



The Open Parasitology Journal

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LETTER

Sexual Choice in Males of the *Triatoma brasiliensis* Complex: A Matter of Maintenance of the Species or Genetic Variability?

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Abstract:

Background:

Members of the *Triatoma brasiliensis* complex can produce experimental and natural hybrids. Crossing experiments performed in the laboratory, with several combinations between species of that complex, revealed a gradient of reproductive affinities among them. However, little is known about the reproductive males' choices when they have the possibility of copulating with females of different species, including interspecific and conspecific females, at the same time. In this unprecedented experiment, the sexual choices of the *T. brasiliensis* complex and *Triatoma infestans* males were observed.

Methods:

Virgin males and females of *T. b. brasiliensis*, *T. sherlocki*, and *T. infestans*, and females of *T. juazeirensis* were used. The experiment was developed in an arena in which one male, one conspecific female, and two non-conspecific females were observed for 15 minutes. The following variables of mating behavior were observed: the male's choice for a female; displacement time (the time it took the male to move from its stall until it reached the female); the copula itself (number of attempts and its occurrence); and the type of rejection of copula by the female.

Results:

Males of *T. sherlocki* were faster in finding the females (conspecific and non-conspecific) than *T. b. brasiliensis* and *T. infestans*. Males of *T. b. brasiliensis* and *T. sherlocki* were able to copulate with conspecific females and other female species: *T. infestans* and *T. b. brasiliensis/T. juazeirensis*, respectively. While *T. infestans* copulated with conspecific females, and *T. juazeirensis* and *T. b. brasiliensis* females.

Conclusion:

The results suggest that the choice for the copula is not always towards conspecific females. In fact, the males of the three different species tested were able to copulate with their conspecific females and also with other female species, which may induce the formation of hybrids and greater genetic diversity. These findings pose new challenges to the understanding of the reproductive behavior and the evolutionary aspects of the Triatominae. Therefore, in areas of sympatry, if no ecological barriers exist, there is the possibility of natural hybridization, which might reflect in the epidemiological risks since the species studied occur in endemic areas for the Chagas disease.

Keywords: Interspecific copula, Chagas disease vectors, Parasitic infections, *Trypanosoma cruzi*, Natural hybrids, Crossing experiments.

Article History

Received: July 1, 2020

Revised: October 20, 2020

Accepted: November 8, 2020

1. INTRODUCTION

Chagas disease is still considered one of the most important parasitic infections in Latin America on account of

its high social and economic impacts. Therefore, multi-disciplinary studies on the triatomines, the vectors of the etiological agent, *Trypanosoma cruzi*, are crucial for understanding, monitoring, and controlling domiciliation processes of the vector species, and also to strengthen the epidemiological surveillance of that pathology [1 - 3].

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In the Triatominae subfamily, the *Triatoma brasiliensis* species complex currently encompasses eight members of distinct epidemiologic importance [4, 5]. The geographic distribution of this species complex is registered from the North of the State of Minas Gerais to the driest areas of Maranhão [4 - 6], and its members can be found associated with different hosts and inhabit several natural and artificial ecotopes [7 - 11]. Among the species of the complex, *T. b. brasiliensis* is considered the main vector, as it shows relevant percentages of domiciliation and natural infection in the different States of Brazil (Alagoas, Ceará, Paraíba, Piauí, Sergipe, Maranhão, and Rio Grande do Norte). The other members are more frequently found in the peridomicile and natural ecotopes [4 - 6, 10,12].

Triatoma brasiliensis complex is a monophyletic group [13, 14] and several studies have been carried out showing distinct degrees of reproductive affinities among the species of this complex. Viable hybrids were obtained from the crosses experimentally performed between different species [15, 16].

Triatomines reach their sexual maturity when they become adults and several morphological and physiological changes occur during ecdysis; however, the males are just able to transfer spermatophore a few days after imaginal molt [17]. The mating behavior of a few species in the Triatominae subfamily has been studied, and it was concluded that it involves a sequence of behavioral steps performed mainly by males. For the females, several types of rejection behavior, in response to male mating attempts, were recorded [18 - 20]. It was demonstrated that age and nutritional status, among other factors, may affect the success of the copula [18 - 23]. A review of sexual behavior, including chemical signals mediating communication between males and females, shows how broad and complex this issue is [22, 24]. The copulation and mate guarding behavior in *T. b. brasiliensis* were studied [25]; however, little is known about the reproductive choices of the males when given the opportunity to copulate with different females species. Our main goal was to describe the male's sexual choices when having the possibility of copulating with multiple female species, including interspecific and conspecific females. To test this, we carried out observations on the males' sexual behavior of two vectors of the *T. brasiliensis* complex, *T. b. brasiliensis*, and *T. sherlocki*, plus *T. infestans* as the external group.

2. MATERIALS AND METHODS

The specimens of the *T. brasiliensis* complex and *T. infestans* came from the triatomine colonies of the Laboratory of Entomological Biodiversity of Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil, and from Dr. Jose Maria Soares Barata Triatominae Collection (CTJMSB) of the São Paulo State University Julio de Mesquita Filho (UNESP), School of Pharmaceutical Sciences (FCFAR), Araraquara, São Paulo, Brazil. Insects were sexed in the 5th instar [26, 27]. Virgin males and females were kept in separate round glass jars (25 cm diameter x 30 cm high), protected against the light by a black card stock applied to the outside of the jars. The insectary room, where the specimens were kept, was artificially lit and acclimatized. The experiments were

carried out under laboratory conditions, during day time (10 AM to 4 PM), with an average temperature of 25.06°C (min. 23.85°C and max. 26.26°C), and relative humidity of 60.74% (min 52%.- max. 69.48%).

The specimens were biweekly fed with live mice, following the protocols for animal welfare defined by FIOCRUZ. The experiment was initiated in the adult phase, counting seven days from the imaginal molt, when the insects were allowed to feed *ad libitum*. All adults were the same age and, after seven days of feeding, specimens were used in the experiment. As a basis stock, 90 virgin males (30 *T. b. brasiliensis*; 30 *T. sherlocki*; 30 *T. infestans*), and 270 virgin females (90 *T. b. brasiliensis*; 60 *T. sherlocki*; 60 *T. juazeirensis*; 60 *T. infestans*) were separated. The specimens were separated into three groups: (1) "TBB" - males of *T. b. brasiliensis* and females (*T. b. brasiliensis*, *T. sherlocki*, *T. juazeirensis* and *T. infestans*); (2) "TS" - *T. sherlocki* males and females (*T. b. brasiliensis*, *T. sherlocki*, *T. juazeirensis* and *T. infestans*); (3) "TI" - males of *T. infestans* and females (*T. b. brasiliensis*, *T. sherlocki*, *T. juazeirensis* and *T. infestans*).

To observe the selective mating behavior of the species used, an acrylic arena with a white square base and four triangular stalls arranged in the vertices of the base (30 X 30 cm) was built. The stalls were connected by halls united in the center of the base. The stalls and halls were black with individual transparent lids, which enabled the observation of the behavior (Fig. 1A). The tests consisted of placing one virgin male of the "A" species with three virgin females of the four species (species "A", "B", "C", and "D") in the arena. The virgin male occupied one of the stalls, the conspecific female, the second stall, and the other two female species were alternated, occupying the remaining stalls. The order of the females in the stalls was randomly changed in each experiment. Once the insects (one male and three females) were placed in each stall and the lid of the male stall was removed, the experiment started being recorded. The male had 15 minutes to get out of its stall, cross the system, and choose one of the females. Once the male moved toward one of the stalls, its lid was opened so that the male could enter and choose to either copulate or not. At the end of the stipulated time, if the copulation was completed, the male was removed, separated, and identified according to the preferred female. After each experiment, the entire system was cleaned with 70% ethanol and left open for 15 minutes so that no chemical residues released by the insects were volatilized and dispersed. The observed insects had no time in advance to explore the arena before being placed in the stalls for the experiment.

The data obtained were recorded according to the observations: (i) Male species; (ii) Displacement time (the time it took the male to move from its stall to that of a female); (iii) Occurrence of copula; (iv) Females' rejection behavior; and (v) Species of a chosen female. This procedure was repeated for all subgroups.

Once the observations of the parameters described were recorded, the male and female were immediately discarded from the remainder of the experiment. The criteria for determining the occurrence of copula were the same as previously described [25]. The experiment was conducted by

means of direct observation from the top of the arena, from where the observer could visually follow and record all movements of the triatomines in detail.

The starting point was considered once the insects were carefully released into the arena. The encounter was considered when the insects touched each other, for example, with their antennae. Percentages and averages were calculated, considering the previously described variables.

All specimens used in the experiment were deposited in the Entomological Collection of Instituto Oswaldo Cruz [28 - 31].

2.1. Statistical Analyses

The male preference for different female species and the types of female rejection behaviors (*i.e.* Flattening, evasion, no rejection) were compared by *Chi*-square. The effect of female species, male species, and the group of female species available for males on time elapsed until copulation was analyzed by ANOVA. When there was a significant difference between treatments, the means were compared by Tukey's honestly significant difference test ($P < 0.05$). Residual plots were checked to confirm no deviation from normality. All statistical analyses were performed in R version 3.3.1 (R Core Team 2015).

3. RESULTS

Sixty-five experiments were carried out, with 16 males of TBB, 30 of TS, and 19 of TI, with conspecific females, plus TJ (Table 1). From the 65 experiments, 16 copulae were recorded: two for TBB, seven for TS, and seven for TI males (Table 1). The results showed that the males of the three species tested (TBB, TS, TI) moved towards not just to encounter their conspecific females but also the ones of distinct species. Males of *T. b. brasiliensis* and *T. sherlocki* were able to copulate with conspecific females and other female species: *T. infestans* and *T. b. brasiliensis*/*T. juazeirensis*, respectively. While *T. infestans* copulated with conspecific females, and *T. juazeirensis* and *T. b. brasiliensis* females (Table 1).

The analysis revealed the time elapsed to encounter females in the arena varied with male species ($F_{2,52}=9.28$, $p < 0.001$). The female species, or the group of female species available in arena, however, did not influence the time expended until the encounters ($F_{3,52}=91.55$, $p=0.212$ and $F_{4,52}=0.25$, $p=0.90$). The shortest average time for a male to find a female was for TS (2.5 min.) while the longest was recorded for TBB (6.71 min). Comparing all experiments together, the shortest time for a male to encounter a female was observed for TS to find a TS female (1.0 min) while the longest time registered was for TBB to find a TI female (11.5 min) (Table 2).

The TBB ($X^2=23.22$, $df=3$, $p < 0.001$ and $X^2=22.16$, $df=3$, $p < 0.001$), TS ($X^2=45.714$, $df=2$, $p < 0.001$; $X^2=14$, $df=2$,

$p < 0.001$; and $X^2=26$, $df=2$, $p < 0.001$) and TI males' choices ($X^2=38$, $df=2$, $p < 0.001$ and $X^2=22$, $df=2$, $p < 0.001$) varied into the females groups (Table 3). Concerning the male's choices to encounter a female, TBB males were more prone to encounter the conspecific ones in both groups of females (43 and 44%), while TS was more prone to find the TBB females (50%) than the conspecific ones in two combinations of the females' groups (10 and 20%). Just in the third females' group combination, in the absence of the TBB females, TS males were more prone to encounter the conspecific ones (50%). TI males showed distinct choices according to the group of females. In the first combination of females, TI males were more prone to encounter the conspecific ones (60%), while in the second combination, in 55% of the times, they were more prone to move toward TBB females (Table 3).

Different females' rejection behaviors were observed when males attempted to copulate, such as flattening, when the female stretches the legs and touches the abdomen on the substrate. This prevents the positioning of the male on the female (Fig. 1b). The evasion/escape happens when, at the male's first sign of attempting to copulate, the female moves away and remains distant. Also, in some situations, there were no male attempts to copulate and no specific behavior of the female. According to the analysis, the types of female rejection behavior on TBB males were different among species in the first and second groups of females ($X^2=48.005$, $df=2$, $p < 0.001$) and ($X^2=210.27$, $df=4$, $p < 0.001$). When TBB males tried to copulate, the females of the three species showed more frequently the flattering behavior than the evasion in the first group of females; TJ females just showed flattering in all attempts. In the second group of females, TS females did not show either flattering or evasion and TI females were more prone to flattering (Table 4). The observations of the TS males' attempts to copulate in the first group of females ($X^2=172.53$, $df=4$, $p < 0.001$), just TJ showed flattering and evasion behavior (17 and 50%, respectively), while TBB and TS showed no specific behavior. In the second group of females ($X^2=152.56$, $df=4$, $p < 0.001$), TI showed, in all males' attempts, flattering and, the other two species, TBB and TS showed flattering and evasion, however, TBB also showed no specific behavior 20% of the times. In the third female group ($X^2=99.046$, $df=2$, $p < 0.001$), just TJ showed flattering in response to the males' TS attempts (Table 5). Observing the TI males' attempts to copulate, it was evidenced that the female species also showed differences in rejection type behaviors in the first and second groups ($X^2=400$, $df=4$, $p < 0.001$ and $X^2=64$, $df=2$, $p < 0.001$, respectively). For the TI males' attempts, TS and TJ females showed no specific behavior, in the first and second group of females, respectively. The TBB females showed flattering and evasion behavior in the first group, and just evasion in the second. While TI females showed flattering behavior 100% of the times in the first group and 50% evasion in the second group (Table 6).

Table 1. Number of males of *Triatoma brasiliensis brasiliensis* (TBB), *T. sherlocki* (TS), and *T. infestans* (TI), that copulated with females of different species and conspecific ones.

Males	N	Females	N
TBB	2	<i>T. b. brasiliensis</i>	1
		<i>T. infestans</i>	1
TS	7	<i>T. b. brasiliensis</i>	3
		<i>T. juazeirensis</i>	2
		<i>T. sherlocki</i>	2
TI	7	<i>T. b. brasiliensis</i>	4
		<i>T. infestans</i>	1
		<i>T. juazeirensis</i>	2
Total	16	-	16

Table 2. Mean (\pm SD) of Time elapsed (in minutes) for the *Triatoma brasiliensis brasiliensis* (TBB), *T. sherlocki* (TS) and *T. infestans* (TI) males to encounter the TBB, TS, *T. juazeirensis* (TJ) and TI females, in the arena. X represents the mean of total time elapsed (in minutes) inside of the different female groups studied.

Males	Females			-
TBB a	TBB	TS	TJ	X
	8.0 (\pm 5.23)	6.5 (\pm 0.71)	2.0 (\pm 0)*	6.71 (\pm 4.31)
	TBB	TS	TI	X
	3.33 (\pm 1.15)	4.0 (\pm 0)*	11.5 (\pm 4.95)	6.17 (\pm 4.75)
TS b	TBB	TS	TJ	X
	2.6 (\pm 1.51)	1 (\pm 0)*	2.75 (\pm 1.7)	2.5 (\pm 1.5)
	TBB	TS	TI	X
	2.4 (\pm 1.94)	3.5 (\pm 0.7)	3.0 (\pm 2.64)	2.8 (\pm 1.87)
	TS	TJ	TI	X
	2.83 (\pm 2.6)	-	3.33 (\pm 0.57)	2.8 (\pm 2.04)
TI ab	TBB	TS	TI	X
	2.66 (\pm 1.5)	3.0 (\pm 0)*	5.66 (\pm 3.3)	4.5 (\pm 2.9)
	TBB	TJ	TI	X
	4.4 (\pm 1.67)	4.5 (\pm 0.7)	3.5 (\pm 0.7)	4.22 (\pm 1.3)

Male species followed by different small letters are significantly different at $P < 0.05$ (Tukey)

The symbol * represents data from only one male sample

Table 3. *Triatoma brasiliensis brasiliensis* (TBB), *T. sherlocki* (TS) and *T. infestans* (TI) males' choices (%) for the TBB, TS, *T. juazeirensis* (TJ) and TI females, in the arena. * NO occurrence.

Males	Females			
TBB	TBB	TS	TJ	NO*
	44%	22%	11%	22%
	TBB	TS	TI	-
	43%	14%	28%	15%
TS	TBB	TS	TJ	-
	50%	10%	40%	-
	TBB	TS	TI	-
	50%	20%	30%	-
	TS	TJ	TI	-
	50%	10%	40%	-
TI	TBB	TS	TI	-
	30%	10%	60%	-
	TBB	TJ	TI	-
	55%	22%	22%	-

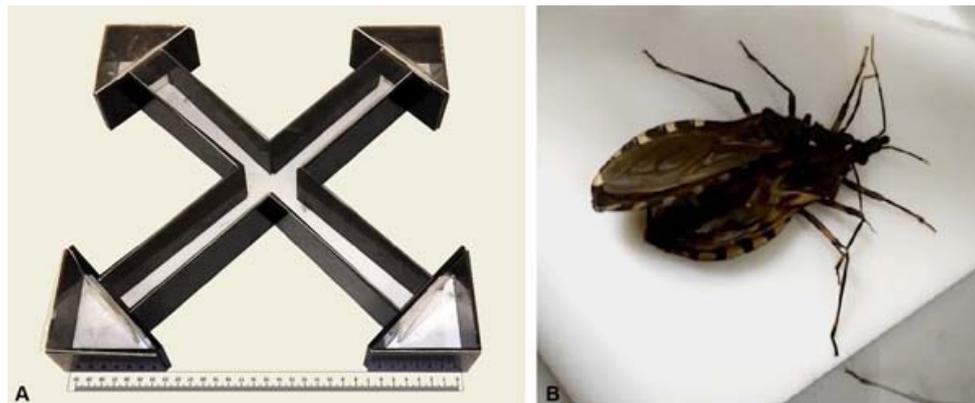


Fig. (1). A- Selective mating arena. ; B- Male of *Triatoma infestans* attempting to copulate with a female of *Triatoma brasiliensis brasiliensis* showing rejection behavior by flattening. Photos: Cauan Antunes.

Table 4. *Triatoma brasiliensis brasiliensis* (TBB), *T. sherlocki* (TS), *T. juazeirensis* (TJ) and *T. infestans* (TI) females' rejection behaviors (%) to the TBB males' copula attempts. F- fluttering; E- evasion; N- none

-	Females		
Rejection	TBB	TS	TJ
F	73%	60%	100%
E	27%	40%	0%
N	-	-	-
Rejection	TBB	TS	TI
F	25%	-	67%
E	30%	-	33%
N	45%	100%	-

Table 5. *Triatoma brasiliensis brasiliensis* (TBB), *T. sherlocki* (TS), *T. juazeirensis* (TJ) and *T. infestans* (TI) females' rejection behavior (%) to the TS males' copula attempts. F- fluttering; E- evasion; N- none

-	Females		
Rejection	TBB	TS	TJ
F	-	-	17%
E	-	-	50%
N	100%	100%	33%
Rejection	TBB	TS	TI
F	40%	17%	100%
E	40%	67%	-
N	20%	16%	-
Rejection	TS	TJ	TI
F	33%	100%	60%
E	67%	-	40%
N	-	-	-

Table 6. *Triatoma brasiliensis brasiliensis* (TBB), *Triatoma sherlocki* (TS), *Triatoma juazeirensis* (TJ) and *Triatoma infestans* (TI) females' rejection behavior (%) to the TI males' copula attempts. F- fluttering; E- evasion; N- none

-	Females		
Rejection	TBB	TS	TI
F	50%	-	100%
E	50%	-	-

(Table 8) cont.....

-	Females		
N	-	100%	-
Rejection	TBB	TJ	TI
F	-	-	-
E	30%	-	50%
N	70%	100%	50%

4. DISCUSSION

Regarding the Triatominae, several studies revealed the complexity of the mating behavior of different species [17 - 23] and explored diverse aspects in this matter [24]. However, experiments were still to be designed to check the males' sexual choices when having the possibility of copulating with multiple female species, including the conspecific ones. In order to test the hypothesis that proposes that males choose to copulate with their conspecific females, an unprecedented experiment was carried out to check the males' choices for copulating with females of different taxa of the *T. brasiliensis* complex, plus *T. infestans* as the external group. Our results suggest that males' choices might not always be conducive to copulating with their conspecific females, indicating the copulation between different species could be a possibility that propitiates genetic diversity rather than the maintenance of the species.

Since previous studies had already demonstrated the viability of experimental hybrids between *T. b. brasiliensis* and *T. juazeirensis*; *T. b. brasiliensis* and *T. sherlocki*; and *T. juazeirensis* and *T. sherlocki* [15, 16] in the methodology used in this study, the observation process was interrupted immediately after copula (as soon as the males and females were separated).

The mating behavioral pattern of *T. brasiliensis* males and females was extensively described and a high level of receptivity of the females for copulating was observed [25]. In our study, the females were more likely to avoid copulating, despite the males' attempts. It is important to mention that the experimental conditions of this study were very different from the ones mentioned in that paper [25]. In the present study, the experiments were carried out during day time (10 AM to 4 PM), while in the other one, they were carried out starting at 6 PM. It is well known that the peak of the activity of the triatomine group starts at sunset [32]. We suppose that the distinct time periods for carrying out the experiments might have affected the females' behavior. Moreover, the format of the arena was very different when compared to the previous study. The shape of the arena might have influenced the behavior of the specimens observed. Despite the low receptivity of the females, it was possible to record 16 copulae: 12 with non-conspecific, and four with conspecific females.

Biological experiments conducted under laboratory conditions, stress may influence biology, behavior, and physiology, among other aspects. The results obtained are unprecedented concerning the triatomine group. However, because of the artificial conditions on which the experiments were carried out, the conclusions should be interpreted parsimoniously. Interestingly, our observations (of males choosing non-conspecific females) are corroborated by recent

studies revealing a natural hybrid zone in which distinct species of the *T. brasiliensis* complex might be reproducing and creating phenotypic variability [33, 34]. It would be important to continue with this method so as to check new possibilities of sexual choices among other species, and the influence of environmental and experimental conditions on the triatomines' sexual behavior.

The results obtained suggest that the males' choice may change according to the group of females available. This can be observed with *T. sherlocki* and *T. infestans*. We point out that the male of *T. b. brasiliensis* was the most conducive for copulating with the conspecific female, while *T. sherlocki* showed a stronger trend for contacting the females of *T. b. brasiliensis*, and there were considerably fewer occurrences of copula attempts with conspecific females (10% and 20%). However, in a third combination - in the absence of *T. b. brasiliensis* -, *T. sherlocki* presented a higher percentage of selective copula attempts with the conspecific female. As already mentioned, *T. b. brasiliensis* presented the highest percentages of selective copula attempts with conspecific females (44% and 43%), depending on the combination of the group of females, while *T. infestans* showed two very different percentages, depending on the group of females (60% and 22%).

Therefore, it seems that according to the mating conditions, except for *T. b. brasiliensis*, the males may choose to copulate with females favoring the appearance of hybrids. This fact would provide a greater genetic variability to the populations, which, in evolutionary terms, can be considered beneficial [34].

Another point to be raised is that, according to the analysis of the mitochondrial DNA sequences of the *Cyt b* gene, *T. b. brasiliensis* is the species that presents the highest genetic variability, while *T. sherlocki* and *T. melanica* are the most restricted ones, not only in terms of genetics, but also in terms of ecological niche [13, 35,36]. The results suggest that, somehow, *T. sherlocki* shows mating behavior that is more conducive to increasing the genetic variability, while *T. b. brasiliensis*, which notably shows a higher genetic variability than the other members of the complex, presents more conducive behavior to copulating with the conspecific female.

Studies on experimental crossings in the *T. brasiliensis* complex and among other taxa have already been conducted to verify the reproductive compatibilities and fertility amongst different species. However, in the analyses performed, neither the male nor the female had a choice [15, 16, 29 - 31]. In this study, a sexual behavior experiment was conducted, for the first time with the triatomine to test the hypothesis that, in a group of females belonging to different species, including conspecific females, the males would choose the conspecific

female for copulation. This would be the expected result. However, the results obtained in this experiment showed that the males do not always choose the conspecific female for copulation, and it seems that the males' choices may vary according to the group of females available for the copula. The analyses of the results suggest that, depending on the species, the selective mating behavior is not directed toward the maintenance of the species, but rather to the creation of genetic variability through the generation of hybrids, which are the raw material of evolution, due to a greater combination of genes provided by the hybridization [37].

Crossing experiments have brought a new perspective to the study of the *T. brasiliensis* complex, both to verify the evolutionary relationships between species and the possibility of the existence of natural hybrids in geographic areas, where related species may occur [33, 34,37,38]. More recently, the susceptibility and elimination capacity of metacyclic forms between the parentals and hybrids obtained from crossings between species of the *T. brasiliensis* complex have been studied. It was found that the hybrids would have greater possibilities of transmitting the *T. cruzi* parasite than their parentals, which could increase the risk of the Chagas disease transmission, posing new challenges to the vectorial surveillance in endemic areas [39].

CONCLUSION

Until the last century, the hybridization between animal species was considered relatively rare and of minor importance for the evolutionary process [40]. Similarly, the speciation related to the gene flow was also considered to be of low relevance [41]. However, these points of view changed in recent decades with broader use of molecular tools to monitor the gene flow among species [37, 38, 41 - 43]. It is important to highlight that hybrids are sometimes fitter than their pure species counterparts [44], as it has been shown for hybrids between *T. juazeirensis* and *T. sherlocki* [45]. Also, the comparative analysis of several biological parameters among some triatomine subspecies of *Meccus phyllosomus* (Burmeister) demonstrated that the hybrid cohorts had better fitness results than the parental cohorts involved in each set of crosses [46].

This study suggests that male's sexual choices, when having the possibility of copulating with multiple female species, including interspecific and conspecific females, can promote an increase in genetic variability, not just the genetic stability of the species, offering new avenues to be explored in terms of vectorial capacity and /or competence and ecoepidemiology of Chagas disease.

LIST OF ABBREVIATIONS

TBB	=	<i>Triatoma Brasiliensis Brasiliensis</i>
TI	=	<i>Triatoma Infestans</i>
TJ	=	<i>Triatoma Juazeirensis</i>
TS	=	<i>Triatoma Sherlocki</i>

AUTHORS' CONTRIBUTIONS

JC and CA designed the experiment, while JO, JAR, and JC provided the insects. CA, CML, and TCMG carried out the

experiments and reared the colonies. CA, GAG, and LPD worked on the obtained data and tables. CA, CEA, TCMG, and JC wrote the first version of the manuscript. All authors collaborated on the text, the discussion, and the revision, and approved the submission of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The specimens were collected in the field with the help of the technicians of the Secretaria Nacional de Saúde and were kept in the laboratory according to the ethical issues approved by the committee of ethical procedures of Instituto Oswaldo Cruz/ Fiocruz Brazil (L-008/2015).

HUMAN AND ANIMAL RIGHTS

No humans were used for the study. All animal experiments were conducted in accordance with the Guidelines for the Treatment of Experimental Animals according to the ethical issues approved by the National Council for Animal Experiment Control (CONCEA no 01200.002529/2014-88 e CIAEP no 01.0234.2014).

CONSENT FOR PUBLICATIONS

Not applicable.

AVAILABILITY OF DATA MATERIALS

The data sets analyzed during the current study are available from the corresponding author upon request.

FUNDING

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for JC and Fundação Oswaldo Cruz for CA. Fundação de Amparo à Pesquisa do Estado de São Paulo (16/08176-9) for CEA. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We would like to thank Dr. Carolina Reigada for the statistical analysis and the valuable comments and suggestions.

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