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Biological differences between two allopatric populations of *Amblyomma cajennense* (Acari: Ixodidae) in Argentina

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Abstract The fertility of hybrids from two distinct populations of *Amblyomma cajennense* from different ecological regions of Northern Argentina was analyzed. Two colonies of *A. cajennense* from El Rey National Park (RNP), Salta Province (24°41′S, 64°36′W), and Copo National Park (CNP), Santiago del Estero Province (25°55′S, 61°43′W) were established infesting rabbits with adults collected from vegetation. Reproductive parameters of the first generation in laboratory of each colony and their crosses were evaluated considering engorged weight of females, engorged period of females, pre-oviposition period of females, minimum egg incubation period, reproductive efficiency index [REI = number of eggs laid/weight of the females in mg], and fertility efficiency index [FEI = number of hatched larvae/weight of the females in mg]. Infestations were made as follows: Group 1) RNP males and females; Group 2) CNP males and females; Group 3) males from CNP and females from RNP; Group 4) males from RNP and females from CNP. The engorgement weight of the females from CNP that mated with males from RNP was significantly lighter than those of the engorged females obtained in the other 3 crosses, and the engorged period of the females from CNP that mated with males from RNP was significantly longer that the engorged period of the females belonging to the remaining groups. The females from group 3 and 4 had a FEI extremely low in comparison with the FEI obtained from the engorged females originated from the groups 1 and 2. Biological implication of these findings is discussed.

Keywords *Amblyomma cajennense* · Biology · Allopatric populations · Cross-mating · Argentina

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Introduction

Amblyomma cajennense (Acari: Ixodidae) is a hard tick widely distributed in the Neotropical Region, from southern United States to northern Argentina (Estrada-Peña et al. 2004). Among the members of the genus *Amblyomma*, this tick is by far the most relevant species in terms of medical importance in Central and South America. In countries of these continents, *A. cajennense* is an usual parasite of domestic animals and the tick with the largest number of records biting humans (Guglielmone et al. 2003, 2006), and it is the main vector of *Rickettsia rickettsii,* the agent of human spotted fever (Labruna 2009).

In Argentina, this species prevails in areas belonging to the phytogeographical province of Chaco (Chaco domain) North 28°S and to the phytogeographical province of Yungas (Amazonic domain), with localities in the administrative provinces of Catamarca, Chaco, Formosa, Jujuy, Misiones, Salta, Santiago del Estero and Tucumán (Estrada-Peña et al. 2004; Guglielmone and Nava 2006). Evidences obtained from population genetic studies suggest that *A. cajennense* is a species complex, with the different clades associated to particular biogeographical regions (Beati et al. 2007). This study delimited two clades for Argentina; the first clade was associated with the dry part of the Chaco region, while ticks from humid forested areas formed the second clade. The information obtained from molecular data is in agreement with the suggestion of Guglielmone et al. (1992) on the potential presence of cryptic speciation within *A. cajennense*, which was based on the marked differences among the life cycles of laboratory *A. cajennense* tick colonies from distinct geographical origins. In view of this situation, the aim of this work is to test the fertility of hybrids from two distinct populations of *A. cajennense* from different ecological regions of Argentina.

Materials and methods

Two colonies of *A. cajennense* were established from adults collected on vegetation in two sites of Northern Argentina, El Rey National Park (RNP), Salta Province (24°41′S, 64°36′W), and Copo National Park (CNP), Santiago del Estero Province (25°55′S, 61°43′W). RNP consists of environmental settings of Chaco and Cloud forest biomes, where the 1,500 mm of annual rainfall are concentrated in spring and summer (Beldoménico et al. 2003). CNP belong to the Western Chaqueño District of the Chaco Phytogeographic Province according to the definition given by Cabrera (1994), and corresponds to the dry area of the Chaco Phythogeographic province. This district lacks permanent watercourses and the 700 mm of annual rainfall are concentrated in spring and summer.

Infestation with adults ticks 15 days old from the first generation in laboratory were performed on rabbits, using a feeding chamber attached on the dorsum of the hosts. Six naïve rabbits (pathogen free) from the Biotherium of the Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Argentina, were used. All rabbits were females of the same age belonging to the "Californian" breed, and they were maintained under the same conditions before and during the experiment. The infestations were made as follow: Group 1) 15 couples of adults form RNP on one rabbit; Group 2) 15 couples of adults from CNP on one rabbit; Group 3) 30 couples with males from CNP and females from RNP on two rabbits; Group 4) 30 couples with males from RNP and females from CNP on two rabbits. The engorged female were kept at 25°C and 83–86% relative humidity, with a daily photoperiod of 12 h light to 12 h dark, and the following biological parameters were

recorded: weight, engorged period, pre-oviposition period, minimum egg incubation period, reproductive efficiency index [REI = number of eggs laid/weight of the females in mg (Drummond and Whetstone 1970)], and fertility efficiency index [FEI = number of hatched larvae/weight of the females in mg (Aguirre et al. 2005)]. Larvae and unhatched eggs were counted as described by Guglielmone et al. (1989). The statistical significance of the differences among the means of the biological parameters was tested by using an analysis of variance (ANOVA) followed by a *posteriori* Tukey test (Zar 1999).

Results and discussion

Number of engorged females recovered from each group and the means (± standard deviation) of the biological parameters evaluated from the crosses among *A. cajennense* adults from RNP and CNP are showed in Table 1. Regarding pre-oviposition period, minimum egg incubation period and REI, no significant statistical differences were found among the four groups. On the other hand, the engorgement weight of the females from CNP that mated with males from RNP was significantly lighter than those of the engorged females obtained in the other 3 crosses, and the engorged period of the females from CNP that mated with males from RNP was significantly longer that the engorged period of the females belonging to the remaining groups. However, the principal difference among the 4 groups is in the FEI. The females from group 3 and 4 had a FEI extremely low in comparison with the FEI obtained from the engorged females originated from the groups 1 and 2. The values of this index indicate that very few larvae hatched from egg masses laid by the females from groups 3 and 4.

Guglielmone et al. (1992) studied the life cycle of *A. cajennense* under laboratory conditions. The ticks used by these authors belonged to a colony originated from engorged females collected on cattle in Cruz Quemada, Salta Province, Argentina. In this way, it is pertinent to mention that the values of the biological parameters found by Guglielmone et al. (1992) are similar to those obtained with the engorged females of the groups 1 and 2.

Compatibility among sexual taxa declines as a result of two distinct processes (prezygotic and postzygotic isolation) during or after speciation, both resulting in a state

Table 1 Means and standard deviation of the biological parameters of engorged females obtained from the crosses of *Amblyomma cajennense* from two allopatric populations of Argentina

	♂♂ RNP ♀♀ RNP (*n*: 8)	♂♂ CNP ♀♀ CNP (*n*: 8)	♂♂ CNP ♀♀ RNP (*n*: 14)	♂♂ RNP ♀♀ CNP (*n*: 19)
Engorgement weight (mg)*	536.61 ± 126.30[a]	460 ± 106.13[a]	411.85 ± 98.48[a]	262.10 ± 60.69[b]
Engorged period (days)*	10.57 ± 1.55[a]	10.14 ± 1.24[a]	13.78 ± 2.59[a]	19.15 ± 2.99[b]
Pre-oviposition period (days)*	6.28 ± 0.40[a]	5.85 ± 0.49[a]	4.41 ± 0.98[a]	5.31 ± 1.2[a]
Minimum egg incubation period (days)*	33.14 ± 1.51[a]	37.00 ± 1.57[a]	37.00**	37.67 ± 0.44[a]
Reproductive efficiency index*	7.7 ± 1.01[a]	8.32 ± 0.91[a]	6.21 ± 1.36[a]	6.81 ± 1.62[a]
Fertility efficiency index	5.60 ± 1.00	6.48 ± 1.53	0.43 ± 0.79	0.04 ± 0.05

RNP El Rey National Park, *CNP* Copo National Park, *n*: number of engorged females recovered from each group

* ANOVA: Numbers within a row not sharing superscripts are significantly different ($P < 0.01$)

** Larvae hatched from 1 of the 14 egg masses

referred as reproductive isolation (Gourbiére and Mallet 2009). Postzygotic isolation is defined as a reduction in fertility or viability in hybrid offspring, including hatching, growth, survival, metamorphosis and cumulative fitness (Edmands 2002). A mechanism of postzigotic isolation appears to be actuating among Argentinean populations of *A. cajennense*, expressed by the small values of the FEI that were recorded when males and females from different ecological regions were crossed (groups 3 and 4). Consequently, these "hybrids" will have low capacity to originate or maintain viable populations in nature. In sharp coincidence with the results reached by Beati et al. (2007), this study suggests that the dissimilarities in the "ecological preferences" of *A. cajennense* populations in Argentina could be indicating a species boundary, although the data obtained are not enough to confirm this hypothesis.

The species concept has been largely discussed, and different definitions (biological, ecological, evolutionary and phylogenetic, among others) were proposed (Wheeler and Meier 2000; Stockman and Bond 2007). In these concepts, the delimitation of the species is based on a single criterion. A most contemporary definition of species is the "unified species concept" (de Queiroz 2005). According to this concept, various contingent properties as phenetic distinguishability, reciprocal monophyly, pre- and postzygotic isolation, fixed character state differences, are important lines of evidence for separation of metapopulations lineages (de Queiroz 2005). Therefore, it is desirable to carry out an extensive study on the morphological, biological and genetic variation of *A. cajennense* along its distribution range to resolve this issue.

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