Effect of feeding frequency on the reproductive efficiency of two species of *Triatoma* with different epidemiological importance

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**ABSTRACT**

**Introduction**: In Triatominae, reproductive efficiency is an important factor influencing population dynamics, and a useful parameter in measuring a species’ epidemiological significance as a vector of *Trypanosoma cruzi* (Chagas, 1909). The reproductive efficiency of triatomines is affected by food availability; hence, we measured and compared the effects of feeding frequency on the reproductive parameters of *Triatoma patagonica* (Del Ponte, 1929) and *Triatoma infestans* (Klug, 1934), and the effects of starvation on *T. patagonica*. **Methods**: Couples from both species were fed weekly, or every 3 weeks; in addition, females in couples of *T. patagonica* were not fed. Each couple was observed weekly and reproductive efficiency was assessed on the following parameters: fecundity (eggs/female), fertility (eggs hatched/eggs laid), initiation and end of oviposition, initiation of mating, number of matings/week, and number of reproductive weeks. Relative meal size index (RMS), blood consumption index (CI), and E values (eggs/mg blood) were also calculated. **Results**: Changes in feeding frequency affected the reproductive parameters of *T. patagonica* only, with a decrease in fecundity and number of reproductive weeks for those fed every 3 weeks, or not fed. The reproductive period, RMS index, and CI were lower for *T. patagonica* than *T. infestans*. However, despite the lower fecundity of *T. patagonica*, this species required less blood to produce eggs, with an E values of 2 compared to 2.94 for *T. infestans*. **Conclusions**: Our results suggest that the differences in fecundity observed between species reflect the availability of food in their natural ecotopes.


**INTRODUCTION**

In Latin America, triatomines (Hemiptera: Reduviidae) are recognized vectors of *Trypanosoma cruzi*, the etiological agent of Chagas disease. Although these hematophagous insects are of wild origin, several species show varying levels of ecological adaptation to human habitats[1,2], and this trait is relevant in characterizing the epidemiological importance of these species[1].

Of the 140 species in this subfamily[3], 18 occupy wild habitats in Argentina, in addition to intradomicile and peridomestic environments[4]. *Triatoma infestans* is one species that has successfully adapted to human habitats. This primary vector has a wide distribution in southern South America, and is the most epidemiologically important species in Argentina, colonizing intradomicile and peridomestic environments, where it is associated with birds, and occasionally mammals[5]. In addition, *Triatoma patagonica* has been shown to have a significant level of natural infection with *T. cruzi*[1,5,6].

*Triatoma patagonica* occupies wild and peridomestic habitats, and has only been reported in Argentina to date, with its southernmost distribution limited to the Chubut province of Patagonia[7]. This secondary vector[8] has been shown to establish significant colonies in peridomestic ecotopes[9] where it feeds on both birds and mammals[7], and a few instances of intradomicile colonization have been recorded[10,11]. *Triatoma infestans* and *T. patagonica* can colonize different peridomestic structures, feeding on both birds and mammals; in rural areas of Argentina, chicken coops are the most common of such structures[12,13], and thus, in these habitats *T. infestans* feeds predominantly on chickens, as opposed to dogs or goats[12,14].

The reproductive efficiency of a species is strongly associated with its potential to colonize or recolonize a habitat; thus, information on its effects on population dynamics may help to determine the epidemiological significance of different triatomine species[15,16]. Reproductive efficiency, as determined by parameters related to fecundity and fertility[15,17], may vary between species of triatomines; however, within species,
this variation is significantly affected by factors such as environment (temperature), physiology (insect age, and gonadotrophic cycle status) and food (volume, quality, and availability)\textsuperscript{18}. Food availability in the wild varies and insects may feed daily or undergo prolonged fasting, with each species having a critical feeding frequency at which reproductive parameters begin to be affected\textsuperscript{20}.

In adult female triatomines, blood meals are essential for egg production, as they stimulate endocrine regulation of ovarian development\textsuperscript{20}. However, females that have high nutritional reserves from the fifth-instar nymph stage can undergo the first cycle of egg production without feeding\textsuperscript{20}. This phenomenon, known as autogeny, has been described for several triatomine species\textsuperscript{21–23}, including \textit{T. infestans}\textsuperscript{23} and \textit{T. patagonica}\textsuperscript{24}. However, reproductive parameters under fasting conditions for the latter have yet to be fully described.

Abalos\textsuperscript{25} has questioned whether \textit{T. patagonica} could replace \textit{T. infestans} as a permanent host of \textit{T. cruzi} in intradomiciliary environments in southern Argentina. Considering that these species, which have a different epidemiological importance, coexist in peridomiciliary habitats, the objective of this work was to compare the effects of different food supply frequencies and starvation conditions on their reproductive parameters.

**METHODS**

\textit{Triatoma patagonica} and \textit{Triatoma infestans} were supplied by the Coordinación Nacional de Control de Vectores (Córdoba province, Argentina). These insects were originally obtained from chicken coops in the San Luis and La Rioja provinces (Argentina). First-generation insects were reared from eggs in our laboratory and maintained under controlled temperature (26 ± 1°C) and relative humidity (60 ± 10%), and a photoperiod of 12:12h (light: dark). Groups of 15 insects were kept in cylindrical (500cm\textsuperscript{3}) glass bottles covered with nylon mesh and containing vertically folded paper to allow insects' access to food and to avoid excess humidity. Triatomines were allowed to feed for 30 min on an immobilized pigeon (\textit{Columba livia} Gamelin) placed on a plastic box. First-instar nymphs of each species were divided into 2 groups and fed either weekly, or every 3 weeks, from the nymphal to the adult stage.

Once triatomines had reached the adult stage (between August and September), couples from each group, for both species, were placed in separate glass bottles until both insects died. The 4 groups were defined as follows: G1: 13 couples of \textit{T. patagonica} fed once a week; G2: 13 couples of \textit{T. patagonica} fed every 3 weeks; G3: 10 couples of \textit{T. infestans} fed once a week; and G4: 13 couples of \textit{T. infestans} fed every 3 weeks.

In addition, an autogenic group comprised 13 couples of \textit{T. patagonica}, which were fed every 2 weeks throughout the nymphal stage, and then females were not fed, and males were fed once a week, for the adult stage.

Reproductive parameters

Couples were observed weekly until females died, and the following data were recorded: initiation and end of oviposition; initiation of mating; matings per week (determined by observation of spermatophores); reproductive weeks, fecundity (number of eggs laid/female); age-specific fecundity (m\textsubscript{w} = mean eggs/female/week); and fertility (number of eggs hatched/number of eggs laid), calculated as: (number of eggs hatched/total eggs) × 100. The length of the adult stage (days) for females was also recorded. Eggs were stored in Eppendorf tubes until nymphs hatched.

**RESULTS**

Only couples in which females laid eggs were considered in the analysis of reproductive efficiency and feeding behavior. All \textit{T. infestans} females (n = 23/23, groups G3 and G4) and 65.38% (n = 17/26, groups G1 and G2) of \textit{T. patagonica} females laid eggs at least once, and data from these groups was further examined (Table 1).

Female longevity was similar within each species for both feeding regimens. However, \textit{T. patagonica} females had a significantly shorter lifespan than \textit{T. infestans} females with a mean age of 77.92 ± 64.81 days compared to 316.60 ± 95.58 days (H = 33.91, p < 0.001).
Reproductive parameters

Fecundity was significantly higher in T. patagonica females fed once a week (G1) than those fed every 3 weeks (G2; p < 0.05; Table 1); however, there were no significant differences between feeding frequency for other reproductive parameters measured for this species (Table 1). For T. infestans there were no significant differences between groups for any of the reproductive parameters measured (Table 1).

Oviposition (H = 16.62, d.f. = 3, p = 0.0008) and mating (H = 16.17, d.f. = 3, p = 0.01) began earlier in T. patagonica than T. infestans; however, fecundity and reproductive weeks were higher for T. infestans (H = 17.62, d.f. = 3, p = 0.005 and H = 25.58, d.f. = 3, p < 0.001) for fecundity and reproductive weeks respectively.

Of all couples observed, 88.2% (n = 15/17) of T. patagonica and 78.3% (n = 18/23) of T. infestans mated at least once, with no difference between species (p = 0.209, Fisher exact test). The mean mating number was significantly higher for T. patagonica (H = 7.94, d.f. = 3, p = 0.04), and the fertility rate was below 62.0%, with no differences between species (U = 132.00, p = 0.307; Table 1).

In T. patagonica reproductive activity began at week 2 and week 3 for G1 and G2 respectively, with the maximum reproductive effort (m*) recorded at week 6 for G1 and week 4 for G2 (mean m* 9.24 ± 6.18 and 5.90 ± 4.52 eggs/female/week for G1 and G2 respectively) with no significant difference between groups (U = 24.00, p = 0.24). From week 7 for G1 and week 13 for G2, m* values began to decrease until females stopped laying eggs; however, there were a number of additional reproductive peaks for G2 (Figure 1A). In T. infestans, reproductive activity began at week 2 for both groups, and mean m* values did not differ significantly between groups (U = 36.00, p = 0.07) (mean m* 7.43 ± 4.51 and 4.56 ± 3.91 eggs/female/week for G3 and G4 respectively). The maximum reproductive effort for this species was recorded at the end of the reproductive period, at week 41 for G3 (26 eggs/female) and at week 65 for G4 (32 eggs/female; Figure 1B).

For both species, the number of matings positively correlated with fecundity (r = 0.64, p = 0.004 and r = 0.56, p = 0.006, for T. infestans and T. patagonica respectively). For T. infestans females, there was also a correlation between initiation of oviposition and mating (r = 0.53, p = 0.013).

Initiation of oviposition (20.23 ± 8.2 days), first mating (14.33 ± 3.70 days), and fertility (53.75 ± 40.85%) for the autogenic group of T. patagonica showed similar values to those for G1 and G2 (H = 3.78, p = 0.15; H = 3.52, p = 0.17 and H = 5.90, p = 0.052 respectively). However, the number of reproductive weeks (2.92 ± 1.75) for the autogenic group was lower than that for other groups (H = 9.07, p = 0.01), and fecundity (16.38 ± 7.81 eggs per female) was significantly lower than G1 and similar to G2 (H = 9.57, p = 0.008).

Relationships between feeding frequency and reproductive parameters

Only G1 and G2 groups were analyzed in terms of relationships between feeding frequency and reproductive parameters.

The weight at moulting of the T. patagonica G1 group was higher than that of G2; however, there were no significant differences between the G3 and G4 groups of T. infestans. For both species, the volume of blood ingested in the adult stage was similar for females fed weekly or every 3 weeks (Table 2). T. infestans females had a higher RMS index and blood CI than T. patagonica, when fed weekly (U = 10.00, p = 0.007 and U = 5.00, p = 0.002 for RMS and CI, respectively) and every 3 weeks (U = 0.00, p = 0.006 and U = 11.00, p = 0.003 for RMS and CI, respectively; Table 2).

For T. patagonica, oviposition began before the first feeding event for 33.3% and 62.5% of females for G1 and G2 respectively; however, for T. infestans, no females laid eggs without being fed at least once.

For T. patagonica females, there were no statistically significant differences for E values in relation to feeding frequency, and standard deviations were high. However, these

### Table 1 - Reproductive parameters for Triatoma patagonica and Triatoma infestans females with different feeding regimens (G1 and G3: fed weekly; G2 and G4: fed every 3 weeks).

<table>
<thead>
<tr>
<th></th>
<th>Triatoma patagonica</th>
<th>Triatoma infestans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1 ($)</td>
<td>G2 ($)</td>
</tr>
<tr>
<td></td>
<td>μ±SD</td>
<td>μ±SD</td>
</tr>
<tr>
<td></td>
<td>n = 9</td>
<td>n = 8</td>
</tr>
<tr>
<td>Initiation of oviposition (days)</td>
<td>31.33 ± 17.65</td>
<td>23.25 ± 6.75</td>
</tr>
<tr>
<td>Fecundity (eggs/female)</td>
<td>72.11 ± 66.0</td>
<td>20.50 ± 14.48</td>
</tr>
<tr>
<td>Reproductive weeks</td>
<td>10.0 ± 7.14</td>
<td>4.12 ± 2.85</td>
</tr>
<tr>
<td>Initiation of mating (days)</td>
<td>31.33 ± 17.65</td>
<td>23.25 ± 6.75</td>
</tr>
<tr>
<td>Number of matings</td>
<td>10.0 ± 7.14</td>
<td>4.12 ± 2.85</td>
</tr>
<tr>
<td>Fertility (%)</td>
<td>72.1 ± 66.0</td>
<td>20.5 ± 14.5</td>
</tr>
</tbody>
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μ±SD: mean ± standard deviation; n: number of insects; U: statistical index of Mann-Whitney test.
Eggs/Female

Weeks

FIGURE 1 - Age-specific fecundity curves ($m_x$) for (A) Triatoma patagonica and (B) Triatoma infestans females with different feeding regimens. Dotted lines: groups fed weekly (G1 and G3); and continuous lines: groups fed every 3 weeks (G2 and G4).

E values were significantly higher than those recorded for both groups of T. infestans females ($H = 20.47$, d.f. = 3, $p = 0.0001$; Table 2).

For both species, the volume of blood ingested during the adult stage correlated positively with fertility ($r_s = 0.62$, $p = 0.030$ for T. patagonica and $r_s = 0.60$, $p = 0.05$ for T. infestans) and reproductive weeks ($r_s = 0.85$, $p = 0.0003$ for T. patagonica and $r_s = 0.54$, $p = 0.05$ for T. infestans).

**DISCUSSION**

Feeding has a direct effect on the reproductive efficiency of triatomines. Variations in reproductive parameters within species relate to food availability, quality, and nutritional status\(^{18}\), whereas differences between species may be due to the relationship between reproductive behavior and the insects’
natural habitat\(^5\). Consensus has not yet been reached in terms of the quality of ingested blood, with some species showing greater reproductive efficiency when fed on mammals or birds\(^{15,17,29}\), but other species not\(^{15}\); variability has even been shown between different studies of the same species\(^{15,29}\). For this reason, our results are discussed in the context of other studies using the same food source. The reproductive efficiency of _T. infestans_ females does not appear to be affected by feeding frequency, as there were no differences between groups for any reproductive parameters. However, the reproductive efficiency of the secondary vector _T. patagonica_ appears to be sensitive to feeding frequency, as fecundity, reproductive weeks, and blood consumption, were lower in females fed every 3 weeks than those fed weekly. Similarly, Zeledón et al.\(^{21}\) and Cabello et al.\(^{19}\) reported an increase in fecundity with increased feeding frequency for _Triatoma dimidiata_ and _Rhodnius neivai_.

On the other hand, the fact that _T. patagonica_ that were starved had similar fecundity and reproductive week values to those of females fed every 3 weeks suggests that this is close to the critical fasting limit for this species in terms of reproductive parameters.

Females of _T. patagonica_ began to lay eggs earlier than _T. infestans_. This may be related to the stimulatory effects of mating on oviposition\(^{28}\), as females of this peridomiciliary species copulated earlier than those of the domiciliary species. In _T. infestans_\(^1\) and other domiciliary triatomine species like _Panstrongylus megistus_\(^{32}\) and _Triatoma brasiliensis_\(^{13}\), virgin females were found to begin laying eggs later than those that had copulated. Accordingly, Carrizo\(^{24}\) reported that virgin females of _T. patagonica_ fed fortnightly began oviposition at 38.2 ± 14.4 days, later than that recorded here.

Fecundity and reproductive weeks were also different between species, with values for both higher in _T. infestans_ than _T. patagonica_. However, the fecundity values under starvation for _T. patagonica_ seen in this study were higher than those reported by Noriega\(^{26}\) for _T. infestans_ under similar conditions. This may be an advantageous characteristic in _T. patagonica_, allowing it to lay many eggs prior to first feeding in a peridomestic habitat where food availability is variable. For both species, fertility rates were similar; however, they were lower than values previously recorded for these species\(^{26,28,33}\), as well as other peridomiciliary and domiciliary species\(^{7,13,34}\), fed on birds blood. This decrease might be due to excessive insect manipulation during the performance of experiments, and is in agreement with results reported by Tolesano et al.\(^{16}\) for _T. infestans_.

Reproductive efficiency in terms of fecundity was compared between species by calculating indices relating egg production to blood consumption. Although _T. patagonica_ laid fewer eggs and had a lower RMS than _T. infestans_, they consumed a smaller volume of blood daily (CI) and were more efficient in converting blood into eggs (E values), producing approximately 2 eggs per mg blood ingested. To produce a similar number of eggs, _T. infestans_ required approximately 4mg of blood when fed weekly. The E values obtained in this work are similar to those reported by Guarneri et al.\(^{12}\) for other domiciliary and peridomiciliary species. These authors indicated that differences in E values between species reflect the food availability of the insects' natural ecotopes. In the natural environments of triatomine species, there are a number of interacting factors that cannot be reproduced in the laboratory, such as behavioral responses to the environment and to different food sources, and genetic variability of populations\(^{13,33}\). Hence, although it is difficult to extrapolate the data presented here directly to populations developing in natural environments, our results do indicate trends in the behavior of these species relative to these environments.

Our comparison of the reproductive efficiency of these two species with different epidemiological importance indicates that, unlike domiciliary species, peridomestic species such as _T. patagonica_ allocate a greater percentage of ingested blood to reproduction than maintenance. This characteristic may be related to the environmental conditions under which this species develops. Peridomiciliary species live in unstable ecotopes, and form small scattered colonies with infrequent access to food sources, whereas domiciliary species live in more stable ecotopes in high densities and with frequent food access.
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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

