

Original paper

Sexual behavior of *Ctenomys pearsoni* (Rodentia: Ctenomyidae) in “Carrasco” population (Montevideo - Uruguay)

Comportamiento sexual de *Ctenomys pearsoni* (Rodentia: Ctenomyidae) en la población “Carrasco” (Montevideo - Uruguay)

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ABSTRACT. *Ctenomys* is a genus of South American subterranean rodents that is distributed from Peru to southern Argentina and Uruguay. The present work studies the reproductive behavior (courtship and copula) of *Ctenomys pearsoni* (Carrasco population, Uruguay). To carry out the characterization, we used an ethogram with 19 behavioral units grouped into different categories. By studying 10 couples we quantified the frequency of occurrence and the time invested in each behavioral unit during the development of the interaction. Through the analysis of the results, the roles of each partner and the importance of each one's role in the development of reproductive behavior are inferred. The results obtained are compared with the data available for other populations of the species and other species of the genus. We discussed the relevance of classifying the reproductive pattern on the basis of the Dewsbury classification. Finally, the importance of each sex in the development and molding of reproductive behavior is discussed.

Key words: courtship; copulatory pattern; sexual selection; Tucu tucu



RESUMEN. *Ctenomys* es un género de roedores subterráneos sudamericanos que se distribuye desde Perú hasta el sur de Argentina y Uruguay. El presente trabajo estudia el comportamiento reproductivo (cortejo y cópula) de *Ctenomys pearsoni* (población Carrasco, Uruguay). Para realizar la caracterización se utilizó un etograma con 19 unidades conductuales agrupadas en diferentes categorías. Mediante el estudio de 10 parejas cuantificamos la frecuencia de ocurrencia y el tiempo invertido en cada unidad de comportamiento durante el desarrollo de la interacción. A través del análisis de los resultados, se infieren los roles de cada miembro de la pareja y la importancia del rol de cada uno en el desarrollo de la conducta reproductiva. Los resultados obtenidos se comparan con los datos disponibles para otras poblaciones de la especie y otras especies del género. Discutimos la relevancia de clasificar el patrón reproductivo sobre la base de la clasificación de Dewsbury. Finalmente, se discute la importancia de cada sexo en el desarrollo y moldeado del comportamiento reproductivo.

Palabras clave: cortejo; patrón copulatorio; selección sexual; Tucu tucu

INTRODUCTION

Reproduction is fundamental to the biology of all organisms. Reproduction is how individuals perpetuate themselves, and it is through differences in reproductive success, that natural selection acts to shape phenotypes and provides us an approximation of individual fitness (Bennett *et al.*, 2000). From an evolutionary perspective, survival is valuable only as it contributes to reproduction (Barnard, 2004). Achieving such reproductive success requires the correct integration of physiological, behavioral, social, and environmental stimuli (Tassinio & Passos, 2010). Reproduction creates a conflict between the members of the couples involved generated by the differences in investment made by each sex from gametes to parental care (Chapman, 2006) because both genders try to maximize their fitness (Krebs & Davies, 1993). It seems likely that reproduction has been more subject to evolutionary pressures than any other physiological system (Weir, 1974). Mating behavior is composed of courtship and copula. Courtship implies the behavior of formation and bonding of pairs (Barrows, 2000); it is modeled through sexual selection, allows coespecific recognition, and provides a mechanism for reproductive isolation (Krebs & Davies, 1993). It is also a form of communication whose primary roles are synchronization, partner spatial orientation, stimulation, and inhibition of aggressive behavior (Dewsbury, 1988; Krebs & Davies, 1993).

Courtship and copulation are highly stereotyped and low-change behaviors within species (Altuna *et al.*, 1991). Instead, it is expected to exhibit high interspecific variability, recognizing increased selection pressure for successful breeding. Courtship shows the components associated with intraspecific competition between the sexes, usually expressed between males regarding access to females and the evaluation that females perform on potential mates (Krebs & Davis, 1993). Recognition and description of behaviors are expressed in behavioral units of an ethogram. Ethograms are species-specific behavioral catalogs that describe an animal's action and also provide standardized labels for behaviors that can be used by different researchers, thus increasing the consistency and repeatability of behavioral studies (Lacey *et al.*, 1991). Dewsbury (1972) proposed a classification system for copulatory behavior based on the presence/absence of four basic parameters for male-female interaction and successful reproduction. Parameters are male

and female and become firmly locked by a robust mechanical connection; pelvic thrusting during insertion; multiple insertions with no sperm transfer and multiple ejaculations might be attained. Copulatory patterns involving prolonged stimulation of females and multiple ejaculations are widespread in rodents (Stockley & Preston, 2004). Prolonged copulatory stimulation by males often takes the form of multiple intrusions, during which no sperm is transferred (Dewsbury, 1972). Stimulation is critical for those species that show induced ovulation, such as the norm in solitary subterranean rodents (Bennett *et al.*, 2000), for which vaginal stimulation is necessary to initiate neuroendocrine activity for ovulation to occur (Fanjul & Zenuto, 2008a). In males, stimulation is related to the amount of sperm ejaculated, facilitates its transport (Toner & Adler, 1986), and participates in the generation and elimination of copulatory plugs (Hartung & Dewsbury, 1978; Stockley & Preston, 2004).

Furthermore, the copulatory series that follows ovulation is essential for the formation of the corpus luteal and the development of pregnancy (Sawrey & Dewsbury, 1985). Different aspects of reproductive behavior have been described in rodents' species, geomids (Schramm, 1961; Andersen, 1978), hystricognaths, including species from different habitats, social organization, and nature of ovulation (Kleiman, 1974). There are also descriptions of copulation and courtship in *Spalax ehrenberghi* Nehring, 1898 (Nevo, 1969) and *Cryptomys* Gray, 1864 (Hickman, 1982).

Tuco-tucos are hystricognath subterranean rodents of genus *Ctenomys* Blainville, 1826; they have Neotropical distribution between 15 ° y 54 ° south latitude (Pearson, 1959). It is a highly polytypic genus in which 70 species are included (Bidau, 2015).

However, new species are often described (Teta & D'elia, 2020); it is considered the genus of mammals with the most prominent species number (Lacey *et al.*, 2000). *Ctenomys pearsoni* Lessa & Langguth, 1983 is a subterranean rodent from southern Uruguay; it lives in the coastal sandy dunes of the Atlantic Ocean and Río de la Plata. Individuals are solitary and territorial and indicate their presence through characteristic acoustic signals (Francescoli, 1999). They present sexual size dimorphism where males are larger than females (Buschiazzo & Izquierdo, 2008). It is an annual monostrian species; the reproductive period occurs in the winter months (June - August), and births occur in late spring, early summer (November, December). Mating occurs inside caves (Altuna *et al.*, 1999). Corneal structures in the penis (Altuna & Lessa, 1985) suggest that females have induced ovulation. Many populations in the south of Uruguay diverge considerably in their karyotypes (Novello & Lessa, 1986; Novello & Altuna, 2002; Villar *et al.*, 2005). They were the species of the genus that had the most remarkable karyotypic diversity ($2n = 56$ to $2n = 70$) (Tomasco & Lessa, 2007). Likewise, differences in behavioral aspects have been observed between different populations of the species (Altuna *et al.*, 1993). The Carrasco population has a population density of 31.7 animals/ha and has a sex ratio of 1: 1.25 (male: female). Observing the natural behavior of subterranean animals in their natural habitat is extremely difficult; behavioral observations of these species are mainly carried out in captivity. This difficulty of seeing organisms creates a challenge between standardizing assays, providing appropriate conditions for animals, and observing and recording behavior. For this reason, the data available on sexual behavior is limited. Sexual behavior has been studied in different species of genus *Ctenomys* in *C. mendocinus* Philippi, 1869 (Camin, 1999), *C. talarum* Thomas, 1898 (Zenuto *et al.*, 2002; Fanjul & Zenuto,

2008b), *C. rionegrensis* Langguth & Abella, 1970 (Tassino & Passos, 2010) and in Solis population of *C. pearsoni* (Altuna *et al.*, 1991). This work aims to determine courtship and copulation behavior in the Carrasco population of *C. pearsoni* through the description of behavioral units and their sequence. We analyze the role of each sex during courtship through quantitative evaluation of different behavioral units and evaluate the different components of the copulatory pattern based on the Dewsbury (1972) classification. According to the theory of Sexual Selection (Darwin, 1871) in animals with differential investment with a polygynous mating system is expected that during courtship, female performed an evaluation of male and there is greater investment in time of stimulatory behaviors than other behaviors. At the same time, it is expected that courtship in subterranean, solitary species as *C. pearsoni* involves different communicative channels, present long duration and exhibit aggressive and appeasement behaviors mainly at the beginning of the interaction.

MATERIALS AND METHODS

Adult *C. pearsoni* specimens were collected in the field (Carrasco, Uruguay, 34° 53' S, 56° 02' W) using Oneida Victor # 0 live capture traps padded with polyurethane foam and adhesive tape placed inside the tubes entrances of the subterranean galleries showing freshly excavated soil. Animals were captured during April-May 2008, before the start of the reproductive season, to avoid the influence of previous reproductive activity in our experiments. All animals were transported to our laboratory. Females and males were kept in different rooms to avoid individual influences between the sexes.

Animals were housed individually in a glass terrarium (60 x 21 x 34 cm) with dry grass as a bed. Fed *ad libitum* with 200g/day of fresh grass (*Cynodon* sp. Richard in Persson, 1805 and *Panicum* sp. Linnaeus, 1758), 50 g /day of Corn (*Zea mays* Linnaeus, 1758), 10 g/day of sunflower seeds (*Helianthus* sp. Linnaeus, 1758), and 50 g/day of carrots (*Daucus carota* Linnaeus, 1758) supplemented with, 3 dandelion leaves/week (*Taraxacum* sp. Weber, 1780). The animals' room was kept at a temperature of 22 ± 1 °C and relative humidity 80-100%, according to the natural conditions in their burrows (Altuna, 1991). The photoperiod was kept natural by using artificial light adjusted regularly according to nature's day/night duration pattern. Before mating tests, females were familiarized with coespecific male odors to promote mating behavior, as Zenuto *et al.* (2007) demonstrated. The selected males had to be 10% larger in mass than the female, according to the sexual size dimorphism observed in the population (Buschiazzo & Izquierdo, 2008). The female was placed in an acrylic device, constructed using two boxes (25 x 25 x 25 cm) connected to a tube (60 x 10 x 10 cm). In one box, the female was placed, and in the other, we placed the male's bed, which was obtained from the terrarium where the male was kept. The female was allowed to meet the male's bed. Acclimatization was carried out for 48 hours, and the male's bed was renewed after 24 hours (Fig. 1).

We conducted 36 male-female interactions using 15 males and 20 females in a neutral glass terrarium measuring 80 x 40 x 40 cm with a dry substrate. Tests were carried out in the animal house under the same environmental conditions in which the acclimatization was carried out. The tests were carried out in a larger neutral glass terrarium (80 x 40 x 40 cm) specially conditioned for

the animals with a wood chip substrate. Both animals were placed in the terrarium separated by a metal grid for 2 minutes of acclimatization and recognition at the beginning of each test (Fig. 2). After this, we allowed free interaction; if copulation started, the observations continued until the standard satiety criterion was reached (30 min without copulation) (Dewsbury, 1975). All tests were performed mid-afternoon and recorded on videotape in white light. Interaction tests that resulted in the absence of reproductive behavior (26/36 cases) were discarded for our analysis. Copulation was observed in 10 pairs involving different males and females. We used the following measures of copulatory pattern: mount latency (ML; time to first mount resulting in copulation) (Fanjul & Zenuto, 2008a); intromission latency (IL; time to first intromission); copulatory series (CS; number and duration of periods that start with penis intromission and ends with the withdrawal that precedes the dismount); intromission with rapid thrusting (IRT; after intromission, the male performs rapid pelvic thrust involving a shallow penis insertion within the vagina) (Dewsbury, 1972; Altuna *et al.*, 1991); intromission with deep thrusting (IDT; after the attaining of intromission, the male performs pelvic thrust with deep insertion within the vagina) (Dewsbury, 1972; Altuna *et al.*, 1991), which are homologous to ejaculatory intromissions (Bignami & Beach, 1968); total number, total duration and frequency (number/total duration) of IRT and IDT during each copulatory series were recorded; cycles IRT-IDT: number of events where IRT and IDT occurred during each copulatory series; inter-intromission interval (III): time period lasting between copulatory series.



Figure 1. Device in which the female was placed to familiarize with male's odor, prior the trials.



Figure 2. Pair of *Ctenomys pearsoni* Carrasco population in the acclimatization period prior to carrying out the sexual behavior tests.

We characterize and analyze courtships using an ethogram with 19 behavioral units. Such units were grouped into different categories considering whether they involve both individuals (interactive) or only one member of the couple (individual). In turn, those involving the two members of the couple were grouped according to the function given to the unit into stimulatory –if the unit reflects the intention to generate sexual stimulation in the other individual; agonistic - if the unit reflects aggressive or rejecting behavior towards the other individual; or binding -if the animal shows intention to bond with the other individual without presenting stimulation or aggressiveness (Table 1).

Statistical analyzes used Kruskal-Wallis (non-parametric ANOVA test) to compare subcategories and the Mann-Whitney test for male and female units. We analyzed the difference among first, middle, and final courtship periods using Friedman (ANOVA non-parametric test for repeated measures). The association between units was analyzed by modifying the Dice association index that takes values between 0 and 2 units (Costa & González, 1986). Statistical associations between units were analyzed using the Chi-square test. We compared the frequency of IRT pairs with one CS and multiple CS using the Mann-Whitney test. In all cases, the critical significance level was established at $P < 0.05$. The results are expressed as means \pm SD.

Table 1. Ethogram of courtship behavior in *Ctenomys pearsoni* population Carrasco. The names of the behavioral units and the description of each of them are observed.

Unit	Description	Category	Function
Effector Allogrooming (EA)	Ritualized grooming with forelimbs, nose and/or incise performed by one partner to the other.	Interactive	Stimulatory
Mount Attempt (MAT)	The male tries copulatory position with or without pelvic movements without achieving intromission. During this unit, the female is considered as allogrooming receptor (AR).	Interactive	Stimulatory
Nose-genital Orientation (NGO)	An individual directs the snout to the anogenital area of another.	Interactive	Stimulatory
Receptor Allogrooming (RA)	Ritualized grooming passively received by one partner from the other.	Interactive	Stimulatory
Allogrooming receptor with displacement (AR-D)	One individual is receiving allogrooming (RA) and simultaneously move in the terrarium.	Interactive	Stimulatory
Mutual Allogrooming (MA)	Both individuals active and simultaneously engaged in ritualized grooming to the other.	Interactive	Stimulatory
Round (R)	Circular motion in which both individuals move in opposite positions with the snout of one individual in contact with the rump or tail of another.	Interactive	Stimulatory
Onslaught (O)	One individual moves quickly and aggressively towards the other.	Interactive	Agonistic
Incisive Display (ID)	One individual shows the teeth to the other, opening mouth and tilting the head upward. The individual may or may not touch the other with their teeth and Vibrissae, and to move in that posture.	Interactive	Agonistic
Frontal Orientation (FO)	Both individuals confront their mouths half showing incisors with his head in an oblique position posture.	Interactive	Agonistic
Submission (S)	The animal turns its body laterally and exposes the neck towards the other individual.	Interactive	Agonistic
Contact (C)	Both individuals are touching each other while in motion.	Interactive	Binding
Contact Stillness (CS)	Both individuals remain still while establishing physical contact	Interactive	Binding

Unit	Description	Category	Function
Genital grooming (GG)	Grooming in genital area, using lips, tongue, and forelimbs.	Individual	Binding
Orientation (O)	Any movement of one animal towards the other.	Individual	Binding
Non-genital grooming (NGG)	Grooming no genital areas, using incisives, tongue, fore and hindlimbs.	Individual	
Snouting (Sn)	Anteroposterior rapid movements of the head by rubbing its snout against the substrate.	Individual	
No oriented locomotion (NOL)	Composed unit which join all the animal movements led to the spatial recognition or simply walking in the terraria.	Individual	
Stillness (St)	The animal is still with the body bent in the form of a ball.	Individual	

RESULTS

Animals have been active since the beginning. Courtship was characterized by presenting a Pre-copulatory Period (PCP), which includes ML and IL (mean time 439.90 sec. \pm 341.63), followed by a variable number of Copulatory Series (CS) (mean 3 \pm 1.76, range 1-6; mean time 55.23 sec. \pm 62.20) alternated by Inter-copulatory Periods (IP) (mean time 221.15 sec. \pm 159.24) (Table 2).

Sexual behavior lasted 17 min. 30 sec. \pm 8 min. 20 sec. The courtship began in general (8/10 cases) with agonistic approaches (FO, ID) only in PCP. This was followed by Binding units (O, C, GG) alternated with stimulatory behaviors (EA, MAt, RA, MA, RA-D, NGO, R). Both sexes showed exclusive behavioral units (MAt and GG males; RA-D females). Both sexes showed different patterns of relationship between units (Fig. 3). The major transitions were statistically significant (Table 3). The main linkage values between units were in males: EA - MAt (0.989) and females MA - RA (0.787); other values were lower than MA - RA. Males executed more time and frequency in effectors units than females ($U = 3$, $P < 0.001$; $U = 7$, $P < 0.005$, respectively). Females spent more time and frequency in receiving units than males ($U = 0$, $P < 0.0001$; $U = 2$, $P < 0.001$). Stimulating units exhibited higher relative time and frequency compared to other groups of interactive units (Kruskal-Wallis test $H(2, n = 27) = 20.2$, $P < 0.00001$; $H(2, n = 27) = 22.3$). Internal comparisons in time showed significant differences between all groups ($U = 0$ to $U = 10.5$; $P < 0.01$ - $P < 0.0001$) while in frequency they showed more significant differences ($U = 0$ to $U = 3$; $P < 0.001$ - $P < 0.0001$) (Fig. 2). Males spent significantly longer in EA (Mann Whitney test, $U = 0$, $P < 0.0001$) and H ($U = 15$, $P < 0.05$) than females; and females spent significantly more time in RA ($U = 0$, $P < 0.0001$) and St ($U = 12$, $P < 0.05$) than males (Fig. 4). During the mating behavior, the interactions exhibited a significant variation mainly in time and frequency making stimulating units (Friedman $X^2 = 7.87$, $P < 0.02$; $X^2 = 9.39$, $P < 0.01$, respectively). The internal inspection showed a decrease in the variation and average from the middle PI. The stimulation performed by the males also showed significant differences (Friedman $X^2 = 6.43$, $P < 0.04$; $X^2 = 9.45$, $P < 0.01$), increased in frequency in the

intermediate PI and significantly decreased at the end (Wilcoxon $T = 10.5$, $P = 0.02$). The mutual stimulation time varied significantly (Friedman $X^2 = 9.25$, $P < 0.01$), decreasing between the beginning and the end ($T = 0$, $P < 0.01$). Agonistic units were only present in the first moments of interactions. The Binding units gradually decrease during courtship; however, no statistically significant differences were detected between periods or in frequency and time (Friedman $X^2 = 0.49$, $P = 0.78$; $X^2 = 0.11$, $P = 0.94$). Comparisons between PCPs, intermediate and final IPs did not show significant differences over time (Friedman $X^2 = 1.17$, $P = 0.56$) (Fig. 2). Each CS begins with a mount and ends with a dismount. The copulatory pattern was characterized by not exhibiting blockage; present pelvic thrusting and guiding intrusion movements in cycles (IRT-IDT), there may be one or multiple intrusions and ejaculations. Once the intrusion was achieved, the males initiated the rapid push (IRT) with a frequency of 0.75-7.90 push / s followed by deep push (IDT) with 0.20-4.33 push / s. The CSS did not show temporal differences (Friedman $X^2 = 5.00$, $P = 0.082$) during the interaction (Table 2). The duration of copulation of couples (3) with a single CS was 55.33 ± 28.30 seconds (range 38 - 88 seconds), while couples with multiple CS (7) was 56.5 ± 50.76 seconds (range 19-167 seconds). Comparisons of the IRTs of both groups of couples did not show significant differences (Mann-Whitney test $U = 124.5$, $P = 0.553$). The interaction ends when the individuals remain in physical contact, resting or moving freely within the terrarium.

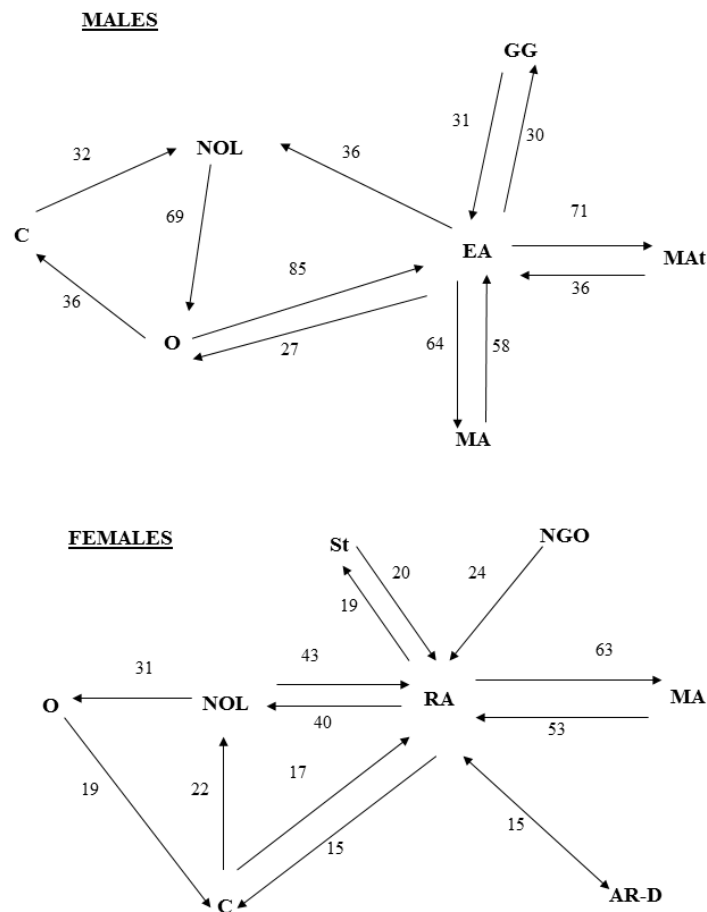


Figure 3. Behavioural unit's Flow diagram presents in Pre-copulatory and Inter-copulatory Periods for both sexes.

Table 2. Measurements of copulatory behavior of *Ctenomys pearsoni* population Carrasco. Data are shown in frequency (events/time) or time (s). ML = mount latency; IL = intromission latency; IP Inter-copulatory period, in other authors = III; IRT = intromission with rapid thrusting; IDT = intromission with deep thrusting.

Couple	ML (s)	IL (s)	Copula duration (s)	Copulatory Serie			IRT			IDT			IP duration (s)	
				Total number	Each series duration (s)	Cycles IRT-IDT number	Total number	Total Duration (s)	f	Total number	Total duration (s)	f		
1	551	559	38	1	11	1	52	9	5,78	3	3	1,0		
							0	145	26	5,58	0	0		
2	294	304	37	4	30	1	169	21	8,05	39	9	4,33	174	
							0	23	7	3,29	0	0		
			33				0	221	33	6,70	0	0		447
			75		52	1	94	46	2,04	5	6	0,83	200	
						0	49	23	2,13	0	0			
3	355	369	88	1	20	2	15	4	3,75	9	16	0,56		
					11	0	14	2	7,00	5	9	0,56		
						0	0	0		15	43	0,35		
			44											
4	458	464	40	1	30	1	60	26	2,31	5	4	1,25		
						0	19	10	1,90	0	0			
5	1190	1194	20	2		0	52	20	2,60	0	0		218	
			47		15	1	6	2	3,00	7	13	0,54		
						0	54	32	1,69	0	0			
6	102	103	187	6	187	1	69	10	6,90	46	177	0,26	271	
			30		12	1	74	11	6,73	2	1	2	35	
						0	94	18	5,22	0	0			
			8			0	43	8	5,38	0	0		53	
			18			0	76	18	4,22	0	0		473	
			18		9	1	53	8	6,63	3	1	3	490	
						0	27	9	3,00	0	0			
7	33	47	194	3	50		228	45	5,07	4	5	0,8	41	
					70	4	149	68	2,19	7	2	3,5		
					58		161	51	3,16	9	7	1,29		
					16		34	9	3,78	5	7	0,75		

Couple	ML (s)	IL (s)	Copula duration (s)	Copulatory Serie		Cycles IRT-IDT number	IRT		f	IDT		f	IP duration (s)
				Total number	Each series duration (s)		Total number	Total Duration (s)		Total number	Total duration (s)		
			27		6		10	3	3,33	3	3	1,00	115
					10	3	21	7	3,00	8	3	2,67	
					11		32	8	4,00	9	3	3	
			280		12		53	8	6,63	5	4	1,25	
					29		123	22	5,59	3	7	0,43	
					8	6	42	6	7,00	8	2	4,00	
					55		204	47	4,34	3	8	0,38	
					82		46	11	4,18	31	71	0,44	
					94		40	14	2,86	27	80	0,34	
	493	496	25		19	1	23	4	5,75	3	15	0,2	443
						0	11	6	1,83	0	0		
8			19	4		0	34	19	1,79	0	0		157
			8			0	21	8	2,63	0	0		93
			35		35	1	69	27	2,56	3	8	0,38	
	721	725	26		15	1	79	10	7,90	11	5	2,2	83
						0	12	11	1,09	0	0		
			53		53	1	102	49	2,08	2	4	0,5	329
			58		21	2	98	17	5,76	3	4	0,75	10
9				5	37		68	33	2,06	3	4	0,75	
			17			0	40	17	2,35	0	0		227
			111		47		67	44	1,52	8	3	2,67	
					23	3	45	20	2,25	2	3	0,67	
					26		70	21	3,33	10	5	2,00	
						0	15	15	1,00	0	0		
	135	138	14		10	1	15	5	3,00	8	5	1,6	158
						0	3	4	0,75	0	0		
10			23	3	17	1	75	14	5,36	2	3	0,67	406
						0	39	6	6,50	0	0		
			20			0	108	20	5,40	0	0		

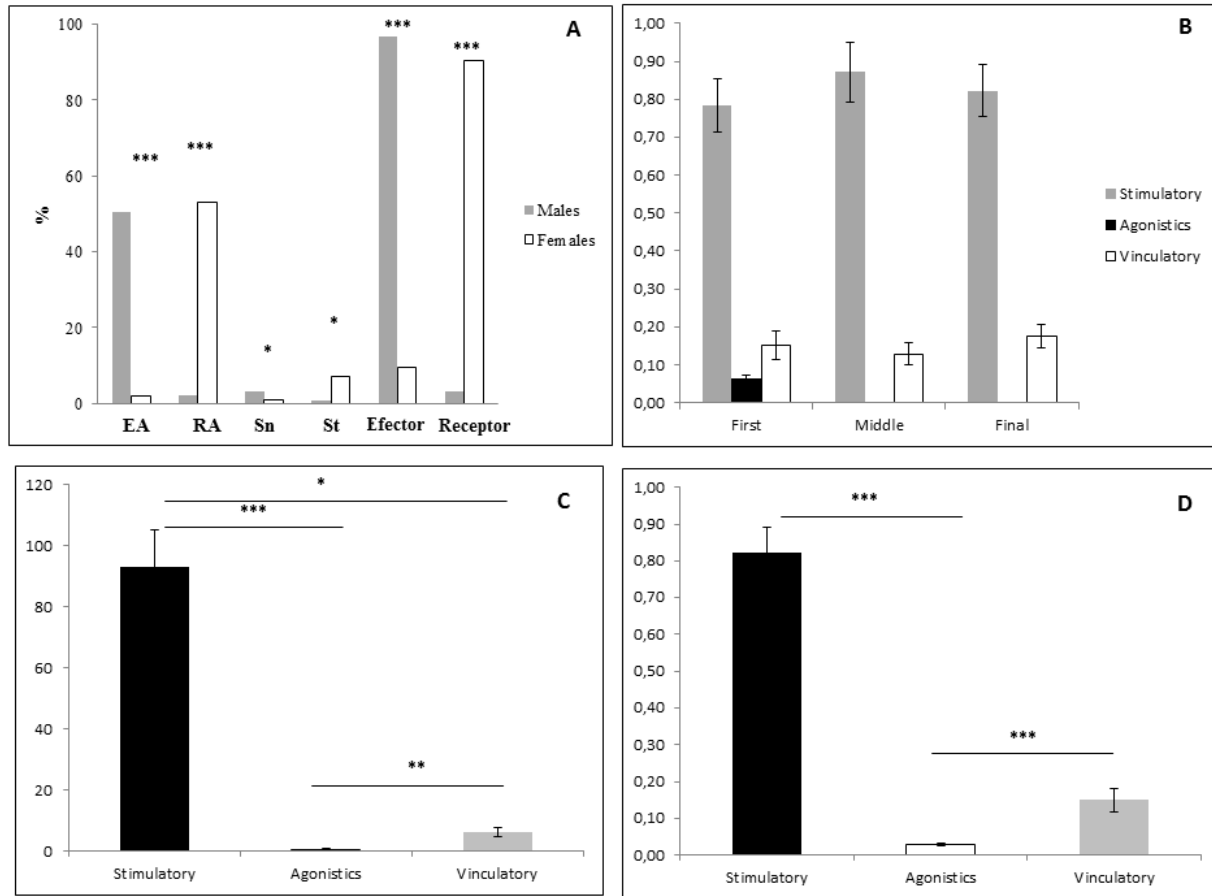


Figure 4. A: Differences in time performing behavioural units and groups of units between sexes. B: Differences in frequency of performing groups of units during time of interaction. C - D: Time and frequency performing each group of units.

Table 3. Main transitions of behavioural units with statistical signification. Statistical associations between units were analyzed using the Chi-square test, $df = 2$, critical significance level was established at $P < 0.05$.

Transition	Statistical signification	
	Male	Female
EA-MA	4.80E-09	NS
EA-MAt	5.15E-31	NS
MA-EA	4.73E-07	NS
MA-RA	NS	0.000132766
RA-MA	7.08E-12	6.11E-11
O-FO	2.50E-06	NS
SN-NOL	NS	4.18E-05
MAt-EA	1.31E-10	NS
NOL-EA	3.56E-07	NS
NOL-SN	NS	1.63E-06
NOL-O	1.72E-29	6.01E-09

Transition	Statistical signification	
	Male	Female
O-EA	1.75E-09	NS
O-C	NS	3.25E-06
OF-E	6.83E-06	NS
OF-S	2.42E-62	1.35E-05
NGO-RA	NS	3.89E-05
St-O	NS	5.22E-05
R-EA	3.46E-05	NS
S-SN	7.64E-06	NS
S-OF	NS	4.25E-06

DISCUSSION

As we have already mentioned, observing any subterranean animal's natural behavior in free life is extremely difficult. For this reason, the behavioral observations of these species are carried out mainly in captivity. Due to the high stereotypy of courtship patterns in different animal species, it is expected that the conditions where it is carried out will not alter their general habits, as long as the animals are in adequate conditions. Similar results have been observed in *C. talarum* (Fanjul & Zenuto, 2008b) using different captivity systems, which would reaffirm this idea, which is why it is advantageous due to the difficulty of working with this type of species in their natural environment. It is assumed that the habituation carried out before the encounters decreased the aggressiveness levels, as in *C. talarum* (Fanjul & Zenuto, 2008b). In solitary species where individuals defend adjacent territories, familiarity is an essential mechanism in recognizing neighbors and selecting pairs (Fanjul & Zenuto, 2008b). Daly (1977) proposes that male markings can familiarize females with their scent so that when both meet, the female will act less aggressively towards the male, copulation is made more accessible. This copulatory behavior has been reported for different species of rodents (Fanjul & Zenuto, 2008b).

Regarding reproductive behavior, *Ctenomys pearsoni* individuals were active from the beginning of the interaction as in other *Ctenomys* species (Camín, 1999; Zenuto *et al.*, 2002; Fanjul & Zenuto, 2008a). Agonistic interactions were followed by the search for chemical information and physical contact, which would indicate that initial inter-individual recognition and evaluation continued to increase during courtship. This evaluation was carried out through Frontal Orientation, Nose-genital Orientation, and Allogroomings, sniffing and touching each other as observed in other tuco-tucos (Camin, 1999; Fanjul & Zenuto, 2008a). Snouting and Nose-genital Orientation are the behavioral units that evaluate chemical information directly about another individual and in the environment. In turn, urine in the terrarium was observed after the tests. Some animals rubbed their perineal region in the terrarium, which together with a perianal gland in *C. pearsoni* (Altuna & Corte, 1989) that would provide chemical information together with the deposition of feces and urine it would support the presence of the chemical communication channel during mating. However, the low frequency of chemical information units, left the role of this type of communication so important in the underground environment, unresolved. Mating continued with an increase in Effector Allogrooming or Mount Attempt performed by males and

movements of the females to avoid mating. Units that involve tactile stimulation interactions appear in greater frequency and duration, in addition to being central in the behavioral sequence. The males are more active as is the Solís population of the species (Altuna *et al.*, 1991) as well as *C. mendocinus* (Camin, 1999) and *C. talarum* (Zenuto *et al.*, 2002; Fanjul & Zenuto, 2008a). Females never mount males as has been observed for *C. mendocinus* (Camin, 1999) and *C. talarum* (Fanjul & Zenuto, 2008a).

In a classical perspective, the succession of behavioral units and their temporal variation throughout the courtship gave rise to the couple's recognition, orientation, stimulation, and synchronization. The more active role of the male in stimulation would suggest that the function of courtship is to appease the aggressiveness of the female and raise her sexual receptivity (Krebs & Davies, 1993). Meanwhile, for the female at this stage, the primary function of courtship would be not only to achieve the necessary stimulation for copulation but also to evaluate the quality of the male. This shows females' active role during courtship, regulating both the display of copulatory behaviors and their duration, both through avoidance and approach behaviors (Dewsbury, 1972); these roles usually tend to be minimized (Fanjul & Zenuto, 2008b). Each sex tries to reduce its costs and maximize its fitness (Krebs & Davies, 1993).

Interestingly, this evaluation is carried out mainly in the intermediate periods that demand more strength from the males. Agonistic units also occurred only initially and were not observed after the first copulation. The high levels of stimulation of the male and the assessment of the female, determine the variability corresponding to induced ovulation (Fanjul & Zenuto, 2008a). In the final stage of the encounter between males and females, individuals usually move to the corners of the terrarium. This could be related to the natural behavior expected in solitary underground rodents. After courting and copulating with the female, the male tends to leave the female's cave before being injured by the female (Bennet & Jarvis, 1988). This type of behavior differs from that observed for *C. rionegrensis* (Tassino & Passos, 2010), where the animals remain together in contact with one individual leaning on the other.

The copulatory pattern in *C. pearsoni* was characterized by the absence of blockage and exhibited pelvic thrusts after achieving intromission. Males with a single intromission could ejaculate once. Considering the Dewsbury classification (1972), where the single prevalence over multiples, we could say that *C. pearsoni* performed pattern 12. However, pattern 12 was conducted by; while pattern 9 (no block, pelvic thrust, multiple intromissions, and multiple ejaculations) was the most common in our study (6/10 cases). It is important to note that pattern 9 is the most common in different rodent species (Dewsbury 1972). The other patterns presented were 10 (twice) and 11 (once). Different types of copulation were observed in *C. pearsoni* (Solís population) and *C. rionegrensis* pattern 9 (Altuna *et al.*, 1991; Tassino & Passos, 2010), *C. mendocinus* patterns 10 in 4 cases and 12 in 14 times (Camin, 1999), and *C. talarum* patterns 9 in 6 cases and 11 once (Fanjul & Zenuto, 2008a). For the Dewsbury classification, the pattern that characterized *C. mendocinus* is 12, *C. talarum* 11, and in our study, *C. pearsoni* performs the pattern 12. As copulation has already been considered, since it has non-ejaculatory and ejaculatory intromissions, it can be regarded in part as a generator of stimulation in the female. Zarrow and Clark (1968) state that ovulation depends significantly on stimulation mechanisms in solitary species. For this reason, induced

ovulation would be an appropriate mechanism in these species where the encounter between potential partners is not frequent. Weir (1974) assumes that females of the genus *Ctenomys* as induced ovulation. This condition would maximize reproductive success by releasing gametes exclusively in the presence of a male that guarantees fertilization. *C. pearsoni* presents a set of penile structures (e.g., spiny bulbs) that could act as a source of cervicovaginal stimulation during mating (Altuna & Lessa, 1985). In females, with or without semen transfer, the copulation itself stimulates neuroendocrine activity (Fanjul & Zenuto, 2008a). Although the ultimate function of copulation is the transfer of sperm from the male to the female, the display, duration, and frequency of the behaviors associated with this process are decisive for both sexes when achieving successful fertilization (Dewsbury, 1972). Reaching different types of copulations (single and multiple) would establish that couples would accomplish the objective of copulation (males, ejaculation, females, ovulation) regardless of the number of copulatory series involved. Males do not require multiple intrusions, which means that the population's copulatory pattern can be variable. The intrapopulation variability may partly reflect the conflict generated by the differences between the interests of males and females (Chapman *et al.*, 2003). Previous studies on sexual selection assume that the male traits they display are invariable as females evaluate them. Each male has a unique trait value that functions their genes, condition, or age. For this reason, previous studies have often focused on finding this outstanding trait value for everyone, and the variation around that value is often seen as noise (Patricelli *et al.*, 2016). If the individual variation results from adjusting displays in response to interactions in mating behavior, this concept could underestimate the role of sexual selection in shaping courtship displays (Patricelli *et al.*, 2016).

Behavioral traits are often variable within and between courtships, making them difficult to quantify and poorly studied (Byers *et al.*, 2010; Kodric-Brown & Nicoletto, 2001). The different observed copulation patterns could be an example of these traits since it varies greatly and adjusts in temporal structure in the rate or intensity of visualization. Dewsbury's point of view focused only on male performance, where simple prevails over multiple was an optimal point of view for the male. However, if we consider the role of the female in mating behavior, the optimum will change and will be determined by the display costs of mating behavior for both sexes. We can observe it in the patterns of a variety of copulation behavior. In some cases, there may be conditional tactics. The optimal viewing level may differ between partners, for example, based on age, condition, experience, local conditions, or rate of encounter with potential mates. We would expect different degrees or types of tactical screen adjustments between individuals (Patricelli *et al.*, 2016). Future studies should focus on the adaptive importance of variability in mating behavior, examine how the social and ecological context influences it and variability in deployments, and how both affect fitness (Bretman *et al.*, 2011).

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