (SLV-960JP; Samsung Corp., Seoul, South Korea) enabled recording the behaviour of nymphs of different groups continuously for 12 h. In all cases, nymphs from different groups were visually distinguished. When necessary, a white spot was painted on the abdomens of insects using acrylic paint to enable their identification.

All the experiments were carried out in an experimental room at 27 ± 1 °C, during the photophase of triatomines (i.e. the period during which they naturally remain inside shelters). In each assay, two groups of 10 nymphs were released into the centre of the arena at the beginning of the photophase by gently tilting a glass jar in which they were contained. Immediately afterwards,

the insects were video-recorded for 12 h while freely moving into or out of the shelter.

Different experiments were designed to address four related questions. These are presented as four separate experiments.

Experiment 1: density-dependent use of shelters in nymphs of *T. infestans* in a competitive environment

To assess competition, individuals must be confronted with a limited resource. In this experiment, the optimal number of individuals able to enter the available space inside the artificial shelter, a scarce resource, was investigated. For this purpose, 10, 20 or 30 fourth instar nymphs of *T. infestans* were released in the experimental arena and the numbers of nymphs occupying the shelter during the 12 h of the experiment were recorded. If space is not a constraint, the percentage of bugs found inside the shelter will be independent of the density of insects released (i.e. the same percentage of nymphs should be found inside the shelter across the different densities). Conversely, if space is scarce, the percentage of individuals inside the shelter will be lower at higher densities.

Based on the results of this experiment, subsequent experiments that aimed to generate a competitive environment were performed and a total of 20 individuals were released into the arena (i.e. two groups of 10 insects each).

Experiment 2: intraspecific comparisons to assess competitive ability in nymphs of *T. infestans* of different instars and nutritional status

This experiment tested whether different individuals of the same species varied in their ability to enter a refuge when space was limited. The competitive abilities of nymphs of *T. infestans* of different instars and nutritional status were compared. The occupancy levels of different instars were assessed by simultaneously releasing 10 fourth (N4) and 10 fifth (N5) instar nymphs of *T. infestans*. To analyse the effect of nutritional status of nymphs on their competitive abilities, 10 unfed fourth (N4) and 10 fed fourth (fed N4) instar nymphs were released together.

Experiment 3: interspecific comparisons to assess competitive ability in nymphs of *T. infestans*, *T. sordida* and *R. prolixus*

In natural environments, different species can cohabit the same area and may in some cases share the same shelters. Therefore, the competitive abilities for shelter of nymphs of three species of triatomines (*T. infestans*, *T. sordida* and *R. prolixus*) were studied by releasing 10 fourth instar nymphs of *T. infestans* with 10 fourth instar nymphs of *T. sordida* or 10 fifth instar nymphs of *R. prolixus*. The numbers of individuals of each group found inside the shared shelter during the experiment were recorded.

Experiment 4: effects of arrival order on the competitive abilities of triatomine nymphs

Reaching a shelter before a competitor may be advantageous if space inside the shelter is limited. In this experiment, two

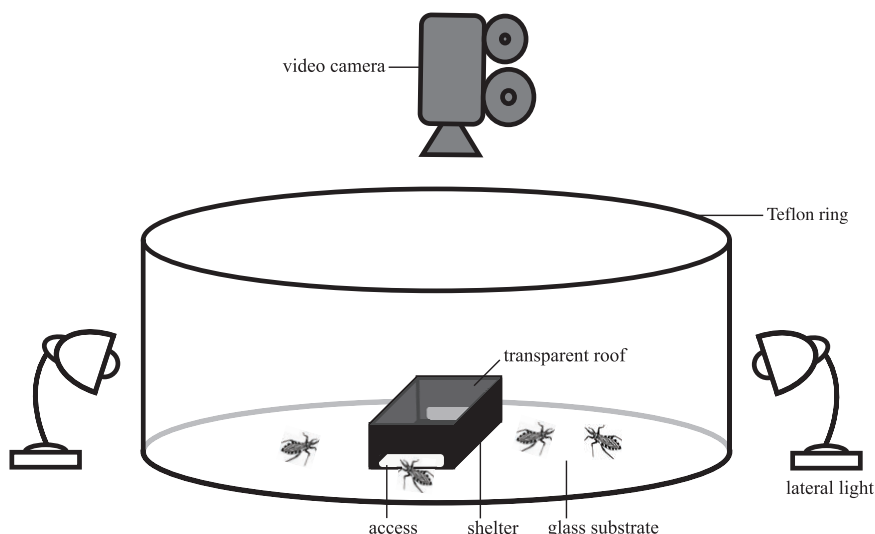


Fig. 1. Experiment arrangement. Different groups of insects were released in the centre of a circular arena containing one artificial space-limited shelter. The number of insects in each group occupying the shelter was registered hourly over 12 h. Ten replicates of each series were performed.

groups of 10 nymphs were released into the experimental arena, but one group was released 3 h after the other. Apart from the time of release, the experimental groups were the same as in Experiments 1, 2 and 3.

Statistical analysis

The numbers of nymphs from different groups found inside the shelter throughout the experimental time were recorded from static images taken from the video films. The first measurement (time 0) was registered 15 min after the insects were released. Subsequently, data were registered at 1-h intervals. One-way analyses of variance (ANOVAs) followed by Tukey's post hoc means comparisons were performed to compare occupancy levels of different groups of insects (Zar, 1984). In all series, the occupancy dynamics of the shelter became stable approximately 3 h after the insects' release. For this reason, the statistical analyses of simultaneous release assays (Experiments 1, 2 and 3) were performed with data obtained between experimental hours 4 and 11. However, in sequential release assays (Experiment 4) statistical analyses were performed with data obtained between experimental hours 7 and 11 (i.e. 3 h after the release of the second group of insects). The last hour was not included in the analyses as insects became more active towards the end of daylight hours. Ten replicates were carried out for each experimental series. Data are given as the mean \pm standard error of the mean (SEM).

Results

In all experiments, nymphs exhibited a high but decreasing level of exploratory behaviour in the arena, accompanied by an increasing level of occupancy of the shelter during the first 3 h of the experiment. Subsequently (i.e. during experimental hours 4–7), the number of individuals inside the shelter remained

constant and was associated with low rates of entry and exit. From 11 h onwards, insects exhibited a slight trend to exit from the shelter, probably in anticipation of the approach of the scotophase, during which they are naturally active.

Experiment 1

When 10 insects were released, 88.80% (8.88 ± 0.23 insects) occupied the shelter. When 20 insects were released, 70.19% (14.04 ± 0.90 insects) remained inside, and when 30 individuals were released, 56.80% (17.04 ± 1.57 insects) occupied the shelter (Fig. 2). The percentage of fourth instar nymphs occupying the shelter varied significantly according to the size of the group (ANOVA $F = 14.62$, d.f. = 2, $P < 0.001$). Post hoc comparisons showed that a higher percentage of bugs remained inside the shelter in the 10-insect group compared with the other two groups (10 insects vs. 20 insects, Tukey's test, $P = 0.011$; 10 insects vs. 30 insects, Tukey's test, $P < 0.001$). No statistical difference in behaviour between the 20-insect and 30-insect groups was found (Tukey's test, $P = 0.081$). These results suggest that when 10–20 insects are released together, they will compete to enter the shelter, but when 20 or 30 individuals were released in the arena, space inside the shelter was already limited. Although the shelter could accommodate at least 17.04 individuals (as observed when 30 were released), only 8.88 were found inside the shelter when 10 individuals were released, which suggests that some process of internal motivation may cause 1.12 of 10 (i.e. 11.20%) bugs to remain outside the shelter even when space is not limited.

Experiment 2

Although both instars occupied the shelter, more N4 (7.71 ± 0.44 insects) than N5 (4.66 ± 0.76 insects) were found in its interior (ANOVA $F = 13.53$, d.f. = 1, $P = 0.001$) (Fig. 3A).

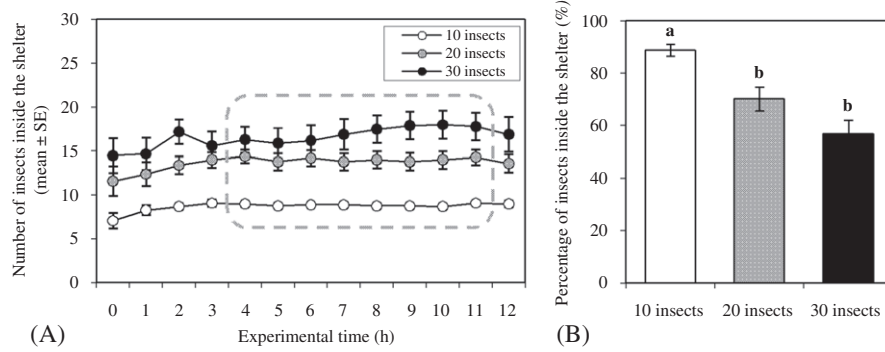


Fig. 2. Experiment 1: setting up a competitive environment. (A) Temporal dynamics of shelter occupancy when groups of 10 (white), 20 (grey) or 30 (black) *Triatoma infestans* nymphs were released in the experimental arena. The grey dotted box clusters the data analysed for each experimental series (i.e. during assay hours 4–11). (B) Percentages of nymphs in each group found inside the shelter during the experiment. Different letters show statistical differences between groups (Tukey's test, $P < 0.05$). Ten replicates were performed for each group.

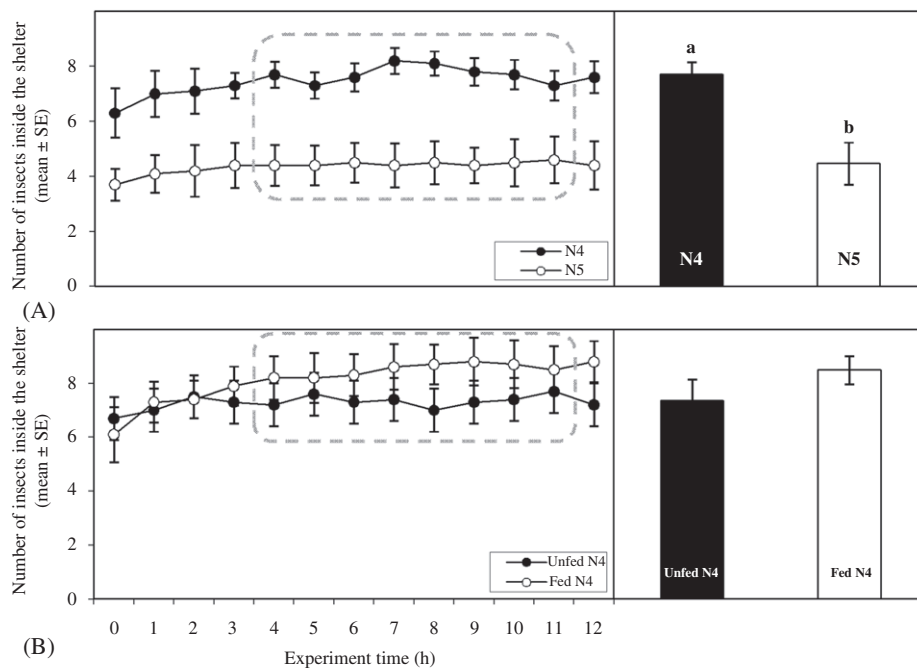


Fig. 3. Experiment 2: intraspecific comparisons in *Triatoma infestans*. (A) Ten fourth instar nymphs (N4) and 10 fifth instar nymphs (N5) were released together. (B) Ten N4 and 10 recently fed N4 were released together. Left panels show the temporal dynamics of shelter occupancy during daylight hours. Right panels show mean numbers of insects in each group found inside the shelter during the experiment. The grey dotted boxes cluster the data analysed for each experimental series during assay hours 4–11. Different letters show statistical differences between groups (ANOVA, $P < 0.05$). Ten replicates were performed for each group.

With regard to nutritional status, there were no statistical differences in the numbers of individuals of each group found inside the shelter (7.36 ± 0.80 N4 insects vs. 8.50 ± 0.53 fed N4 insects; ANOVA $F = 1.51$, d.f. = 1, $P = 0.233$) (Fig. 3B).

Experiment 3

Significantly more *T. sordida* (7.69 ± 0.76 insects) than *T. infestans* (3.60 ± 0.49 insects) nymphs were found inside the shelter (ANOVA $F = 20.27$, d.f. = 1, $P < 0.001$) (Fig. 4A). This result suggests that *T. sordida* can occupy low-space shelters

more efficiently than *T. infestans*. Conversely, when *T. infestans* and *R. prolixus* were released together, significantly more *T. infestans* (8.08 ± 0.44 insects) than *R. prolixus* (2.30 ± 0.40 insects) nymphs occupied the shelter (ANOVA $F = 94.12$, d.f. = 1, $P < 0.001$) (Fig. 4B), which suggests the former species exploits its opportunities better than the latter.

Experiment 4

When two equal groups of 10 fourth instar nymphs of *T. infestans* were released at different time-points, 7.48 ± 0.45

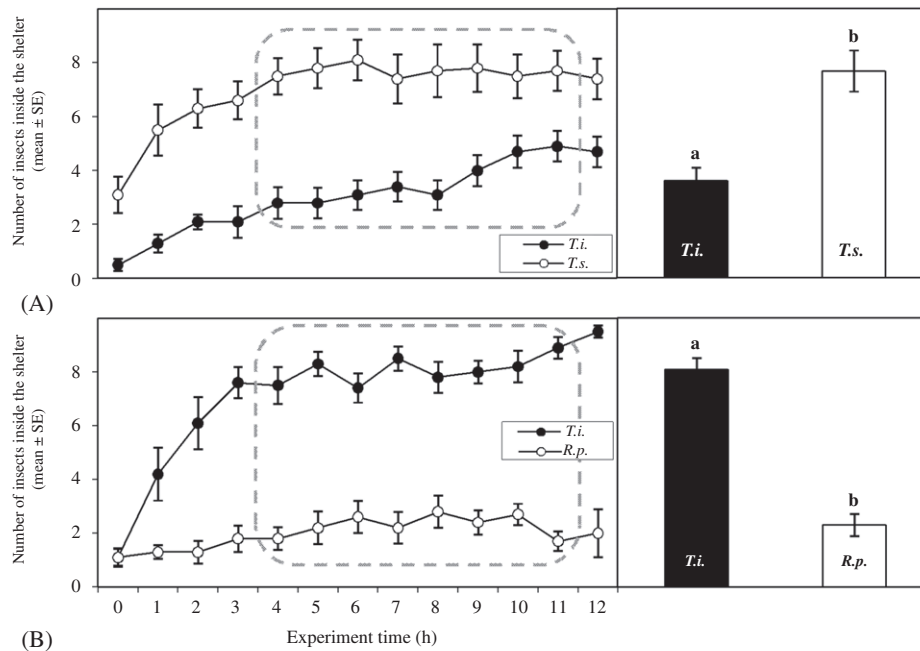


Fig. 4. Experiment 3: interspecific comparisons. (A) Ten *Triatoma infestans* and 10 *Triatoma sordida* nymphs were released together. (B) Ten *T. infestans* and 10 *Rhodnius prolixus* nymphs were released together. Left panels show the temporal dynamics of insects inside the shelter during daylight hours. Right panels show mean numbers of insects in each group found inside the shelter during the experiment. The grey dotted boxes cluster the data analysed for each experimental series during assay hours 4–11. Different letters show statistical differences between groups (ANOVA, $P < 0.05$). Ten replicates were performed for each group.

insects from the first group to be released (N_{4_1}) and 6.14 ± 0.41 insects from the group released 3 h later (N_{4_2}) were found inside the shelter (Fig. 5A). Bugs that arrived earlier occupied the shelter in higher proportions than those released later (ANOVA $F = 4.82$, d.f. = 1, $P = 0.041$). However, the effect of arrival order disappeared when nymphs of different instars were released sequentially (Fig. 5B, C, right). More fourth than fifth instar nymphs occupied the shelter when they were released together (Fig. 3A, right). This result did not change when fourth instar nymphs were given first access to the shelter (8.50 ± 0.27 N4 vs. 1.90 ± 0.38 N5; ANOVA $F = 202.91$, d.f. = 1, $P < 0.001$) (Fig. 5B, right), nor when fourth instars were given later access than fifth instar nymphs (3.14 ± 0.70 N5 vs. 5.32 ± 0.65 N4; ANOVA $F = 5.23$, d.f. = 1, $P = 0.034$) (Fig. 5C, right). These results suggest that fourth instar nymphs have better competitive abilities for scarce shelters than fifth instar nymphs, regardless of their arrival order. Figure 5 (B, C, left) shows that more fourth instar nymphs entered the shelter during all assays.

Fed and unfed insects occupied the shelter at similar rates when they were released simultaneously (Fig. 3B). However, differences associated with their nutritional status appeared when sequential releases were performed. When released first, the fed group remained inside the shelter in higher proportions than the unfed group (9.12 ± 0.22 fed N4 vs. 3.98 ± 0.78 N4; ANOVA $F = 40.51$, d.f. = 1, $P < 0.001$) (Fig. 5E). Surprisingly, even when released in second place, fed insects were able to occupy the shelter significantly more often than unfed insects (3.44 ± 0.79 N4 vs. 9.16 ± 0.17 fed N4; ANOVA $F = 50.51$,

d.f. = 1, $P < 0.001$) (Fig. 5D). These results indicate that fed nymphs are somewhat more effective than starved nymphs in occupying shelters, regardless of the order in which they are released. The temporal dynamics of occupation of the shelter illustrated in Fig. 5 (D, E, left) show in detail the occupancy advantage of fed N4 over unfed N4 in the sequential release assays, even when the former arrived second.

As shown before, more *T. sordida* than *T. infestans* were found inside the shelter in simultaneous release assays (Fig. 4A). This difference was maintained in sequential release assays in which *T. sordida* was released first (8.36 ± 0.57 *T. sordida* vs. 5.34 ± 0.84 *T. infestans*; ANOVA $F = 8.83$, d.f. = 1, $P = 0.008$) (Fig. 6B) or second (6.62 ± 0.88 *T. infestans* vs. 9.24 ± 0.18 *T. sordida*; ANOVA $F = 8.57$, d.f. = 1, $P = 0.008$) (Fig. 6A). Analyses of the temporal dynamics of the use of the shelter show in detail how *T. sordida* occupies the shelter more often than *T. infestans* throughout the assay (Figs 4A and 6A, B, left), even when the former arrives in second place.

The analysis of shelter use in *T. infestans* and *R. prolixus* showed that *T. infestans* occupies significantly more of the shared shelter than *R. prolixus* when both species are released together (Fig. 4B), but also when *T. infestans* is released in either first (8.24 ± 0.35 *T. infestans* vs. 0.74 ± 0.12 *R. prolixus*; ANOVA $F = 416.73$, d.f. = 1, $P < 0.001$) (Fig. 6C) or second (2.18 ± 0.45 *R. prolixus* vs. 5.64 ± 0.79 *T. infestans*; ANOVA $F = 14.54$, d.f. = 1, $P = 0.001$) place (Fig. 6D). Figures 4 (B) and 6 (C, D, left) show the temporal dynamics of shelter use and evidence higher levels of occupancy for *T. infestans* across all assays, even when this species is released in second place.

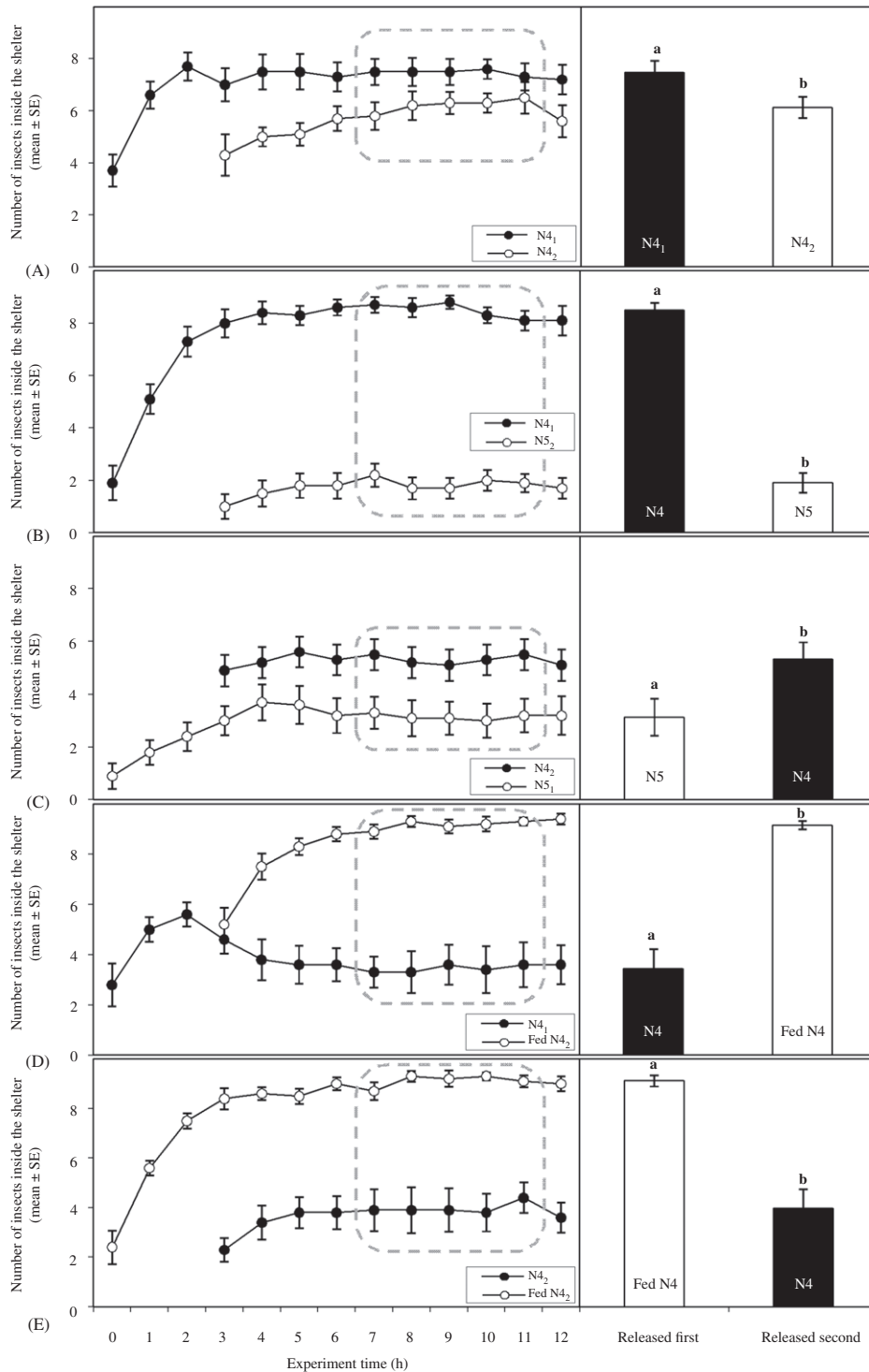


Fig. 5. Experiment 4: arrival order and intraspecific comparisons in *Triatoma infestans*. (A) Ten fourth instar nymphs (N4₁) were released first and another 10 N4₂ were released 3 h later. (B) Ten N4 were released first and 10 fifth instar nymphs (N5) were released 3 h later. (C) Ten N5 were released first and 10 N4 were released 3 h later. (D) Ten unfed N4 were released first and 10 recently fed N4 were released 3 h later. (E) Ten fed N4 were released first and 10 unfed N4 were released 3 h later. Left panels show the temporal dynamics of shelter occupancy during daylight hours. Right panels show mean numbers of insects in each group found inside the shelter during the experiment. The grey dotted boxes cluster the data analysed for each experimental series during assay hours 7–11. Different letters show statistical differences between groups (ANOVA, $P < 0.05$). Ten replicates were performed for each group.

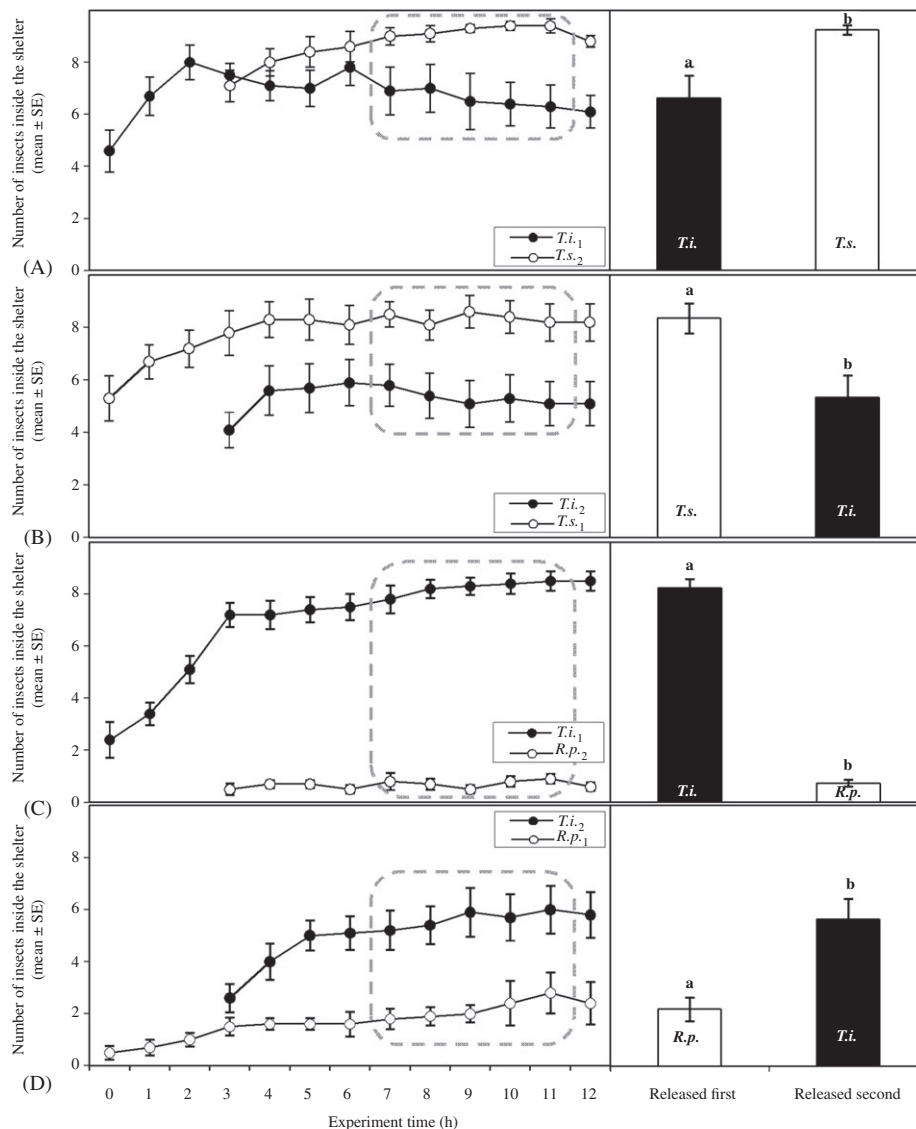


Fig. 6. Experiment 4: arrival order and interspecific comparisons (A) Ten *Triatoma infestans* (*T.i.*) were released first and 10 *Triatoma sordida* (*T.s.*) were released 3 h later. (B) Ten *T. sordida* were released first and 10 *T. infestans* were released 3 h later. (C) Ten *T. infestans* were released first and 10 *Rhodnius prolixus* (*R.p.*) were released 3 h later. (D) Ten *R. prolixus* were released first and 10 *T. infestans* were released 3 h later. Left panels show the temporal dynamics of shelter occupancy during daylight hours. Right panels show mean numbers of insects in each group found inside the shelter during the experiment. The grey dotted boxes cluster the data analysed for each experimental series during assay hours 7–11. Different letters show statistical differences between groups (ANOVA, $P < 0.05$). Ten replicates were performed for each group.

The results presented here show that, although arriving first provides advantages in accessing a shared shelter, differences between instars, species and in nutritional status are far more relevant to competitive ability in accessing shelter in a limited space.

Discussion

The present study analyses the intra- and interspecific competitive interactions involved in the use of shelters by nymphs of several triatomine species. Nymphs of *T. infestans* showed a

density-dependent use of a space-limited artificial shelter when 20 individuals were released together. In addition, the competitive abilities of these insects for a limited space were affected by their nutritional status, nymphal stage, the presence of individuals of other species and arrival order.

The temporal dynamics of shelter occupancy were similar in the insects in all experimental groups. During the first 3 h after their release, the numbers of individuals inside the shelter increased to a constant value. During this period, most insects explored the experimental arena in walks that included entry to and exit from the shelter. Then, general activity levels and entry and exit rates decreased to maintain a stable level of

shelter occupancy over the subsequent 8 h. This suggests that at the beginning of the photophase, nymphs may engage in a decision-making process for a period of 2–3 h in which they decide whether or not to occupy a potentially adequate shelter. Although no other choice was offered (e.g. a second shelter) in these experiments, in nature these insects would probably test alternative shelters before choosing the most adequate one. In any case, once they have chosen to do so, they enter and remain inside the shelter for the rest of the photophase.

The results of Experiment 1, in which only 8.88 of the 10 insects released used the shelter, suggest the natural occurrence of exploratory behaviour that results in willingness to stay outside the shelter in a certain proportion of insects. Higher numbers of insects outside the shelter were observed when more were released inside the experimental arena, which may be attributable to the shelter's limited capacity to accommodate them all. An increase in exploitation interactions inside the shelter may also occur and may force some insects to exit in search of more space. Moreover, the number of insects outside may determine the level of shelter occupancy.

Furthermore, arrival order may confer certain occupancy advantages for triatomines. Insects released 3 h before another group may decide to occupy a shelter while the second group is still exploring the area. This advantage remains evident for the rest of the photophase for identical groups, but can revert when other factors (e.g. stage, nutritional status or species) are involved in motivation for shelter occupancy.

In addition to nymphal stage, nutritional status and species, natural variations in size between treatment groups may affect their abilities to compete for shelter space. For instance, nymphs of the fifth stage of *T. infestans* are larger than those of the fourth stage, and fed nymphs are larger than unfed nymphs. However, although in one instance smaller individuals (i.e. fourth instars rather than fifth instars) were more often found inside the shelter, larger insects were found in another (i.e. fed rather than unfed insects). Similarly, although the fourth instar *T. infestans* is slightly bigger than the fourth instar *T. sordida* and fifth instar *R. prolixus*, in one instance smaller insects occupied the shelter (*T. sordida* rather than *T. infestans*), but larger insects did so on another occasion (*T. infestans* rather than *R. prolixus*). These overall results suggest that size is not the most important characteristic affecting competitive ability to occupy shelters. Although clear differences relating to differences in physiological status and species were demonstrated here, no aggressive or displacement behaviours were observed. Further studies are required to unveil which competitive mechanisms are involved in producing these results.

The feeding status of individuals modulated their ability to occupy a limited space. Triatomines are extremely vulnerable in the post-prandial hours because they normally consume large amounts of blood in each feeding event, which dramatically reduces their mobility. When fed to repletion, *T. infestans* nymphs may increase their weight by 10 times and the volume of their abdomen by up to four times. Similarly, *R. prolixus* nymphs may increase their own weight by up to nine times (Friend *et al.*, 1965). Diuresis continues for 3 h following a bloodmeal, during which time insects excrete rapidly, but this decreases over the subsequent days (Maddrell, 1963). Therefore, finding a safe place in which to perform diuresis and digestion, and to

overcome the reduction in mobility, is essential to their survival. When fed and unfed groups of insects were released simultaneously, insects used the shelter in similar proportions, but findings tended to show more fed insects inside the shelter. In the sequential release assays, more fed insects remained inside the shelter regardless of whether they had arrived in first or second place, suggesting a partial occupancy advantage for engorged over unfed individuals. Fed insects are known to be less prone to exiting shelters and exploring their surroundings during digestion, when their motivation to feed is low (Lorenzo & Lazzari, 1998).

Intraspecific competitive interactions and the ways in which insects exploit available spaces may determine the survival success of a species in a given environment. Studying these types of interaction helps to elucidate the behaviour of triatomine bugs in relation to the use of shelters by individuals bearing different characteristics within the same population (e.g. in age, size, reproductive status, nutritional status and gender). However, if the niche is shared by more than one species, interspecific comparisons of competitive interactions for a limited space can provide valuable information about the potential effects of a decrease or even the total removal of a particular species of triatomines in a particular area. In previous work carried out in artificial environments, *T. infestans* was capable of displacing populations of other species such as *T. sordida* (Bar *et al.*, 1994). Displacement was suggested to mainly represent a consequence of the better efficiency in alimentation exhibited by *T. infestans* in comparison with *T. sordida* (Bar *et al.*, 1994). Many authors have reported that, in natural environments, after massive removal events of *T. infestans* from its niches (mainly human dwellings that were sprayed with pyrethroids), the free space became occupied by secondary species such as *P. megistus* (Dias, 1955; Pereira *et al.*, 1995), *T. sordida* (Schofield, 1980; Bar *et al.*, 1994; Oscherov *et al.*, 2001), *T. brasiliensis* and *T. pseudomaculata* (Silveira *et al.*, 1984). In the present study, *T. sordida* was more competitive than *T. infestans*. Moreover, when *T. sordida* was released in the experimental arena with *T. infestans*, it presented exactly the same pattern of shelter use as when it was released alone (data not shown). In other words, *T. sordida* did not modify its use of the shelter in the presence of *T. infestans* whether the two species were released simultaneously or sequentially. Even when released in second place, *T. sordida* presented higher rates of occupancy than *T. infestans* and achieved the same level of occupancy as it did when it was alone. Conversely, occupancy levels in *T. infestans* were dramatically decreased by the presence of *T. sordida*. These insights might help in understanding how competition processes can govern levels of occupancy of domiciliary or peridomiciliary environments by *T. infestans* and *T. sordida*. If *T. infestans* occupies dwellings more efficiently than *T. sordida*, this may not come about because the former species displaces the latter or interferes with the limited space, but may involve another interaction. As the present paper shows, the coexistence of two populations of different species may be conditioned by context and the resources available. The ways in which populations partition resources may modulate competitive behaviour towards another species and determine the level of coexistence. Contrarily, *R. prolixus* showed lower levels of shelter occupancy in comparison with *T. infestans* when both species were released together or sequentially. It is worth noting that *R. prolixus* uses

the artificial shelter in very low proportions, even in the absence of another species (data not shown) and these values do not change even in the presence of *T. infestans*. Although the geographical distributions of *T. infestans* and *R. prolixus* do not overlap in nature, they are both better adapted to living in households than other species of triatomines. Both species remain very close to their hosts, also live under peridomestic conditions and are the most epidemiologically important species because of the high densities they attain, their good adaptation to intra-domestic habitats, wide geographical distributions and high levels of vector efficiency (Zeledon & Rabinovich, 1981). Hence it appears that, at least in the context of use of a space-limited shelter, *T. sordida* presents greater competitive abilities than *T. infestans* and the latter has greater abilities than *R. prolixus*.

Triatomines use their shelters as places in which to hide during unfavourable times of the day. During daylight hours, their hosts are normally awake and hence become very difficult to feed upon. Moreover, as mammals or birds, these hosts may even become predators of triatomines. The appropriate occupancy of a shelter during the day then presents a key adaptation for survival. If hiding places are significantly reduced, refuges can become a limited resource for which insects compete. Those insects that remain inside a shelter or are capable of removing others from it are more likely to survive. Further understanding of the ways in which triatomines exploit their shelters may offer valuable contributions to the prevention, reduction or even prediction of future infestation and re-infestation events in dwellings, and thereby to avoidance of the transmission of the parasite *T. cruzi* by these vectors of Chagas' disease.

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