Singing, allogrooming, and allomarking behaviour during inter- and intra-sexual encounters in the Neotropical short-tailed singing mouse \( (Scotinomys teguina) \)

M. Fernández-Vargas\(^1,3)\), Z. Tang-Martínez\(^1)\) & S.M. Phelps\(^2,4)\)

\(^1\) Department of Biology, University of Missouri-St. Louis, St. Louis, MO 63121, USA; \(^2\) Department of Biology, University of Florida-Gainesville, Gainesville, FL 32611, USA

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Summary

In this study we determine whether brief interactions with unfamiliar conspecifics stimulate audible singing behaviour in the Neotropical short-tailed singing mouse \( (Scotinomys teguina) \). Specifically, we examine whether intra- or inter-sexual interactions elicit singing in males in a neutral-arena design. We conducted two experiments. In experiment 1, we recorded singing behaviour of male subjects both before and after a brief exposure to a female mouse. Males significantly increased their singing behaviour after the exposure to the female, as compared to prior to the exposure. In experiment 2, we compared the singing behaviour of male test subjects after a brief exposure with one of three different treatment animals: a male, a non-oestrous female and an oestrous female. We found that males are most likely to sing after an interaction with a female, regardless of her reproductive condition. Male subjects sang significantly less following an interaction with another male. Although spontaneous singing is known to occur in males and females, opposite sex elicited-singing behaviour was found to be sexually dimorphic. An interaction with a male was not effective in eliciting singing in females. In experiment 2, we also recorded incidences of allogrooming and allomarking by males during the interactions with males, non-oestrous females, and oestrous females. Male allogrooming and allomarking behaviours using the mid-ventral sebaceous gland tend to occur more frequently during interactions with females as compared to males, but were significantly different only in the case of allogrooming. Thus, this study clearly suggests sex

\(^3\) Corresponding author’s current address: Department of Psychology, Cornell University, Ithaca, NY 14853, USA, e-mail: mf463@cornell.edu

\(^4\) Current address: Section of Integrative Biology, University of Texas, Austin, TX 78712, USA.
differences in singing, allogrooming and allomarking, and a likely relationship between these behaviours and courtship in this Neotropical rodent.

Keywords: audible singing, rodent, oestrus cycle, allogrooming, allomarking, Neotropical short-tailed singing mouse, Scotinomys teguina.

1. Introduction

Bird and anuran vocalizations are among the most studied animal signals in the field of animal communication. Rodent vocalizations, although less often studied, show a wide frequency spread, including both audible and ultrasonic components, during different contexts (Sales, 1972a,b; Brown, 1976; Portfors, 2007). Currently, there is more information on ultrasonic than on sonic (audible) vocalizations. Ultrasonic vocalizations (USV) have been studied in infant rodents (e.g., Brudzynski et al., 1999; Blake, 2002; Hashimoto et al., 2004; Sales, 2010; Grimsley et al., 2011) and in laboratory-bred adult rats (Rattus norvegicus), house mice (Mus musculus), and golden hamsters (Mesocricetus auratus) (e.g., Sales, 1972a,b, 2010; Nyby et al., 1977a,b; Floody et al., 1977; Floody & Pfaff, 1977a,b; White et al., 1990; Holy & Guo, 2005; Scattoni et al., 2009), and, to a lesser extent, in wild species such as deer mice, Peromyscus spp. (Kalcounis-Rueppell et al., 2006), voles, Microtus spp. (Pierce et al., 1989; Kapusta et al., 2007; Kapusta & Sales, 2009) and recently the wild-derived house mouse (Musolf et al., 2010).

In general, male rodents tend to emit USV during sexual contexts. For example, such vocalizations may be used to attract mates or to facilitate copulatory activities (Cherry, 1989; Pierce et al., 1989; Musolf et al., 2010), and may also occur post-ejaculation (e.g., rats: Barfield & Geyer, 1972; White et al., 1990). Adult male rats also may vocalize ultrasonically in anticipation of social contact or sexual activity, and during agonistic encounters (Sales, 1972a; Haney & Miczek, 1993; Bialy et al., 2000; Brudzynski & Pniak, 2002). Likewise, in house mice, it appears that USV are typically related to courtship behaviours and sexual arousal (Whitney et al., 1973; Nyby et al., 1977a,b; Nyby, 1983; Holy & Guo, 2005; Musolf et al., 2010), and may also serve to inhibit female aggression (Whitney et al., 1973).

Females are also capable of producing USV. For example, female rats emit vocalizations at all stages of the oestrous cycle but greatly increase
Anecdotal reports of audible vocalizations in rodents go back for more than a century (Sidebotham, 1877; Dice, 1932). However, there is a dearth of studies on audible communication in rodents, with most of the few studies that do exist examining calls produced during agonistic encounters and distress, or as alarm calls during threatening situations (e.g., Shelley & Blumstein, 2004; Barros et al., 2010). With the exception of alarm calls in sciurids, only a relatively small number of species such as the grasshopper mouse (*Onychomys* spp.) (Hafner & Hafner, 1979), the Mexican harvest mouse (*Reithrodontomys mexicanus*) (Miller & Engstrom, 2010) and the Neotropical short-tailed singing mouse (*Scotinomys teguina*) (Hooper & Carleton, 1976; Miller & Engstrom, 2007; Crino et al., 2010; Pasch et al., 2011) have been studied for their audible vocalizations. For example, the most common vocalization type spontaneously emitted by the grasshopper mice vary among individuals, sex and species; suggesting a function in conspecific recognition (Hafner & Hafner, 1979). Moreover, given that audible calls in these rodents tend to be stereotyped, low in frequency, frequency modulated and emitted on a regular basis, it has been hypothesized that they could be used to maintain contact among individuals and facilitate location of the caller (Hafner & Hafner, 1979; Miller & Engstrom, 2007, 2010).

In this paper, we examine the context and possible functions of sonic (audible) communication in the short-tailed singing mouse (*S. teguina*), a cricetid rodent from the highlands of Central America. *Scotinomys teguina* emits sustained songs simultaneously containing both sonic and ultrasonic components (Hooper & Carleton, 1976; Miller & Engstrom, 2007). During
periods of highest activity in the laboratory, both males and females vocalize frequently and spontaneously (Hooper & Carleton, 1976). The song produced by *S. teguina* is modulated in frequency, amplitude and time (Miller & Engstrom, 2007). It is characterized by a repetitive number of notes (10–170 notes) with a whole song duration of 1–16 s. Each note has a broad range of frequencies from 8 to 50 kHz, beginning approximately in the 30–50 kHz range and falling to frequencies that the human ear can hear (approximately 8–20 kHz). A significant amount of the song’s energy is invested on this terminal audible spectrum of the note (see Figure 7 in Miller & Engstrom, 2007 for a spectrogram of a typical *S. teguina* song). Song variation has been demonstrated to be highly correlated with geographical and genetic distance, suggesting evolution by genetic drift (Campbell et al., 2010).

There is sexual dimorphism in the singing behaviour of *S. teguina*, particularly in the temporal characteristics of songs (Miller & Engstrom, 2007). Songs produced by males are approximately 40% longer than those produced by females (Miller & Engstrom, 2007) and males tend to spontaneously sing more frequently than females (Hooper & Carleton, 1976, pers. obs.). With regards to context, singing appears to be common after intersexual pairings (Hooper & Carleton, 1976). Therefore, although the exact context and function of singing behaviour are not known, a role in male sexual behaviour is suspected. In fact, in the absence of gonadal androgens in males, calling rate drastically decreases and the song becomes shorter, lower in power, higher in frequency and less stereotyped (Pasch et al., 2011). This pattern is consistent with a sexual function in normal males.

In addition to vocalizing, *S. teguina* also employs chemical communication, using the scent produced by a mid-ventral sebaceous gland (Fernández-Vargas et al., 2008). Allogrooming may result in allomarking by males, using the secretions of this scent gland. Allogrooming and allomarking can occur in social encounters and also may be part of this species’ courtship behaviour. Although the mating system of this species remains unknown, a promiscuous mating system has been suggested in the long-tailed singing mouse (*Scotinomys xerampelinus*), the most closely related species to *S. teguina* (Blondel et al., 2009).

Our study is based on the observations made by Hooper & Carleton (1976), who reported that males sing following an encounter with a female. Specifically, in this study we staged male-male and male-female dyadic encounters to investigate possible behavioural contexts in which singing takes
place. We also examined the effects of female reproductive condition on male singing. Thus, this study is the first to experimentally manipulate sexual contexts in an attempt to elucidate a possible function of audible singing in this species. In addition to investigating singing behaviour, we also recorded male allogrooming and allomarking behaviours during brief social encounters between males and females.

2. Materials and methods

2.1. Laboratory colony, housing and subjects

The *S. teguina* used in this study came from a colony housed at the University of Florida-Gainesville. This colony was started with wild *S. teguina* collected from Monteverde, Costa Rica in 2003. The animals used in this study were F4 and F5 generations in the laboratory. The use of live animals and the procedures used in these experiments were approved by the IACUCs of the University of Missouri-St. Louis and the University of Florida-Gainesville. All housing and experimental manipulations met legal standards of animal care and use.

The animals were housed in a climate-controlled environment with a constant temperature of 21±2°C and on a 12:12 L/D cycle, with lights on at 8:00 h EST. Animals were housed individually in glass terrariums (51 × 35 × 26 (height) cm) containing sterile bedding, sterile moss (to provide adequate humidity), a running wheel for exercise, and a ‘log’ for shelter (half of an 18 cm long white PVC tube cut transversally). Water and food (a mixture of cat chow, nuts and seeds) were provided ad libitum. To conform with their insectivorous diet, each mouse received mealworms three times a week.

In summer 2005, we used 10 males (subjects) and 10 females (stimuli) in experiment 1 (E1). The average ages (±SE) were: males 6.67 ± 0.87 months, females 9.5 ± 1.46 months. In summer 2006, we used 11 males (subjects) and 14 females (stimuli) in experiment 2 (E2). The average ages (±SE) were: males 15.9±1.32 months, females 19.6±1.72 months. Because of the limited number of animals in the colony, six of the males used in E1 were also used a year later in E2. No animal was ever used more than once for the same experimental trial.

We randomly selected the pair of subject-stimulus mice except for controlling for genetic relatedness (only unrelated mice were paired) and familiarity (only non-familiar mice were paired). Because of the limited sample
size, we controlled for possible effects of size and age by randomly assigning the mice that were used as the subject and stimulus for each pairing. This ensured that there were no systematic biases with regards to age or size in these pairings. Moreover, there were no noticeable or obvious differences in size.

All mice were sexually mature at the time of all experiments (females mature at 28–39 days of age; males at 6–8 weeks of age: Hooper & Carleton, 1976). All females were sexually naïve and housed singly; except for 3 females in E2 who had had previous breeding experience. Two males in E1 and eight males in E2 had had breeding experience. There was no obvious difference in the behaviour of mice with and without breeding experience. Mice in both groups engaged in similar behaviours. Experiments were not performed during a specific breeding season since S. teguina lacks a clearly delimited breeding seasonality, rather breeding and giving birth during the whole year (Hooper & Carleton, 1976).

2.2. Experimental procedures

We conducted two different experiments. Both experiments consisted of focal sampling tests in which the subject (all males) was allowed to interact briefly with a stimulus animal (either a male or a female). Trials were conducted in the afternoon between 12:00 and 6:00 pm. We used the same experimenter in all experimental trials of the study. The experimenter recorded all behaviours (see below) by taking note of direct observations. Singing behaviour was easy to record because, as we mentioned above, we could hear the animal vocalize given that most of the energy of the song is in the audible spectrum of the song. Secondly, it was possible to unambiguously determine the identity of a caller because, when singing, males or females adopt a conspicuous posture with their snout upwards and their mouth open. Also, during singing, mice either would rise on their hind legs, assuming a bipedal stance, or stay immobile on all four legs (Hooper & Carleton, 1976; Miller & Engstrom, 2007). Additionally, when S. teguina assumes this characteristic posture during singing, it does not normally engage in other behaviours (e.g., sniffing, grooming, running) at the same time.

Our experimental apparatus consisted of two black Plexiglas boxes (30 × 30 × 19.5 cm) each covered with a transparent Plexiglas lid. The boxes were connected by a sliding, opaque, door that could be opened or closed by the
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experimenter without removing the lids. The experimenter was positioned in front of the two boxes, and approximately 25 cm away. This distance allowed the experimenter to have a clear view of both boxes and to observe and listen to the animals’ behaviours through the transparent lids. Between trials, all equipment was washed with hot soapy water and rinsed with 70% ethanol to eliminate any residual odours. Boxes were allowed to dry completely before a new trial was conducted.

2.3. Experiment 1 (E1): male behaviour pre-, during and post-interaction with a female

In E1, we tested 10 male subjects with 10 female stimuli mice. We did not control the reproductive condition of these females because in this initial test we were most interested in determining the accuracy of Hooper & Carleton’s (1976) preliminary report that males tended to sing after encountering a female.

Before each experimental trial, we moved the cages housing the subject and stimulus animals into the experimental room. This room had the same temperature and light conditions as the animal colony room. Animals were allowed an acclimation period of at least 20 min. After the acclimation period, we marked the male subjects with a dab of ‘colour powder radiant colour’ (T1-OR6714). We dipped a watercolour brush in the powder and passed it once over the rump of the subject. As a control, we also passed a clean watercolour brush (with no colour) once over the rump of the stimulus mouse. This marking technique is used routinely in this laboratory and has never been found to alter the behaviour of these animals.

The test subject was then placed in one box of the testing apparatus and the stimulus mouse was placed in the other box. We randomly assigned the left or right box to each of the animals. After the subject and stimulus were placed in the experimental apparatus, we allowed an additional 2 min of acclimation before starting the experiment. This pre-test acclimatisation time was roughly equal to the duration of a session and comparable to the acclimation periods used in other behavioural experiments of this type (e.g., Nyby et al., 1977a,b; Maggio & Whitney, 1985). Consequently, we considered this acclimation period long enough to allow the animal to recover from handling; during this time the animals investigated the box without showing any signs of agitation or anxiety, such as freezing or frantic running. We also
ran the experiments under red light to minimize disturbance to the animals by the presence of the experimenter.

There were 3 sessions in each trial of this experiment and each session was 3 min long. During each session we recorded by direct observation the type and number of audible songs produced by the subject (see description of singing posture, above). At the end of the first 3 min (the pre-interaction session), we immediately lifted the sliding door separating the two boxes, allowing the mice to enter each other’s boxes and interact (the interaction session). At the end of these 3 min, we again separated the two mice by closing the sliding door so that each mouse was in a different box for 3 additional min (the post-interaction session). The animals were usually moving from one box to the other (e.g., one following the other). Therefore, at the end of the interaction session, we would wait until one animal was in one box while the other animal was still in the other, to close the sliding door. We never had to handle the animals to separate them and there was no indication that this procedure disturbed the animals in any way. In fact, to prevent any disturbance due to handling during the trial, the box assignment during the post-interaction session was totally random for each mouse.

2.4. *Experiment 2 (E2): male behaviour after an interaction with a male, a non-oestrous female or an oestrous female*

In this experiment the apparatus used was the same Plexiglas box used in E1. Male subjects \((N = 11)\) were exposed to 3 min interactions with three categories of stimulus animals: (a) male, (b) female in non-reproductive (non-oestrus) condition; and (c) female in reproductive (oestrus) condition. Each male subject was tested in each of the three experimental conditions in a repeated measures design. However, there was an interval of two days between tests using each of the three different conditions. The order of experiments was random for each test subject and no subject was ever paired with the same stimulus animal in more than one experiment (i.e., the oestrous vs. non-oestrous females were always different individuals).

After the 3-min interaction, we separated the subject from the stimulus animal and recorded the number of songs each subject sang in the first 3 min following separation. During the interaction session, in addition to number of songs, we also recorded the amount of time (duration in s) that the male subject spent allogrooming the stimulus animal and number of allomarks
the male subject performed on the stimulus animal. We recorded these behaviours by direct observation and using a stopwatch. Allomarking is a discrete behaviour, so it was only measured as counts. It was during E1 that we became aware of the frequency with which these behaviours occur, and decided to quantify them during E2.

Female reproductive condition was determined before each experimental trial. We assessed the reproductive condition of females by following the protocol for the cytological assay of vaginal smears for *S. teguina* developed by Fernandez-V (2006). *S. teguina*, like many other rodents, is a spontaneous ovulator showing 4–5-day oestrous cycles. The cycle consists of a sequence of phases: oestrus (ES), metestrus (ME), diestrus (DI) and proestrus (PRO). We determined the phase of the cycle by collecting a vaginal smear on the day of each test. The exact phase of the cycle was determined for each female on a daily basis, during a period of 20 days before the experiment to corroborate the regularity of the cycle.

Details of the cytological assay to determine the phase of the oestrous cycle are described in Fernández-Vargas (2006). In summary, vaginal swabs were taken and a vaginal smear was prepared on a glass slide, using mammalian ringer solution (Carolina Biological Supply). The layer of cells on the slide was then observed under a microscope. Cornified epithelial cells, leukocytes (white blood cells) and nucleated epithelial cells were the three main cell types visible on the smears. Based on the changes in relative abundance of each cell type on the whole slide, we determined the four phases of the oestrous cycle (ES, ME, DI and PRO) (Nelson, 2005).

We considered a female to be in a non-oestrous condition when her vaginal smear showed a phase in transition of ME-DI, DI or transition DI-PRO. We determined that a female was in oestrus when the vaginal smear showed a transition PRO-ES or ES. In *S. teguina* during this phase, nucleated epithelial cells outnumbered cornified epithelial cells to the point that they completely dominated the smear. This same pattern of cell types and vaginal phase (oestrus) has been demonstrated to coincide with the behavioural oestrus in the rat (Long & Evans, 1922), mouse (Fowler & Edwards, 1957) and golden hamster (Kent, 1968).

2.5. Behaviours measured

Behaviours measured in these experiments were:
1. Type-I stereotypic song: Consists of a series of frequency modulated pulses ranging from approximately 8–50 kHz, with 10–170 notes and 1–16 s duration (Miller & Engstrom, 2007). Throughout this paper, we refer to this Type-I stereotypic song simply as the ‘song’ produced by subjects in the experiments. As described previously, the mouse normally assumes the characteristic singing posture while performing this song.

2. Short song: This type of vocalization consists of a short excerpt of a Type-I song with generally fewer than 5 notes. Relative to Type-I stereotypic song, this short song is vocalized quickly and at low intensity, during intersexual encounters (these songs are likely the same as those described as ‘strophes’ by Miller & Engstrom (2007)).

3. Allogrooming: One mouse grooms the fur of another one, using its mouth and teeth. Commonly, allogrooming starts behind the ears and head of the conspecific and proceeds to other parts of the body.

4. Allomarking: One individual first rubs its mid-ventral sebaceous gland with its forepaws and mouth and then rubs its paws over a conspecific’s fur while simultaneously using its mouth and teeth in allogrooming.

5. Agonistic behaviours: These include chases, threats (mouse stands up on its hind legs facing its opponent with its fore paws held high and extended), and brief fights (mice locked together and rolling around in the box).

2.6. Statistical analysis

We assessed normality of the data distributions by using the Shapiro & Wilk test (JMP 7.0, SAS Institute, 2007). Because our data did not meet the assumptions of normality, we used non-parametric statistics for repeated measurements (Siegel, 1956; InStat, GraphPad Software, 1998). In E1, we compared the singing behaviour between the pre- and post-interaction sessions using a Wilcoxon signed-rank test (test statistic \( t \)). We used this test to compare pre- and post-interaction only because we found that males did not vocalize Type I song during any of the interaction with the female. In E2, we compared differences in male singing behaviour after interacting with a male, a non-oestrous female, and an oestrous female using a Friedman Test (test statistic \( Q \)). We also used the Friedman test to analyze differences in allogrooming, and allomarking during the interaction sessions in E2. Dunn’s multiple comparison tests were used to analyze post hoc pairwise comparisons when the Friedman showed significant differences. In all comparisons, the \( p \) values are two-tailed and the critical level of \( \alpha \) was set at 0.05.
3. Results

Experiment 1 (E1): This experiment examined male singing before, during and after encounters with females. Male subjects sang Type I stereotypic songs during the pre- and post-interaction sessions of the experiment. However, only a few songs were produced during the pre-interactions and the number of Type I songs was significantly higher during the post-interaction condition (Figure 1, $T = 10.05$, df = 9, $p < 0.03$). Males never sang Type-I stereotypic songs during the interaction with the female. However, a few males (3 out of 10) did produce short songs or strophes. Females never vocalized during any of the sessions.

Experiment 2 (E2): This experiment was designed to compare the singing behaviour of males after encounters with the three categories of stimulus mice. Male subjects produced Type I stereotypic songs after (post) the interaction with a female, regardless of her reproductive condition. Male–male encounters elicited significantly fewer songs during the post sessions as compared to the higher number of songs produced after male–female encounters (Figure 2, $Q = 7$, $p = 0.03$). As in E1, very few songs were produced in the pre-encounter tests, and males never sang Type-I stereotypic songs during any of the interactions with either another male, or with an oestrous or

![Figure 1](image.png)

**Figure 1.** Experiment 1. Number of Type-I stereotypic songs produced by male subjects ($N = 10$) in pre-, during- and post-interaction sessions with a female whose stage in the oestrous cycle was not controlled ($p = 0.03$). Results were analyzed with a Wilcoxon signed-rank test. We compared pre- and post-interaction only because males did not vocalize Type-I song during interaction with a female. The box represents the interquartile range (IQR, measure of variability); it contains the middle 50% of the data. The horizontal line inside the box is the median value, the dots are data points and the dots outside the whiskers are extreme values.
Figure 2. Experiment 2. Number of Type-I stereotypic songs produced by male subjects \((N = 11)\) in post-interaction trials with another male; a non-oestrous female (Female NE) and an oestrous female (Female E). Male subjects sang significantly more after the interaction with a female (regardless of her reproductive condition) as compared to a male \((p = 0.03)\). Results were analyzed with the non-parametric Friedman Test. The box represents the interquartile range (IQR, measure of variability); it contains the middle 50% of the data. The horizontal line inside the box is the median value, the dots are data points and the dots outside the whiskers are extreme values.

a non-oestrous female. A few males (3 out of 11) did produce short songs or strophes. These short songs were completely absent in interactions with males, and occurred more frequently with oestrous, as compared to non-oestrous females. However, due to the low number of males who exhibited this behaviour, we were unable to compare the treatment groups statistically.

We never observed or heard females vocalizing sonically during any of the sessions.

In E2, male subjects spent more time allogrooming females than grooming males during the interaction sessions (Figure 3A, \(Q = 12.76, p = 0.002\)). In fact, only rarely did males groom other males and attempts to groom males were often met with agonistic behaviours. Post-hoc tests showed that the greatest difference in the time spent allogrooming was between the oestrous female and the male stimulus \((p < 0.01)\). There was no significant difference between the male stimulus and the non-oestrous female \((p > 0.05)\), or between the two categories of females \((p > 0.05)\). We observed only one non-oestrous female allogrooming a male and she did it only for 5 s. Thus, male allogrooming of females, and not vice versa, was most common.

During the intersexual encounters, we observed agonistic behaviours only in one trial between a male and non-oestrous female. She displayed threats to the male twice. On the other hand, during male–male encounters, threats
were displayed in 64% of the trials and brief fights were registered in 27% of the trials. During agonistic encounters, no Type-I songs were recorded.

Lastly, male subjects spent much more time allomarking oestrous and non-oestrous females as compared to males (Figure 3B). However, there was much individual variation and only 7 out 11 males allomarked. Only one male allomarked another male. These differences were not significant ($Q = 4.36$, $p = 0.113$). Although females have a midventral sebaceous gland (smaller as compared to males), they were never observed allomarking a male.
4. Discussion

Because singing by *S. teguina* often seems to occur spontaneously and at random, whether in the laboratory or field, there has been uncertainty about the possible functions of this behaviour, and few studies have experimentally examined the factors or stimuli that elicit singing (see Pasch et al., 2011). Our findings now suggest that, at least in males, audible singing may have a sexual function. Specifically, we found that males are most likely to sing after they have been separated from a female that they had previously encountered. This elicited-singing behaviour does not occur during or after encounters with other males. Typically, only squeaks of variable intensity are heard during agonistic encounters (Hooper & Carleton, 1976).

It is unlikely that the singing was induced by stress produced by the separation or by the novelty of the environment. It has been recently demonstrated in this species that there are no significant differences in faecal corticosterone (CORT) between open-field singers or isolated spontaneous singers vs. non-singers. Interestingly, in response to restraint stress, open-field singers actually showed a lower rise in plasma CORT compared to non-singers (Crino et al., 2010). Moreover, stress due to our testing situation, or to the novelty of our apparatus or procedures would have been the same under all three conditions, but males vocalized mostly after encounters with females. Moreover, even after male–male encounters in which aggression occurred (presumably a stressful situation) males rarely vocalized. Thus, these various lines of evidence strongly argue against stress as a factor responsible for singing behaviour.

Several rodent species such as male house mice, *Mus musculus* (Nyby, 1983), deer mice, *Peromyscus maniculatus* (Pomerantz et al., 1983), and rats, *Rattus norvegicus* (Barfield & Geyer, 1972), vocalize ultrasonically during sexual contexts. In contrast, *S. teguina* males vocalized Type I songs only after the encounter, when the female was removed. In a similar study on male golden hamsters (*M. auratus*), Floody et al. (1977, 1987) also found that females stimulated high rates of USV in males after an encounter. Moreover, oestrous female hamsters stimulated significantly more USV from males than did non-oestrous females (Floody et al., 1977). Although *S. teguina* males can distinguish between non-oestrous and oestrous females, and show a preference for the odours of the latter (Fernández-Vargas et al., 2008), in this study, males vocalized as much after encounters with non-oestrous as with oestrous females.
Of particular interest is that, while male house mice did not call in response to male urine, males did call indiscriminately in response to female urine from different stages of the oestrous cycle, and even to ovariectomized female urine (Nyby et al., 1979; Whitney & Nyby, 1979; Nyby, 2010). These results are directly comparable to what we found in our experiment. Thus, we hypothesize that male *S. teguina* vocalize after encounters with any female to ‘call back’ or entice the female to return. Because female *S. teguina* have short cycles and enter oestrus every four days, the males’ behaviour could be an adaptive response (i.e., may result in increased male reproductive success if the female does return in response to the male’s singing), whether or not the encountered female is in oestrus. Nyby (2010) proposed a similar hypothesis, suggesting that, because of the short oestrous cycle in the house mouse, a communicatory bond may keep a non-receptive female around until she becomes receptive. These hypotheses remain to be tested experimentally in both species.

Our results also suggest that male *S. teguina* use two different audible songs in different sexual contexts. During initial encounters with females, they may use a more variable, brief and low intensity call (the short song or strophe). However, we observed this behaviour only in a few males. After being separated from the female, males typically emit the more common, highly stereotyped and longer Type 1 song (see also Miller & Engstrom, 2007). Context-specific vocalizations also have been described in the common marmoset (*Callithrix jacchus*). In this species, newly-paired individuals produce calls that are different from those made by the same individuals when they are physically separated from their mate (Norcross et al., 1999). It would be valuable to know whether such variation in the calls used in different contexts (during vs. post-interaction) is common in other vocalizing species, particularly among rodents. The existence of context-specific differences in vocalizations may facilitate communication and provide more finely tuned information (e.g., motivational state, social status, individual identity) to conspecifics.

The function of allogrooming differs from species to species, but copulation in many rodents is characterized by allogrooming, which is almost exclusively performed by the male on the female (Dewsbury, 1974a,b). This asymmetry of allogrooming also has been observed in the herb-field mouse (*Apodemus microps*) in which this trait may be under sexual selection (Stopka & Graciasová, 2001; Polechova & Stopka, 2002). In *S. teguina*,
our findings suggest a possible role of allogrooming in male courtship behaviour. Specifically, males initiated allogrooming of females and spent significantly more time allogrooming oestrous females than males. Females appeared receptive to being allogroomed and no agonistic responses were apparent. Male allogrooming did not differ between oestrous and non-oestrous female stimuli. As mentioned above, it is possible that it is advantageous for a male to groom a female, even though she is not in oestrus, because she can be expected to come into oestrus in fewer than four days. Therefore, male courtship may begin as soon as a male encounters a female. Based on our results, we hypothesize that, in this species, allogrooming is used as a courtship behaviour with females. Additional studies on the components of male courtship in *S. teguina* are necessary to test this hypothesis.

Scent marking on objects or substrates is a common behaviour among mammals that possess specialized scent glands. In addition, scent marks are sometimes placed on other animals such as mates, colony members and offspring, possibly for sexual advertisement or social recognition (see examples in Johnson, 1973; Brown, 1979). Females are marked by males in the African giant rat (*Cricetomys gambianus*) (Ewer, 1967) and the European badger (*Meles meles*). Badgers directly allomark conspecifics with subcaudal gland secretions and males allomark significantly more often than females (Buesching et al., 2003). In *S. teguina*, we observed males using their mid-ventral sebaceous gland to allomark conspecifics and the behaviour occurred most frequently during male–female interactions. Although the differences were not statistically significant, males very rarely even attempted to allomark other males. Therefore, it is possible that this is another behaviour that functions to facilitate courtship. The glandular secretions of *S. teguina* communicate information about sex and reproductive condition (Fernández-Vargas et al., 2008), and may also convey information on individual identity. Although the latter function has not been studied in this species, males may allomark females to ‘label’ them with the male’s individual odour. Clearly, this possibility begs for additional studies.

An interesting finding of our study was that females were silent before, during, and after interactions with males. Yet females are known to sing spontaneously, albeit less than males when they were housed separately from males but in the same colony room (Hooper & Carleton, 1976, pers. obs.). Although in house mice it is not possible to visually discriminate the identity of the caller during intersexual pairings, it is generally accepted that the vocalizations recorded during courtship are produced by the male (Sales,
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1972b; Whitney et al., 1973; Nyby et al., 1977a; Nyby, 1983); females tend to vocalize during other social context such as female–female encounters (e.g., Moles et al., 2007). Because *S. teguina* assume a characteristic singing posture, it was very easy to see, in our study, that only males were singing. It is not clear, however, whether the failure of female *S. teguina* to vocalize during this study has any adaptive function; the conditions and contexts that elicit female singing require further study. However, as in the house mouse (Nyby et al., 1977a), *S. teguina* exhibits sex differences in singing behaviour.

In general, more research is needed to more certainly determine the function of singing, allogrooming and allomarking during sexual encounters in this species. Studies on the possible role of vocalizations in other contexts would also be valuable. For example, it is not clear whether song plays a role in an agonistic context; our results do not indicate such a role, but our experiments were not designed to shed light on this question. Interestingly, it has been recently demonstrated that in the laboratory a playback of a male conspecific Type-I song elicits a singing response in males and androgens have a strong effect on the latency of this response (Pasch et al., 2011). Although males clearly respond to recordings of other males’ songs, it is still not completely certain whether singing responses to playbacks are motivated primarily by agonism or if they may additionally indicate male–male competition for females. Our study, in conjunction with that of Pasch et al. (2011) suggests the hypothesis that the calling behaviour of male *S. teguina* may have dual functions in sexual behaviour and in male–male competition.

In summary, our results indicate that one important function of female elicited-singing in males is related to sexual and courtship activities, and that males may sing to re-establish contact with a previously encountered female, thereby facilitating reproduction. Allomarking and allogrooming behaviours may be additional components of courtship that make searching easier and facilitate recognition. Furthermore, opposite-sex elicited-singing, allogrooming and allomarking are sexually dimorphic behaviours that males engage in substantially more often than females. Our study provides new insights into the possible contexts in which *S. teguina* uses its multi-modal communication systems, and on the functions of its behaviours.

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