

Twenty years of monitoring of mortality and fecundity of *Triatoma infestans* in the laboratory

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Summary

A colony of *Triatoma infestans* (Klug) has been maintained continuously in the laboratory “Colonia de Vinchucas” of the Faculty of Medicine of the University of Chile since 1949, under a constant regime of temperature and humidity, recording weekly since 1983 the oviposition and deaths of individuals. This constitutes an invaluable record of a single long-term colony maintained in the same place and under the same environmental conditions for a number of years. The aim of this report was to study mortality and fecundity rates in this population of *T. infestans* maintained under constant environmental conditions, which may serve as a standard of comparison for other species or the same species under other environmental conditions. Fecundity and mortality time series showed seasonal fluctuations confirmed with the ARIMA model. The mean fecundity of *T. infestans* varied from averages of 0.39 eggs/female/week in autumn to 1.63 eggs/female/week in spring, with maximum values of 7.59 eggs/female/week in spring and 5.83 eggs/female/week in summer. Maximum mean mortality rates were in spring and summer. Mortality rates of males and females were not different. Fluctuations of fecundity were of greater magnitude than mortality rates. The highest fecundity rates were also in spring and summer.

Keywords: Fecundity, Mortality, Triatomines

Introduction

Chagas' disease, caused by the flagellate protozoan *Trypanosoma cruzi*, affects more than 8 million people in America (WHO 2013). Its main vector in South America is the kissing bug *Triatoma infestans* (Klug 1834) (Hemiptera, Reduviidae), because of its wide distribution range and its capacity to maintain high intra-domiciliary population densities (Lent & Wygodzinsky 1979, Zeledón & Rabinovich 1981, Canals et al. 1991a,b, 1993, 1994, 1997, 1999). This insect has a relatively low intrinsic rate of increase, great longevity and resistance to starvation, maintaining populations near the saturation level (Rabinovich 1972, Canals et al. 1998). In Chile *T. infestans* can live in domestic and peri-domestic habitats with a preference for warm micro-environments (24 °C) (Canals et al. 1997); it is able to survive, mate and rear in human dwelling environments (Canals et al. 1994), and blood of humans represents about 68% of its diets (Schenone et al. 1985, Gürtler et al. 2014). In Chile it inhabits the Mediterranean climate, experimenting large fluctuations of temperature and humidity; although the vector chain of transmission of Chagas' disease has been broken since 1999 (Lorca et al. 2001), recently sylvatic population foci have been reported in the central zone of Chile (Bacigalu-

po et al. 2006, 2010), in Bolivia (Cortez et al. 2006, 2007, 2010, Buitrago et al. 2013) and in Argentina (Gurevitz et al. 2011).

Fecundity and mortality rates are the two of the most fundamental factors of the demographic regime of a population, and are of great interest in populations of vectors such as *T. infestans* (Rabinovich et al. 2011, Medone et al. 2015). Stochastic, seasonal and secular variations in these variables may result in fluctuations if the population size or the proportions of individuals in each age class, extinctions or plagues (Canals et al. 1991 a,b). These fluctuations are mostly induced by climatic variations. For example, temperature and humidity variability affect population parameters such as the basic reproductive rate (R_0), the pre-imaginal period and the generation time (Canals et al. 1992, 1994), and also infection indexes of vectors (Canals et al. 1998).

A colony of *Triatoma infestans* (Klug) was maintained continuously in the laboratory “Colonia de Vinchucas” of the Faculty of Medicine of the University of Chile since 1949, under a constant regime of temperature and humidity, recording weekly since 1983 the oviposition and deaths of individuals; it was suspended in 2003 because the loss of interest as a result of the interruption of the vector transmission chain in Chile. This constitutes

an invaluable record of a single long-term colony maintained in the same place and under the same environmental conditions for several years. The aim of this report was to study mortality and fecundity rates in this population of *T. infestans* maintained under constant environmental conditions, which may serve as a standard of comparison with other species or the same species under other environmental conditions.

Material and methods

The weekly record of the fecundity (number of eggs) and adult deaths of *T. infestans* from 1983 to 2003 was analyzed. Adult insects were maintained in groups of 200 individuals (100 males and 100 females) in glass jars of 18 liters. There were three to eight jars in the analyzed period. They were fed weekly on adult hens. The insects were maintained in a climatic chamber of 2 x 2 x 2 m (8m³) (Jordon Commercial Refrigerator, Model D.46.8, UK) at 24 ± 2.5 °C, with a maximal range between 18 and 28 °C and a relative humidity of $50 \pm 8\%$. The climatic chamber remained with the door open 30 cm, allowing indirect light entrance provided by a window of the laboratory and an artificial light only during working hours. The density of individuals in each glass jar was maintained constant; dead individuals were replaced weekly by new ones from the breeding jars. The count of individuals and eggs was performed weekly. Fecundity (number of eggs /female week) and sex-specific mortality (dead individuals/ individual week) rates were calculated. Time series of 20 years (1040 weeks) of each variable were obtained. The seasonal behavior of the series was described graphically and with descriptive statistics. Periodogram, autocorrelation (ACF) and partial autocorrelation (PACF) functions of the time series were analyzed and a seasonal integrated autoregressive and moving average model, ARIMA, (P,D,Q)x(Ps,Ds,Qs) was adjusted to probe the seasonal component, where P, D and Q represent autoregressive, difference and moving average parameters, respectively, and "s" indicates the seasonal component-.

Results

Both time series showed seasonal fluctuations (Fig. 1). The ARIMA model confirmed the seasonal

component and a non-seasonal autoregressive parameter that accounted for the dependence of both variables with themselves in the immediately preceding week (Table 1). Mortality rates of males and females were not different ($t = -0.168$, $p = 0.867$). Fluctuations in fecundity ($CV = 0.59$) were of greater magnitude than those of mortality rates ($CV = 0.34$) (Bartlett test = 14.37, $p = 0.0002$). The highest values of fecundity and mortality rates were in spring and summer (Table 2).

	Mortality			Fecundity		
	Value	SE	t-test	Value	SE	t-test
P1	0.980	0.008	129.4*	0.961	0.010	95.88*
Q1	0.494	0.036	13.9*	0.523	0.031	16.86*
Ps1	0.895	0.089	10.0*	0.139	0.033	4.21*
Qs1	0.824	0.107	7.7*	-	-	-

Table 1. Parameter values of the seasonal ARIMA model of the mortality and fecundity time series for *Triatoma infestans* under constant laboratory conditions. P1, Q1, Ps1 and Qs1 are non-seasonal and seasonal (s) auto-regression and moving average parameters, respectively. Se indicates standars error of the parameters and* indicates a p-value <0.001 in a t test.

	Fecundity (eggs/female week)	Mortality (deaths/individual week)
Spring	1.63 ± 1.13 [0.00-7.59]	0.059 ± 0.035 [0.00-0.155]
Summer	1.37 ± 1.05 [0.00-5.83]	0.071 ± 0.039 [0.01-0.184]
Autumn	0.39 ± 0.36 [0.00-2.13]	0.044 ± 0.034 [0.00-0.205]
Winter	0.66 ± 0.48 [0.02-2.78]	0.031 ± 0.024 [0.00-0.103]

Table 2. Seasonal variation of mortality and fecundity in *Triatoma infestans* under constant laboratory conditions for twenty years. Range in brackets.

Discussion

The mean fecundity of *T. infestans* reported in this study varied from 0.39 eggs/female week (eggs/female/week) in autumn to 1.63 eggs/female/week in spring, with maximum values of 7.59 eggs/female/week in spring and 5.83 eggs/female/week in summer. Other reports of fecundity for this species are 1.9 eggs/female/week at 15-32 °C and 40 to 90% relative humidity (RH) (Canals et al. 1992), 1.0 eggs/female/week at 25 °C and 75% RH (Canals et al. 1991), 5.4 eggs/female/week at 26 °C and 60% RH (Rabinovich 1972), between 5.95eggs/female/week and 13.65 eggs/female/week at 28 °C and 60% RH (Espinoza et al. 2011), fecundities ranging from

1.16 and 4.07 eggs/female/day under controlled conditions (Nuñez & Segura 1987) and 3.68 ± 2.39 and 3.79 ± 2.94 eggs/female/week for individuals fed on humans and hens respectively, at 28 °C and 40% RH (Medone et al. 2015). The results of the last three authors were obtained in laboratory but for shorter periods than that reported in our study, which explains that these values are within the range of our reported maximum values. This species may have high fecundity for short periods (Fig. 1). Mortality rates were similar to previous reports: 0.0083 deaths/individual week at 25 °C and 75% RH (Canals et al. 1991) and 0.0070 deaths/individual week at 15-32 °C and 40-90% RH (Canals et al. 1992).

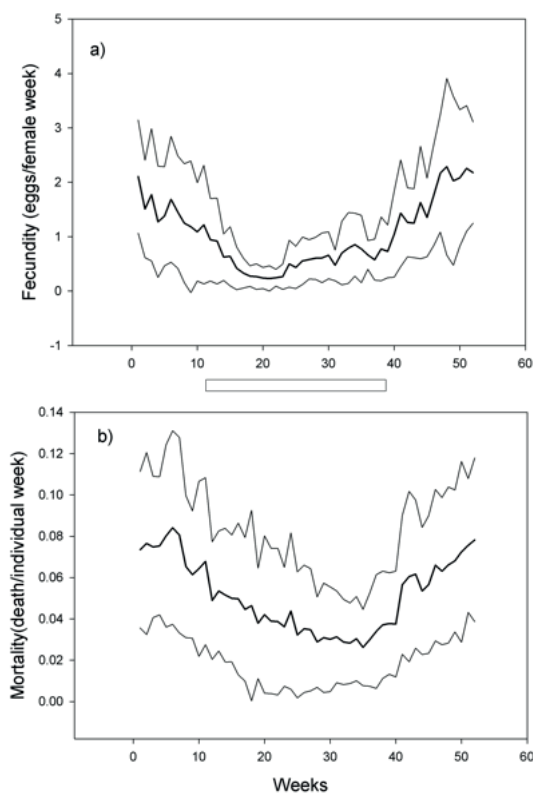


Figura 1. Annual variation of fecundity and mortality rates (b) in *Triatoma infestans* under constant laboratory conditions for twenty years. Thick lines represent the average for each week from the week one to week 52. Bold lines indicate ± 1 standard deviations. The white bar indicates the cold period of the yaers (Autumn and Winter).

Fluctuations in fecundity are an expected result in insect populations in natural conditions because they are strongly influenced by environmental conditions (Carey 2001, Peterson et al. 2009). Particularly it has been reported that *T. infestans* has high sensitivity to temperature, especially below 20 °C.

In constant temperature and humidity conditions there should not be a direct effect of these variables on fecundity. Probably seasonal variations of light trigger or module endogenous population cycles related to mating and oviposition; most insects adapt to seasonal changes by a direct response to photoperiod (Miyasaki et al. 2014, Meireles-Filho & Kiriakou 2013, Numata et al. 2015). For example in the two most epidemiologically relevant species, *Rhodnius prolixus* and *T. infestans*, locomotor activity and other behaviors such as foraging, breeding, oviposition, dispersion and thermopreference are controlled by the circadian clock (Ampleford & Steel 1982, Ampleford & Davey 1989, Lazzari 1992, Reisenman et al. 1998, 2002, Minoli & Lazzari 2003, Bodin et al. 2008, Valentinuzzi et al. 2014). *T. infestans* is a nocturnal species (Canals et al. 1997) with its locomotor activity driven by the circadian clock mainly synchronized by light as the predominant zeitgeber (Lazzari 1992, Lorenzo & Lazzari 1998, 1999). The light dependence *T. infestans* shows in the laboratory may be similar to what *T. infestans* experiments in natural conditions because this species has adapted to living in houses, where they feed on humans and domestic animals (Vazquez-Prokopec et al. 2002). The fact that the studied population was acclimated to the same conditions for a number of generations may make it particularly sensitive to small light changes into the laboratory.

Population cycles may have an endogenous cause generated in the population related to the density of individuals, or an exogenous environmental cause. For the seasonal cycle of mortality rates we cannot propose a population fluctuation modulated by the density of individuals because the density was maintained constant (200 individuals), although there may exist an effect of delayed density dependence as result of the high density of immature stages in the breeding jars, since these were maintained in fluctuating populations that reached 400 individuals per jar. In this case the mechanism would be a greater mortality rate in adults from high densities of offspring due to a nutritional deficit in the immature stages. However it is unlikely that

these induced cycles would have a one year period. Another possibility to explain an endogenous cycle may be a fluctuation induced by a generation time of one year. In the case of *T. infestans* the generation time has been estimated as 7.1 (Rabinovich 1972) and 14.7 months (Canals et al. 1992), so this is a real possibility.

A more reasonable possibility is that the cycle of mortality is environmentally induced. This can occur because the high mortality period coincides with the reproductive period. Mating and oviposition are behaviors with high energetic requirements; the probability of individual death increases, which is known as the cost of reproduction -a trade-off between reproduction and survival-, which has been shown to exist in virtually all insect studies (Bell & Koufopanou 1986, Roitberg 1989, Carey 2001). A final possibility to explain this cycle is a periodic increase in pathogens in spring and summer. Although the temperature was maintained constant inside the climate chamber, it was open, communicating with the laboratory which had variations in temperature and humidity. This may have provided a possible entry of pathogens to the chamber, where microorganisms would find an environment of stable and high temperature and humidity favoring their development. (Rabinovich 1972) reported that the high humidity in the jars is a potential danger for *T. infestans* because of possible colonization by fungi, which are a natural agent of mortality for insects (Varley et al. 1973). This agrees with reports that domestic, peridomestic and sylvatic habitats of triatomines have favorable conditions for entomopathogenic fungi during rainy periods in Latin America (Romaña 1992, Romaña & Fargues 1987, Luz et al. 1994) and that entomopathogenic fungi have been proposed as a cause of mortality in triatomines (Fargues & Luz 2000).

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