

Copulatory pattern of the subterranean rodent *Ctenomys talarum*

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Abstract

We describe qualitatively and quantitatively the copulatory pattern of *Ctenomys talarum* (tuco-tuco) and evaluate it according to that expected for induced ovulators. Mating trials were performed in an experimental apparatus consisting of three chambers. Copulatory pattern of *C. talarum* is characterized by no lock, pelvic thrusts after intromission and multiple ejaculations. Males may not require multiple intromissions to ejaculate. Courtship starts with agonistic approaches, followed by recognition behaviors, gentle bites, snout pushes and neck exposure. Male mount attempts are frequent (mean 1.2 ± 0.85 attempts/min), while females try to interrupt the mount, suggesting that evasive behavior is an important feature of female *C. talarum* courtship. The number of copulatory series is variable among couples, but 2–3 are the more frequent cases. Once intromission is achieved, the male starts rapid pelvic thrusts (12 movements/s) of moderate intromission. Later, the male proceeds with deep pelvic thrusts, characterized by deeper intromission and longer duration, (1 or 2 movements/s). The duration of copulatory series is influenced by both the evasive movements of the female and the male interest in continuing mating. Results of this study suggest that the amount of vaginal stimuli provided during mating is crucial for the occurrence of ovulation in *C. talarum*.

Keywords: copulatory pattern; *Ctenomys*; induced ovulation; subterranean rodents.

Introduction

The primary function of copulation is clear, sperm is transferred from male to female. However, several parameters of male copulatory pattern are of great functional significance for reproductive success in mammals (Dewsbury 1972). Intromission pattern is related to the occurrence of ejaculation, affects the amount of semen transferred, facilitates its transport (Toner and Adler 1986, Stockley 1997), and participates in removing copulatory plugs (Dewsbury 1981, Stockley and Preston 2004). For females, copulation is important in stimulating neural and hormonal activity, triggering pseudopregnancy and milk secretion in species with spontaneous ovulation (Dilley

and Adler 1968, Dewsbury 1972, Lombardi 1988). Copulation is also critical for those species showing reflex or induced ovulation, for which vaginal stimulation is necessary for initiating the neuroendocrine activity for the occurrence of ovulation (Sawrey and Dewsbury 1985, Ramirez and Soufi 1994). The probability of ovulation relies on the amount of copulatory stimulation in some species, while for others, a small amount of cervical stimulation is effective. Furthermore, copulatory series following ovulation are important for *corpora lutea* formation and pregnancy support (Sawrey and Dewsbury 1985).

Copulatory behavior in a number of taxonomically unrelated subterranean rodents has been rated as similar, mainly showing no lock, thrusting during intromission, multiple intromissions, and multiple ejaculations (pattern 9 from Dewsbury 1975, Bennett et al. 2000). Differences and similarities found in some components of copulatory pattern were explained by phylogenetic affinities, as well as to differences in social biology and the structure of the tunnel system where copulation takes place (Hickman 1982).

Mating behavior has been described for a number of hystricognath rodents (Kleiman 1974), including species from different habitats (subterranean, surface, aquatics), social organization (social or solitary), and nature of ovulation (spontaneous or induced). *Ctenomys*, the most speciose genus of subterranean genera (almost 60 species, Woods 1993), belongs to this group. However, copulatory patterns have been described only for two species, *C. pearsoni* (Altuna et al. 1991) and *C. mendocinus* (Camin 1999), which presented patterns 9 and 12, respectively. *Ctenomys talarum* (Thomas 1898) is a highly territorial subterranean rodent that occupies burrows solitarily; individuals do not share burrows, except when mating occurs and the mother and the young during the rearing period, when the mother and the young occupy the same burrow (Busch et al. 1989). Populations are characterized by a polygynous mating system (Zenuto et al. 1999), and males never exert preparental or parental care (Zenuto et al. 2004 **((author: 2001 is not listed in the list but 2004 is. Please confirm if this change is correct)))**). During courtship, mate assessment includes not only the use of chemical cues, but also body contact and a particular vocalization for each sex (Zenuto et al. 2002, 2007, Schleich and Busch 2002, Zenuto and Fanjul 2002, Fanjul et al. 2003). According to Weir (1974), this species shows induced ovulation, as only mated females showed *corpora lutea*.

The study of mating behavior in solitary subterranean rodents is a challenging task due to their secretive habits in the wild and difficulties in rearing them in captivity. For this reason, the data available on this topic are limited in terms of both quantity and quality. The present work provides detailed data on the general copulatory pattern in *C. talarum*, with the aim of relating behavioral and phys-

iological patterns in order to contribute to the general knowledge of the reproductive behavior in this particular group of rodents. The specific objectives of the present work were: a) to describe qualitatively and quantitatively the copulatory pattern of *C. talarum*, and b) to evaluate the different components of copulatory pattern of this species according to those expected for induced ovulators. Given the stimulatory requirements of induced ovulators (Ramirez and Soufi 1994), we predict that the copulatory pattern in *C. talarum* includes a conspicuous stimulatory phase (i.e., thrusting) and a longer duration of copula when compared to species with spontaneous ovulation.

Materials and methods

Animals and housing

Mature *C. talarum* individuals were collected in the field (Mar de Cobo, Argentina, 37°45' S, 57°56' W) using plastic live traps located in burrow openings that showed fresh excavated soil. According to the reproductive period reported for the same population (Malizia and Busch 1991, Fanjul et al. 2006), females were captured during their non-breeding season (March–May 2005) to avoid the influence of previous reproductive activity in our mating trials. Males were captured during their reproductive season (June–December 2005). All animals were transported to our laboratory, where each individual was housed in a plastic box measuring 42×34×26 cm, with 3 cm of wood shavings for bedding. The animal room was maintained within the thermoneutrality range of the study species (23±1°C; Busch 1989). Food was provided *ad libitum* and consisted of carrots, sweet potatoes, catalogna chicory (*Cichorium intybus*), corn, mixed grasses, and sunflower seeds. Because *C. talarum* does not drink free water, fresh vegetables were provided every day to secure water provision. Males were allowed to acclimate to captive conditions for at least 4 days prior to participation in mating trials.

Mating trials

During each mating trial, a male and a female were placed together in a Plexiglas chamber (described below). Before the mating trials, *C. talarum* females were allowed to choose, between two males, the one that would act as their partner (for details see Zenuto et al. 2007). Previous studies demonstrated that when potential partners were randomly assigned, little reproductive activity occurred in *C. talarum* (Zenuto et al. 2007) and *Spalax ehrenbergi* (Shanas et al. 2005 (**author: This reference is not listed in the reference list**)), while when a female was allowed to choose the male, mating success was enhanced in both cases. During the preference trials, a female was placed in a Plexiglas chamber (45×30×30 cm) connected to a T-maze with arms of equivalent length (25 cm) and diameter (10 cm). One male was confined into each arm of the maze using a mesh screen. Females could obtain chemical information directly from each male and from additional soiled bedding (placed at the distal portion of each arm) collected

from the boxes of each individual. Preference tests started when the female entered the arms that contained the males, and lasted 15 min. We defined the preferred male for each female as the one with which she spent more time in close contact and/or towards which she showed more interest (indicated by sniffing odor cues and showing interest to contacting the male's body; Zenuto et al. 2007).

Mating trials were performed in an experimental apparatus consisting of three Plexiglas chambers (45×30×30 cm) connected to each other by a Plexiglas tube (10 cm diameter, 20 cm length). The test female and her preferred male were individually confined (1-h acclimation time) in one of the chambers, which contained soiled shavings from their respective housing boxes. At the start of each trial, both animals were allowed to enter the central cage (neutral space), containing clean shavings. Pairs had to wait for 30 min to initiate copulation. If copula was initiated, observations continued until reaching standard satiety criterion (30 min with no copulation; Dewsbury 1975). Chambers and tubes used during female preference tests and mating trials were carefully washed with tap water and odorless glassware cleaner, wiped with alcohol, and then allowed to air dry to ensure that no odors from previous trials remained. Latex gloves were worn when handling animals and collecting odors to avoid human scent transfer. A video camera was used to record mating activity. Videotapes were then viewed to record the copulatory parameters detailed below.

Mating trials resulting in the absence of copulation (13/20 cases) were discarded, and these individuals were included in other interactions after at least 15 days between subsequent trials. A total of 20 females and 16 males were used. Copulation was observed in 7 mating pairs, involving different females and males. Each mating was assessed qualitatively and quantitatively. We used the following measures of copulatory pattern: mount latency (ML; time to first mount resulting in copulation); 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 and 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.

Once a mating trial ended, a vaginal smear sample was taken for each female (detailed in Fanjul et al. 2006). Presence of spermatozoa in the smears proved that ejaculation occurred. As part of another ongoing project, qualitative histological observations of the ovaries from a subgroup of females (n=4) of this study were used to

confirm the presence of *corpora lutea* as indicator of ovulation occurrence (Fanjul 2006). Ovaries were removed the 5th day after the mating trial, fixed in formalin, treated by standard histological techniques (Drury and Wallington 1967) and observed under microscope (100 \times).

Results

Courtship started with agonistic approaches (threatening and showing incisors), followed by recognition behaviors, such as sniffing each other's genitalia. Moreover, both sexes sniffed actively the urine left in the substrate by both partners. Those behaviors were followed by gentle bites along the neck area, snout pushes and neck exposure. Both males and females usually vocalized during the courtship. Mating trials lasted 42.87 ± 12.32 min (mean \pm SD), in which male mount attempts (non-intromitting mounts) were frequent (mean 1.2 ± 0.85 attempts/min, range 16–85). Females usually hindered the mounts by moving away (7 females), suggesting that evasive behavior is a characteristic of female *C. talarum* courtship. Nonetheless, when males showed decreased mating interest, females solicited courtship by pushing, chasing and frequently mounting the male. During a successful mount, the male mounted the female from behind, grasping her shoulders with his forelimbs and tightly biting her neck. Once they reached this position, males pressed the female hindquarters with their back limbs, restraining her movements. This process lasted 1–11 s, while females tried to interrupt the mount (Table 1). Copulatory series started with the mount, followed by the first intromission until the final withdrawal of the penis, generally taking place a few seconds before dismounting. Copulation was attained both in the chambers as well as inside the tunnels of the experimental apparatus.

Mating behavior was highly variable between couples and this particularly concerned mount and copulation. Also, the number of copulatory series recorded was variable (range 1–7), though 2 or 3 series were more frequent. Once intromission was achieved, males started rapid thrusts (IRT), where intromissions were moderate and sometimes the penis was exposed completely outside the vagina. Those pelvic movements were performed with a frequency of 4–12 thrusts/s (Table 1). Later, deep thrusts (IDT) were observed, characterized by deeper intromissions into the vagina and longer in duration (1–2 movements/s; Table 1). In some cases, ejaculation during deep thrusts was clear, denoted by a brief period of female immobility while the male grasped her neck tightly and his tail assumed an upright position. The ending of IDT, as well as the duration of CS and III was conditioned by both the female avoiding movements and the male attempting to continue; resulting in a high variance in copulatory activity between the observed couples (Table 1). After dismounting, both partners frequently stayed in close proximity and males dedicated to self-groom their abdominal and perigenital area. Following that, they restarted courtship and/or copulation. Sperm transfer was verified by the presence of spermatozoa in the vaginal smears in all copulated females, except for

the one with the highest number of copulatory series which were of brief duration and lacked of deep pelvic thrusts. Yet, that female appeared to have received the required stimuli to reach ovulation, as *corpora lutea* was present in the ovaries. Ovulation was not verified for one couple showing a copulation consisting in one CS and absence of IDT, even though sperm transfer was detected (Table 1). Following the last CS, males groomed their genital area and were unresponsive to female solicitations. Soliciting behavior by females was usually displayed until males showed no interest which consisted of showing his rump, resting, or moving away. Some couples remained in physical contact resting or self-grooming their genitalia.

Discussion

Ctenomys talarum courtship behavior is prolonged and complex, as indicated by its duration and the wide repertoire of behaviors displayed (Zenuto et al. 2002, 2007). During courtship, mating partners experience a lengthy period of assessment and familiarization until aggression diminishes and they engage in body contact. This is consistent with other studies involving solitary species of subterranean rodents, such as *C. pearsoni*, *S. ehrenbergi*, *G. ((author: Please provide full species name)) capensis*, and *H. ((author: Please provide full species name)) argenteocinereus* (Altuna and Lessa 1985, Bennett and Jarvis 1988, Gazit and Terkel 2000, Šumbera 2001).

The basic copulatory pattern of *C. talarum* was characterized by several copulatory series (CS), in which males performed pelvic thrusts at a very high frequency (range 5–12 thrusts/s, to date the highest frequency observed in a subterranean rodent according to Šumbera 2001), leading us to propose a stimulatory function both for the male (to achieve ejaculation) but also for the female (to reach ovulation). Afterward, a decrease in frequency and increase in depth of pelvic thrusts were recorded, and this period could be assigned to ejaculatory intromissions. According to our observations, the intensity of vaginal stimulation might be of great importance for *C. talarum*, as *corpora lutea* were observed only in those females that experienced at least three copulatory series. Indeed, it is noteworthy that one female reached ovulation after multiple copulatory series but without deep thrusts, suggesting the importance of intromissions with rapid thrusting in inducing ovulation.

Copulatory pattern in *C. talarum* was characterized by no lock, pelvic thrusts after the intromission was achieved, and multiple ejaculations (considering deep thrusts as ejaculatory series; Bignami and Beach 1968). Males did not require multiple intromissions to ejaculate, but this must be confirmed with a larger sample size. Hence, copulatory pattern of *C. talarum* conforms to pattern 11 of Dewsbury classification, as males did not require multiple intromissions to achieve ejaculation. The pattern 9 (no lock, pelvic thrust, multiple intromissions, and multiple ejaculations) is the most common pattern reported for rodents (Dewsbury 1972) and was also found for several subterranean species belonging to phy-

Table 1 Measures of copulatory pattern of *Ctenomys talarum*.

Couple	ML	IL	Copulatory series		Cycles IRT-IDT	IRT		IDT		III (s)	Spermatozoa	Corpora lutea
			Total number	Each series duration (s)		Total number	Total duration (s)	Total number	Total duration (s)			
1	155	162	3	20	1	136	15	8	4	64	Present	Yes
				96		2	211	23	20			
2	165	167	2	7	0	30	4	0	0	1048	Present	ND
				63		1	214	21	24			
3	528	538	3	20	0	71	15	0	0	64	Present	Yes
				9		1	60	7	4			
4	1080	1083	7	3	0	18	3	0	0	26	No detected	Yes
				46		1	100	14	4			
5	1275	1276	1	2	0	6	1	0	0	218	Present	No
				11		0	53	10	0			
6	365	376	2	5	0	24	4	0	0	19	Present	ND
				8		0	29	7	0			
7	1673	1681	2	6	0	ND	ND	0	0	53	Present	ND
				6		0	31	5	0			
Mean	748.71	755.14	3	7	0	38	5	0	0	316	Present	Yes
				26.4		0	38	6	0			
SD	595.84	595.50	2.3	64	1	34	7	50	57	77	Present	ND
				27.05		1	51	7	17			
				46	ND	ND	ND	ND	ND	65	Present	ND
				55	ND	ND	ND	ND	ND	194.3		
				27.05	ND	65.66	9.05	9.11	17	214.4		
						61.53	6.15	14.82	17.61			

For each couple, detailed information is presented: ML: mount latency, IL: intromission latency, IRT: intromission with rapid thrusting; IDT: intromission with deep thrust; III: inter-intromission interval; ND: not determined; f: frequency (total number/total duration); s: seconds. See materials and methods for more details.

Table 2 A compilation of available data on duration of copula and copulatory pattern in subterranean rodent taxa and non-subterranean hystricognath rodents. **(author: Please confirm that spellings of the families listed below are correct, as some were incorrect)**

Suborder family: subfamily	Species	Subterranean habit	Social organization	Duration of copula	Ovulation	Copulatory pattern	References
Hystricognath							
Bathyergidae: Bathyergidae	<i>Cryptomys hottentotus</i>	Yes	Social	1–5 s	Induced	9	Hickman 1982
	<i>C. h. natalensis</i>	Yes	Loosely social		Induced	9	Jackson and Bennett 2005, Bennett et al. 2000
	<i>C. h. pretoriae</i>	Yes	Loosely social		Induced		Malherbe et al. 2004, Bennett et al. 2000
Bathyergidae: Heterocephalinae	<i>Fukomys damarensis</i>	Yes	Highly social		Spontaneous	10	Bennett and Jarvis 1988
	<i>Heliphobius argentocinereus</i>	Yes	Solitary	10–30 s			Sumbera 2001
	<i>Heterocephalus glaber</i>	Yes	Highly social	<15 s	Spontaneous		Jarvis 1991
Octodontidae: Octodontidae	<i>Ctenomys talarum</i>	Yes	Solitary	2–64	Induced	11	This study
	<i>C. pearsoni</i>	Yes	Solitary	26–65	Induced	9	Altuna et al. 1991
	<i>C. mendocinus</i>	Yes	Solitary	25–237		12	Camín 1999
Chinchillidae	<i>Spalacopus cyanus</i>	Yes	Social	<15 s			Begall et al. 1999
	<i>Octodon degu</i>	No	Loosely social	10 s	Induced	9	Kleiman 1974, Weir 1974
	<i>Chinchilla</i>	No	Social	5 s	Spontaneous	9–10	Kleiman 1974
	<i>Cavia</i>	No	Social	5 s	Spontaneous	12	Kleiman 1974
	<i>Microcavia</i>	No	Social	2–6 s	Spontaneous	9–10	Kleiman 1974
Myomorpha	<i>Nannospalax ehrenbergi</i>	Yes	Solitary	2 s–several minutes	Induced	10	Nevo 1969, Shanas et al. 1995
	<i>Georychus capensis</i>	Yes	Solitary		Induced	9	van Sandwyk and Bennett 2005
	<i>Thomomys talpoides</i>	Yes	Solitary	30–900		9	Andersen 1978

logenetically distant groups (Table 2). Furthermore, patterns 9 or 10 were reported for aboveground hystricognaths as *Chinchilla*, *Cavia*, and *Microcavia* (Kleiman 1974). Among members of the family Octodontidae, patterns 9, 11, and 12 were reported (Table 2). Therefore, solitary subterranean rodents conform to patterns 9–12 of Desvurbian classification, as they failed to exhibit a lock, but show clear pelvic thrusts. However, whether multiple intromissions are required to achieve ejaculation and multiple ejaculations are detected show a more variable pattern.

The amount of stimuli required to reach ovulation is highly variable in different mammal species (Ramirez and Soufi 1994). Indeed, prolonged mating proved to increase the probability of ovulation but also the number of embryos produced (Roberts et al. 1999). Females of the subterranean solitary *S. ehrenbergi* showed no ovulation after only one CS, but they did after two or more CS (Shanas et al. 1995). Besides the amount of CS performed, the total or partial duration of each CS could be critical for female stimulation. Copulation duration in *C. talarum* (2–96 s) varied among couples, but the range found was similar to that reported for other subterranean rodents (see Table 2 for references). Much shorter CS was reported for other hystricognaths (less than 10 s; Kleiman 1974); however, these species are spontaneous ovulators. It appears that copulatory stimulation, considering the length and/or the patterns of intromissions, would be crucial for induced ovulators. In the case of social subterranean hystricognaths that ovulate spontaneously, intromission times reported were brief (*Cryptomys hottentotus*, Hickman 1982; *Heterocephalus glaber*, Jarvis 1991; Table 2). According to our observations in the present study, the amount of vaginal stimuli was critical for *C. talarum* successful breeding. Only females that engaged in at least three CS presented *corpora lutea*, further suggesting that it may be a prerequisite for females to ensure ovulation during interactions with unfamiliar males, but this observation must be confirmed with a larger sample size.

Nearly all solitary subterranean rodents are induced ovulators, as most of solitary mammals. Intuitively, ovulation in response to mating appears to be the more efficient system to ensure egg fertilization in non-gregarious species, for which encounters between potential partners may not be frequent (Zarrow and Clark 1968). Some solitary mammals developed penises endowed with spikes, spiny bulbs, and fingerlike projections that may help males to trigger a physiological response in the female, whereas in other species, females only require a more subtle source of stimuli, as urine pheromones (Lombardi 1988). Cervicovaginal stimulation has been proposed as a mechanism to trigger ovulation in *Ctenomys* (Weir 1974). Presence of spines covering the *gland penis* surface was described for *C. talarum*, which may contribute to enhance the vaginal friction during copulation. Moreover, the intromittent sac presented spikes, which are protruded during copulation and provide stimulation inside the deeper portion of the vagina (Spotorno 1979, Balbontin et al. 1996). Similar structures were reported for *C. pearsoni*, but spikes were functionally replaced by the presence of spiny bulbs (Altuna and Lessa 1985).

In many rodent species, sperm competition is considered a source of selective pressure favoring shorter copulation latencies, multiple brief intromissions and multiple ejaculations (Stockley and Preston 2004). The copulatory pattern evidenced for *C. talarum* suggests that sperm competition may not impact its copulatory behavior, given the long copulation latencies and the pattern of intromissions. Further, previous studies reported low testicular size (Zenuto et al. 1999) and low sperm count (Zenuto et al. 2003), two characteristics that may not be consistent with sperm competition.

In many species, the intensity of sexual behavior relates to the development of social bond between mating partners (Féron and Gouat 2007). Male presence, either by direct or indirect contact (i.e., odors), generates familiarization and may influence female mate choice and reproductive success in both sexes (Daly 1977, Tang-Martinez et al. 1993). *Ctenomys talarum* females familiarized with male odors showed some reduction of their aggression during courtship but did not shorten the latency to copulation. Furthermore, female interest for unfamiliar males could be interpreted as part of an inbreeding avoidance strategy (Zenuto et al. 2007).

The stimulation provided by several copulatory series, the pattern of pelvic thrust, and the number and length of intromissions observed in *C. talarum*, are in agreement with predictions for an induced ovulator species. Hence, we conclude that the amount of vaginal stimuli provided during the sexual encounter may be crucial for the occurrence of ovulation in *C. talarum*.

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