When allowed, females prefer novel males in the polygynous subterranean rodent *Ctenomys talarum* (tuco-tuco)

Maria Sol Fanjul*, Roxana Rita Zenuto

Grupo Ecología fisiológica y del comportamiento, Instituto de Investigaciones Marinas y Costeras (IIMyC), Universidad Nacional de Mar del Plata, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Box 1245, 7600 Mar del Plata, Argentina

ABSTRACT

The ability to recognize familiar conspecifics plays an important role at the time of choosing a mating partner in rodents. A laboratory study using preference test was used in order to test the hypothesis that, in the polygynous subterranean rodent *Ctenomys talarum*, females prefer novel males when offered two odors, or individuals (one familiar and one novel) limited in their movements so that male aggression is prevented. Our findings show that females prefer novel tuco-tucos at three levels of male assessment: odor samples (consisting of shavings soiled with urine, feces, and presumably, other body secretions collected from the male home cage), confined males behind a wire mesh, and full contact with tethered males. Previous studies of this species demonstrated that in the wild, male–male competition and male coercion severely limit the possibility of females mating non-neighbors, i.e. novel males. Females mating neighbors to whom they are familiarized, obtain high quality mating since they are territorial, highly competitive males. Nonetheless, when females have the opportunity, as shown in the two-choice experiments, they choose novel males, probably benefiting their progeny from novel genetic combinations. Hence, combining evidence from laboratory and field studies in *C. talarum*, it is possible to better understand female preferences in modeling individual reproductive strategies.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Intersexual communication plays a crucial role in determining the outcome of reproductive encounters. Signals produced by one sex and received by the other contain valuable information about a potential mate quality (Andersson, 1994). For many mammals, particularly for rodents, chemical signals constitute a major communication channel (reviewed in Eisenberg and Kleiman, 1972; Hurst, 2009). Scents convey a wide array of information about the sender, such as species, sex, individual identity, kinship, reproductive status, genetic profile, diet quality, health and vigor, and testosterone levels (e.g. Ferkin et al., 1997; Milinski, 2006; Candolin, 2003; Colombelli-Negrel and Gout, 2006; Johansson and Jones, 2007; Thom et al., 2008; Hurst, 2009). Moreover, many animals are able to discriminate between a scent they have previously encountered (familiar), from novel (unfamiliar) ones (Gosling and Roberts, 2001; Mateo, 2002; Johnston, 2003; Todrank and Heth, 2003; Thom and Hurst, 2004; Holmes and Mateo, 2007). Familiarization with scents is a common event for many vertebrates; also, the ability to discriminate familiar from novel scents is critical in two main

contexts during their life: territoriality and mate choice (Gosling, 1990; Cheetham et al., 2008).

Familiarity is reached through direct association with an animal or its odors. Recognizing the individual, or at least that the odor matches a previously encountered odor, allows the avoidance of potentially hazardous and energetically demanding interactions with a territory owner and/or a neighbor, as proposed by the dear enemy hypothesis (Temeles, 1994). However, some territorial animals do not show lesser aggression toward neighbors; such difference may be due to the type of territory defended (feeding, breeding or multipurpose territories; Temeles, 1994), instability of territories (Lachish and Goldizen, 2004), and changes in their social environment (Hyman, 2005). Also, animals use odor familiarity when choosing a mate. Female choice of males varies in relation to familiarity; some studies have reported the preference of mating familiar males, while others, unfamiliar ones. Differences in this preference rely on the species' social structure, mating system and female reproductive condition (Cheetham et al., 2008; Brandt and Macdonald, 2011). For monogamous individuals, familiarity allows the recognition and selection of previous partners for mating purposes, while for those that benefit from multiple mating, avoidance of familiar males favors the acquisition of rare genotypes (e.g. Shapiro et al., 1986; Patris and Baudoin, 1998; Cheetham et al., 2008; Richankova et al., 2007; Lucia and Keane, 2012). Familiarity

* Corresponding author. Fax: +54 223 4753150.
  E-mail address: msfanjul@mdp.edu.ar (M.S. Fanjul).

also acts as a proxy of male competitive quality, since animals that successfully defend their territories maintain their scents available for female assessment and familiarization (Rich and Hurst, 1998). Benefits of mating preferences for territorial males can be direct, such as the provision of high quality resources for females or their pups, and/or secure mating, since the risk of getting infections during copulation is diminished (Andersson, 1994). Indirect benefits are represented by the inheritance of good genes for territorial ability in the progeny (Gosling, 1990; Johansson and Jones, 2007).

Subterranean rodents are a diverse group of species worldwide showing convergences in many physiological, morphological and ecological traits associated to the particular habitat in which they evolve (Nevo, 1999). Despite the fact that most of these species are solitary, reproductive biology and behavior have only been well studied in social and eusocial species (Lacey et al., 2000; Begall et al., 2007), probably as a result of both logistic difficulties associated to studying secretive animals in the wild and low success reached when breeding solitary species in captive conditions. Life underground imposes specific environmental constraints on some chemical channels – particularly the use of visual signals – and concurrently favors the use of chemical communication. Chemical cues left by individuals on the substrate persist in the environment in the absence of the sender providing an effective mode of communication for solitary subterranean species (Francescoli, 2000). This is the case in *Ctenomys talarum* (talas tuco-tuco, Thomas, 1898) a species that belongs to a highly diversified genus of herbivorous subterranean rodents from South America inhabiting coastal dunes in southeast Argentina (Reig et al., 1990; Woods, 1993). Both sexes are highly territorial, live solitarily (except for adult male and female during mating and for mother and pups until weaning), and aggressively defend their own burrow systems (Busch et al., 1989, 2000). Breeding season extends from autumn to midsummer (Malizia and Busch, 1991) and females adjust their reproductive activity according to temperature and food availability (Fanjul et al., 2006). Male odors do not stimulate changes in vaginal smear parameters indicative of receptive condition in females (Fanjul and Zenuto, 2008a). *C. talarum* is a copulation induced ovulator, for which the manifestation of different degrees of reproductive activity would be the result of their reproductive physiology (i.e. hormonal levels). Male presence and interaction with females affect hormone levels (progesterone and oestradiol) and vaginal cytology but follicular development is not stimulated (Fanjul and Zenuto, 2012). DNA fingerprinting analyses suggest that *C. talarum* is polygynous (Zenuto et al., 1999a), and consistent with genetic data, males are bigger than females (Zenuto et al., 1999b). In the wild, adult females outnumber adult males and the burrows of each male are regularly surrounded by other burrow systems, each individually occupied by females (Busch et al., 1989). Chemical communication may be important for tuco-tucos. They have the ability to discriminate not only familiar from novel scents, but also gender and reproductive condition from metabolic chemical cues in urine, feces and other bodily fluids (Zenuto and Fanjul, 2002; Fanjul et al., 2003; Zenuto et al., 2004). Scent marking behavior and the rubbing of the anogenital area were observed for both sexes in laboratory studies on reproductive behavior (Zenuto et al., 2002a, 2007). During mating aggression must be decreased, and chemical communication provides an excellent way to convey individuals' willingness to mate. Mate assessment and courtship starts with agonistic approaches (showing incisors and chasing) followed by recognition behaviors (visual and body contact, sniffing each other bodies and the urine left in the substrate). Once the couple reaches close contact, the female usually exposes her neck and back area and raises her tail. Later, males exhibit mounting attempts and copulation (Fanjul and Zenuto, 2008b, 2012). In the wild, animals frequently store soil heavily mixed with urine, feces and small bits of grass forming a mound next to the burrow entrance (authors' personal observation). Even though tuco-tucos perform most of their activities inside their own burrow systems, they make short and fast outside trips to collect plants that they later consume inside the tunnels. Conspecifics exposed to those scented mounds during their foraging trips or patrolling activities would obtain information about the burrow owner. Thus, familiarity by odor cues is a regular phenomenon in tuco tucos' lives. Using this information, both sexes modulate their aggressiveness during territorial and reproductive activities; males respond less aggressively when confronted with familiar males (Zenuto, 2010) and females also show low aggression during courtship when encountering familiar males (Zenuto et al., 2007).

Although living solitarily in their own burrow systems, female tuco-tucos are regularly exposed to odors of males that occupy burrows located in their surrounding area. Familiarity by odor cues may allow them to recognize neighbors and use that information for mate choice. Zenuto et al. (2007) demonstrated that the familiarization of females with male odors affects their subsequent reproductive behavior. Familiarized females direct lower aggression toward males during courtship, but sexual behavior is not increased in relation to those females that interact with novel males. Moreover, females that are not previously exposed to male odors, copulate repeatedly with the same partner in subsequent trials. Overall, these results impose the necessity to evaluate the possibility that females of *C. talarum* show preferences for novel males when confronted simultaneously with a familiar and a novel one. Even though limited mobility of individuals, male size and aggression could limit the expression of female preferences in the wild. Nonetheless, as females are the sex that invests more energy in reproduction (Zenuto et al., 2002b), female choice is expected when male aggression and/or coercion are prevented. In this study, we used preference tests to determine whether females display preference at three levels of male assessment: odor samples, confined males behind a wire mesh, and full contact with tethered males. The aim of this study is to evaluate whether familiarity with odor cues of potential mates affects the mating preferences of females. Even though a previous study showed that familiarity with potential partners may reduce mating costs decreasing aggression during mating encounters, novel genotypes appear as valuable traits for females; hence we predicted that females would show preference for novel males.

2. Materials and methods

2.1. Animal capture and housing conditions

We used live traps to collect mature individuals of *C. talarum* at Mar de Cobo, Argentina (37°45′S, 57°26′W). Females were captured during their non-breeding season (March–May 2006, 2007 and 2009) to avoid the influence of previous reproductive activity while males were captured during the reproductive season (June–December). All animals were transported to the laboratory, where each tuco-tuco was individually housed in a plastic cage with a wire-mesh lid measuring 42 cm × 34 cm × 26 cm. Wood shavings were provided for bedding. Fresh food (carrots, sweet potatoes, Catalogna chicory, corn, mixed grasses, and sunflower seeds) was provided ad libitum every day to supply *C. talarum* with a water source since this species does not drink free water. Photoperiod and temperature were automatically controlled (12:12 L:D; 25 ± 1 °C). Experiments were carried out throughout the light period during the breeding season. Males were allowed to adapt to captivity for at least 5 days before mating trials. Animals were randomly assigned to each preference test. At the end of the experiments, the animals were returned to their site of capture. Disposable

gloves were used in all instances of sample collection and during experimental trials. All equipment used during the study was washed with tap water, and odorless glassware cleaner, was wiped with alcohol and allowed to air dry to ensure that no trace odors from previous trials remained. A total of 72 females and 144 males were used to conduct a total of 72 trials. Only trials in which females showed active and displayed reproductive behaviors (Zenuto et al., 2007) were included (37 trials; 37 females and 74 males). To assess whether female familiarization with conspecific male odors influences reproductive behavior in female tuco-tucos, each female was familiarized with male odors and a preference test was subsequently carried out. Preference tests were used to determine whether females displayed sexually distinctive preference responses to three levels of male assessment: (a) odors (n = 9, 9 females and 18 males), (b) confined males through a wire mesh (n = 11, 11 females and 22 males) and (c) full contact with tethered males (n = 14, 14 females and 28 males). Females were not hormonally induced. In each trial, females were simultaneously exposed to: a familiar male versus a novel male (or their odors). Pairs of males were randomly assigned to each female but their body mass did not differ by more than 5%. Animals participated only once as test animals and odor donors. The possibility that females and males used in this study could be relatives was not analyzed conducting parallel studies involving genetic markers. Nonetheless, this was unlikely to occur since males are the more dispersive sex and low coefficients of relatedness between males were found for the same population in previous studies (Malizia et al., 1995; Zenuto et al., 1999a). Also, during assays, females were never offered with a male neighbor in the wild, so that the likelihood that subjects were related and/or familiar was minimized.

2.2. Familiarity

Females were familiarized with a randomly chosen male by exposing them to a male odor sample placed in her home cage during 4 consecutive days (time required to reach familiarization, Zenuto and Fanjul, 2002; Fig. 1). Odor samples consisted of shavings soiled with urine, feces, and presumably other bodily secretions collected from the corners of the male home cage, where the bedding had not been changed for at least 7 days. Odor samples were presented to test animals in PVC dishes (10 cm diameter) covered by a wire mesh with an opening (3 cm diameter) that allowed females to lick and touch shavings and hence to use both the main olfactory epithelium and the vomero-nasal organ to assess odor source. Odor samples were replenished every day.

2.3. Preference test

Preference tests were carried out the day after familiarization concluded (see above). The experimental apparatus consisted of a Perspex box/es (45 cm × 30 cm × 30 cm) and a Perspex Y maze (10 cm diameter and 25 cm length each arm). Prior to preference tests, each female and/or males were placed individually in the corresponding box for at least an hour to be habituated to it before the test begun (Zenuto and Fanjul, 2002).

During the odor preference test (a), the female box was connected to the Y maze. At the end of each maze arm a sample of male odor was placed as an end-cap: one belonging to the familiar male and the other to a novel male (Fig. 1). Trials started when the test female entered the maze and lasted for 7 min (Fanjul et al., 2003; Zenuto et al., 2004). We recorded the interest of a female in each sample (the familiar and the novel male odor) as the total time devoted to sniffing each sample (information gathering) and the number of times the test female visited each arm (frequency).

In preference tests using males confined through a wire mesh (b), the female box was connected to the Y maze and each maze arm was connected to each male's tube: the familiar male and a novel male (Fig. 1). The entrances of both males' tubes were covered with wire mesh, which prevented animals from being in full contact, but allowing females to see, sniff and hear each male. Shavings from the respective male housing cage were placed next to the mesh at each maze arm. Trials started when the females entered the Y pipe and lasted 30 min. Trials were recorded with a HD camcorder (Sony™ HDR-XR100). Videotapes were observed afterwards. To determine the relative interest test females showed for each male, the total time they stayed active in each arm was recorded. Frequencies of behaviors (recognition, aggression soliciting, and mutual courtship, Table 1) females displayed toward each male according to previous studies (Fanjul and Zenuto, 2012) were also recorded.

In preference tests involving full contact with tethered males (c), the stem of the Y maze was connected to the female cage while the arms were connected to the cages, each containing an individual male (the familiar and a novel one). Male cages were provided with a transversal rod and a movable line of stainless steel fishing leader ending in a snap swivel that could be attached to a self-locking nylon tie worn by the male as a collar (Fig. 1). Males were individually tethered to their cage before habituation and collars were removed immediately after trials. Trials started when females entered the Y pipe and lasted 30 min. They were recorded with a HD camcorder and videotapes were observed afterwards. During preference tests on male odors (a), the frequency of visits females performed to each maze arm, as well as the time spent sniffing the odor sample of familiar and novel males, were recorded. To determine the relative interest of females toward each male, both confined (b) or tethered (c), the following variables were recorded: frequency of visits females performed to the tube/cage of each male, total time females stayed active moving around the box, frequency of behaviors displayed only by females, or the interacting couple (described in Table 1). We recorded the frequency of behaviors the females displayed toward each male separately, the familiar and the novel male.

To avoid analyzing too many variables, the behaviors measured during each test were pooled and analyzed according the type of behavior as described in Table 1: recognition, aggression, soliciting, and mutual courtship. Once videos of trials involving confined and tethered males were analyzed, we found that females displayed most behaviors during the initial 10 min. Females performed 63.7% of behaviors (accumulated frequencies) during the first 10 min of trials using confined males through a wire mesh (26.4 ± 13.4 behaviors of 41.4 ± 26.2 during the whole 30-min trials). Similarly, females performed 53.2% behaviors of during the first 10 min of the trials using tethered males (25.7 ± 14.2 behaviors of 48.3 ± 30.5 during the whole 30-min trials). Therefore, we decided to report data on frequencies and duration of behaviors for the initial 10 min of trials.

Female interest to each odor/male was tested by comparing each behavior with Paired-sample t-test or its non-parametric analog, if assumptions were not met (Zar, 2010). Results were reported as means ± SD. In all cases, the critical significance level was set at $p \leq 0.05$. Along with the exact p values, the observed effect sizes according to the suggestions of Nakagawa (2004) and Stoehr (1999) for behavioral sciences were reported. Cohen (1992) defined effect sizes as 'a scale-free value that measures, in terms appropriate to it, the discrepancy between H_0 and the H'_1. Cohen (1992) proposed the benchmark values for what are considered to be 'small', 'medium' and 'large' effects, which are 0.2, 0.5 and 0.8, respectively. We calculated effect size, as standardized Cohen's d_z, for paired samples using the free program GPower 3.1.1 (by F. Faul, Universität Kiel, Germany;
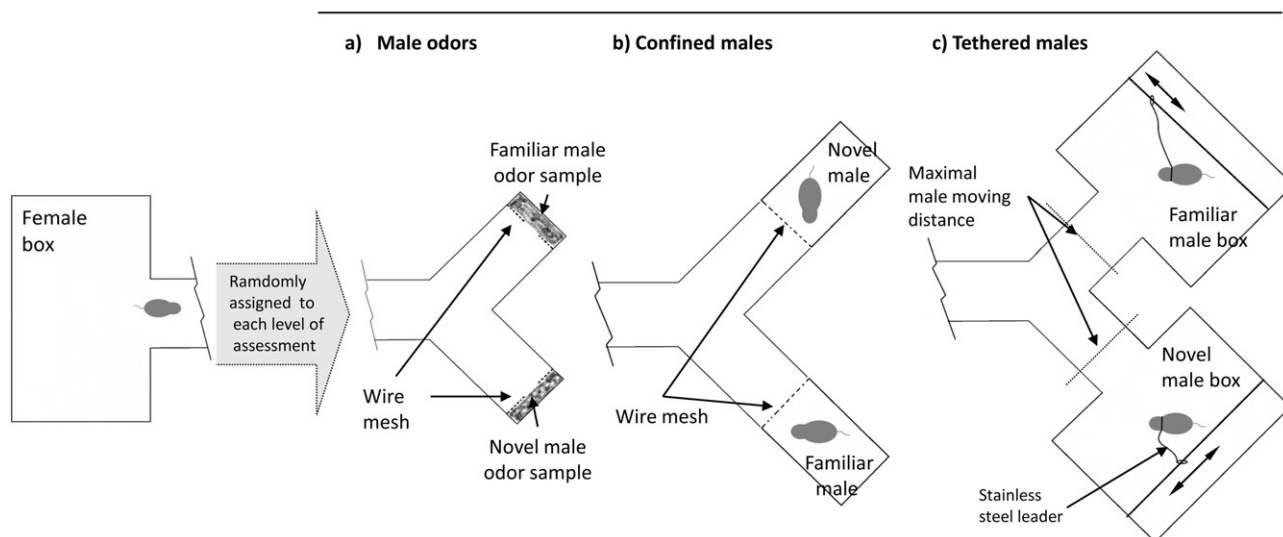
Preference tests

Fig. 1. Schematic representation of the Y-shaped mazes used for female preference trials for familiar and novel individuals (randomly assigned to each Y-maze arm) involving three levels of assessment (a) male odors, consisting of shavings soiled with urine, feces, and presumably other bodily secretions collected from males' housing cage ($n = 9$), (b) confined males through a wire mesh ($n = 11$), and (c) full contact with tethered males ($n = 14$). Female becomes familiar to a male by being exposed to their odor during 4 days previous to preference trial.

http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/) (McDonald, 2009).

2.4. Ethical note

We adhered to the guidelines for the Use of Animals in Research and Teaching (ASAB/ABS 2003). The use of collars did not affect the exploring behavior of males, which interacted with the choosing females and even mated them.

3. Results

3.1. Preference of male odors

Females visited each arm of the maze in the same frequency (familiar males: 6.2 ± 2.4, novel males: 6.4 ± 1.8; Paired t-test: $t = -0.29$, $df = 8$, $p = 0.77$, Cohen's $d_z = 0.09$). However, females devoted more time in sniffing novel male odors from soiled shavings (familiar males: 81.1 ± 67.1 s, novel males: 203.7 ± 130.9 s; Paired t-test: $t = -2.45$, df = 8, $p = 0.03$, Cohen's $d_z = 1.06$).

3.2. Preference of confined males

Females visited each arm of the maze in the same frequency (familiar males: 7 ± 4.7, novel males 6.7 ± 4.4; Paired t-test: $t = 0.38$, $df = 10$, $p = 0.71$, Cohen's $d_z = 0.17$). However, females explored longer the arm occupied by novel males than that occupied by familiar ones (familiar males: 103.1 ± 87.6 s, novel males: 248.6 ± 179 s; Paired t-test: $t = -2.26$, $df = 10$, $p = 0.04$, Cohen's $d_z = 0.72$). However, no significant differences were detected for the frequencies in which females sniffed the two offered males through the mesh and by its soiled shavings (Fig. 2; Paired t-test: $t = -1.12$, $df = 11$, $p = 0.28$, Cohen's $d_z = 0.27$). Females displayed soliciting behaviors toward novel males more frequently than toward familiar males (Fig. 2; Paired t-test: $t = -2.19$, df = 11, $p = 0.05$, Cohen's $d_z = 0.66$).

3.3. Preference of tethered males

Females visited each box in the same frequency (familiar males: 5.4 ± 2.1 visits, novel males: 5.5 ± 2.5 visits; Wilcoxon matched-pair test: $T = 23$, $df = 13$, $p = 0.54$, Cohen's $d_z = 0.62$). However,

Table 1
Description of behaviors recorded for female preference for familiar and novel confined or tethered males.

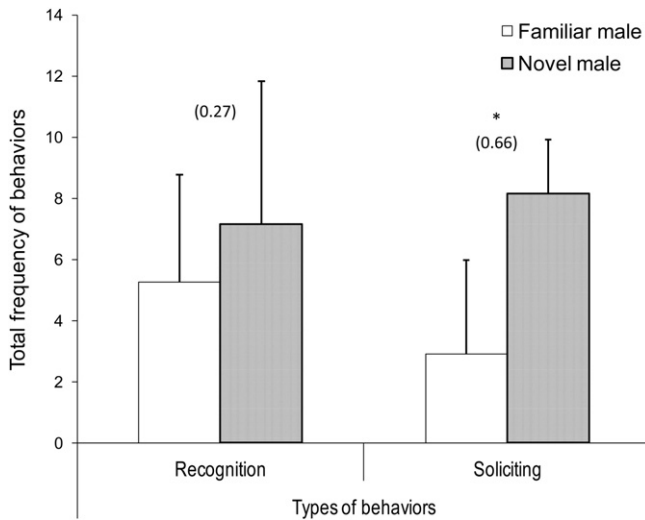| Type of behaviorBehavior	Description	Preference trial
Recognition		
Sniff male	Female sniffs male, mainly hindquarters or genitalia	Confined males/tethered males
Sniff shavings	Female sniffs soiled shavings obtained from male cage	Confined males
Aggression		
Show incisors	Female raises head and shows incisors	Tethered males
Soliciting		
Snout push	Female pushes flank of partner, promoting close contact	Tethered males
Expose neck	Female exposes neck to male who bites it smoothly	Tethered males
Expose rump	Female shows rump when encounters male	Confined males/tethered males
Raise tail	Female raises tail exposing genitalia to male	Tethered males
Female mount	Female mounts male.	Tethered males
Mutual courtship		
Spin	Male and female sniff ano-genital area and/or attempt to mount each other at same time resulting in circling movement	Tethered males
Male mount	Male mounts female tightly biting her neck	Tethered males
Copulation		Tethered males

Fig. 2. Mean frequency of types of behaviors (±SD) displayed by tuco-tuco females at the arms of the Y-maze where familiar and novel males were confined through a wire mesh. Effect sizes' d_z (within parenthesis) are reported for each Paired *t*-tests or its non-parametric equivalent (Wilcoxon matched-pair tests) when assumptions were not met ($n = 11$). *$p \leq 0.05$; **$p \leq 0.01$.

females spent more time investigating boxes belonging to novel males than familiarized males (familiar males: 124.6 ± 54.4 s, novel males: 218 ± 126 s; Paired *t*-test: $t = -2.29$, $df = 13$, $p = 0.03$, Cohen's $d_z = 0.77$). Even though females sniffed (recognition behavior) the novel male more frequently than they did familiar ones, such difference was not statistically significant (Fig. 3; Paired *t*-test: $t = -1.43$, $df = 13$, $p = 0.17$, Cohen's $d_z = 0.45$). Females showed their incisors (aggression) more frequently to novel males than to familiar males (Fig. 3; Paired *t*-test: $t = -2.51$, $df = 11$, $p = 0.02$, Cohen's $d_z = 0.72$). Females displayed soliciting behaviors to novel males more frequently than they did to familiar males (Fig. 3; Paired *t*-test: $t = -3.81$, $df = 14$, $p = 0.002$, Cohen's $d_z = 1.458$). Courtship behaviors with novel males were more frequent (Fig. 3; Wilcoxon matched-pair test: $T = 33.50$, $df = 14$, $p = 0.02$, Cohen's $d_z = 0.60$). Copulation was recorded in 3 couples during the whole 30 min trials, in which 2 females mated the novel male and one female

mated both the novel and the familiar male (Fig. 3; Wilcoxon matched-pair test: $T = 3$, $df = 14$, $p = 0.5$, Cohen's $d_z = 0.29$).

4. Discussion

Our findings in the solitary and polygynous subterranean rodent *C. talarum* show that females prefer novel tuco-tucos at three levels of male assessment: odor samples, confined males behind a wire mesh, and full contact with tethered males. Proximity or spatial association with males is often used as a measure of mating preference (Thom et al., 2004); however some authors consider that spatial association represents social preferences, not always a reliable indicator of mating preference (Gubernick and Addington, 1994; Castelli et al., 2011; Chesh et al., 2012). In this sense, it is recognized that some females need the opportunity to associate with several males until mating or are reluctant to mate until the pair bond is well established (Gubernick and Addington, 1994). In part, this may be the reason why female preferences showed in laboratory conditions do not result in similar quantities of copulations. However, our experimental approach includes female odor investigation, social preferences and also the display of soliciting behaviors. Experiments were performed in captivity but during their breeding season, when females are searching for males in the wild. As shown in *Micromys minutus*, females react differently toward familiar or novel males according to their oestrus condition (Brandt and Macdonald, 2011). Nonetheless, in our study, some females did not show reproductive interest on offered males. That could have been be due to the fact that females of *C. talarum* are copulation-induced ovulators and their reproductive behavior is variable according to hormonal status (Fanjul and Zenuto, 2012).

In the present study, females investigated both familiar and novel male odor samples, but spent significantly more time investigating novel ones. For *C. talarum*, as it is common for mammals (Cheetham et al., 2008), relatively long times are usually devoted to investigate novel odors (Zenuto and Fanjul, 2002). Animals usually spend time investigating novel cues, but later reduce their interest in future encounters since most of the available information has been obtained; this is the basis of the habituation–dishabituation test (Gregg and Thiessen, 1981). Hence, the sole assessment of males by means of their odors is not enough to distinguish that females prefer novel males. Using confined males, females explored both males but devoted more time in association with novel ones.
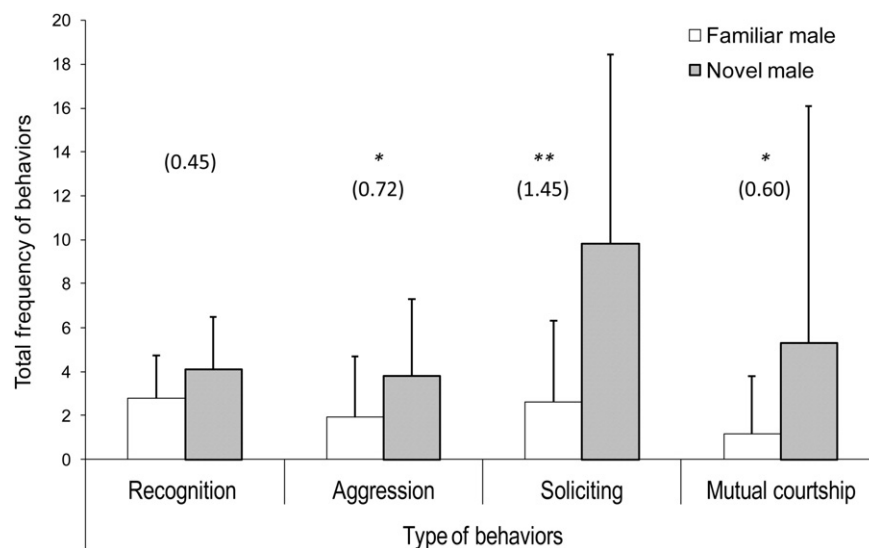

Fig. 3. Mean frequency of types of behaviors (±SD) displayed by tucos-tuco females toward familiar or novel tethered males. Effect sizes' d_z (within parenthesis) are reported for each Paired *t*-test or its non-parametric equivalent (Wilcoxon matched-pair tests) if assumptions were not met ($n = 14$). *$p \leq 0.05$; **$p \leq 0.01$.

Table 2
Summary of female preferences for familiar or novel males in previous studies of rodent species.

Species	Social organization	Mating system	Female preference	Reference
Fam. Cricetidae				
Myodes glareolus (bank vole)	Social	Pr	Novel males (TI) No increased aggre to novel males	Kozakiewicz et al. (2009)
Fam. Muridae				
Dicrostonyx groenlandicus (collared lemming)	Solitary-social	Po	Familiar males (O), (FI)[a] + Aggre to novel males	Huck and Banks (1979)
Microtus pennsilvanicus (meadow vole)	Solitary	Po/Pr	Familiar males (TI) + Aggre to novel males	Parker et al. (2001)
Microtus ochrogaster (praire vole)	Pairs	Mo	Familiar males (O) Familiar males (TI) Aggre not reported	Newman and Tang-Halpin (1988) Shapiro et al. (1986)
Microtus montanus (montane vole)	Solitary	Po	Novel (TI) Aggre not reported	Shapiro et al. (1986)
Microtus arvalis (common vole)	Social	Mo?	Familiar males (TI) + Aggre novel males (TI)	Richankova et al. (2007)
Peromyscus californicus (california mouse)	Pairs	Mo	Familiar males (TI) Aggre not reported	Gubernick and Addington (1994)
Mus spicilegus (mound-builder mice)	Pairs	Mo	Familiar males (CI)	Patris and Baudoin (1998)
Mus musculus domesticus (house mouse)	Social	Po/Pr	Novel (CI)	Patris and Baudoin (1998)
M. m. domesticus (non comensal house mice)	Social	Po/Pr?	Novel (CI)	Frynta et al. (2010)
M. musculus musculus (comensal house mice)	Social	Po/Pr	Novel (CI)	Frynta et al. (2010)
Mesocricetus auratus (golden hamster)	Solitary	Pr	Familiar males (FI)[a] + Aggre to novel males	Tang-Martinez et al. (1993)
Micromys minutus (harvest mice)	Solitary	Pr?	Familiar (0) No preference (CI) – Aggre familiar males (FI)[a] No preference (O) Familiar males (TI) Novel males (TI)1 Aggre not reported	Roberts and Gosling (2004) Brandt and Macdonald (2011)
Fam. Heteromyidae				
Dipodomys ingens (kangaroo rat)	Solitary	Mo/Po/Pr	Familiar males (FI) – Aggre to familiar males	Randall et al. (2002)
Dipodomys heermanni (kangaroo rat)	Solitary	Po	Familiar males (FI)[a] – Aggre to familiar males	Thompson et al. (1995)
Fam. Bathyergidae				
Heterocephalus glaber (naked mole-rat)	Eusocial	Mo/Pa	No preference (O) Novel males (CI) (FI[b]) Aggre not reported	Clarke and Faulkes (1999)
Fam. Ctenomyidae				
Ctenomys talarum (talas tuco-tuco)	Solitary	Po	Novel males (FI)[a] – Aggre to familiar males (FI)[a] Novel Males (O), (CI), (TI) – Aggre to familiar males	Zenuto et al. (2007) This study

Po: polygynous, Pr: promiscuos, Mo: monogamous, Pa: polyandrous, O: Experiments concerned females choosing male odors. FI: Experiments concerned females choosing free-moving male individuals. CI: Experiments concerned females choosing confined male individuals. TI: Experiments concerned females choosing tethered male individuals.
1: non oestrus females. +/– Aggre: more/less aggressive (for experiments involving contact with individuals; i.e. FI or TI).

[a] Females interacting with single males separately.
[b] Females interacting with multiple males in a colony.

Also, females scratched and bit the mesh that separated the males, probably with the aim of contacting them. Male presence, although confined, may contribute in eliciting female interest to assess male quality. Furthermore, other sources of mate quality assessment than familiarity by odors, such as visual and/or auditive signals, may mask female preference for familiar or novel partners. When females interacted with tethered males, they visited and sniffed equally both kinds of males, but showed more soliciting behaviors toward novel males. In the present study, females had unlimited access to all cages while the special collars worn by males of *C. talarum* prevented them from leaving their cages, reducing the effects of male competition and male coercion as confounding factors in mate choice (Clutton-Brock and McAuliffe, 2009).

4.1. Familiarity: decreased aggression between neighbor or proxy of male quality?

Individuals of *Ctenomys talarum* are solitary and live underground in adjacent tunnel systems. Limited above-ground activity constrains the occurrence of encounters between individuals, but available odor cues deposited around the openings of the tunnels may allow neighbor recognition, even in absence of the sender. Hence, familiarity by odor cues may serve to recognize neighbors and to display appropriate behaviors to specific individuals during interactions with conspecifics. Preference for familiar individuals (Table 2) is often accompanied by lower aggression toward neighbors, to whom females are usually familiarized by means of odors or short individual encounters, a fact that is expected as an extension of dear enemy phenomenon if territories are stable (Temeles, 1994). Using free-moving males interacting with females (Zenuto et al., 2007), low aggression displayed by females toward familiar males was detected; mainly involving aggressive behaviors that represented low risk of injury (the female flees from the male or presented their rump). In the two-choice preference test, males were restricted in their movements, so females modulated their reproductive behavior much more than their aggressive behavior, simply assessing males and leaving them if they were not interested.

As already seen, familiarity is related with the capacity of individuals to maintain their scents in their territory (Hurst and Rich,

1999). Thus, female preference for familiar males is often related to obtaining direct (e.g. resources for the female and/or offspring) and indirect benefits (e.g. good genes for the progeny) by copulating with high quality males. Preference for familiar males is clear for monogamous species, but is also found in those being solitary or showing some pattern of association between sexes with high variability in their mating systems (Table 2). Females of *C. talarum* did not increase their reproductive behavior when encountering familiar males, but showed interest when they did so with novel males, even if these encounters were more aggressive (Zenuto et al., 2007). When both kinds of males were offered simultaneously in a two-choice experiment and females were able to display their preferences without male coercion risk, we confirmed that they showed clear interest in novel males, displaying more soliciting behaviors toward them.

4.2. Novelty: inbreeding avoidance?

Preference for novel individuals is often found in species under high risk of inbreeding such as those showing group-living social systems (Table 2); for these species, sex-biased dispersal, avoidance of kin as mates, and extra-group copulations contribute to minimize inbreeding (Pusey and Wolf, 1996). Furthermore, familiarity is used as a mechanism of kin avoidance of mate (Cheetham et al., 2008; Lucia and Keane, 2012). Inbreeding avoidance may be considered as a factor influencing mating preferences in females of *C. talarum*. Both sexes disperse (Malizia et al., 1995) but it seems that males move longer distances than females since DNA fingerprinting analyses revealed higher band-sharing values among females than among males (Zenuto et al., 1999a). Concomitantly, a pronounced genetic structure was described at a local geographic scale (Cutrera et al., 2005). This fact may be a consequence of a polygynous mating system, where males exert male-male competition and female coercion during mate acquisition (Zenuto, 1999; Zenuto et al., 2002a). Under this scenario, it is expected that females benefit looking for mates carrying novel genetic combinations (Tregenza and Wedell, 2000). However, a recent study on this species (Cutrera et al., 2012), where major histocompatibility complex-based mate choice was assessed, reported that females did not show preferences for males carrying dissimilar genotypes, a strategy which is expected when inbreeding avoidance is important. In contrast to expectations, males carrying specific rare alleles or MHC heterozygous seemed to be preferred by females under laboratory and field conditions. As shown in other studies (Eizaguirre et al., 2009), the more dissimilar mate may not always represents the best choice. Indeed, intermediate levels of dissimilarity implies the double advantage of retaining useful genetic combinations plus the benefit of new ones, a strategy expected in species like *C. talarum*, in which parasitism represents a moderate selective pressure (Rossin and Malizia, 2002; Cutrera et al., 2011).

4.3. Conclusions

Studies of familiarity affecting reproductive behavior in rodents are not abundant and most of them only tested one level of male assessment (Table 2). As discussed above, odor preferences for novel males are of limited value. The use of confined males, and even more, tethered males, allow females for a more complete assessment of male quality with the benefit of avoiding their aggression. In this way, female preferences and their reproductive tactics could be identified. However, the interplay between both male and female choice occurs in the field, and females may have to trade benefits identified in a particular potential mate against the risk of injury to themselves or their offspring (Ophir and Galef, 2003). The structure of mammalian breeding systems and the prevalence of male coercive tactics lead to a frequent underestimation of the impact of female mate preference in the mating system and each sex reproductive strategies (Clutton-Brock and McAuliffe, 2009). Consequently, there is a common perception that female mate choice is less developed, and at the same time is less related to genetic benefits for the offspring in mammals than in birds (Clutton-Brock and McAuliffe, 2009). Such vision could be modified when more information on each sex mating tactics obtained from both laboratory and field studies were combined.

Data from studies in wild populations of *C. talarum*, showed that offsprings in this species are fathered by one male that inhabit a burrow system located close to the mother's burrow (Zenuto, 1999). Also, behavioral interactions registered in laboratory enclosures resembling the natural complexity of burrows and female skewed sex ratio in wild populations, showed that dominant males monopolize the reproductive activity after aggressively deterring subordinated males (Zenuto et al., 2002a). Hence, male-male competition and male coercion severely limits the possibility of females to mate non-neighbors, novel males. Females mating those territorial, highly competitive males, may obtain high quality mating with the additional benefit that, being neighbors, low aggression is expected during the encounter. Nonetheless, when females have the opportunity to exert preferences by soliciting mating, as shown in the present study, they preferred novel males, emphasising that costs and benefits of choosing particular categories of males may differ in individuals under diverse captive or wild conditions. Hence, combining evidences from controlled conditions in the laboratory and field studies it is possible to better understand individual reproductive strategies in *C. talarum*.

References

Andersson, M., 1994. Sexual Selection. Princeton University Press, Princeton, NJ.
Begall, S., Burda, H., Schleich, C., 2007. Subterranean Rodents, News from Underground. Springer, Germany.
Brandt, R., Macdonald, D.W., 2011. To know him is to love him? Familiarity and female preference in the harvest mouse, *Micromys minutus*. Anim. Behav. 82, 353–358.
Busch, C., Malizia, A.I., Scaglia, O.A., Reig, O.A., 1989. Spatial distribution and attributes of a population of *Ctenomys talarum* (Rodentia: Octodontidae). J. Mammal. 70, 204–208.
Busch, C., Antinuchi, C.D., Del Valle, J.C., Kittlein, M.J., Malizia, A.I., Vassallo, A.I., Zenuto, R.R., 2000. Population ecology of subterranean rodents. In: Lacey, E.A., Patton, J.L., Cameron, G.N. (Eds.), Life Underground: The Biology of Subterranean Rodents. The University of Chicago Press, Chicago, IL, pp. 183–226.
Candolin, U., 2003. The use of multiple cues in mate choice. Biol. Rev. Camb. Philos. Soc. 78, 575–595.
Castelli, F.R., Kelley, R., Keane, B., Solomon, N.G., 2011. Female prairie voles show social and sexual preferences for males with longer *avpr1a* microsatellite alleles. Anim. Behav. 82, 1117–1126.
Cheetham, S.A., Thom, M.D., Beynon, R.J., Hurst, J.L., 2008. The effect of familiarity on mate choice. In: Hurst, J.L., Beynon, R.J., Roberts, S.C., Wyatt, T. (Eds.), Chemical Signals in Vertebrates, vol. 11. Springer Science+Business Media, New York, USA, pp. 271–280.
Chesh, A.S., Mabry, K.E., Keane, B., Noe, D.A., Solomon, N.G., 2012. Are body mass and parasite load related to social partnerships and mating in *Microtus ochrogaster*. J. Mammal. 93, 229–238.
Clarke, F.M., Faulkes, C.G., 1999. Kin discrimination and female choice in the naked mole-rat *Heterocephalus glaber*. Proc. R. Soc. Lond. B: Biol. Sci. 266, 1995–2002.
Clutton-Brock, T., McAuliffe, K., 2009. Female mate choice in mammals. Q. Rev. Biol. 84, 3–27.
Cohen, J., 1992. A power primer. Psychol. Bull. 112, 155–159.
Colombelli-Negrel, D., Gout, P., 2006. Male and female mound-building mice, *Mus spicilegus* discriminate dietary and individual odours of conspecifics. Anim. Behav. 72, 577–583.

Cutrera, A.P., Lacey, E.A., Busch, C., 2005. Genetic structure in a solitary rodent (*Ctenomys talarum*): implications for kinship and dispersal. Mol. Ecol. 14, 2511–2523.

Cutrera, A.P., Zenuto, R.R., Lacey, E.A., 2011. MHC variation, multiple simultaneous infections and physiological condition in the subterranean rodent *Ctenomys talarum*. Inf. Gen. Evol. 11, 1023–1036.

Cutrera, A.P., Fanjul, M.S., Zenuto, R.R., 2012. Females prefer good genes: MHC-based mate choice in wild and captive tuco-tucos. Anim. Behav. 83, 847–856.

Eisenberg, J.F., Kleiman, D.G., 1972. Olfactory communication in mammals. Ann. Rev. Ecol. Syst. 3, 1–32.

Eizaguirre, C., Yeates, S.E., Lenz, T.L., Kalbe, M., Milinski, M., 2009. MHC-based mate choice combines good genes and maintenance of MHC polymorphism. Mol. Ecol. 18, 3316–3329.

Fanjul, M.S., Zenuto, R.R., Busch, C., 2006. Seasonality of breeding in wild tuco-tucos *Ctenomys talarum* in relation to climate and food availability. Acta Theriol. 51 (3), 283–293.

Fanjul, M.S., Zenuto, R.R., 2008a. Reproductive responses to photoperiod of females tuco-tucos (*Ctenomys talarum*). Acta Theriol. 53, 73–85.

Fanjul, M.S., Zenuto, R.R., 2008b. Copulatory pattern of the subterranean rodent *Ctenomys talarum*. Mammalia 72, 102–108.

Fanjul, M.S., Zenuto, R.R., 2012. Female reproductive behaviour, ovarian hormones and vaginal cytology of the induced ovulator, *Ctenomys talarum*. Acta Theriol. 57, 15–27.

Fanjul, M.S., Zenuto, R.R., Busch, C., 2003. Use of olfaction for sexual recognition in the subterranean rodent *Ctenomys talarum*. Acta Theriol. 148, 35–46.

Ferkin, M.H., Sorokin, E.S., Johnston, R.E., Lee, C.J., 1997. Attractiveness of scents varies with protein content of the diet in meadow voles. Anim. Behav. 53, 133–141.

Francescoli, G., 2000. Sensory capabilities and communication. In: Lacey, E.A., Patton, J.L., Cameron, G.N. (Eds.), Life Underground: The Biology of Subterranean Rodents. The University of Chicago Press, Chicago, pp. 111–144.

Frynta, D., Volfova, R., Francova-Novakova, M., Stejskal, V., 2010. Oestrous females investigate the unfamiliar male more than the familiar male in both commensal and non-commmensal populations of house mice. Behav. Proc. 83, 54–60.

Gosling, L.M., 1990. Scent-marking by resource holders: alternative mechanisms for advertising the costs of competition. In: MacDonald, D.W., Muller-Schwarze, D., Natynczuk, S.E. (Eds.), Chemical Signals in Vertebrates, vol. 5. Oxford University Press, pp. 315–358.

Gosling, L.M., Roberts, S.C., 2001. Testing ideas about the function of scent marks in territories from spatial patterns. Anim. Behav. 62, F7–F10.

Gregg, B., Thiessen, D.D., 1981. A simple method of olfactory discrimination of urines for the Mongolian gerbil, *Meriones unguiculatus*. Physiol. Behav. 26 (6), 1133–1136.

Gubernick, D.J., Addington, R.L., 1994. The stability of female social and mating preferences in the monogamous California mouse, *Peromyscus californicus*. Anim. Behav. 47, 567–599.

Holmes, W.G., Mateo, J.M., 2007. Kin recognition in rodents—critical issues, empirical evidence and model systems. In: Wolff, J.O., Sherman, P.W. (Eds.), Rodent Societies: An Ecological and Evolutionary Perspective. The University of Chicago Press, Chicago, USA, pp. 216–228.

Huck., U.W., Banks, E.M., 1979. Behavioral components of individual recognition in the collared lemming, *Dicrostonyx groenlandicus*. Behav. Ecol. Sociobiol. 6, 85–90.

Hurst, J.L., Rich, T.J., 1999. Scent marks as competitive signals of mate quality. In: Johnston, R.E., Müller-Schwarze, D., Sorensen, P. (Eds.), Advances in Chemical Communication in Vertebrates. Kluwer Academic, NY, pp. 209–223.

Hurst, J.L., 2009. Female recognition and assessment of males through scent. Behav. Brain Res. 200, 295–303.

Hyman, J., 2005. Seasonal variation in response to neighbors and strangers by a territorial songbird. Ethology 111, 951–961.

Johansson, B.G., Jones, T.M., 2007. The role of chemical communication in mate choice. Biol. Rev. 82, 265–289.

Johnston, R.E., 2003. Chemical communication in rodents: from pheromones to individual recognition. J. Mammal. 84, 1141–1162.

Kozakiewicz, M., Choluj, A., Kozakiewicz, A., Sokol, M., 2009. Familiarity and female choice in the bank vole—do females prefer stangers? Acta Theriol. 54, 157–164.

Lachish, S., Goldizen, A.W., 2004. Responses to neighbours and non-neighbours in the buff-banded rail (*Gallirallus philippensis*): no dear–enemy relationships. Aust. J. Zool. 52, 369–378.

Lacey, E.A., Patton, J.L., Cameron, G.N., 2000. Life Underground: The Biology of Subterranean Rodents. Univ. of Chicago Press, Chicago.

Lucia, K.E., Keane, B., 2012. A field test of the effects of familiarity and relatedness on social associations and reproduction in prairie voles. Behav. Ecol. Sociobiol. 66, 13–27.

McDonald, J.H., 2009. Handbook of Biological Statistics, 2nd ed. Sparky House Publishing, Baltimore, MD.

Malizia, A.I., Busch, C., 1991. Reproductive parameters and growth in the fossorial rodent *Ctenomys talarum* (Rodentia: Octodontidae). Mammalia 55, 293–305.

Malizia, A.I., Zenuto, R.R., Busch, C., 1995. Demographic and reproductive attributes of dispersers in two populations of *Ctenomys talarum*. Can. J. Zool. 73, 732–738.

Mateo, J.M., 2002. Kin recognition abilities and nepotism as a function of sociality. Proc. R. Soc. Lond. B 269, 721–727.

Milinski, M., 2006. The major histocompatibility complex, sexual selection and mate choice. Ann. Rev. Ecol. Evol. Syst 37, 159–186.

Nakagawa, S., 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. Behav. Ecol. 15, 1044–1045.

Nevo, E., 1999. Mosaic Evolution of Subterranean Mammals: Regression, Progression, and Global Convergence. Oxford University Press, Oxford.

Newman, K.S., Tang-Halpin, Z., 1988. Individual odors and mate recognition in the prairie vole, *Microtus ochrogaster*. Anim. Behav. 36, 1779–1787.

Ophir, A.G., Galef, B.G., 2003. Female Japanese quail that eavesdrop on fighting males prefer losers to winners. Anim. Behav. 66, 399–407.

Patris, B., Baudoin, C., 1998. Female sexual preferences differ in *Mus spicilegus* and *Mus musculus domesticus*: the role of familiarization and sexual experience. Anim. Behav. 56, 1465–1470.

Parker, K.J., Phillips, K.M., Lee, T.M., 2001. Development of selective preferences in captive male and female meadow voles, *Microtus pennysilvanicus*. Anim. Behav. 61, 1217–1226.

Pusey, A., Wolf, M., 1996. Inbreeding avoidance in animals. TREE 11, 201–206.

Randall, J.A., Hekkala, E.R., Cooper, L.D., Barfield, J., 2002. Familiarity and flexible mating strategies of a solitary rodent, *Dipodomys ingens*. Anim. Behav. 64, 11–21.

Reig, O.A., Busch, C., Ortells, M.O., Contreras, J.R., 1990. An overview of evolution, systematics, population biology, cytogenetics, molecular biology, and speciations in *Ctenomys*. In: Nevo, E., Reig, O.A. (Eds.), Evolution of Subterranean Mammals at the Organismal and Molecular Levels. Wiley-Liss, New York, pp. 71–96.

Rich, T.J., Hurst, J.L., 1998. Scent marks as reliable signals of the competitive ability of mates. Anim. Behav. 56, 727–735.

Richankova, V., Sumbera, R., Sedlacek, F., 2007. Familiarity and partner preferences in female commons, *Microtus arvalis*. J. Ethol. 25, 95–98.

Rossin, M.A., Malizia, A.I., 2002. Relationship between helminth parasites and demographic attributes of a population of the subterranean rodent *Ctenomys talarum* (Rodentia: Octodontidae). J. Parasitol. 88, 1268–1270.

Roberts, S.C., Gosling, L.M., 2004. Manipulation of olfactory signalling and mate choice for conservation breeding: a case study of the harvest mouse. Conserv. Biol. 18, 548–556.

Shapiro, L.E., Austin, D., Ward, S.E., Dewsbury, D.A., 1986. Familiarity and mate choice in two species of voles (*Microtus orchrogaster* and *Microtus montanus*). Anim. Behav. 34, 90–97.

Stoehr, A., 1999. Are significance thresholds appropriate for the study of animal behaviour? Anim. Behav. 57, F22–F25.

Tang-Martinez, Z., Mueller, L.L., Taylor, G.T., 1993. Individual odors and mating success in the golden hamster *Mesocricetus auratus*. Anim. Behav. 45, 1141–1151.

Temeles, E.J., 1994. The role of neighbors in territorial systems: when are they dear enemies? Anim. Behav. 47, 339–350.

Thom, M.D., Macdonald, D.W., Mason, G.J., Pedersen, V., Johnson, P.J., 2004. Female American mink (*Mustela vison*) mate multiply in a free-choice environment. Anim. Behav. 67, 975–984.

Thom, M.D., Hurst, J.L., 2004. Individual recognition by scent. Ann. Zool. Fenn. 41 (6), 765–787.

Thom, M.D., Stockley, P., Beynon, R.J., Hurst, J.L., 2008. Scent, mate choice and genetic heterozygosity. In: Hurst, J.L., Beynon, R.J., Roberts, S.C., Wyatt, T. (Eds.), Chemical Signals in Vertebrates, vol. 11. Springer Science+ Business Media, New York, USA, pp. 271–280.

Thompson, K.V., Roberts, M., Rall, W.F., 1995. Factors affecting pair compatibility in captive kangaroo rats, *Dipodomys heermanni*. Zoo Biol. 14, 317–330.

Todrank, J., Heth, G., 2003. Odor-genes covariance and genetic relatedness assessments: rethinking odor-based "recognition" mechanisms in rodents. Adv. Study Behav. 32, 77–130.

Tregenza, T., Wedell, N., 2000. Genetic compatibility, mate choice and patterns of parentage. Mol. Ecol. 9, 1013–1027.

Woods, C.A., 1993. Suborder Hystricognathi. In: Wilson, D.E., Reeder, D.M. (Eds.), Mammal Species of the World: A Taxonomic and Geographic Reference. Smithsonian Institution Press, Washington, DC, pp. 771–806.

Zar, J.H., 2010. Biostatistical Analysis, 5th ed. Prentice Hall, New Jersey.

Zenuto, R.R., 1999. Sistema de apareamiento en *Ctenomys talarum* (Rodentia: Octodontidae). Doctoral thesis. Universidad Nacional de Mar del Plata, Argentina.

Zenuto, R.R., 2010. Dear enemy relationships in the subterranean rodent *Ctenomys talarum*: the role of memory of individual odours. Anim. Behav. 79, 1247–1255.

Zenuto, R.R., Fanjul, M.S., 2002. Olfactory discrimination of individual scents in the subterranean rodent *Ctenomys talarum* (tuco-tuco). Ethology 108, 629–641.

Zenuto, R.R., Lacey, E.A., Busch, C., 1999a. DNA fingerprinting reveals polygyny in the subterranean rodent *Ctenomys talarum*. Mol. Ecol. 8, 1529–1532.

Zenuto, R.R., Malizia, A.I., Busch, C., 1999b. Sexual size dimorphism, relative testes size and mating system in two populations of *Ctenomys talarum* (Rodentia: Octodontidae). J. Nat. Hist. 33, 305–314.

Zenuto, R.R., Vasallo, A.I., Busch, C., 2002a. Comportamiento social y reproductivo del roedor subterráneo solitario *Ctenomys talarum* (Rodentia: Ctenomydae) en condiciones de semicautiverio. Rev. Chil. Hist. Nat. 75, 165–177.

Zenuto, R.R., Antinuchi, C.D., Busch, C., 2002b. Bioenergetics of reproduction and strategy of pup development in the subterranean rodent *Ctenomys talarum*. Physiol. Biochem. Zool. 45, 469–478.

Zenuto, R.R., Fanjul, M.S., Busch, C., 2004. Subterranean rodent *Ctenomys talarum* (tuco-tuco) use chemical communication during breeding season. J. Chem. Ecol. 30, 2111–2126.

Zenuto, R.R., Estavillo, C., Fanjul, M.S., 2007. Familiarity and mating behaviour in the subterranean rodent *Ctenomys talarum* (tuco-tuco). Can. J. Zool. 85, 944–955.