

Comparative population dynamics of the bug *Mepraia spinolai*, a sylvatic vector of Chagas' disease, in different hosts

M. ACUÑA-RETAMAR^{1,2}, C. BOTTO-MAHAN³, M. CANALS³,
J. P. CORREA¹ and P. E. CATTAN¹

¹Departamento de Ciencias Biológicas Animales, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile, ²Department of Pathology, University of New Mexico, Albuquerque, New Mexico, U.S.A. and ³Departamento de Ecología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

Abstract. The aim of this work was to determine the impact of specific hosts on a population of *Mepraia spinolai* (Porter) (Hemiptera, Reduviidae), a sylvatic vector of Chagas' disease in Chile. We assessed whether a recently introduced host could be an important epidemiological factor in maintaining Chagas' disease in Chile. The study stressed the variations in survival, individual weight and fecundity in the insect population when the vector was raised with a species-specific food supply. The study compared the European rabbit *Oryctolagus cuniculus*, introduced in Chile ≤ 150 years ago, with a wild endemic rodent, *Octodon degus*. Hosts were placed separately in experimental glass chambers. Groups of insects maintained with rabbits had greater fecundity than those raised with rodents, but both groups had similar survival times and average weights. Both *O. degus* and the European rabbit appear to be suitable hosts for *M. spinolai*, but the rabbit is better than the rodent. Additional research is needed to determine which factors of *O. cuniculus* produce the observed results.

Key words. *Mepraia spinolai*, Chagas' disease, European rabbit, food source, host preferences, Chile.

Introduction

Chagas' disease is a parasitic zoonosis which has great social and economic impact in several Latin American countries (Schofield, 1994). The causative agent, *Trypanosoma cruzi*, is transmitted by a variety of triatomine insects, most of them sylvatic species. In Chile, there are two sylvatic species: *Mepraia spinolai* (Porter, 1834) and *Mepraia gajardoi* (Frías). The latter, *M. gajardoi*, has been only recently described (Frías *et al.*, 1998) and there are few studies on its vector capacity (but see Botto-Mahan *et al.*, 2008). *M. spinolai* is a vector for Chagas' disease and is subject to controversial epidemiological interest as a result of the scarcity of its populations near human dwellings (Schenone *et al.*, 1980; Canals *et al.*, 1999a). This species depends on wild rodents, rabbits and other vertebrates as hosts (Canals *et al.*, 2001). However, its importance as a vector will depend on human–insect contact in zones where there is considered to be risk. This contact may be increased when the domestic vector (*Triatoma infestans*) is eradicated,

allowing the sylvatic vector to colonize human dwellings, and when a sylvatic host makes a bridge between field and domestic habitats. Such colonization has been reported in Uruguay with the sylvatic triatomine *Triatoma rubrovaria* (Salvatella *et al.*, 1991, 1994) and in Bolivia with *Rhodnius stali* (Matias *et al.*, 2003).

The lifecycle and population dynamics of triatomines depend on their interaction with their hosts (Schofield, 1994). For this reason it is important to study the habitats and ecology of host species, especially when these habitats coincide with those of their parasites (Canals *et al.*, 1998). This is the case in the species tested in this contribution. In recent years, several studies have been conducted to elucidate aspects of the biology and ecology of *M. spinolai*, particularly its genetics (Frías *et al.*, 1998; Moreno *et al.*, 2005), population biology (Canals *et al.*, 1994), microhabitat selection (Canals *et al.*, 1998), behaviour (Canals *et al.*, 1999b), alimentary profile (Cruzat, 1997; Canals *et al.*, 2001; Molina *et al.*, 2004), *T. cruzi* infection (Botto-Mahan *et al.*, 2006) and host availability (Botto-Mahan *et al.*, 2005). However, studies

Correspondence: Pedro E. Cattán, Departamento de Ciencias Biológicas Animales, Universidad de Chile, Casilla 2 Correo 15, Santiago, Chile. Tel.: + 56 2 978 5629; Fax: + 56 2 978 5526; E-mail: pcattan@uchile.cl

comparing population dynamics (variations in fertility, survival and population growth) in association with a unique and specific blood-source host are lacking (Wolff *et al.*, 2004). Blood characteristics and host behaviour may affect the population dynamics of insects and, consequently, their fitness. These factors will be of relevance in determining the comparative importance of a host (Schofield, 1979; Lehane, 1991). The purpose of this study was to compare the effect of two different sources of food on the survival, individual weight and fecundity of *M. spinolai* cohorts.

Materials and methods

Experimental design

Individuals of *M. spinolai* (fourth and fifth instars and a few adults) were collected at Las Chinchillas National Reserve, IV Region, Chile (31°30' S, 71°06' W). The experiment was carried out under laboratory conditions where temperature (range 25–27 °C), relative humidity (65–70% RH) and photoperiod (light : dark [LD] cycle 12 : 12 h) were controlled (Schaub & Lösch, 1989; Ehrenfeld *et al.*, 1998). We built five experimental glass chambers of 100 × 50 × 50 cm; RH was controlled using water containers. To one of the 50 × 50 cm walls of each chamber we attached a plastic jar (15 × 10 cm) to be used as a refuge for the insect colony. Insects entered the chamber through a hole in the wall of the jar. The hole was protected with wire mesh to prevent the host from entering the jar.

One host was placed in a chamber at a time. Juvenile rabbits (*Oryctolagus cuniculus*, average weight 300 g) and wild rodents (*Octodon degus*, average weight 185 g) were used. Animals were sourced from stocks reared in the laboratory and had had no previous contact with the insects. For each replicate, four animals were used in a rotation period. Each animal stayed in the chamber for 1 week to avoid habituation to insect bites and potential injuries. Hosts were provided with food and water *ad libitum*. Forty starved insects (45 days of starvation in the laboratory) were placed in each jar. The initial average weight of the insects was 87.2 ± 49 mg. This initial colony was composed of three adults marked with permanent non-toxic marker (one male and two females) and 37 fourth and fifth instars. The five simultaneous experiments were maintained for 14 weeks because, in laboratory conditions, fifth instars moult to adults over a period of 5–13 weeks (Ehrenfeld *et al.*, 1998).

For each host species, two replicates were performed; these were designated DC1 and DC2 in the rodent colonies, and LC1 and LC2 in the rabbit colonies. Moreover, as a control, an identical colony in terms of number of individuals and laboratory conditions was maintained in a similar experimental chamber without a food source for a similar period of time. This was the 'reference colony' (RC, hereafter). It allowed us to compare the effect of starvation on population growth.

Statistical analyses

Survival and moulting were recorded weekly in each of the experimental chambers. New adults resulting from moulting

were removed from the colonies to maintain the initial number of adult insects; this extraction was considered in survival curves. Curves of cumulative chance of survival (CCS) (Almeida & Billingsley, 1998) were estimated for each colony. Mortalities were compared with the Mantel–Haenszel test. Multiple comparisons were performed with bootstrap analysis, and odds ratios (ORs) were calculated for each colony. Each insect was weighed weekly in an analytical scale (0.1/0.01 mg repeatability; Denver Instruments Co., Denver, CO, U.S.A.) and an average weight was calculated weekly for each colony.

To evaluate the effect of host on average weight a general linear model (GLM) was used with time as covariant. Multiple comparisons were calculated subtracting the covariant effect by a Tukey test. Additionally, the homogeneity of slopes was studied with the GLM and multiple comparisons were performed with Bonferroni correction (Zar, 1996).

Fecundity was expressed as the number of eggs/number of adult females/week, but only the initial two adult females were considered in this rate. This ratio was compared using nested analysis of variance (nested ANOVA), with fecundity as response variable, type of host as factor, and colonies nested within each host species (Zar, 1996). All statistical analyses were performed with STATISTICA 6.0 (StatSoft, Inc., Tulsa, OK, U.S.A.).

Results

Survival

All the colonies remained until the end of week 14 of the experiment, but they differed significantly in population survival. Adults (both genders) survived throughout the experiment, whereas nymphs showed differences in mortality rates among the colonies. The mortalities of the colonies associated with both mammals were statistically different from those in the RC (Mantel–Haenszel test, $\chi^2_4 = 22.074$, $P < 0.05$). When the mortality curve for the RC (iterative method) was removed, there was no statistically significant difference between insects fed on rabbit or rodent ($\chi^2_3 = 4.32$, $P > 0.05$). The ORs of the experimental groups showed no significant differences with the RC, but the insects fed on rabbits showed a slight tendency to have a lower risk of death (OR LC1 0.085, OR LC2 0.076) than the insects fed on rodents (OR DC1 0.176, OR DC2 0.160).

Average weight

The RC showed a slow decay in average weight throughout the experiment, whereas the weights of DCs and LCs showed an increment (Fig. 1). The ANCOVA showed an association between average weight of colonies and type of host ($F_{4,64} = 27.7$, $P < 0.001$), with the RC differing from the LCs and DCs. One of the DCs was similar to the LCs, but, in general, the average weight of LCs was greater than the average weight of DCs. The homogeneity of slopes test showed that average weight is associated with time, and differences in slopes of weight change (Host*Age [slopes] $F_{2,64} = 73.4$, $P < 0.001$; Age $F_{1,64} = 41.4$, $P < 0.001$). The slopes corrected with the Bonferroni criterion

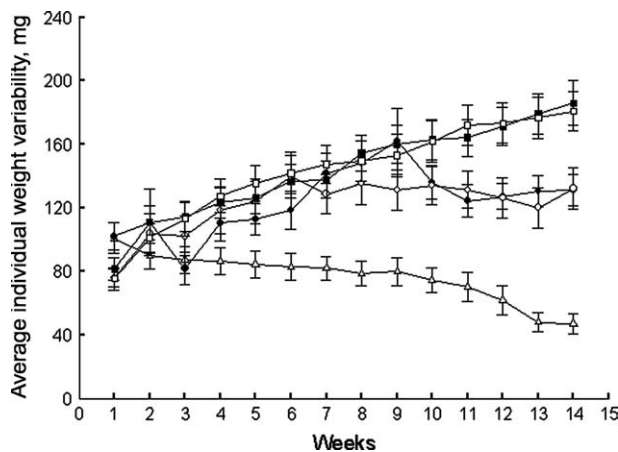


Fig. 1. Average individual weight variability of specimens in experimental colonies of *Mepraia spinolai*. Δ , control (reference) colony; $\bullet$, rodent colony 1 (DC1); $\circ$, rodent colony 2 (DC2); $\blacksquare$, rabbit colony 1 (LC1); $\square$, rabbit colony 2 (LC2). Vertical bars show standard errors.

showed that, although the RC was different from treatment colonies (DCs and LCs), treatment colonies were similar (RC–DCs $T_{38} = 4.99$, $P < 0.05$; RC–LCs $T_{38} = 22.53$, $P < 0.05$; DCs–LCs $T_{52} = 0.44$, $P > 0.05$). In summary, while RC loses weight, DCs and RCs gain weight.

Fecundity

Fecundity was higher for LCs compared with DCs ($F_{1,52} = 12.17$, $P < 0.001$). Plotting the cumulative eggs per week, we observed that LCs maintained an increase from week 5 up to the end of the experiment, whereas those from the DCs stopped reproducing by week 7 (Fig. 2).

Discussion

The results of this study show that *Mepraia spinolai* is able to survive starvation for ≥ 2 months. This fact has been demonstrated in other triatomines, some of which have survived ≤ 12 weeks without being fed (Schofield, 1994) and ≤ 25 weeks in *Tr. rubrovaria* (Almeida *et al.*, 2003). Colonies of *M. spinolai* were affected by starvation from week 8 onwards and became nearly extinct by week 15. Adaptation to starvation could be considered a result of selective pressure in view of the habitat of this insect (e.g. rock crevices, stone quarries), which confronts remarkable seasonal cycles in its host availability (Lima *et al.*, 1999). Moreover, the prey offered is highly variable within the habitat itself. This explains why a large number of insects captured in each collecting session were unfed (Rengifo, 2000). The qualitative (nutritious) aspect of the blood source is an important factor for these insects. Guarneri *et al.* (2000a) demonstrated that survival is higher in nymphs fed on rats (*Rattus* sp.) compared with those fed on doves (*Columba* sp.). Comparing cohorts fed on rabbits or on hens (*Gallus* sp.), Cabello *et al.*

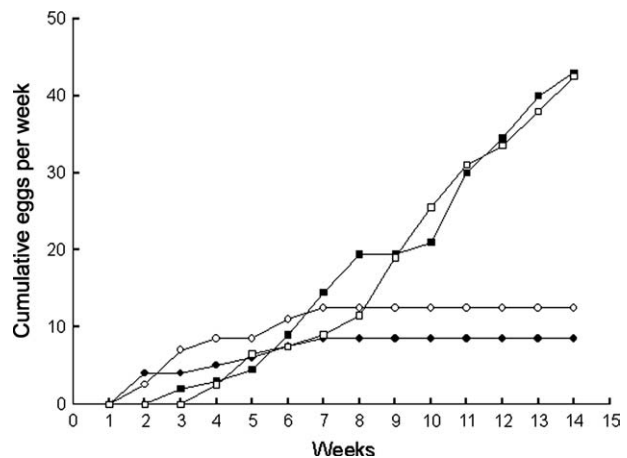


Fig. 2. Cumulative eggs per week in experimental colonies of *Mepraia spinolai*. $\bullet$, rodent colony 1 (DC1); $\circ$, rodent colony 2 (DC2); $\blacksquare$, rabbit colony 1 (LC1); $\square$, rabbit colony 2 (LC2).

(1988) found that life expectancy and survival were higher in insect cohorts fed on rabbits. In this study, we compared rodent vs. rabbit as host, and our results showed a remarkable effect of rabbit on insect fecundity, but not on average weight or survival.

Individual weight is affected by blood source. Crocco & Catalá (1997), comparing insects fed on doves and rodents, found that meal average weight was greater for insects feeding on rodents. However, the relationship between weight changes and the quantity of blood ingested has not yet been established (Wisnivesky-Colli *et al.*, 1995; Pereira *et al.*, 1998; Guarneri *et al.*, 2000b; Soares *et al.*, 2000). The most immediate question concerns the differences between these two host mammal species that might explain the present results. One important effect, following Schofield's (1994) argument, is host irritability. A host that presents a minor level of irritability when sucked by an insect would be preferred. Studying the feeding behaviour of three species of triatomines, Guarneri *et al.* (2000b) established that both time devoted to test the host and interruptions during the sucking period are important factors that differentiate hosts. Accordingly, we need to assess first whether the testing time for rabbits is shorter than that for wild rodents, and whether the number of interruptions caused by rabbit irritability is lower compared with the number occurring on wild rodents.

The high fecundity observed in colonies fed on rabbits may be explained by two non-mutually exclusive hypotheses. The first of these suggests that, as rabbit irritability is low, insects can increase the amount of blood ingested and this will be reflected in the number of eggs produced. According to Schofield (1994), a reduction of 20 mg of blood ingested by *Tr. infestans* means a reduction of one egg during oviposition. The second hypothesis supposes that rabbit blood is of higher nutritional quality than the blood of wild rodents. Differences in concentrations of ATP (adenosine triphosphate) in the red cells may explain why nymphs feeding on different hosts showed notorious differences in their moulting periods (Schofield, 1979). Blood

viscosity will also be important; low viscosity (a lower haematocrit level) will support a shorter period of bloodsucking, which may fail to elicit the behavioural response by the host. If the bloodmeal depends on red cell size and viscosity, we may expect that *M. spinolai* will select the host with the smaller red cell size and lower haematocrit level, maximizing the amount of blood that can be ingested in the shortest period of time. Given that haematological values for red cell size and haematocrit in rabbits are lower than those in wild rodents (Fowler, 1986), it is not surprising that *M. spinolai* shows more fecundity when it feeds on this host.

Although our results do not show a significant difference in survival or mortality risk between insects fed on rodents and those fed on rabbits, there is a slight indication that insects fed on rabbits have a lower risk of mortality than insects fed on rodents. This situation could be elucidated with a larger dataset or a longer experimental period.

Our results show evidence that the European rabbit should be considered as an important host for the population dynamics of *M. spinolai*. Given that this introduced species has colonized central Chile, including various habitats occupied by the wild insect, and 37.9% of *T. cruzi*-infected European rabbits in the wild (Botto-Mahan *et al.* 2009, in press), we propose that this mammal is a recent but probably one of the most important reservoirs of Chagas' disease, and that it may have great impact on the epidemiology of the disease in Chile.

Acknowledgements

This research was funded by Fondo Nacional de Desarrollo Científico y Tecnológico grants 1980768 and 1040711 to PEC. CB-M was supported by Comisión Nacional de Investigación Científica y Tecnológica - Programa Bicentenario de Ciencia y Tecnología (PSD66). The authors thank Katy Mirowsky for helpful suggestions to improve the manuscript.

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Accepted 23 August 2008