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A vocal ethogram for *Fukomys micklemi* (Bathyergidae, Rodentia).

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Contents

1. Introduction

It is generally assumed that sociality in African mole-rats has evolved along pathways predicted by the Aridity Food Distribution Hypothesis (AFDH) (Lovegrove, 1991; Jarvis et al., 1994; Faulkes et al. 1997; Spinks et al., 2000). The AFDH states that there is a correlation between the level of sociality and increasing habitat aridity (Jarvis et al., 1994; Spinks et al., 2000).
In arid regions a lot of water is lost through evaporation and rainfall is unpredictable. The costs of burrowing greatly increase during dry periods, when the soil is hard and difficult to excavate. This implies that burrowing activity peaks during the sparse periods when the soil is moist. During these short times, it is necessary that resources are optimally used. Due to the energetic costs of burrowing, the maximal rate of incisor growth and heat load, there are limits to the work one single mole-rat can do in a day. In addition, the food resources (geophytes) tend to be of poor quality, and are often clumped or widely dispersed (Jarvis et al., 1998). In this way, living and foraging in social groups spreads the energetic costs of burrowing and increases the chances of finding food. Once food has been discovered, it is often sufficient to support large groups of animals because of the clumped nature of these food resources (Sichilima et al., 2008).

Successfully integrating individuals into groups and arranging communal activities, such as foraging, requires the development of signals (Bradbury & Vehrencamp, 1998 in Schleich et al., 2007). The ability of animals to recognize conspecifics is also important in a wide range of contexts including parental care, competition and mating (Waldman, 1988). Communication is however severely constrained by the physical properties of the subterranean habitat and by regressed and/or narrowly specialized organs (Credner et al., 1997). Most mammalian species exploit four different types of communication, namely olfaction, touch, vision and vibration. This is also the case for subterranean rodents, though olfaction, touch and vibration are used more than vision. Evidently, in the darkness of the subterranean milieu, there are considerable visual restrictions (Begall et al., 2007). Most species of mole-rats are rarely exposed to light stimuli and hence live in continuous darkness. Studies on *Spalax ehrenbergi* show that this species lacks all visual ability, but that the regressed eye may have been retained for photoperiodic perception. For *Cryptomys* sensu lato (including *Fukomys*, see below) there are indications of a more elaborate visual system and the possible retention of basic visual capacities (Peichl et al., 2004).

There is considerable evidence that subterranean rodents use chemical signals (olfaction) for communication (Francescoli, 2000). The use of tactile signals requires close contact between individuals. As a result, this kind of communication will not be used extensively in dangerous situations such as predator detection or aggressive encounters with conspecifics. On the other hand, tactile input is likely to be quite useful in navigation through dark, subterranean tunnels. Some forms of tactile communication are limited to specialized contexts, such as mating, or to social species, where there is frequent physical contact among burrowmates (Francescoli, 2000). Communication by means of vibration comprises of two primary types of signals: acoustic and seismic signals (Francescoli, 2000). The latter consists of substrate-born vibrations that are received as somatosensory signals, independently from the auditory mechanism (Nevo et al., 1991). These may function as a major communication channel and can even be enhanced by the solid medium. However the use of seismic signals varies greatly among subterranean rodents, not all genera emit them (Francescoli, 2000).

In this study, we are interested in the second type of communication by vibration, acoustic signalling. Acoustic signals are transmitted as sound waves and are received and decoded using specialized sound receptors, such as ears (Francescoli, 2000). Animals use vocal signals for many different purposes: to elicit specific behaviours, initiate contact with conspecifics, identify individuals or species, signalize status (reproductive, dominance or territorial), solicit food, warn against predators and coordinate reproductive efforts (Schleich et al., 2007).

Vocal communication is of great importance to rodents who live in subterranean tunnels, where sounds of relatively low frequencies propagate best (are least attenuated). Certain frequencies might even be enhanced by the environment. Consequently, audition is shifted mainly to the low-frequency range, and the hearing apparatus is tuned to these sounds (Begall & Schleich, 2007). The first noticeable feature of the hearing apparatus of subterranean rodents is the size-reduction of the pinnae (external ears) (Francescoli, 2000; Begall et al., 2007). Many explanations for this reduction have been proposed. According to Burda et al. (1989 in Begall et al., 2007) it seems that binaural sound localization, which is the normal function of the pinnae, is of little use in underground burrows. Furthermore, the outer ear canals of *Spalax ehrenbergi* and *Fukomys anselli* have a relatively small lumen and are filled with cerumen. These features prevent dirt from piling up in the outer ear canal, and thus protect the ears. However, at the same time they also reduce hearing sensitivity (Burda, 2006).

Investigations of the middle ear transmitting chain of subterranean rodents have pointed out that none of the components are reduced or degenerated. The enlargement of some parts, primarily the incus and the stapedial footplate, leads to reduced sensitivity of the hearing apparatus (Burda, 2006; Begall et al., 2007). The middle ear of *Spalax* and *Cryptomys* s.l. can therefore be perceived as a low-frequency tuned apparatus (Burda, 2006). When looking at the inner ear, it seems that the cochlea is specialized for sensitive perception and high resolution of low frequencies (Begall et al., 2007). Compared with *Rattus norvegicus*, the cochleae in mole-rats are higher, more coiled and exhibit relatively longer cochlear ducts. Furthermore, longer basilar membranes can accommodate more hair cells, enabling a better frequency and intensity discrimination (Burda, 2006).

When trying to figure out what these underground animals can really hear in their natural surroundings, one has to take into account not only the capabilities of their auditory systems, but also the acoustic characteristics of their tunnels (Begall & Schleich, 2007). Heth et al. (1986) investigated the propagation of air-borne sounds in burrow systems of subterranean rodents and discovered the enhancement of some frequencies (around 440 Hz) and the attenuation of others. They suggested that this finding can be extrapolated to other subterranean rodents, assuming a rather uniform acoustic environment for all subterranean species. Lange et al. (2007) studied the existence of this so-called "stethoscope effect", in other words the selective amplification of certain frequencies (200, 400 and 800 Hz) in the tunnel systems. For their research they investigated the tunnels of blind mole-rats (*Spalax ehrenbergi*). The earlier mentioned frequency tuning of vocalizations, hearing and ear morphology correlates with the frequency transmission characteristics in burrows. The lowered hearing sensitivity may help to compensate for amplified sounds, avoiding overstimulation due to the stethoscope effect (Lange et al., 2007). Hence, both frequency tuning and low sensitivity of hearing can be considered convergent adaptive specializations to a unique acoustic environment (Begall et al., 2007). In other words, *Cryptomys* and *Spalax* share the same specializations to the underground environment. In their sensitivity to low-frequencies (below 1 kHz)

mole-rats resemble aboveground living rodents such as kangaroo rats or gerbils. Compared to aboveground living generalists like rats, a lot of differences can be noted (Brückmann & Burda, 1997; Begall et al., 2007). The hearing of subterranean rodents is, however, much less sensitive above 1 (to 4) kHz (Brückmann & Burda, 1997).

Acoustic signals are also an important factor in reproductive isolation and speciation processes (Schleich et al., 2007). Results by Nevo et al. (1987) highlight vocalization as an important pre-mating isolation mechanism in blind mole-rats, where visual cues do not operate. They compared the physical structure of male courtship calls of mole-rats belonging to four chromosomal species of *Spalax ehrenbergi*. Each chromosomal species appeared to have a vocal dialect significantly different from all others (Nevo et al., 1987).

Recently, molecular and phylogenetic studies have revealed that the genus *Cryptomys* (Bathyergidae, Rodentia) actually comprises two different genera (Faulkes et al. 2004; Ingram et al., 2004). As a consequence *Cryptomys* sensu lato is now split into *Cryptomys* and the new genus *Fukomys* (Kock et al., 2006; Van Daele et al., 2007b). *Fukomys* is considered one of the most speciose rodent genera. The genus encompasses several species of subterranean rodents that are endemic to Sub-Saharan Africa. It is the most derived lineage within the Bathyergidae, comprising several social and at least one eusocial species (*Fukomys damarensis*). A major problem when describing the genus *Fukomys* is that most species are morphologically very similar. This is why traditional morphology based systematic studies of the genus yield no clear results. Consequently, species limits should be established using multiple sources of evidence which includes data from the molecular, cytogenetic and organismal levels (Van Daele et al., 2004, 2007a,b).

This thesis is an extension of existing ethological work performed on *Fukomys micklemi*. A non-vocal ethogram has been constructed (Bouly, 2005; Desmet masterthesis, 2009; Van Daele et al. in prep.) Adding vocalizations and hence creating a vocal ethogram for *Fukomys micklemi* (and comparing this to vocal ethograms for other *Fukomys* species) can be instrumental for gaining further insights into the complex specific relations within the genus.

2. Objectives

In spite of the constraints posed by an underground lifestyle, the study of vocal communication of mole-rats is an interesting field of research. Hence, the main purpose of this thesis is to construct a vocal ethogram for *Fukomys micklemi*. This will be a useful addition to current research, which has already yielded a non-vocal ethogram for this species.

2.1 General questions

Because sociality implies an extensive system of communication, an elaborated vocal repertoire may be expected in spite of the limitations posed by the subterranean milieu.

Q   Which vocalizations can be recorded for *Fukomys micklemi*?

Q   Which behaviour(s) can be associated with the production of each vocalization?

Underground sound propagation and regressed and/or narrowly specialized organs pose some constraints on the characteristics of the produced vocalizations. Sounds of relatively low frequencies are propagated best in subterranean tunnels. It can thus be hypothesized that mole-rats, who spend most of their lives underground, will mainly use low-frequency vocalizations.

Q   Are most vocalizations produced by *Fukomys micklemi* situated in the low-frequency area of the spectrogram?

2.2 Specific questions

To record an elaborate vocal repertoire, not only ad libitum samplings are needed. Different experiments, simulating different natural circumstances, are needed to grasp the range of vocalizations of the study species.

2.2.1   Mating

Q   Which are mating specific vocalizations?

Q   Are they produced by the male or the female?

2.2.2   Intracolonial pairing

Q   On an intracolonial level, which are the vocalizations associated with contact?

Q   Does the placing of a piece of food elicit agonistic behaviour and associated calls?

2.2.3   Homospecific encounters

Q   Which vocalizations and behaviours are used during homospecific encounters on an intercolonial level?

2.2.4   Heterospecific encounters

Q   Which vocalizations and behaviours are used during heterospecific encounters?

Q   Are these behaviours (and associated vocalizations) generally more agonistic than the behaviours (and vocalizations) seen during homospecific encounters?

2.2.5   Juveniles and mother

Q   Which types of maternal behaviour can be observed?

Q   Which types of vocalizations are produced by the juveniles?

Q   Can these vocalizations be clearly distinguished from the adult calls?

2.2.6   Juveniles separation

Q Do juveniles emit specific vocalizations when they are separated from their mother?

3. Material and Methods

3.1 Study specimens

3.1.1 Species

The study specimens belong to the Sekute cytotype (2n = 56) of *Fukomys micklemi* (see figure 1) (Bathyergidae, Rodentia). The latter comprises a chromosomally very diverse, central Zambezian lineage (2n = 42-68) (Van Daele et al., 2007b).

Figure 1. *Fukomys micklemi* in the nest with neonates

3.1.2 Molarium

The specimens were kept in a molarium at Ghent University. This is an animal room with a constant climate, a temperature of 22°C ± 2°C and a relative humidity of maximally 60%. The different mole-rat colonies were kept in separate terraria. Wood shavings were provided as bedding and pieces of shredded paper as nesting material. No windows were present in the molarium, so the animals live in darkness for 24 hours/day. During experiments and maintenance, the room was lit using a fluorescent light. The animals were fed potatoes and carrots ad libitum, their diet was complemented once or twice a week with muesli, and apples, cucumber or lettuce. As mole-rats don't drink free water, no additional water was administered. All animals over 50 grams carry a subcutaneous microchip, which was read using a Trovan Unique™ LID-572 Pocket Reader. This allowed for an efficient recognition of the animals. A picture of the molarium can be found in Appendix 1.

3.1.3   Health status

An animal's mass was used as a proxy for its health (see table 1), as was the incidence of dermatitis in the corners of their mouths. The latter stayed fairly constant during the research period. Four out of six males (numbers 24, 43, 44 and 65) had a little dermatitis in the corners of their mouths, especially number 65. The mole-rats were weighed on February

Table 1. Colony FMI-04 - Weight			
Individual	Start weight (in g)	End weight (in g)	Difference
05	81,40	90,60	9,2 (+)
23	96,00	99,70	3,7 (+)
24	101,00	106,10	5,1 (+)
29	81,00	95,20	14,2 (+)
43	120,50	142,10	21,6 (+)
44	87,60	92,20	4,6 (+)
50	125,00	113,60	11,4 (-)
64	93,30	91,00	9,2 (+)
65	125,00	134,10	9,1 (+)
67	77,20	91,00	13,8 (+)
74	86,30	112,70	26,4 (+)
75	101,00	120,40	19,4 (+)

11th before starting the actual recording and again on April 2nd, the last day of recording. Most animals gained a little weight, probably as a result of the re-introduction of apples into the diet, and the use of cucumber during experiments. It must be noted that numbers 74 and 75 gained a lot of weight, because they were not completely mature when the recording sessions started. The queen (50) is the only one that lost weight after giving birth to three juveniles during the research period.

3.2 Data collection

Between February 9th and April 2nd 2010 sound recordings and behavioural observations were carried out on colony FMI-04. For some experiments, other colonies or individuals were used. During the intercolonial, heterospecific experiments some *Fukomys whytei* specimens were also used. For a complete overview of used colonies and animals, see Appendix 2. The first week of the data collection period was used to test the material and to get familiar with the behaviour of the animals. The actual data collection was done over a seven-week period, with a total of 28 days of recording. During ad libitum sampling, data were entered in an MS excel worksheet on a laptop. During focal experiments, because of the great number of vocalizations, data were written down on a notepad and later entered in the worksheet.

3.3 Sound recording

Recording of the vocalizations was done using a SENNHEISER ME 67 microphone, connected to an EDIROL R-09 recording device. The EDIROL R-09 was set to a sampling frequency of 48 kHz and recording mode WAV 16 bit, because not every computer was able to play samples recorded in WAV 24 bit. To limit the loss of quality, the WAV-format was preferred to the MP3-format. Each recording session lasted approximately five minutes. In total 874 sessions were carried out. Every time an animal vocalized five variables were noted: time, individual, behaviour, note and camera (for an example of a worksheet see Appendix 3). Sessions without vocalizations were discarded.

3.4 Behaviour

During the sound recording sessions, a Sony camera was used to film relevant behaviour in the terrarium. In this way, all behavioural observations could be double-checked when constructing the vocal ethogram. Labelling of the performed behaviours was done according to the ethogram constructed during previous research (Bouly, 2005; Desmet, 2009; Van Daele et al. in prep). Behaviours which had not yet been included in the ethogram were added.

3.5 Setup

The data collection was subdivided into ad libitum sampling and focal experiments (see below) in order to collect a varied amount of vocalizations (Martin & Bateson, 1993). A complete time-line of the different sampling days can be found in Appendix 4.

3.5.1   Ad libitum sampling

This type of observing and recording means that no systematic constraints were placed on what is recorded or when (Martin & Bateson, 1993). All vocalizations produced by the entire colony were recorded and written down.This entailed solely observing, thus not manipulating the animals. Two periods of ad libitum sampling were carried out. The main reason for this second period was the birth of three juveniles, of which two survived.

3.5.2   Focal experiments

During this type of recording some manipulation of the animals was necessary, off course always keeping in mind health and welfare. Six different experiments were carried out.

Mating experiment   A male specimen (FMI-P5MN1) that resided alone in a terrarium, was placed together with a female (14) from colony FMI-03. The three following days sounds were recorded, with specific attention for vocalizations associated with mating.

Pairing experiment   The first part of this experiment was designed to elicit contact calls. Pairs of individuals from colony FMI-04 were placed together in a separate cage. All sounds were recorded 15 minutes subsequent to joining the individuals. During the second part, a little piece of cucumber was placed in the cage, in order to check for agonistic reactions. When choosing the pairs, both male and female, breeding and non-breeding individuals were used (for a list of the pairs see Appendix 5).

Homospecific encounters   Specimens of colony FMI-04 and *Fukomys micklemi* specimens from other colonies were placed together in pairs to check for intercolonial aggression or contact calls (for a list of the different pairs see Appendix 5).

Heterospecific encounters   Specimens of colony FMI-04 and specimens of the species *Fukomys whytei* were placed together in pairs to check for interspecies interactions (for a list of the different pairs see Appendix 5).

During the encounter experiments, a 2nd researcher was present as a precautionary measure in case of interspecific fights. If two animals started fighting, they were immediately taken out of the arena, before they could hurt each other.

Juveniles and mother   During this experiment, the two juveniles and their mother were taken out of the colony and placed in a separate cage. This was done in order to focus on the interactions between mother and pups, and to record juvenile specific vocalizations. The experiment was carried out three times for 15 to 30 minutes. Longer periods might have harmed the health of the juveniles due to the risk of hypothermia.

Juveniles separation   To take a closer look at the interaction between the juveniles and to check for distress calls, the pups were taken out of the colony and placed in a separate cage without their mother. This experiment was carried out two times for 15 minutes (cf. supra).

3.6 <u>Analysis</u>

All five minute soundtracks were first cropped to the actual recording of vocalisations. For the analyses, spectrograms were generated for part of the vocalizations. All sound editing was done using BatSound Pro (version 3.31b by Pettersson Elektronik AB). In total 711 spectrograms were made and 2208 sounds were analysed. If a track consisted of more than one vocalization, each vocalization was analysed separately. The spectrogram analysis entailed the noting of seven variables: number of vocalizations present in the track, duration of each separate vocalization, start/peak/end frequency of the ground tone, the number of harmonics, and whether the sound was produced by an adult animal or a juvenile. When counting the number of harmonics of a certain vocalization, the ground tone was considered as the first harmonic, following Pepper et al. (1991).

The calls were classified into types based on their physical characteristics, as read from the spectrograms (see Appendix 6). At all times the behavioural contexts were linked to specific vocalizations.

4. Results

Sixteen different adult specific calls and four juvenile specific calls could be distinguished based on their physical characteristics derived from the spectrograms. In total 2208 calls were analysed in spectrograms. The calls could be subdivided into 1801 adult specific vocalizations and 407 juvenile specific vocalizations. Firstly, a general description of the different vocalizations will be given. Both the structure of the call and the associated behaviour will be explained. Every call-type will be illustrated by a spectrogram, in which the X-axis represents time and the Y-axis the frequency reach of the calls. Secondly the results of the different experiments will be discussed.

4.1 Vocalizations (overview see Appendix 7)

Before exploring the different types of vocalizations, it must be noted that the nomenclature follows Pepper et al. (1991) and Credner et al. (1997) where possible. Calls that were not present in these studies were given a new name. For a sound file of each call see Appendix A on the CD-ROM. An example of the spectrogram analysis worksheet can be found in Appendix 8[1].

4.1.1   Adult specific calls

4.1.1.1   Loud call I (Fig. 2)

This type of call was recorded 426 times, which makes it the most common adult specific call (23.6% of the adult specific calls, 19.3 % of all calls). The peak of the fundamental frequency lied between 2.0 – 3.5 kHz. The *loud call I* lasted approximately 40 – 100 ms. The number of harmonics was variable and ranged from three to eight. The high intensity of the call made it sound quite loud, hence the name. The *loud call I* was produced in different situations (see section 4.1.1.2).

4.1.1.2   Loud call II (Fig. 3)

The *loud call II* was recorded 197 times and is very similar to the *loud call I*. Most *loud calls II* lasted 40 – 100 ms. Here, the peak of the fundamental frequency was 3.5 – 4.5 kHz, which is higher than what is observed in the *loud call I*. The number of harmonics was similar to the *loud call I* and ranged from three to eight.

The distinction between *loud call I* and *loud call II* is actually arbitrary with respect to loudness, with 3.5 kHz as a boundary frequency. The data show that the *loud calls* show a continuous spectrum, but with great differences at both ends of the spectrum, hence the distinction between both calls. Both the *loud call I* and *II* are used in a number of different behaviours, and are hence difficult to distinguish purely based on behaviour.

Both calls were very common during interactions between animals that were unfamiliar. When unfamiliar mole-rats sniffed each other, *loud calls* were sometimes produced, either singly or in the form of high pitched squeaking or vocalized hopping. The latter is the behaviour in which an individual moves its head and rump with short jerks and makes a high pitched sound (see ethogram

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[1] Supplementary materials: primary data (available in copies for examinators (Appendix B on CD-ROM); available on request from the author).

in Appendix 9). During this behaviour the animal is almost hopping on all fours. Vocalized hopping also occurred as a consequence of agonistic interactions such as another animal thrust-gaping or tail pulling. Thrust-gaping is defined as a gape, an opening of the mouth while rapidly inhaling and exhaling through the mouth, accompanied by a lunge towards another mole-rat (see ethogram in Appendix 9). While trust-gaping the animals produced a very typical sound which is described lower (see 4.1.1.9). High pitched squeaking on the other hand can be described as producing a series of loud, high calls, but without a hopping movement.

General disturbances in the nest also elicited *loud calls*, e.g. when a resting animal was awoken by another mole-rat entering the nest. Also when a disturbance out of the nest occurred, startling an animal, a single *loud call I* was emitted. *Loud calls I* were also very frequent when trying to protect a piece of food from another mole-rat, or during a tug-of-war. During a tug-of-war two animals hold the same piece of food between their incisors and pull it while bracing themselves against the substrate (see ethogram in Appendix 9).

When being allogroomed, some animals also produced *loud calls*, though not exclusively. Sparring, a social interaction between two animals in which they keep their incisors locked together and then shove back and forth against each other, also elicited *loud calls*.
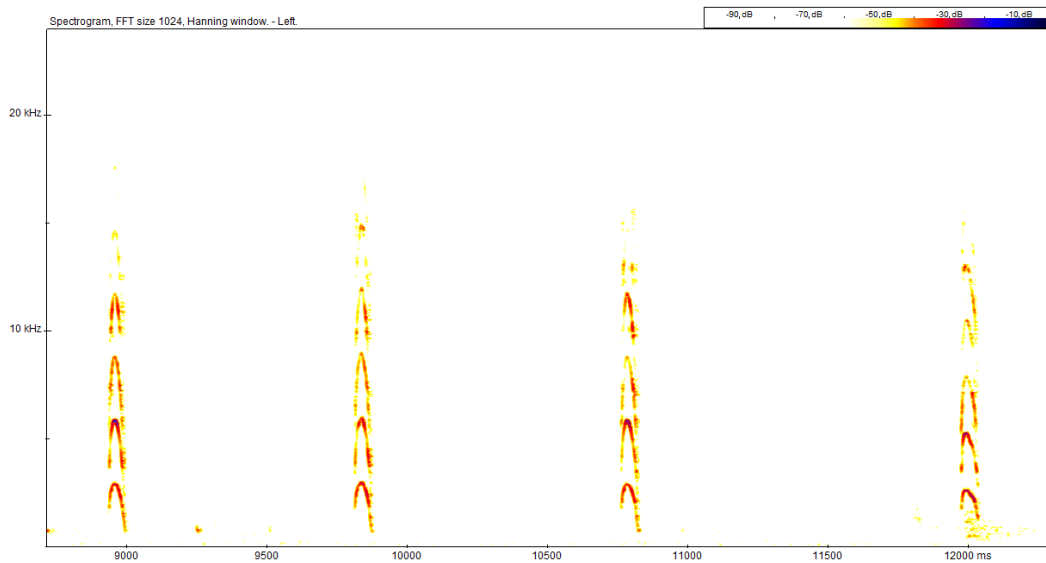
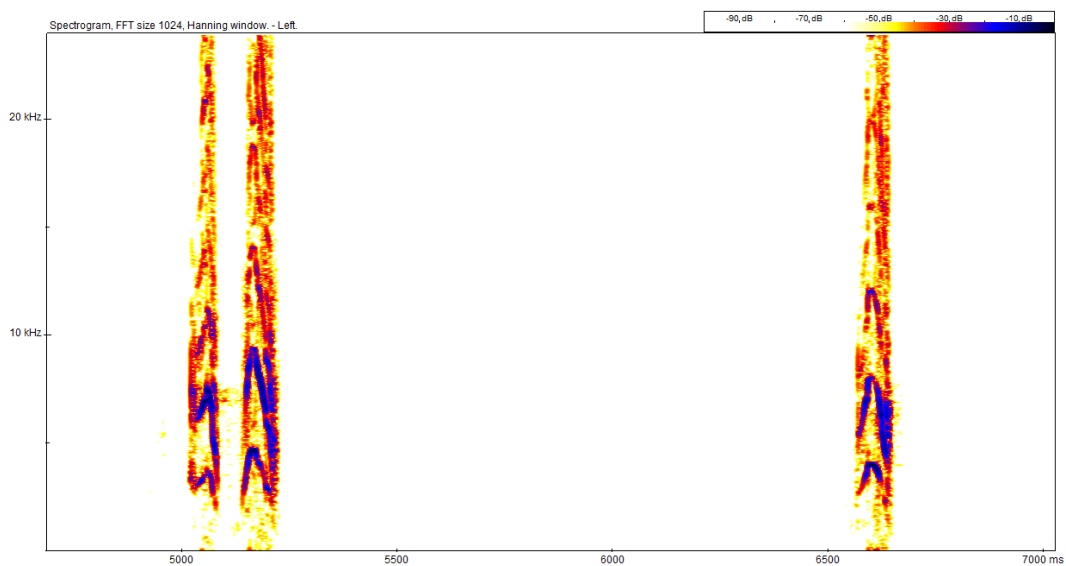Figure 2. *Loud call I* – four different calls

Figure 3. *Loud call II* – three different calls

4.1.1.3   Soft call I (Fig. 4)

The *soft call I* was recorded 251 times and lasted approximately 40 – 100 ms. The peak frequency of the key-note was 2.0 – 3.5 kHz, analogous to the *loud call I*. There are two main differences between the *soft call I* and the *loud call I*. Firstly the number of harmonics in the *soft call I* is limited to one or two. Secondly the intensity of the call is much lower in the *soft call I*. Exceptionally a call with three harmonics was noted as *soft call I*, when the intensity of the call was very low.

4.1.1.4   Soft call II (Fig. 5)

This call was recorded 96 times. The peak of the fundamental frequency was 3.5 – 4.5 kHz and one or two harmonics could be counted. Just as in the *loud calls*, the distinction between the *soft call I* and *II* is arbitrary. The difference is merely based on physical characteristics instead of behavioural ones. Since the discrimination of both is rather vague, the data on the associated behaviour of both calls are discussed together. Most *soft calls* were produced during encounters with familiar animals, while sniffing each other. *Soft calls* were also common when an animal was passed over by another animal. In combination with unfamiliar animals (both homospecific and heterospecific) *soft calls* were also produced. When assuming a submissive position the call was produced mostly in combination with the *cluck* and the *shriek* (see 4.1.1.11 and 4.1.1.13). During incisor fencing it was sometimes combined with the *cluck*. The animals also produced *soft calls* when being allogroomed in the nest.

4.1.1.5   High call (Fig. 6)

The *high call* was counted 47 times. It resembled the *soft calls*, but the frequency of the key-note was generally higher (5.0 – 7.0 kHz). The *high call* lasted 40-90 ms and was produced either singly or in short series of three or four. Mostly these calls preceded or followed *loud calls* and *soft calls*. The number of harmonics was never more than two.

Most *high calls* were produced during contact with an unfamiliar individual (homospecific and heterospecific). During some episodes of vocalized hopping, *high calls* were combined with *loud calls*. These episodes took place mainly after an incident of thrust-gaping or in a heterospecific encounter with *Fukomys whytei* (see 4.2). Further, *high calls* were sometimes registered when animals were disturbed while resting.
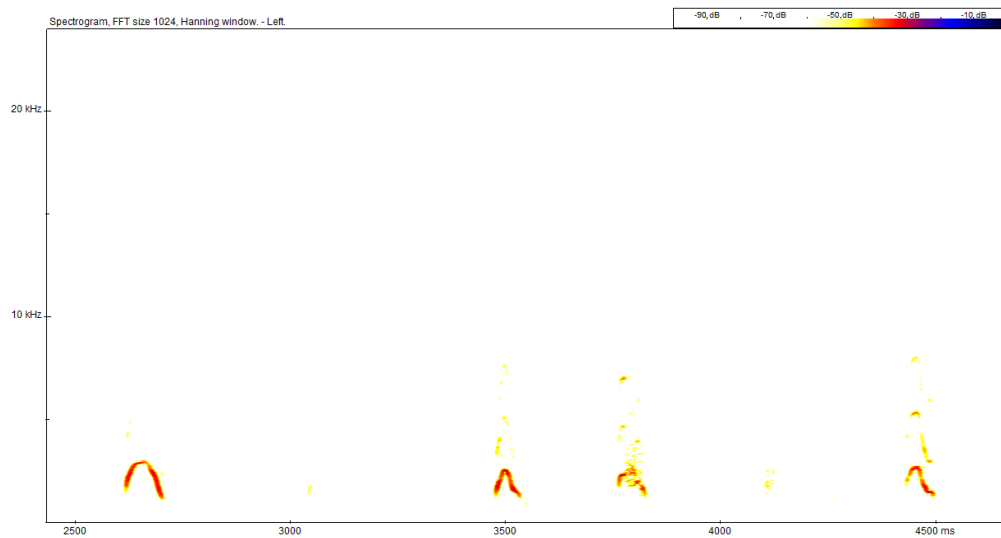
Figure 4. *Soft call I* – four different calls
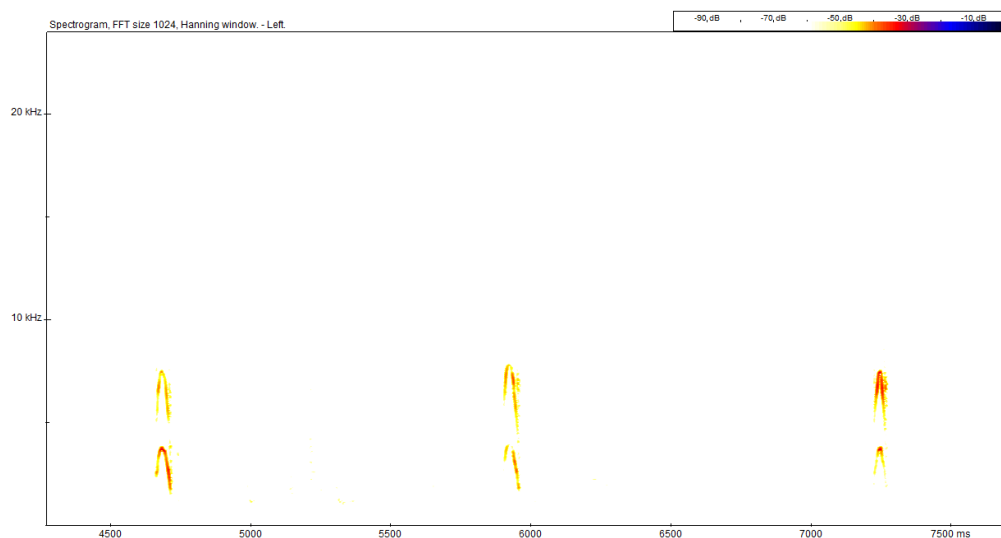
Figure 5. *Soft call II* – three different calls
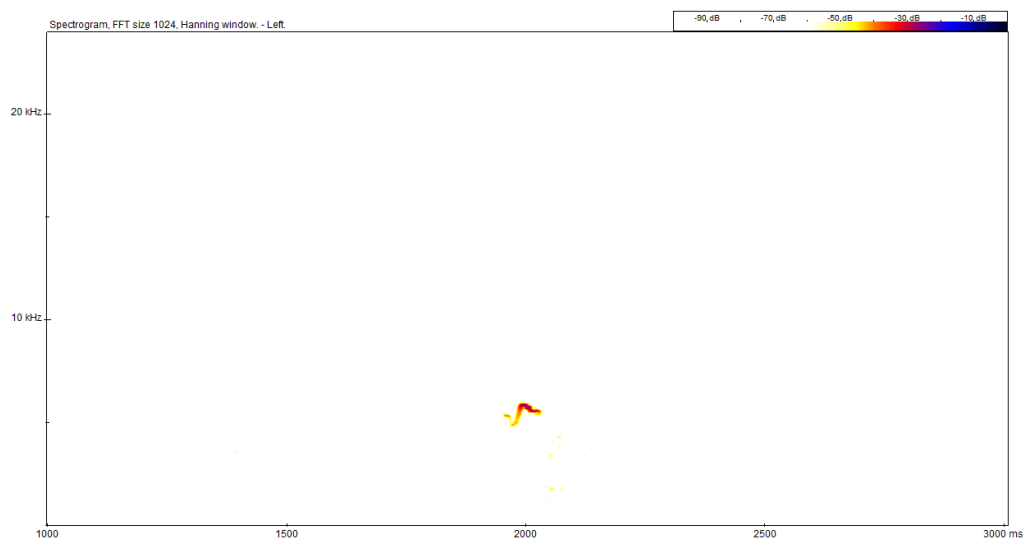
Figure 6. *High call*

4.1.1.6   Loud squeak (Fig. 7)

The *loud squeak* was recorded 67 times. This call resembled the *loud call I* and *II* and sounded similar to the human ear, though the sonogram showed consistent differences. Usually none or only one clear frequency band was visible. The rest of the sound consisted of a loud broadband sound. The *loud squeak* lasted approximately 40 – 90 ms, with a frequency range of 2.0 to 20.0 kHz. Most of the time the *loud squeak* was produced in combination with *loud calls* (and some *high calls*), but sometimes there were multiple *loud squeaks* in a series. The associated behaviours were similar to the ones associated with the *loud calls*, though the *loud squeak* is much less frequent. It was used in combination with *loud calls* during vocalized hopping, high pitched squeaking and protection of food. Rarely, it was also used when interacting with the pups, or receiving allogrooming. Disturbance in the nest while allogrooming or resting also elicited *loud squeaks*.

4.1.1.7   Soft squeak (Fig. 8)

This vocalization was recorded 82 times and sounded a lot like a *soft call*, but without clear frequency bands. Sometimes one frequency band was visible, but only to devolve into broadband sound. This call lasted approximately 40 – 90 ms. The frequency of the call ranged from 2.0 kHz to 7.5 kHz, in one case the upper boundary was 10.0 kHz. Most *soft squeaks* were produced in the same context as *soft calls*. They were heard during encounters with both familiar and unfamiliar animals, though the first case was more common. The *soft squeak* was also produced when being passed over or when resisting a pass-over by another individual. During vocalized hopping, high pitched squeaking, disturbance while resting or allogrooming and tug-of-war behaviour soft squeaks were also infrequently observed.

4.1.1.8   Chevron (Fig. 9)

The *chevron* was counted fifteen times, and looked like a version of the *loud call*. It contained a lot of harmonic frequencies, five to seven. The fundamental frequency peaked at 1.0 – 1.5 kHz and the call lasted 40 to 80 ms. The *chevron* was produced in situations of disturbance, or as a part of vocalized hopping and high pitched squeaking.
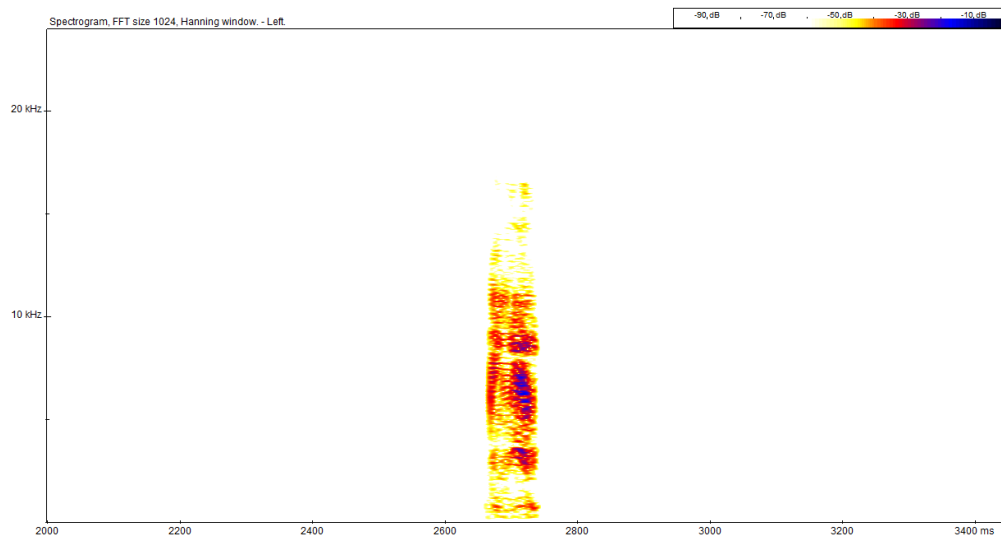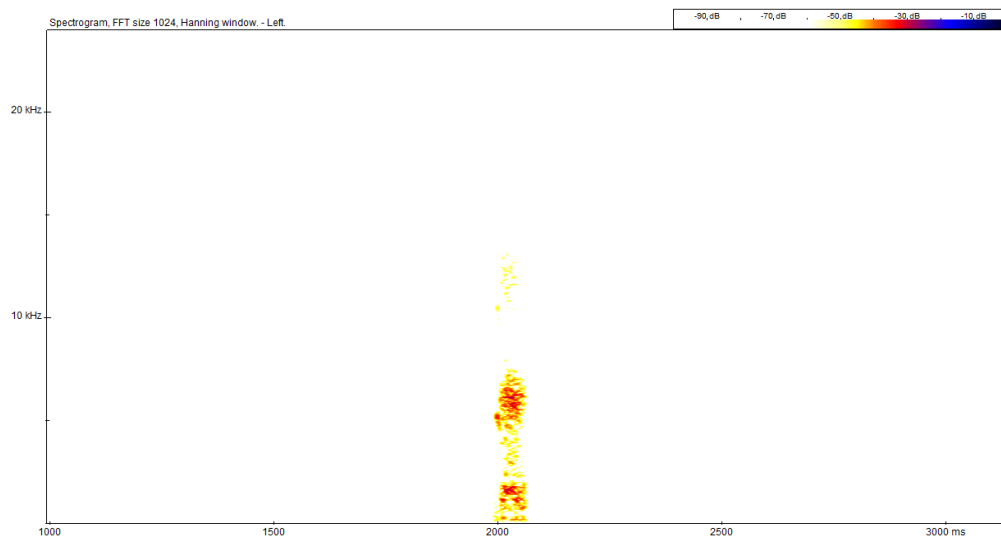
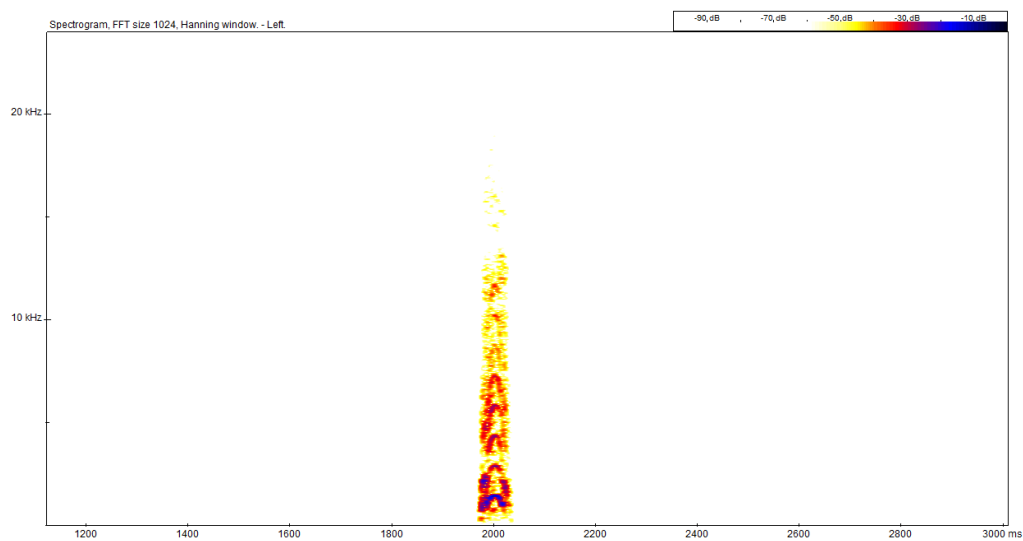Figure 7. *Loud squeak*

Figure 8. *Soft squeak*

Figure 9. *Chevron*

4.1.1.9   Grunt (Fig. 10)

The easily recognizable *grunt* was counted 107 times. Sometimes it was produced singly, but mostly in bouts. The *grunt* showed a broad frequency range of 0.3 – 20.0 kHz, with intensity peaks around 0.3 – 0.4 kHz and 0.7 – 0.8 kHz. Distinct frequency bands were present up to about 2.5 kHz and again between 4.5 – 6.5 kHz. Duration of the call varied between 50 and 260 ms, but usually hovered around 100 ms. It is clearly a vocalization associated with agonistic behaviour, as it was produced during open-mouth gaping and thrust-gaping. Often it was also used to scare other individuals away from a piece of food. Right before attacking another mole-rat, there was also a significant amount of *grunting* by the attacking animal. Most *grunts* were produced by the queen of the study colony.

4.1.1.10   Squeal (Fig. 11)

The *squeal* was counted thirteen times. The length of the call was variable and ranged from 75 to 550 ms. It consisted of a key-note peaking at 2.5 – 4.0 kHz and three to five harmonics. The intensity of this vocalization was comparable to the intensity seen in the *loud calls*. Most *squeals* were recorded during homospecific, intercolonial encounters while sniffing and high pitched squeaking. Some *squeals* were also emitted while grooming pups, during an episode of vocalized hopping and while protecting food.

4.1.1.11   Cluck (Fig. 12)

This low frequency call was produced 92 times, usually in bouts of several calls, sometimes in combination with short *shrieks* (see section 4.1.1.13) or *soft calls*. The peak of the fundamental frequency could be found at 1.5 – 2.0 kHz. The duration of the call was rather short, usually 20 – 60 ms. There were never more than two harmonics present.

The *cluck* was produced by male specimens in a submissive position (usually during or after a power-struggle in which dominance was established, see 4.2). This call was produced during both heterospecific and intercolonial homospecific encounters.
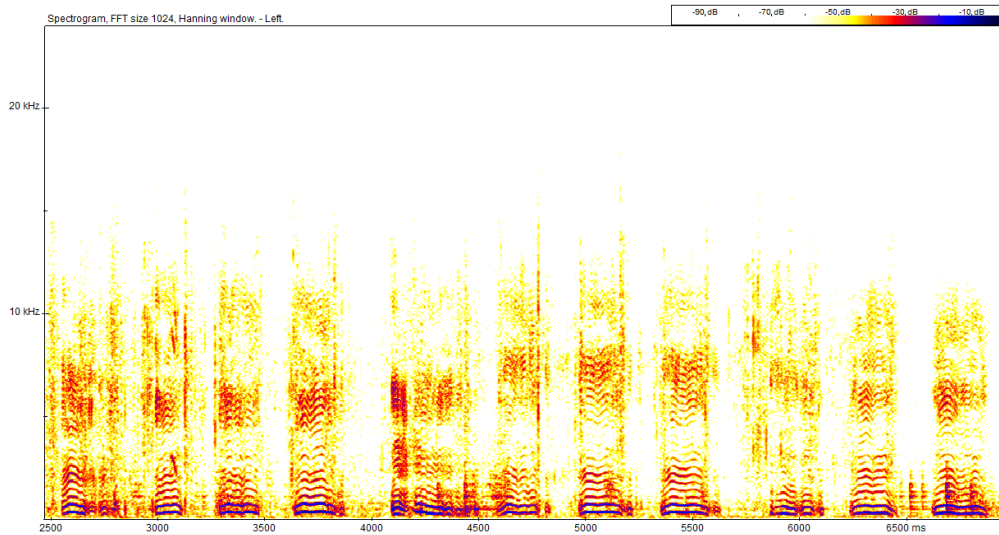
Figure 10. *Grunt* – twelve different calls
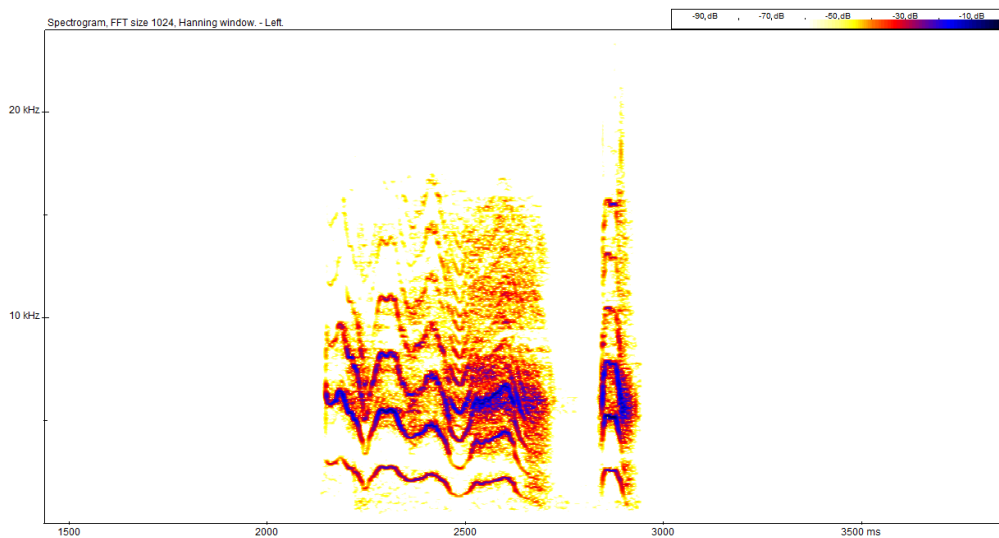
Figure 11. *Squeal* (followed by *loud call*)
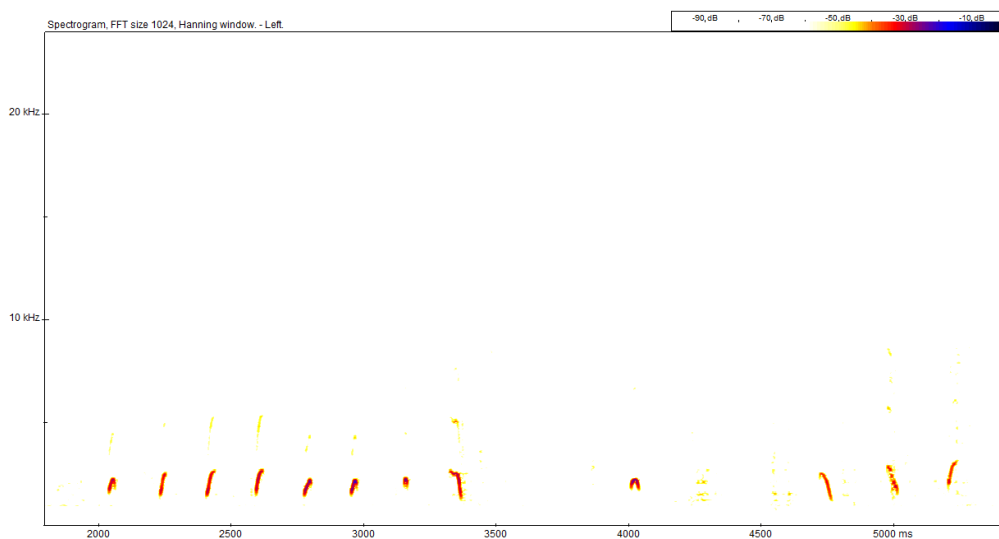
Figure 12. *Cluck* – twelve different calls

4.1.1.12   Female cluck (Fig. 13)

The *female cluck* was produced 241 times during pre-mating behaviour: anogenital sniffing, chasing and lordosis. Generally this call was low in frequency and intensity, with the peak of the fundamental frequency lying at 1.5 – 2.0 kHz. This short vocalization (20-60 ms) consisted of only one or two harmonics. The *female cluck* was produced in bouts of several calls and was often, but not always, combined with *shrieks* (see section 4.1.1.13).

4.1.1.13   Shriek (Fig. 14)

This call was recorded 134 times. *Shrieks* were produced by both males and females, but in different contexts. Females produced it during anogenital sniffing, chasing and lordosis. In this context it was always produced in combination with the *female cluck*. However, males also produced this call when they assumed a submissive position. This happened during both heterospecific and intercolonial homospecific encounters. The duration of the *shriek* was very variable, ranging from 40 to 700 ms. Usually *shrieks* were not produced singly, but in bouts. The peak of the fundamental frequency lied between 1.5 and 2.5 kHz.

4.1.1.14   Whistle (Fig. 15)

The *whistle* was recorded fourteen times. This call was produced by subordinate male individuals during intercolonial, homospecific encounters. The duration of this call was quite variable, the shortest one being 43 ms and the longest 579 ms. Most calls were between 200 – 400 ms long. The bottom harmonic peaked around 0.5 – 3.0 kHz. It was often hard to distinguish the peak frequency or the number of harmonics because of the compact nature of the call.
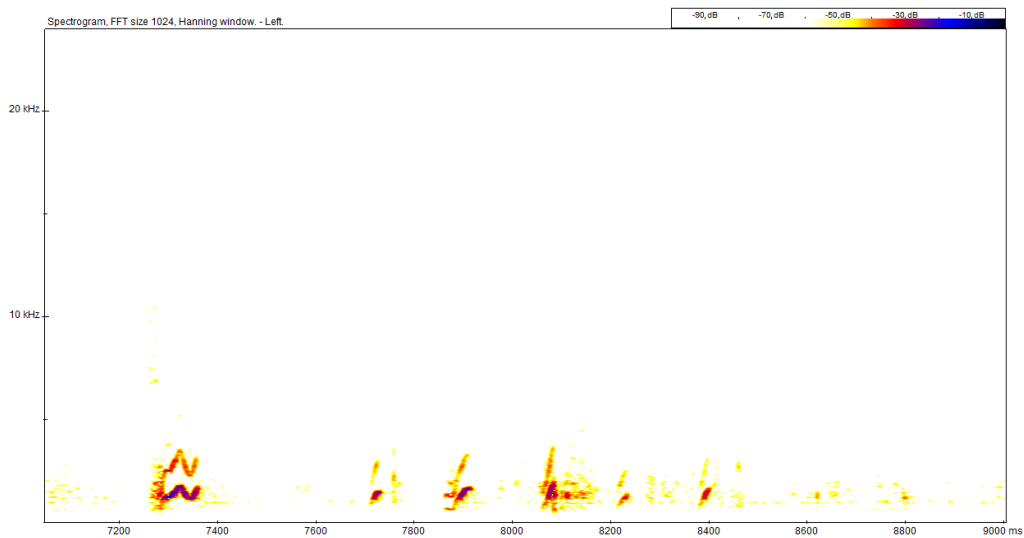
Figure 13. *Female cluck* – six different calls
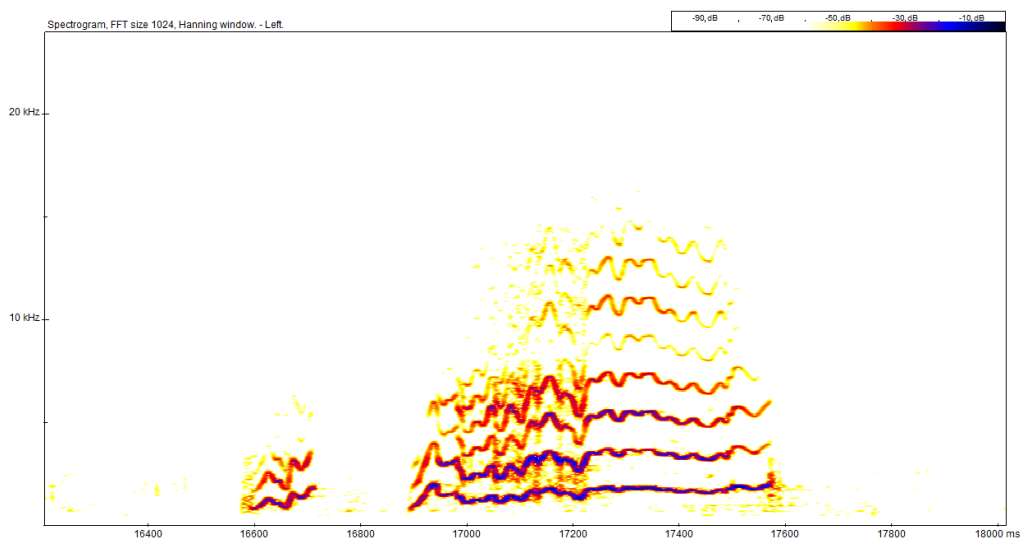
Figure 14. *Shriek* – two different calls
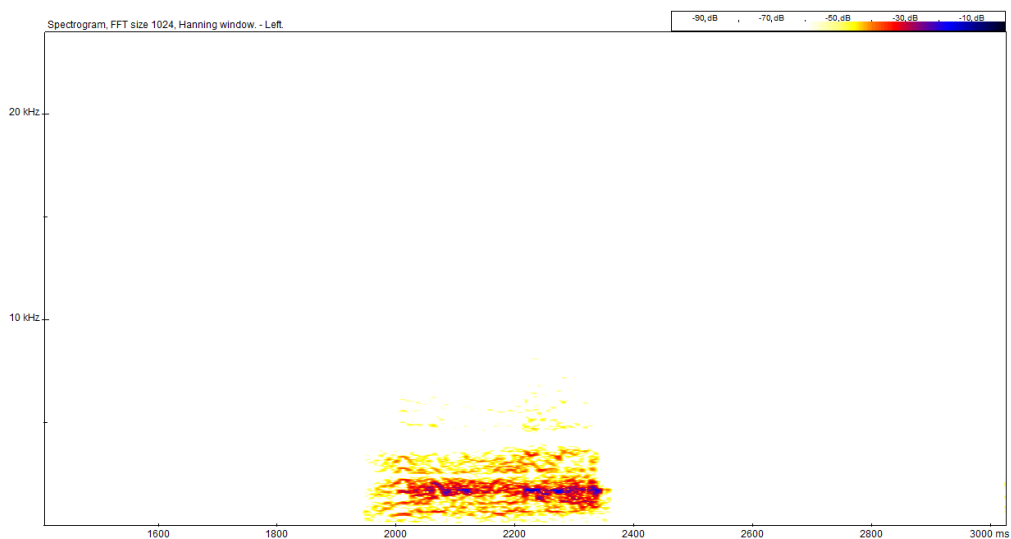
Figure 15. *Whistle*

4.1.1.15   Adult chirp (Fig. 16)

The *adult chirp* was registered only seven times. It can be described as a *loud call* or *squeal* that fades out near the end. It was produced by both males and females, during conflicts involving food, as a part of high pitched squeaking, or when encountering unknown conspecifics. Its length varied between 100 and 500 ms. Three to seven clear harmonics were visible, but in the part of the call that faded out, more frequency bands were present. The key-note peaked around 1.5 – 3.0 kHz. The *adult chirp* was produced singly, usually in between a series of loud calls.

4.1.1.16   Tooth grinding (Fig. 17)

Twelve episodes of *tooth grinding* were investigated. It was actually not a true vocalization, but a mechanical sound. The mole-rats produced this sound by rubbing the upper and lower incisors together. Both long and short strokes could be distinguished from the spectrogram. Long strokes lasted around 100 ms, short strokes only 20 ms. Long strokes were produced two or three times per second, short strokes up to six times per second. *Tooth grinding* usually lasted a few minutes, periods of 30 seconds to 5 minutes were registered. Its function is the sharpening of the incisors (Lacey et al., 1991).
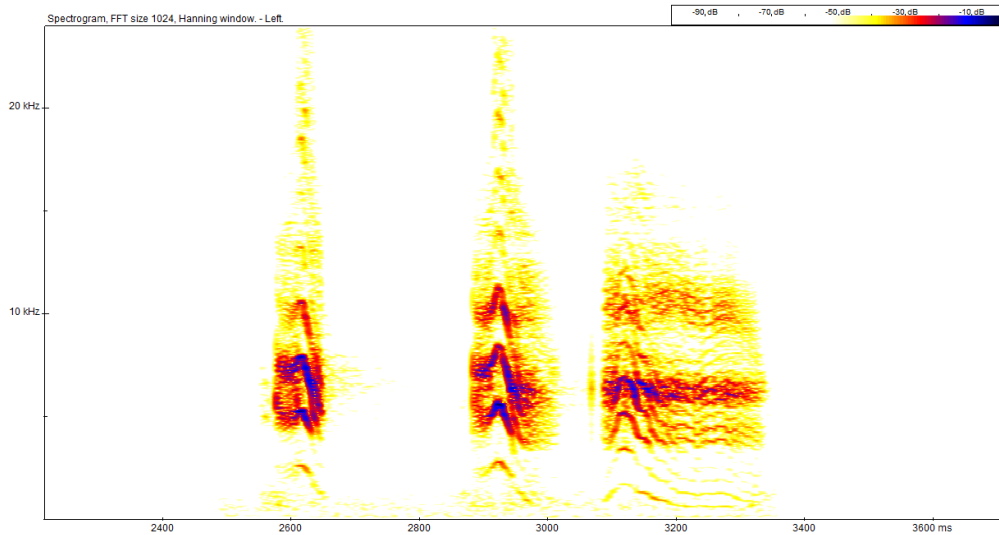
Figure 16. *Adult chirp* (and two *loud calls*)
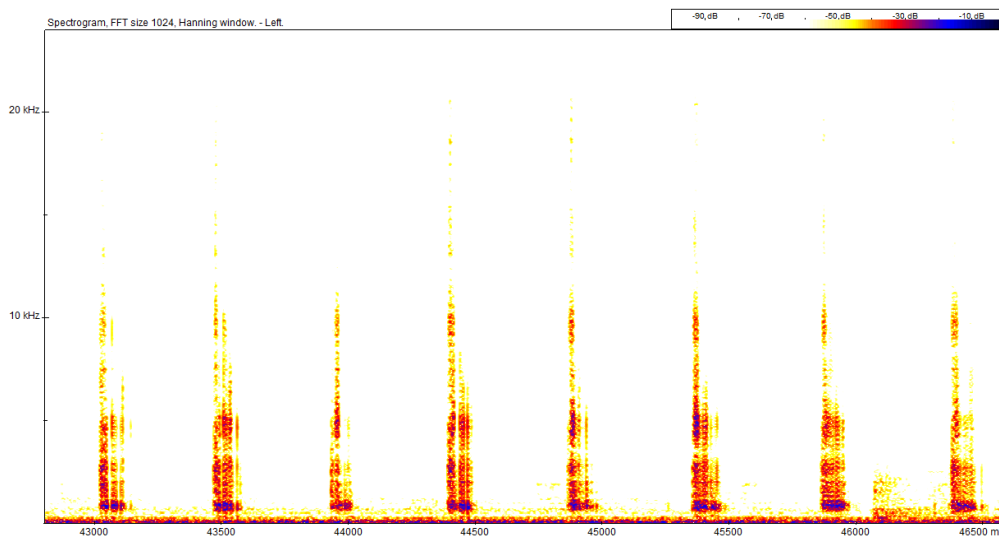
Figure 17. *Tooth grinding* – long strokes
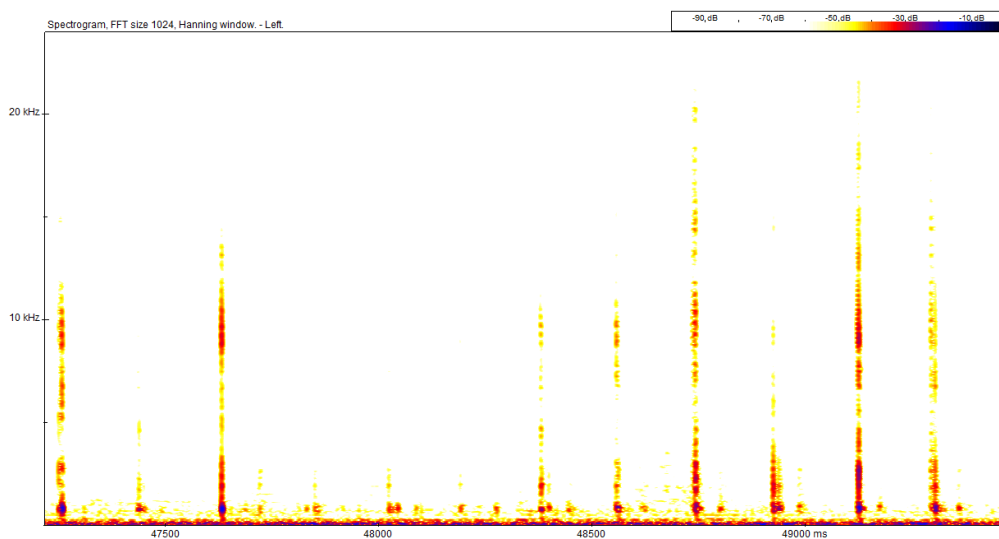
Figure 18. *Tooth grinding* – short strokes

4.1.2   Juvenile specific calls

4.1.2.1   *Juvenile loud call (Fig. 19)*

This call was registered 302 times, which made it the most frequent juvenile specific call. It was produced in different kinds of contexts: during playful interactions among the juveniles and during interactions with the mother or other colony-members. It is very similar to the adult *loud calls*, but more structurally variable. The peak of the fundamental frequency was 2.0 – 4.0 kHz, and consisted of 3 – 7 harmonics. The call lasted between 40 – 100 ms. The *juvenile loud call* was produced both singly and in series, sometimes combined with *juvenile soft calls*.

4.1.2.2   *Juvenile soft call (Fig. 20)*

The *juvenile soft call* was counted 74 times and lasted 30 – 80 ms. The peak of the fundamental frequency was 2.0 – 4.0 kHz, and consisted of one or two harmonics. Sometimes a call with three harmonics, but of very low intensity was named a *juvenile soft call*. Differentiating between *juvenile loud* and *soft calls* was an arbitrary process (cfr *loud call I* and *II* and *soft call I* and *II* in adults). The calls actually formed a continuous spectrum, instead of two clear categories. *Juvenile soft calls* were produced during playful interactions among the juveniles and during interactions with adult animals.

4.1.2.3   *Juvenile chirp (Fig. 21)*

The *juvenile chirp* was registered 27 times. It was a more lengthy juvenile call that lasted 70 – 150 ms. The peak frequency lied at 2.5 – 3.5 kHz. Most of the *juvenile chirps* were recorded during interactions with a parental animal (mostly the mother). The maternal behaviour that evoked most *juvenile chirps* was allogrooming. Some occasional *juvenile chirps* were also elicited during playful interactions among the juveniles, such as batting and sparring.

4.1.2.4   *Juvenile squeak (Fig. 22)*

This call was the least common juvenile call, and was only heard four times. The peak frequency of the key-note was 2.5 – 3.0 kHz. The call lasted 80 – 150 ms. It was produced during playful interactions among the juveniles, such as batting and sparring, and during interactions with a parental animal.

Figure 19. *Juvenile loud call*

Figure 20. *Juvenile soft call*

Figure 21. *Juvenile chirp*

Figure 22. *Juvenile squeak*

4.2 Experiments

4.2.1   Mating experiment

Two types of mating vocalizations were recorded, the *female cluck* and the *shriek*. Both were produced by the female. The vocalizations were not emitted during the actual copulation, but in the pre-mating phase, during anogenital sniffing, chasing and lordosis. No male courtship calls were recorded.

4.2.2   Pairing experiment

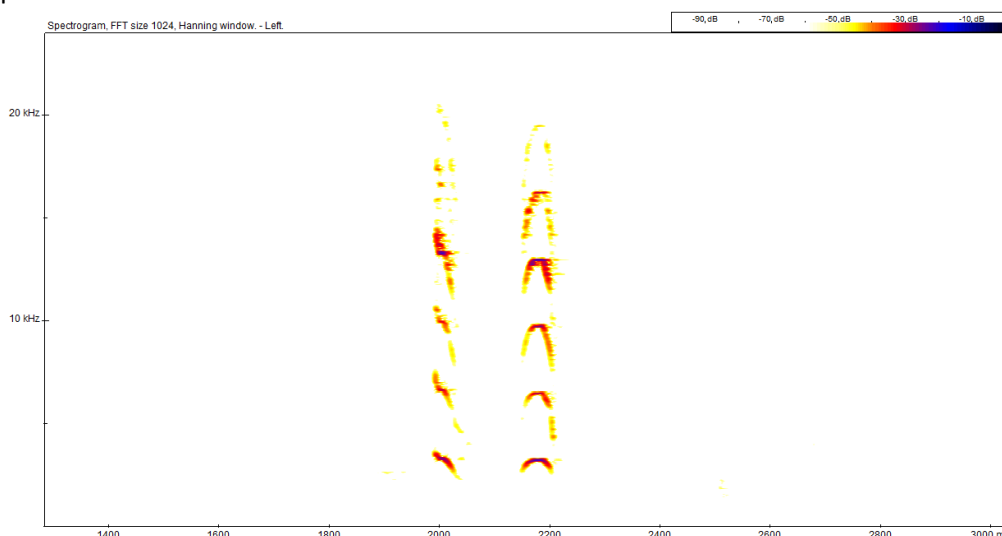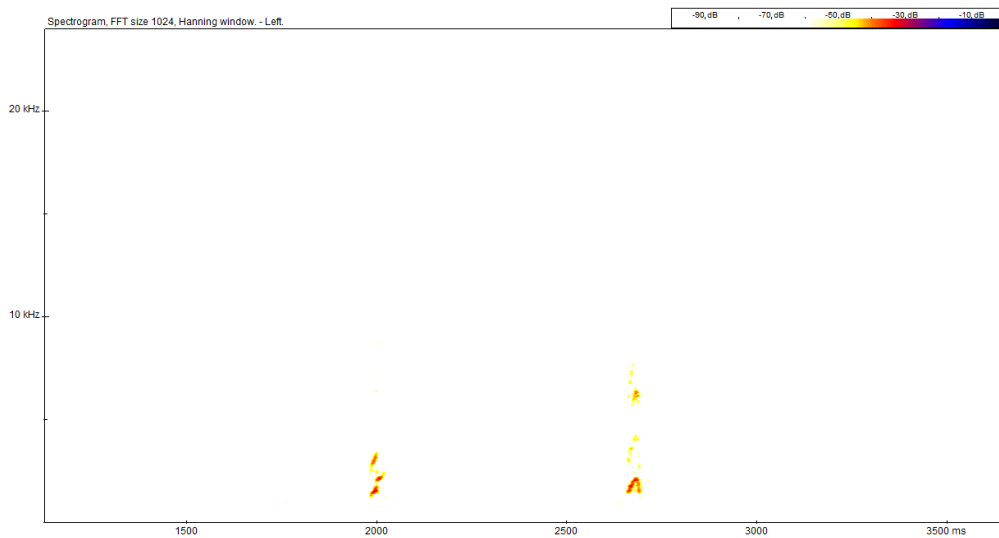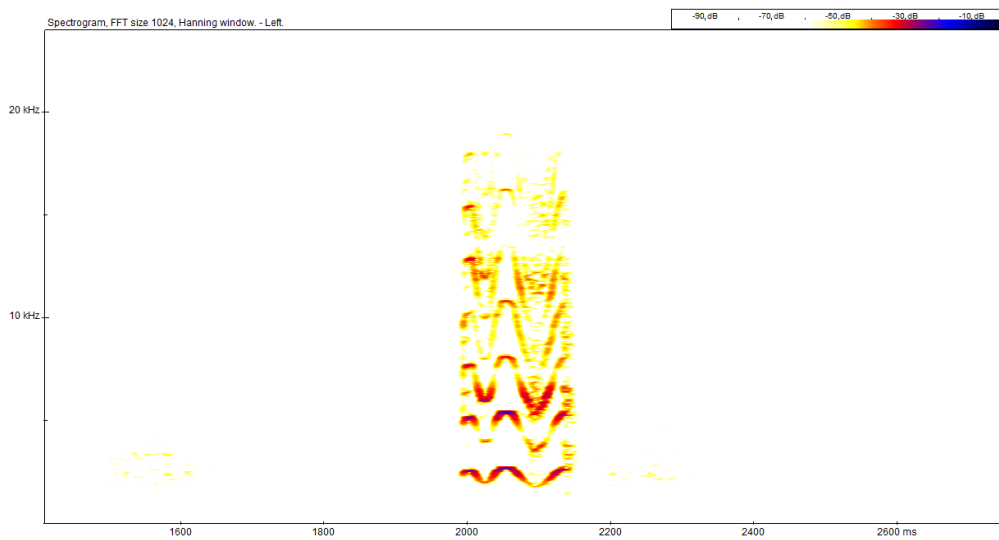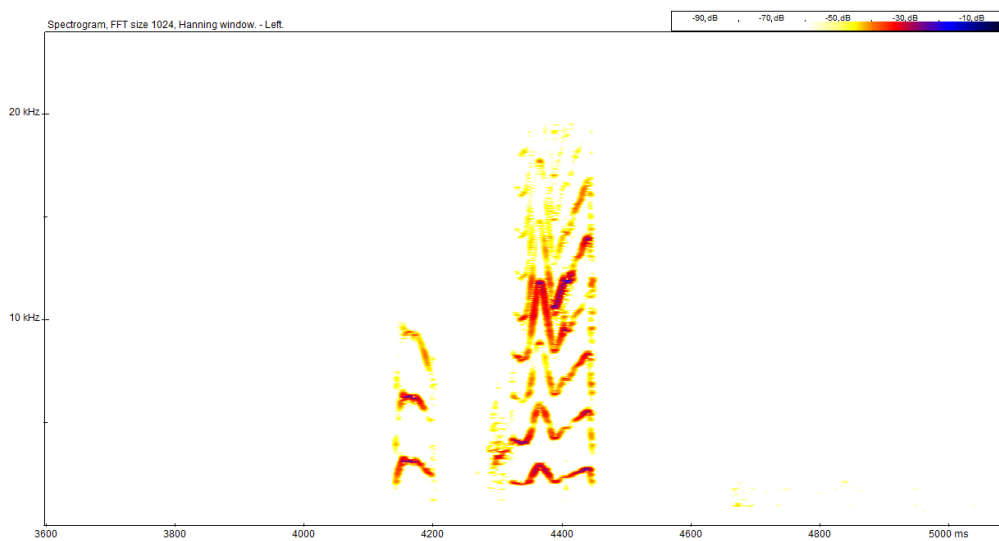During the intracolonial homospecific experiments, a number of different calls were recorded. During the first part of the experiment, the majority of the calls were *soft calls I* and *II*, with an occasional *soft squeak*. In this phase of the experiment the animals were often in close contact with each other, sniffing each other and passing each other sideways.

In the second part of the experiment, when a piece of cucumber was placed near the individuals, the calls were more varied. There were still a lot of *soft calls I* and *II*, but also a lot of *loud calls I* and a few *squeals*, *adult chirps* and *soft squeaks* during tug-of-war behaviour. When trying to protect the food from the other mole-rat, some *grunts* were emitted. A common reaction to the *grunt* was vocalized hopping, this could consist of a single call, but mostly a whole series of calls were emitted. For the most part, the emitted calls were *loud calls I* and *II*, but also some *soft calls* and *high calls* were emitted. An individual could also perform vocalized hopping when another individual occupied the food in such a way that it wasn't available for the first one to eat.This happened when the occupying individual took the food between the incisors and kept on turning its head and body away from the other individual. Even when the individuals managed to share the food, a lot of *loud* and *soft calls* were produced.

4.2.3   Homospecific encounters

As there were considerable sexual differences during the encounters, a distinction is made between female-female, male-male and female-male interactions. The interactions between females tended to be the more aggressive.

Females usually started by extensively sniffing each other, with one female often being a little more enthusiastic or curious, and the other one constantly turning away. While turning away, there was often high pitched squeaking and vocalized hopping. Almost every female-female encounter ended in a fight, with one individual attempting to bite the other. The individual trying to bite, emitted one or several *grunts*, while the other one resorted to vocalized hopping.

During the male-male encounters, there usually was a short power-struggle in which dominance was established. After this, two things could happen. In some cases the subordinate individual remained passive while being pushed over by the dominant one. In other cases the subordinate individual assumed the classic lordosis position, as seen by a female during mating. When assuming these submissive positions the animal emitted both *clucks* and *shrieks*, very similar to the sounds emitted by a female during mating. The dominant individual remained silent, except for one time, when an individual produced *tooth grinding* as an intimidation sound.

During the female-male interactions, there was some pre-mating behaviour, but never an actual copulation. Behaviours remained limited to anogenital sniffing, chasing and lordosis. During these behaviours the female emitted *female clucks* and *shrieks*.

4.2.4   Heterospecific encounters

The individuals of *Fukomys whytei* were dominant, except during the male-male encounters. Generally, *F. micklemi* individuals were more vocal than *F. whytei* individuals. For similar reasons as mentioned for homospecific encounters a distinction is made between female-female, male-male and female-male couples.

During the female-female interactions, the *F. micklemi* females seemed very stressed by the presence of a *F. whytei* female. Every time a *F. whytei* specimen approached too close, the *F. micklemi* females started vocalized hopping and as a consequence produced a lot of *loud calls I* and *II*, and some *loud squeaks*.

The male-male encounters were very similar to the homospecific male-male encounters described above. A power-struggle determined which individual was dominant. This could be either the *F. whytei* or the *F. micklemi* and was quite unpredictable. Again it was always the subordinate individual that produced vocalizations. These vocalizations were the same ones as produced during the homospecific, intercolonial encounters: *clucks* and *shrieks*.

Two different *F. micklemi* females were placed with *F. whytei* males, and both reacted in a completely different way. The first female (64) continuously performed vocalized hopping, thereby producing mainly *loud calls I* and some *loud squeaks*. The second female (67) barely vocalized, and when she did, she produced almost exclusively *soft calls* (*I* and *II*).

Because there was only one female *F. whytei* specimen suitable for these experiments, she was only placed once with a male *F. micklemi*, to limit stress on this individual. She produced sounds that were very alike to the mating sounds produced by the *F. micklemi* females. The *F. whytei* sounds were not part of this study and were not further investigated.

4.2.5   Juveniles and mother

During the short periods of time in which mother and pups were isolated from the rest of the colony, the mother carried the pups, pushed them around and groomed them. As mentioned earlier, four different vocalizations were produced by the juveniles. It was not possible to couple one specific vocalization with a behaviour, because most calls served multiple purposes. The *juvenile loud call* and the *juvenile soft call* were produced during all of the above mentioned behaviours. The *juvenile chirp* and the *juvenile squeak* were mostly associated with grooming behaviour, but were also heard during ad libitum sampling when the animals were batting and sparring.

4.2.6   Juveniles alone

The juveniles did not produce any audible sounds during these experiments (n = 2).

5. Discussion

In a recording period of 28 days, more than 4000 vocalizations were recorded, 2209 of these were analysed in spectrograms. Furthermore, the behaviour of *Fukomys micklemi* was investigated. Both behaviours and vocalizations were put together to construct a vocal ethogram. An effort was made to record vocalizations under different circumstances which are likely to occur in nature. The limited range of stimuli present in an artificial, captive environment should be taken into account when analysing the vocal repertoires of mole-rats in general. Therefore, in their natural underground habitat additional calls may have been sampled, e.g. agonistic behaviour and the corresponding calls under the presence of a predator.

During previous studies, an ethogram for *Fukomys micklemi* has been constructed, two behaviours were added to the existing ethogram (Bouly, 2005; Desmet, 2009 and Van Daele et al. in prep.; for an expanded ethogram see Appendix 9). *Nest behaviour* deserves a separate categorical description. This behavioural category consists of all types of resting, but also passing over, passing sideways, passing under and tail pulling, actions that took place in the nest on a regular basis. Most of latter behaviours also occurred outside the nest and have hence been described in the existing ethogram (Bouly, 2005; Desmet, 2009 and Van Daele et al. in prep; see Appendix 9).

Though it was not always clear which behaviour the animals were exactly displaying in the nest, some behaviours were not readily characterised by the above descriptions: namely *disturbed while resting* and *pushing for a good nest place*. *Disturbed while resting* occurred when a mole-rat was resting in the nest and was suddenly awoken by another mole-rat moving or entering the nest. The awoken animal usually emitted the *loud call* (*I* or *II*). *Pushing for a good nest place* was most often performed by the breeding animals, more specifically the breeding female. This fits with other observations: though the configuration of the huddle in the nest is constantly changing, it is not fully random. The breeding female (i.e. the queen) is a focal point of the resting pile, being huddled by all other animals and particularly by older males (Brückmann & Burda, 1997).

Three other behaviours, observed during the focal experiments could be added to the ethogram. As mentioned above, during the male-male intercolonial encounters, there usually was a *power-struggle* to determine the dominance of the animals. This kind of behaviour can be described as two male mole-rats pushing each other, after which one mole-rat will lay on its back or assume the lordosis position, while producing submissive calls. The second behaviour was seen both during ad libitum sampling and during intracolonial encounters. After placing a piece of food in the arena the *protecting food*-behaviour, in which one mole-rat took the food between the incisors and tried to protect it from another mole-rat by turning its head and body away, could often be observed. The mole-rat that couldn't reach the food would usually emit loud calls. The third behaviour observed during experiments was *high pitched squeaking*, which resembles the sound made during vocalized hopping, but without the clear hopping movement.

There were two main challenges when naming the different calls. Firstly there was the process of categorising the different call-types. Some of the calls (more specifically the *loud call I*, *loud call II*, *soft call I*, *soft call II* and the *high call*) physically formed a continuous spectrum, but showed consistent differences in peak frequencies. E.g. while *loud calls* could be distinguished from *soft calls* on a behavioural basis and sounded different, the differences between *loud call I* and *loud call II* could only be identified by physical characteristics of the spectrograms. Secondly there was the need

to use a nomenclature consistent with the literature. During the present study, spectrograms of the recorded vocalizations and their associated behaviours were compared to existing literature. Vocalizations that are had a similar structure and function across species were given published names, but how to name a call that doesn't exist in another species or genus? In the latter case, names were chosen based on how the calls sounded or in some cases based on names of rather similar calls in the literature, of which names were adapted to clearly state the difference (e.g. a *cluck* in Credner et al. (1997) and the *female cluck* in the present study).

5.1 Social versus solitary

Credner et al. (1997) state that *Fukomys*, more specifically *Fukomys anselli* and *Fukomys mechowii* are fairly vocal animals. This study on *Fukomys micklemi* confirms the existence of an elaborate vocal repertoire for *Fukomys* species. There seems to be a correlation between the degree of social organization and the diversity of the vocal repertoire (Schleich et al., 2007). Keeping this in mind, it can be expected that social mole-rat species are more vocal than their solitary counterparts. In other words, a sociality continuum of vocalizations may exist in Bathyergidae.

The vocalizations produced by *Fukomys micklemi* could be subdivided into twenty different types of vocalizations. Sixteen of these were produced by adult animals, four by juvenile animals, a repertoire similar to earlier studies on the vocal repertoire of other (eu)social mole-rats, such as *F. anselli* and *H. glaber*. Credner et al. (1997) distinguished fourteen different adult specific sounds and three juvenile specific sounds in a sister species, the social *Fukomys anselli*. Pepper et al. (1991) found that the eusocial naked mole-rat (*Heterocephalus glaber*) produces seventeen types of calls. Twelve of these were adult specific vocalizations, five were produced exclusively by juveniles

In a study on *Ctenomys talarum* (Ctenomidae, Rodentia), a solitary species, only four types of adult specific calls were recorded. Two of these types were associated with agonistic behaviour, the other two were mating-specific vocalizations. No contact calls or distress calls were reported, which has been related to a lesser degree of social organisation (Schleich & Busch, 2002). The main social activities of solitary species are related to territorial defence and mating coordination (Schleich et al., 2007).

Other factors than sociality may have to be considered when explaining evolution of the communicative complexity in mole-rats. It might be interesting to further investigate morpho-physiological or behavioural differences that could have influenced the evolution of communicative complexity in the different species of mole-rats (Blumstein & Armitage, 1997).

5.2 Call categories

In Pepper et al. (1991) and Credner et al. (1997), all adult calls were subdivided into general categories such as: contact calls, mating calls, agonistic calls and distress calls. For the sixteen different adult calls recorded for *Fukomys micklemi* this seemed like a daunting task, since many vocalizations served multiple purposes. Some vocalizations were easy to classify, such as the *grunt*, which clearly was an agonistic vocalization. The *loud call* (*I* and *II*) on the contrary were used as both contact calls (e.g. during allogrooming) and distress calls (e.g. during vocalized hopping), making them a lot harder to categorize.

Therefore instead of placing each call-type into only one category, it seemed more useful to ascribe a call to different categories when necessary (Table 2). As opposed to e.g. Credner et al. (1997) 'submissive call' is added as a fifth category. Below, a comparison is made to both *F. anselli* and *H. glaber*, based on the structures derived from the spectrograms. For a general comparison of the calls of *F. micklemi*, *F. anselli* and *H. glaber* see Appendix 10.

Table 2. Call categories					
Call type	**Contact call**	**Mating call**	**Distress call**	**Agonistic call**	**Submissive call**
Loud call I	X		X		
Loud call II	X		X		
Soft call I	X				X
Soft call II	X				X
High call	X		X		
Loud squeak			X		
Soft squeak	X				
Chevron			X		
Grunt				X	
Squeal			X		
Cluck					X
Female cluck		X			
Shriek		X			X
Whistle					X
Adult chirp			X		
Tooth grinding				X	

5.2.1   Contact calls

Contact is defined as an initial and/or friendly encounter between two animals (Credner et al., 1997). The contact calls recorded for *Fukomys micklemi* were very different from the ones recorded for *Fukomys anselli*. The latter has two types of contact calls: a *grunt*[2] and a *twitter*. The grunt was recorded in the breeding pair during greeting and anogenital sniffing (Credner et al., 1997). For *Fukomys micklemi*, however, no specific type of greeting call could be distinguished for the breeding pair. All contact calls that were registered in the breeding pair, were also used by the other animals in the colony. The second type of contact call found by Credner et al. (1997) was a *twitter*, which accompanied the meeting of an adult animal with an unfamiliar juvenile. We did not record a *twitter*, but no experiment placing together an adult with unfamiliar juveniles was performed, due to the fact that we wanted to limit the stress on the only two juveniles present in the colonies. We found six different call-types in *F. micklemi* that functioned as contact calls, albeit not exclusively.

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[2] In Credner et al. (1997) three types of grunts were described (*grunt*, *grunt I* and *grunt II*). The type of *grunt* that corresponds to the *grunt* discovered in this study is a *grunt II*.

The calls that were most used as a greeting call were the *soft call I* and *II*, especially when encountering a familiar animal. This is similar to the contact calls described for *Heterocephalus glaber* (Pepper et al., 1991; the so-called routine vocalizations). In *H. glaber* a *soft chirp* functioned as the most common contact call and was structurally very similar to the *soft call II* recorded for *Fukomys micklemi*. It has been suggested that such *soft chirps* function in social rank recognition, rather than in individual identification (Yosida & Okanoya, 2009). The other calls that functioned as contact calls for *F. micklemi* were the *loud call I* and *II*, the *high call* and the *soft squeak*. These calls were either non-existent for *F. anselli* or *H. glaber* or were used in completely different contexts, in this case they will be discussed in one of the other categories.

5.2.2   Mating calls

Mating was usually accompanied by intense vocalization. Two mating calls could be distinguished and both were produced by female specimens: the *female cluck* and the *shriek*. The term *female cluck* is used in order to avoid confusion with a *cluck* described for *F. anselli* (Credner et al., 1997) which is a courtship call produced by males only. Although both calls look structurally similar, they are produced by different sexes and are rather best kept separate for future reference. The *shriek* on the other hand, is produced by females of both *F. micklemi* and *F. anselli*. However, the latter species also produced a *cry*, a call that is uttered near the end of the copulation (Credner et al., 1997). This type of call was not recorded for *Fukomys micklemi*. In general no sounds were recorded during the copulation itself, only during the pre-mating phase of anogenital sniffing, chasing and lordosis. In *Heterocephalus glaber*, only one mating vocalization was found, a *V-trill*. Though the structure of the call was very different from the mating calls recorded for *Fukomys micklemi*, the behavioural context was very similar. A *V-trill* was produced by sexually receptive breeding females, especially while soliciting copulations (Pepper et al., 1991). These differences among mating calls indicate that vocalizations can play an important role in reproductive isolation. This was already mentioned by Nevo et al. (1987) who found that vocalizations are an important pre-mating isolation mechanism in different chromosomal species of *Spalax ehrenbergi.*

5.2.3   Agonistic calls

Only one agonistic vocalization was recorded for *Fukomys micklemi*, the *grunt*. For *Fukomys anselli* six different aggressive calls have been found. A *whistle*, two versions of a *trill*, a *hiss* and two versions of a *grunt* (Credner et al, 1997). The *whistle* has also been recorded for *Fukomys micklemi* but was placed under the category of submissive calls because it was never followed by an attempt to bite or attack another mole-rat. A *grunt II* recorded by Credner et al. (1997) is very similar to the *grunt* recorded for *Fukomys micklemi*. A *hiss* has not been recorded, but was heard once, during feeding, after the colony had been disturbed by an experiment. For *Heterocephalus glaber* four different agonistic vocalizations have been recorded: a *grunt*, a *hiss*, an *upsweep trill* and a *loud chirp*. The latter is very similar to the *loud call I* and *II* in *F. micklemi* , though these didn't seem to serve an agonistic purpose for *Fukomys micklemi*. As mentioned, the *loud call I* and *II* were mostly used as distress calls and, to a lesser extent, as contact calls. Nothing similar to the *upsweep trill* was recorded during this study, but in the experiment by Pepper et al. (1991) this call was associated with very violent behaviour.

When comparing all vocalizations found across different studies, it seems the *grunt* (named *grunt II* in Credner et al. (1997)) is a very conserved vocalization. It is virtually the same across all studied

social species, and is used in the same agonistic context. Credner et al. (1997) not only investigated *Fukomys anselli*, but also did a relatively short study on giant mole-rats (*Fukomys mechowii*). Both species used the *grunt* in aggressive situations, as did *Heterocephalus glaber* and *Fukomys micklemi*. The universal nature of the *grunt* might be explained by its use on an interspecific level. Being able to communicate aggressiveness might be useful when encountering a different species of mole-rat or a predator.

5.2.4   Distress calls

Seven calls seemed to function as distress calls: the *loud call I* and *II*, the *high call*, the *loud squeak*, the *chevron*, the *squeal* and the *adult chirp*. Generally these calls can be described as having a wide frequency range and high intensity. Two distress calls were recorded for *Fukomys anselli*, a *loud call* and a *scream*. A *loud call* of *F. anselli* was very similar to the *loud calls* recorded for *Fukomys micklemi*. A *scream* in *F. anselli* appeared to be a variation of a *loud call*, produced when the animals seemingly experienced severe pain. At no time did the specimens in this study appear to experience severe pain, therefore the call could not be registered. The vocal ethogram of *Heterocephalus glaber* revealed four distress calls: a *tap*, a *sneeze*, a *low-pitched chirp* and a *scream* (Pepper et al., 1991). None of these were heard in *Fukomys micklemi*.

5.2.5   Submissive calls

Yosida & Okanoya (2009) state that a *soft chirp* produced by *Heterocephalus glaber* might function as an affiliative (promoting social cohesion) or submissive signal. In the present study submissive calls are retained as a full category. Five submissive calls were recorded: the *soft call I* and *II*, the *cluck*, the *shriek* and the *whistle*. Generally, all submissive calls were either low-frequency or low-intensity. As mentioned earlier, the *soft calls* also served an important function as contact calls. The *shriek* was also used for mating purposes, but the *cluck* and the *whistle* were exclusively submissive. It may be noteworthy that all submissive calls were produced by male specimens.

An analogy could be detected between mating calls and submissive calls. Though both categories were produced by different sexes, they indicated a certain level of subordinance. In case of mating behaviour this submission was directed towards the male. In case of a power-struggle between unfamiliar individuals, the subordinate male expressed his submission towards the dominant male.

Nevo et al. (1987) already came to this conclusion when investigating the courtship call of *Spalax ehrenbergi* (Spalacidae, Rodentia). They noticed that this call was used primarily by the submissive but also by the dominant partner during fights. Here, a mating call thus also served as an appeasement call (Nevo et al., 1987).

5.3 Investigation of behaviours

5.3.1   Which behaviours were more commonly associated with vocalizations?

Vocalizations were observed only when mole-rats were in the vicinity of other mole-rats. Physical contact in particular elicited vocalizations. These physical contacts could be described as "friendly", including sniffing, passing each other sideways, passing over and allogrooming and "hostile", including thrust-gaping and biting. In fact a third category of physical contact can be added in which

the contact is neither friendly nor hostile, but submissive in order to avoid possible attacks by another mole-rat.

Vocalizations further occurred commonly during interactions in a foraging context: in particular during tug-of-war behaviour and efforts to keep another mole-rat from stealing food. Also pre-mating behaviour was commonly associated with vocalizations. However, there were some interindividual differences as to the intensity of the calls and the ratio of *shrieks* to *female clucks*. Stress situations were commonly associated with vocalized hopping and high pitched squeaking.

5.3.2   Functionality of sound frequency

Morton (1977) proposed that, for the vocal repertoire of some birds and mammals, harsh, low-frequency sounds are used in "hostile" situations and pure, tone-like, relatively high-frequency sounds in "friendly" situations (Morton, 1977 in Schleich et al., 2007). Applied to the above data, this hypothesis doesn't generally hold for observations in *F micklemi*. The submissive calls, used in the intercolonial encounters were mainly low-frequency, but these weren't hostile per se. Probably the animals were under some kind of stress, but they didn't behave truly agonistic towards each other, except for some of the females. For the only agonistic call recorded, Morton's rule most certainly applied. The *grunt* can be described as a harsh, low-frequency sound. Whether or not the higher calls were used in more friendly situations, remains a question. The *loud calls* for example can be described as pure, tone-like, relatively high-frequency calls, but are used both in cases of friendly contact and distress. For the soft calls then, Morton's rule does seem to apply. In general, Morton's rule is not applicable to all investigated vocalizations in the present study.

5.4 Low frequency range in relation to underground communication

Because of the acoustic characteristics of the subterranean milieu, vocalizations in all subterranean rodents investigated up to date, are shifted towards the middle to low frequency range (Pepper et al., 1991; Credner et al., 1997). The frequencies of the recorded sounds for *Fukomys anselli* mainly ranged between 0.5 and 16.0 kHz, with fundamental frequencies below 2.5 kHz (Credner et al., 1997). Most of the vocalizations produced by *Fukomys micklemi* were situated in a frequency range of 0.5 – 17.0 kHz, though some had harmonic frequencies that extended to more than 20.0 kHz. The peaks of the fundamental frequencies ranged from 0.5 – 4.5 kHz, with exception of the *high call* that could have a peak fundamental frequency up to 7.0 kHz. Generally, it might be stated that the vocalizations of *Fukomys micklemi* were situated in the low to middle frequency range (as expected for subterranean rodents), of course with some exceptions to this rule (e.g. the *high call*). Comparing this to the vocalizations of *Fukomys anselli*, as described by Credner et al. (1997), the results seem quite similar. However, the fundamental frequencies of the vocalizations by *Fukomys anselli* were situated below 2.5 kHz (Credner et al., 1997), which was generally not the case for *Fukomys micklemi*. Only four calls (the *chevron*, the *cluck*, the *female cluck* and the *whistle*) had a fundamental frequency that was always lower than 2.5 kHz. The other calls, with the *loud call II* and *soft call II* as exceptions, were sometimes situated below 2.5 kHz but not as a general rule. This corresponds with results found in studies that investigated hearing in other obligatory subterranean rodents. The frequency range of best hearing in naked mole-rats (*Heterocephalus glaber*) is found between 4.0 and 6.0 kHz (Yosida et al., 2007). In the morphologically convergent coruros (*Spalacopus cyanus*, Octodontidae, Rodentia), which do forage above ground, optimal hearing was recorded at frequencies between 1.25 and 1.6 kHz (Begall et al., 2004).

All recordings have been made under open-field conditions, both in an artificial nest and in open terraria, i.e. not in burrows. According to Credner et al. (1997) this probably doesn't make a difference, because it is highly improbable that mole-rats would display different types of vocalizations aboveground and underground. It is on the other hand highly convenient to record under open-field circumstances as this excludes echoes and distortions introduced by the tunnel-system (Credner et al., 1997).

5.5 General comparison to other rodent and mammal species

Mammals vocalize in quite a number of behavioural contexts. The main purpose of produced sounds is the alteration in behaviour of members of their own or other species (Ehret, 1980). According to Heffner & Heffner (2008) a general feature of mammals is their good high-frequency hearing, an exception to this rule is the group of subterranean mammals. The latter are adapted to the one-dimensional world of an underground habitat. Hence they have little use for sound localization and are therefore released from the selective pressure to hear high frequencies. For other rodents, the production of ultrasonic vocalizations and hence a good high-frequency hearing, is not that unusual (Heffner & Heffner, 2008). An extensive amount of literature is available on the description of ultrasonic calls in several species of rodents. However, high-frequency communication is less relevant here, especially ultrasonic communication. The type of communication very typical for subterranean rodents is low-frequency communication, in spite of small differences in the frequency range (Schleich et al., 2007). In Heffner & Heffner (2001) audiograms of five species of rodents were investigated. This study included the groundhog (*Marmota monax*), the chipmunk (*Tamias striatus*), Darwin's leaf-eared mouse (*Phyllotis darwini*), the golden hamster (*Mesocricetus auratus*) and the Egyptian spiny mouse (*Acomys cahirinus*). They found that there was a considerable amount of variation in sensitivity to high frequencies and an even greater variation in low-frequency hearing. With regard to this low-frequency hearing the animals fell into two groups, those with extended low-frequency hearing (chipmunks, groundhogs and hamsters) and those with restricted low-frequency hearing (spiny and leaf-eared mice). Interestingly, the animals with extended low-frequency hearing are all burrowing rodents (Heffner et al., 2001). This research indicates once more the importance of low-frequency acoustic communication in the underground environment.

5.6 Do intercolonial, same sex-encounters lead to more agonistic behaviour (and associated vocalizations) than intracolonial encounters? Is there a difference between homospecific and heterospecific, same sex- encounters?

Intercolonial encounters did not lead to more agonistic behaviour per se. It is a fact that the behaviour performed during intracolonial and intercolonial encounters differed greatly, but both were not truly agonistic. During the first part of the intracolonial experiment, in which pairs of individuals from colony FMI-04 were placed in a separate arena, a lot of soft contact calls were produced and there was absolutely no sign of aggression. When a piece of food was placed in the arena the interactions became a little more competitive (tug-of-war and the protection of food) and the calls became more varied. *Soft calls I* and *II* were still produced, but combined with *loud calls I* and *II*, *squeals*, *adult chirps* and some *soft squeaks*. Exceptionally some *grunts* were emitted to scare the other individual away from the food. Generally the intracolonial encounters were gentile, probably because of the relatedness that exists within the colony.

Most agonistic interactions were seen during the homospecific, intercolonial encounters among females. Initially these encounters stayed friendly, with a lot of sniffing, but they always escalated into a fight, after one specimen emitted *grunts*. Ganem & Bennett (2004) focused on the tolerance towards unfamiliar conspecifics in female mole-rats. The study was performed on four species of mole-rats, ranging from solitary to eusocial (*Georychus capensis*, *Cryptomys hottentotus pretoriae*, *Fukomys darlingi* and *Fukomys damarensis*). They found that social mole-rats discriminate against unfamiliar conspecifics, but that they are able to show great tolerance to unfamiliar animals. They can even cope with such events without apparent physiological stress. Apparently this duality is adaptive for a social mole-rat . The ability to discriminate against unfamiliar conspecifics allows them to protect their colony from intruders, while tolerance to unfamiliar conspecifics may allow a social mole-rat to remain in the colony when the breeder has to be replaced, as well as be beneficial during dispersal to ensure regrouping (Ganem & Bennett, 2004).

During the heterospecific female-female encounters, there was no real aggression. The *Fukomys whytei* specimen usually sniffed the *Fukomys micklemi* female, who responded with vocalized hopping and high pitched squeaking. No real conclusions can be drawn from this experiment, due to the fact that only one *F. whytei* female could be used for the encounters.

The male-male intercolonial encounters were even less aggressive. After and during a power-struggle to determine the dominance of the animals, one animal produced submissive calls (a combination of *soft calls I*, *soft calls II*, *clucks*, *shrieks* and *whistles*) and there were no fights or attacks. Male intercolonial encounters have been investigated by Jacobs & Kuiper (2000). Though the setting of their experiment was different, their conclusion remains more or less valid for the present study. They found that males directed significantly more agonistic behaviour towards foreign males (and male colony mates from which they had been separated) than towards colony-mates (that weren't estranged) (Jacobs & Kuiper, 2000). In this study males were usually not aggressive, but showed a clear power-struggle when encountering an unfamiliar male. Homospecific and heterospecific male-male encounters elicited the same behaviour and associated vocalizations.

5.7 Juvenile vocalizations

Four forms of juvenile tending, namely nursing, pushing, grooming pups and carrying pups, were observed, most of which were performed by the mother (Bouly (2005); Desmet (2009) and Van Daele et al. in prep.; Appendix 9). During the ad libitum sampling phase other adults than the queen were regularly observed taking care of the juveniles. Nursing was of course exclusively a maternal behaviour. The other three behaviours were performed both during the isolation of mother and juveniles and during the ad libitum sampling. All of them elicited vocalizations from the juveniles, but none of them were clearly linked to one specific vocalization. The *juvenile chirp* was usually produced during grooming of the juveniles, but grooming also elicited other calls. When comparing the juvenile specific vocalizations to the adult specific calls, it seems that the *juvenile chirp* and the *juvenile squeak* were clearly different from all adult calls. The *juvenile loud call* and the *juvenile soft call* were very similar to respectively the *loud calls* and the *soft calls* produced by the adult animals. They probably represent an immature form of these adult specific calls.

5.8 Future vocalization research?

Pepper et al. (1991) already mentioned that sounds produced by juveniles were generally less stereotyped and more structurally variable than adult vocalizations. The present study confirms this, especially the *juvenile loud call* was structurally rather variable. It might be interesting for future research to investigate vocalization-ontogeny, in other words, how do vocalizations change during the development from juvenile to adult.

An interesting addition to the current research, as already mentioned by Credner et al. (1997) are playback tests. These can be done to assess whether calls alone can trigger an action in colony mates or whether they are just accompanying or supporting elements of particular behaviours (Credner et al., 1997; Schleich & Busch, 2002).

A limitation to the present study was that the recording material didn't allow us to record sounds above 20.0 kHz. As a consequence no clear statement can be made about the presence of ultrasonic calls. It is possible that in addition to the vocalizations described in this thesis, adult or juvenile specimens are capable of producing ultrasonic calls in particular circumstances. Although pure ultrasonic vocalizations have not been registered either during earlier trials with a bat detector (Van Daele, personal communication) or in this study, several calls including the *loud call I* and *II* in this study show spectrograms with ultrasonic harmonics. For now, no information is available on the production of ultrasonic calls in mole-rats, so this would be an interesting field of research.

During future playback or ultrasonic experiments, extra attention must be paid to the presence of these ultrasonic harmonics. An interesting question is whether these are a by-product of produced sonic vocalizations or if they have a specific functionality. This question might be answered by comparing playback experiments in which these ultrasonic harmonics are correctly recorded (by using equipment suitable for ultrasonic recording) and experiments in which these harmonics are cut off.

6. Conclusions

Vocal repertoire   This study revealed an extensive vocal repertoire for *Fukomys micklemi*. Sixteen adult specific calls and four juvenile specific calls were recorded. These adult call-types were: the *loud call I, loud call II, soft call I, soft call II, high call, loud squeak, soft squeak, chevron, grunt, squeal, cluck, female cluck, shriek, whistle, adult chirp* and *tooth grinding*. Though the latter is not a true vocalization, but a mechanical sound, it was included in the vocalizations because of its common nature. This vocal repertoire is in accordance with previous studies, which already indicated that a social lifestyle implies a more extensive communication system than a solitary one. This is also visible in the Bathyergidae, where social species generally have a more elaborate vocal repertoire than solitary species. These calls were further subdivided into five call-categories: contact calls, mating calls, distress calls, agonistic calls and submissive calls. Certain call-types served multiple functions and hence belonged to more than one call-category at a time.

The juvenile call-types were the *juvenile loud call*, *juvenile soft call*, *juvenile chirp* and the *juvenile squeak*. Juvenile calls were mainly produced during playful interactions among juveniles and interactions with adult animals. The latter consisted of the grooming, carrying, pushing and nursing of the pups by their parents or other colony-members.

Frequency range   In accordance to previous research concerning underground vocalizations, the vocalizations of *Fukomys micklemi* were situated in the low to middle frequency range.

Ethogram   Based on ad libitum sampling, one behavioural category should be added to the existing ethogram of *Fukomys micklemi*, namely *nest behaviour*. Most behaviours that occur in the nest were already described in different contexts, but two behaviours can be added. These are the *disturbed while resting*-behaviour and *pushing for a good nest place*-behaviour. During focal experiments, three other new behaviours were added to the existing ethogram, the *power-struggle* among males, the *protecting food*-behaviour and *high pitched squeaking*.

Encounters   Interactions between two mole-rats differed greatly depending on the nature of the encounter. The performed behaviours and associated vocalizations differed greatly between sexes, colonies and species. Interindividual variation is also present, but wasn't investigated in this study and should be included in future research.

7. Summary

7.1 English summary

The Aridity Food Distribution Hypothesis (AFDH) offers a valuable model for the evolution of sociality in African mole-rats (Bathyergidae). It states that there is a correlation between the level of sociality and habitat aridity. Due to the unpredictability of the arid environment and the widely dispersed food sources, living and foraging in social groups will reduce both risks and costs in comparison to a solitary lifestyle. To integrate individuals into a social group, the development of communication signals is necessary. However, communication is severely constrained by the properties of the subterranean habitat and by the regressed and/or narrowly specialized auditory system. From the four main types of communication generally used by mammals, subterranean rodents mainly exploit olfaction, touch and vibration. The fourth one, vision, is of lesser importance to subterranean rodents, given the constant darkness in this ecotope. This study focused on communication by vibration, more specifically by acoustic signalling.

Because low-frequency sounds are better propagated in subterranean tunnels, audition in mole-rats is shifted to the low-frequency range and the hearing apparatus is tuned to these sounds. This correlates with the so-called 'stethoscope effect' in burrows, by which there is selective amplification of certain frequencies.

Acoustic signals are considered an important factor in reproductive isolation and speciation processes. Molecular and phylogenetic studies have revealed that the genus *Cryptomys* (Bathyergidae, Rodentia) comprises two different genera. Consequently, *Cryptomys* s.l. was split into *Cryptomys* and *Fukomys*. The latter is considered one of the most speciose genera. Because of this, current research is focusing on describing the different species within the genus *Fukomys*; a challenging task because most species are morphologically very similar. The species limits should be established using multiple sources of evidence which include data from the molecular, cytogenetic and organismal levels.

This thesis is an extension of current phylogenetic and behavioural studies. The main objective is to construct a vocal ethogram of *Fukomys micklemi* and make a comparison with vocal ethograms already constructed for other mole-rat species. A non-vocal ethogram has been characterized in previous studies, but adding vocalizations can be instrumental for gaining further insights into the complex interspecies relations within the genus.

Vocalizations were recorded under different circumstances (i.e. experimental settings) that are likely to occur in nature. Specimens used for ad libitum sampling all belonged to *Fukomys micklemi*. This species was also used for all focal experiments. For heterospecific encounters *F. micklemi* specimens were confronted with specimens of *Fukomys whytei*.

Vocalizations

Sixteen different adult specific calls and four juvenile specific calls were distinguished based on their physical characteristics derived from the spectrograms and the associated behaviours. These calls were recorded during ad libitum sampling as well as focal experiments. The multitude of vocal signals confirms previous research that describes social mole-rats as relatively vocal animals.

Adult calls

The sixteen adult calls were the *loud call I*, *loud call II*, *soft call I*, *soft call II*, *high call*, *loud squeak*, *soft squeak*, *chevron*, *grunt*, *squeal*, *cluck*, *female cluck*, *shriek*, *whistle*, *adult chirp* and *tooth grinding*. Categorising these calls was at times difficult, but was accomplished by investigating both the physical properties of the calls and the associated behaviours. The first five calls mentioned above physically formed a continuous spectrum, but could be distinguished by combining the audible sounds, their characteristics in the spectrograms and the associated behaviours. The sixteen call-types were further divided into five call-categories: contact calls, mating calls, distress calls, agonistic calls and submissive calls. Some calls belonged to more than one category, as they served multiple functions.

Contact calls were defined as vocalizations accompanying an initial and/or friendly encounter between two mole-rats. We found six calls that functioned as contact calls: the *loud call I*, *loud call II*, *soft call I*, *soft call II*, *high call* and *soft squeak*. The most common greeting call was the *soft call* (*I* and *II*). Contact calls were registered during ad libitum sampling, part one of the intracolonial pairing experiment and once during an intercolonial, heterospecific male-female encounter, in which the female was a *Fukomys micklemi* specimen.

Mating was usually accompanied by intense vocalization. Two **mating calls** were identified: the *shriek* and the *female cluck*. Both were produced during the pre-mating phase of anogenital sniffing, chasing and lordosis; no sounds were recorded during the copulation itself. All mating vocalizations were produced by the female specimen. Mating sounds were recorded both during the mating experiment and the intercolonial, homospecific male-female encounters.

Only one **agonistic call** was recorded for *Fukomys micklemi*: the *grunt*. This call seems much conserved among different species. Previous studies identified it as part of the vocal repertoire of *Heterocephalus glaber*, *Fukomys anselli* and *Fukomys mechowii*. Agonistic calls were produced during ad libitum sampling, the second part of the intracolonial encounters and during intercolonial, homospecific female-female encounters.

Seven calls functioned as **distress calls**: the *loud call I*, *loud call II*, *high call*, *loud squeak*, *chevron*, *squeal* and the *adult chirp*. All distress calls had a wide frequency range and high intensity. Distress calls were heard during ad libitum sampling, intercolonial, heterospecific female-female encounters and once during an intercolonial, heterospecific male-female encounter, in which the female was a *Fukomys micklemi* specimen.

The category of **submissive calls** has never been used before, but here consisted of five different calls: the *soft call I*, *soft call II*, *cluck*, *shriek* and the *whistle*. This category of calls was recorded during the intercolonial male-male encounters, both homospecific and heterospecific. We also recorded it during male-female heterospecific encounters, but only when the male was a *Fukomys micklemi* specimen. There was an analogy between mating calls and submissive calls. Though both categories were produced by different sexes, they indicated a certain level of subordinance. During mating behaviour this submission was directed towards the male mate. In case of a power-struggle between unfamiliar individuals during the intercolonial male-male encounters, the subordinate male expressed his submission towards the dominant male.

Juvenile calls

Juvenile calls were recorded both during ad libitum sampling and an experiment in which mother and juveniles were separated from the colony for short periods of time. The vocalizations produced by the juveniles were the *juvenile loud call*, *juvenile soft call*, *juvenile chirp* and the *juvenile squeak.* The *juvenile loud call*, *juvenile soft call* and the *juvenile squeak* were produced mainly during playful interactions among the juveniles and during interactions with adult animals. The *juvenile chirp* was mostly made by juveniles that were being allogroomed by the mother.

Low frequency range

Underground sound propagation and the mole-rats' specialized hearing organs pose some constraints on the characteristics of the produced vocalizations. The vocalizations of *Fukomys micklemi* were situated in the low to middle frequency range, in accordance to what can be expected for subterranean rodents. Most vocalizations were situated in a frequency range of 0.5 – 17.0 kHz, but some had harmonic frequencies that extended into the ultrasonic range.

Ethogram

In previous research, a non-vocal ethogram was constructed for *Fukomys micklemi*. In the present study, the ethogram was further extended using analyses of vocalisations. One category was added namely *nest behaviour*, consisting of some formerly described behaviours and two newly described behaviours: *disturbed while resting* and *pushing for a good nest place*. During the experiment three specific behaviours were added, the *power-struggle*, the *protecting food-* behaviour and *high pitched squeaking*.

In future research, it would be interesting to further expand the vocal ethogram of *F. micklemi*. During the present study it was not possible to record sound in the ultrasonic range (i.e. 20.0 kHz and higher). The performance of playback tests would be instrumental to assess whether calls (alone) can trigger a response in colony mates or whether they are just accompanying or supporting elements of particular behaviours.

7.2 Nederlandse samenvatting

De Aridity Food Distribution Hypothesis (AFDH) biedt een verklaring voor de evolutie naar socialiteit in Afrikaanse molratten (Bathyergidae). Dit model beschrijft de correlatie tussen de socialiteit van bepaalde soorten en de ariditeit van de habitat waarin ze leven. Het leven in een ariede omgeving brengt enkele risico's en kosten met zich mee. Deze zijn gekoppeld aan de onvoorspelbaarheid van dergelijke omgeving waarin regenval beperkt is en voedselbronnen een verspreide verdeling kennen. Molratten zouden onder druk van die omstandigheden in sociale groepen zijn gaan fourageren en leven.

Groepsactiviteiten vereisen een vorm van coördinatie, maar hiertoe is een goed ontwikkeld communicatiesysteem nodig. Dit wordt enerzijds bemoeilijkt door de beperkingen van het ondergronds habitat en anderzijds door de geregresseerde en/of gespecialiseerde gehoororganen die de dieren bezitten. In het algemeen maken zoogdieren gebruik van vier communicatietypes: tast, geur, vibratie en zicht. Deze laatste is van minder groot belang in de ondergrondse habitat, waar het continu donker is. Bijgevolg maken ondergrondse knaagdieren vooral gebruik van tast, geur en vibratie. In deze studie werd gefocust op communicatie door middel van vibratie en meer specifiek akoestische communicatie.

In het ondergrondse tunnelsysteem waarin de molratten leven, worden lage frequenties het beste gepropageerd; hogere frequenties gaan vaak verloren door attenuatie. Het tunnelsysteem zal er zelfs voor zorgen dat bepaalde frequenties selectief versterkt worden, het zogenaamde 'stethoscoop effect'. Bijgevolg zijn zowel het gehoor als de oorstructuren in molratten afgestemd op deze lage frequenties.

Akoestische signalen zijn een belangrijke factor in reproductieve isolatie en speciatieprocessen. Het genus *Cryptomys* (Bathyergidae, Rodentia), nu aangeduid als *Cryptomys* s.l., is recent gesplitst in het genus *Cryptomys* en het genus *Fukomys*. Aangezien *Fukomys* een zeer soortenrijk genus blijkt te zijn, heeft het huidig onderzoek zich toegespitst op het verwerven van inzichten in de fylogenie en soortvorming binnen *Fukomys*.   Dit is een hele uitdaging, daar de meeste soorten morfologisch zeer gelijkend zijn. Bijgevolg moet de diagnostiek van de soorten gebeuren op basis van zeer uiteenlopend onderzoek, waaronder genetisch, cytogenetisch en op niveau van het organisme (bv craniale morfologie en ethologie).

Deze thesis is een aanvulling op het huidig ethologisch onderzoek dat momenteel plaatsvindt op *Fukomys micklemi*. Tot nu toe was er voor deze soort al een niet-vocaal ethogram opgesteld, dat in dit onderzoek uitgebreid wordt met een vocaal ethogram en vergeleken wordt met bestaande onderzoeken. Op deze wijze hopen we uiteindelijk een beter inzicht te verkrijgen in de complexe relaties die bestaan tussen de verschillende soorten in het genus *Fukomys* en Afrikaanse molratten (Bathyergidae) in het algemeen.

De geanalyseerde vocalisaties in deze studie zijn opgenomen onder verschillende experimentele omstandigheden, die in de mate van het mogelijke de natuurlijke situatie weergeven. De studiespecimens die gebruikt werden bij zowel een ad libitum sampling fase als de focal experimentele fase behoren tot *Fukomys micklemi*. Bij de interspecifieke confrontaties werden specimens van *Fukomys whytei* gebruikt.

Vocalisaties

In deze studie werden twintig verschillende vocalisaties beschreven voor *Fukomys micklemi*, waarvan zestien adulte en vier juveniele vocalisaties. Het karakteriseren van deze vocalisaties gebeurde op basis van zowel de fysische kenmerken die we konden afleiden uit de spectrogrammen, als gedragsobservaties. Dit aantal gevonden vocalisaties bevestigt de resultaten van gepubliceerd onderzoek waarin molratten beschouwd worden als relatief vocale dieren.

Vocalisaties van adulten

De zestien adulte vocalisatietypes waren de *loud call I*, *loud call II*, *soft call I*, *soft call II*, *high call*, *loud squeak*, *soft squeak*, *chevron*, *grunt*, *squeal*, *cluck*, *female cluck*, *shriek*, *whistle*, *adult chirp* en *tooth grinding*. Het toewijzen van namen aan de verschillende vocalisaties werd gedaan op basis van de kenmerken in het spectrogram en geassocieerde gedragingen. De *loud call I*, *loud call II*, *soft call I*, *soft call II* en de *high call* vormden in werkelijkheid een continu spectrum, maar vielen toch te onderscheiden op basis van het gehoor, spectrogramkenmerken en geassocieerde gedragingen. De vocalisatietypes werden naar voorbeeld van de bestaande literatuur opgedeeld in vocalisatiecategorieën. In deze studie werd gebruik gemaakt van vijf vocalisatiecategorieën: contactvocalisaties, paringsvocalisaties, alarmvocalisaties, agressieve vocalisaties en onderdanige vocalisaties. Aangezien sommige vocalisatietypes meer dan één functie vervulden, maakten deze deel uit van meerdere categorieën.

De **contactvocalisaties** kunnen best omschreven worden als vocalisaties die geuit worden bij een initieel en/of vriendelijk contact tussen twee molratten. Zes vocalisaties werden in deze categorie geplaatst: de *loud call I*, *loud call II*, *soft call I*, *soft call II*, *high call* en de *soft squeak*. De *soft call I en II* leken een belangrijke functie te vervullen als groetsignaal. Contactvocalisaties waren veel voorkomend en werden opgenomen tijdens de ad libitum sampling, de intraspecifieke ontmoetingen en ook eenmaal gedurende interkoloniale, heterospecifieke ontmoeting tussen een mannelijk en een vrouwelijk specimen.

Twee verschillende **paringsvocalisaties** behoren tot het vocaal repertoire van *Fukomys micklemi*: de *shriek* en de *female cluck*. Beide werden uitsluitend geproduceerd door het vrouwtje. Gedurende het copuleren zelf werden er geen geluiden geregistreerd, maar wel gedurende de fase net voor het paren. Deze fase bestond uit het mannelijke en vrouwelijke specimen die elkaar anogenitaal besnuffelden en achtervolgden, waarna het vrouwtje de lordosis-positie aannam. Deze paringsvocalisaties werden opgenomen gedurende het paringsexperiment en de interkoloniale, homospecifieke ontmoetingen tussen een mannelijk en vrouwelijk specimen.

Tot de categorie van **agressieve vocalisaties** behoorde enkel de *grunt*. Deze vocalisatie maakte ook deel uit van het vocaal repertoire van *Heterocephalus glaber*, *Fukomys anselli* en *Fukomys mechowii*. Een mogelijke verklaring voor de conservatieve natuur van deze vocalisatie is dat het uitdrukken van agressie niet enkel van nut is binnen een bepaalde soort, maar ook wanneer er zich conflicten voordoen tussen verschillende species. Deze agressieve vocalisatie werd geproduceerd tijdens de ad libitum sampling, deel twee van het intrakoloniale experiment en gedurende de interkoloniale, heterospecifieke ontmoetingen tussen vrouwelijke specimens.

De **alarmvocalisaties** waren vrij eenvoudig te herkennen dankzij hun groot frequentiebereik en hoge intensiteit. Zeven vocalisatietypes behoorden tot deze categorie: de *loud call I*, *loud call II*, *high call*, *loud squeak*, *chevron*, *squeal* en de *adult chirp*. Deze werden opgenomen gedurende de ad libitum sampling, tijdens de interkoloniale, heterospecifieke ontmoetingen van vrouwelijke specimens en eenmaal tijdens een dergelijke ontmoeting tussen een mannelijk en een vrouwelijk specimen, waarbij het vrouwtje tot *Fukomys micklemi* behoorde.

De laatste categorie, de **onderdanige vocalisaties**, wordt hier toegevoegd aan de klassieke categorieën en bestaat uit vijf verschillende vocalisaties: de *soft call I*, *soft call II*, *cluck*, *shriek* en de *whistle*. Onderdanige vocalisaties werden enkel gebruikt in zeer specifieke omstandigheden, namelijk gedurende zowel de homospecifieke als heterospecifieke, interkoloniale ontmoetingen van mannelijke *Fukomys micklemi* specimens. Er viel een analogie te detecteren tussen de onderdanige vocalisaties en de paringsvocalisaties. Niet enkel waren sommige vocalisaties zeer gelijkend of identiek, ze drukten beide een vorm van onderdanigheid uit. Gedurende de paringen vertoonde het vrouwtje een zekere onderdanigheid ten opzichte van het mannetje. Op gelijkaardige wijze was er tijdens de machtstrijd tussen onbekende mannetjes, ook altijd één mannetje dat zich ondergeschikt toonde door het produceren van onderdanige geluiden en het aannemen van onderdanige posities.

Juveniele vocalisaties

Juveniele geluiden werden opgenomen gedurende de ad libitum sampling en het experiment waarin moeder en juvenielen tijdelijk uit de kolonie gehaald werden. De juvenielen produceerden vier types vocalisaties: de *juvenile loud call*, *juvenile soft call*, *juvenile chirp* en de *juvenile squeak.* De *juvenile loud call*, *juvenile soft call* en de *juvenile squeak* waren geassocieerd met speels gedrag van de juvenielen onderling en interacties met adulte molratten. De *juvenile chirp* werd vooral geproduceerd wanneer de juvenielen door de moeder geallogroomed werden.

Laag frequentiegebied

Aangezien de ondergrondse levenswijze van molratten en hun aangepast gehoor enkele beperkingen met zich meebrengen, heeft dit een invloed op de kenmerken van de geluiden die ze produceren. In de literatuur situeert men de vocalisaties van alle onderzochte soorten tot nu toe in het lage tot middelmatige frequentiegebied. Deze studie bevestigt dit. Het merendeel van de vocalisaties was gesitueerd in het frequentiegebied tussen 0.5 en 17.0 kHz, hoewel sommige geluiden harmonischen bevatten in het ultrasone gebied.

Het bestaande ethogram voor *Fukomys micklemi* is aangevuld met een vocaal ethogram. Bovendien werden een aantal specifieke gedragingen aan het ethogram toegevoegd. De (nieuwe) categorie *nestgedrag* werd toegevoegd. Nieuwe specifieke nestgedragingen zijn *verstoord tijdens het rusten* en *het bemachtigen van een goede ligplaats in het nest*. Verder werden de gedragingen *machtsstrijd*, *beschermen van voedsel* en *hoog piepen* toegevoegd aan het ethogram. Het zou interessant zijn om dit vocaal ethogram in de toekomst verder uit te breiden. Gedurende deze studie was het niet mogelijk om ultrasone geluiden te registreren, gezien de beperkingen van het opnamemateriaal. Een verdere aanvulling zou het uitvoeren van 'playback' testen kunnen zijn, om na te gaan of geluiden op zich bepaalde gedragingen kunnen uitlokken of ze eerder een ondersteunend element zijn bij bepaalde gedragingen.

8. Acknowledgements

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10. Appendices

Appendix 1. Picture of the molarium at Ghent University

Appendix 2. Overview of used animals

Table 3. *Fukomys micklemi*	
Colony	**Individual**
FMI-04	05
	23
	24
	29
	43
	44
	50
	64
	65
	67
	74
	75
	juv1
	juv2
FMI-P3	V1KAL04
FMI-2KAL	V03
FMI-2KAL	V04
FMI-MP1	V2KAL10
FMI-01	51
FMI-P1	oN9
FMI-B	19
FMI-B	20
FMI-P3	oN5
FMI-MP2	MKAT02
FMI-03	14
FMI-P5	MN1

Table 4. *Fukomys whytei*	
Colony	**Individual**
FWH-F	F
FWH-01a	0006B7D453
FWH-01a	0006A41115
FWH-01a	0006A3F3F4

Appendix 3. Observation worksheet

Appendix 4. Timeline of observations

Light green: data not used for analysis; Dark green: data used for analysis; F.E.: Focal experiments; Balloons indicate a change in type of observation.
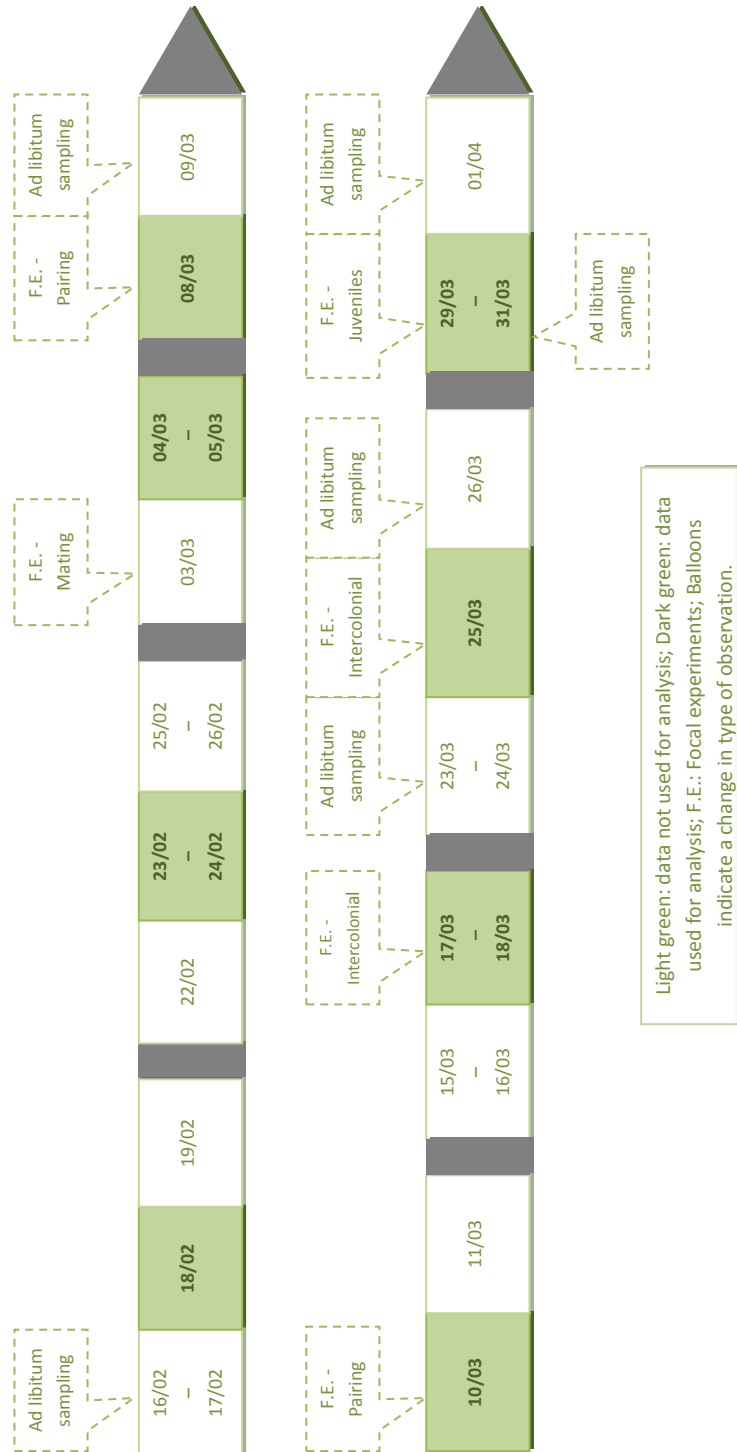
Appendix 5. Overview of used animals during mating experiment, intracolonial encounters and intercolonial encounters.

Table 5. Intracolonial encounters			
Individual 1	**Sex1**	**Individual2**	**Sex2**
75	M	23	F
44	M	67	F
74	M	43	M
24	M	44	M
05	F	64	F
65	M	67	F
50	F	23	F
65	M	75	M
29	F	24	M
64	F	29	F
43	M	50	F

Table 6. Intercolonial, heterospecific encounters					
Colony1	**Individual1**	**Sex1**	**Colony2**	**Individual2**	**Sex2**
FMI-04	74	M	FWH-F	F	F
FMI-04	64	F	FWH-01a	0006B7D453	M
FMI-04	67	F	FWH-01a	0006A41115	M
FMI-04	74	M	FWH-01a	0006A3F3F4	M
FMI-04	05	F	FWH-F	F	F
FMI-04	23	F	FWH-F	F	F
FMI-04	67	F	FWH-F	F	F
FMI-04	24	M	FWH-01a	0006A3F3F4	M
FMI-04	44	M	FWH-01a	0006B7D453	M

Table 7. Mating experiment					
Colony1	**Individual1**	**Sex1**	**Colony2**	**Individual2**	**Sex2**
FMI-03	14	F	FMI-P5	FMI-P5MN1	M

Table 8. Intercolonial, homospecific encounters					
Colony1	**Individual1**	**Sex1**	**Colony2**	**Individual2**	**Sex2**
FMI-04	67	F	FMI-P3	V1KAL04	F
FMI-04	05	F	FMI-2KAL	V03/V04	F
FMI-04	75	M	FMI-MP1	V2KAL10	F
FMI-04	74	M	FMI-01	51	M
FMI-04	24	M	FMI-P1	oN9	M
FMI-04	43	M	FMI-MP1	V2KAL10	F
FMI-04	43	M	FMI-B	19	M
FMI-04	43	M	FMI-B	20	M
FMI-04	43	M	FMI-P3	oN5	M
FMI-04	23	F	FMI-2KAL	V03/V04	F
FMI-04	64	F	FMI-2KAL	V03/V04	F
FMI-04	24	M	FMI-2KAL	V03/V04	F
FMI-04	65	M	FMI-2KAL	V03/V04	F
FMI-04	65	M	FMI-P3	V1KAL04	F
FMI-04	43	M	FMI-MP3	MKAT02	M

Appendix 6. Example of a spectrogram (*loud call I*) and analyzed variables.

Duration: length of the call; 1st harmonic: fundamental frequency, Other harmonics: multiples of the frequencies of the fundamental tone; Start frequency: frequency of fundamental tone at which the call starts; Peak frequency: highest frequency of the fundamental frequency; Stop frequency: frequency of fundamental tone at which the call stops.
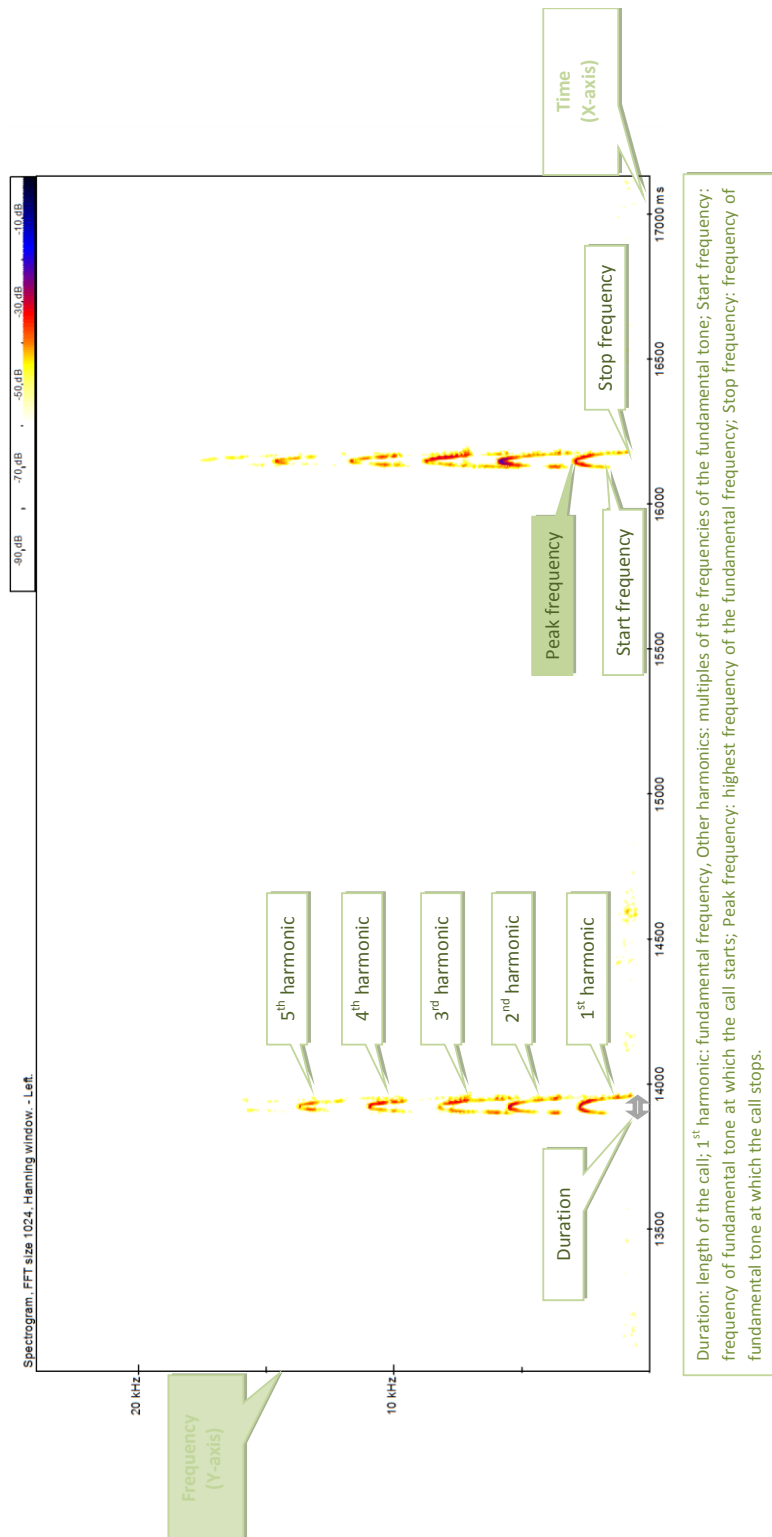
Appendix 7. Table 9. Overview of call-types (F.F.: fundamental frequency; F.R.: frequency reach; I.P.: intensity peak; n.a.: not applicable)

Call-type	Number	Duration (in ms)	Peak of F.F. (in kHz)	Nr of harmonics	Behaviour
loud call I	426	40 - 100	2.0 - 3.5	3 - 8	vocalized hopping, high pitched squeaking, disturbed while resting, startling, protecting food, tug-of-war, being allogroomed, sparring
loud call II	197	40 - 100	3.5 - 4.5	3 - 8	
soft call I	251	40 - 100	2.0 - 3.5	1 - 2	familiar encounters, being passed-over, subordinance during unfamiliar encounters, incisor fencing, being allogroomed
soft call II	96	40 - 100	3.5 - 4.5	1 - 2	
high call	47	40 - 90	5.0 - 7.0	1 - 2	unfamiliar encounters, vocalized hopping, disturbed while resting
loud squeak	67	40 - 90	n.a. (F.R. 2.0 - 20.0)	n.a.	vocalized hopping, high pitched squeaking, protection of food, interaction with pups, being allogroomed, disturbance in the nest
soft squeak	82	40 - 90	n.a. (F.R. 2.0 - 7.5)	n.a.	familiar and unfamiliar encounters, being passed over, resisting a pass-over, vocalized hopping, high-pitched squeaking, disturbance in the nest, tug-of-war
chevron	15	40 - 80	1.0 - 1.5	5 - 7	disturbance, vocalized hopping, high pitched squeaking
grunt	107	50 - 260	n.a. (I.P 0.3 - 0.4; 0.7 - 0.8)	n.a.	open-mouth gaping, thrust-gaping, fight
squeal	13	75 - 550	2.5 - 4.0	3 - 5	unfamiliar encounters, high pitched squeaking, grooming pups, vocalized hopping, protecting food
cluck	92	20 - 60	1.5 - 2.0	1 - 2	subordinance
female cluck	241	20 - 60	1.5 - 2.0	1 - 2	pre-mating phase (anogenital sniffing, chasing, lordosis)
shriek	134	40 - 700	1.5 - 2.5	1 - 5	pre-mating phase (anogenital sniffing, chasing, lordosis)
whistle	14	200 - 400	0.5 - 3.0	n.a.	subordinance
adult chirp	7	100 - 500	1.5 - 3.0	3 - 7	unfamiliar encounters, protecting food, high pitched squeaking
tooth grinding	12	30000 - 300000	mechanical	mechanical	sharpening of incisors
juvenile loud call	302	40 - 100	2.0 - 4.0	3 - 7	playful interactions among juveniles, interactions with adult
juvenile soft call	74	30 - 80	2.0 - 4.0	1 - 2	playful interactions among juveniles, interactions with adult
juvenile chirp	27	70 - 150	2.5 - 3.5	1 - 6	interactions with parental animal, mostly allogrooming
juvenile squeak	4	80 - 150	2.5 - 3.0	2 - 4	playful interactions among juveniles, interactions with parental animal

Appendix 8. Example of worksheet for spectrogram analysis (numbers in the column category correspond to the different call-categories: loud call I = 1; loud call II = 2; soft call I = 3;...in accordance to the order in the section Results).

Track N°	Voc N°	Duration (in ms)	Start freq	Peak freq	Stop freq	Harmonics	at frequency	Category	Adult/Juvenile
170303_0-55	1/13	55	5.5	5.8	5.6	1	5.6; 8.0	7	A
	2/13	39	1.7	2.7	2.7	3		1	A
	3/13	105	1.7	2.8	2.2	4	5.6; 8.3; 11.0	1	A
	4/13	137	1.6	2.6+ 2.6	1.4	4	5.3; 8.1; 10.6	10	A
	5/13	77	1.4	1.5	1.2	9	.5; 6.0; 7.5; 9.0; 10.5; 12.0	8	A
	6/13	70	1.7	2.4	1.3	5	4.7; 7.2; 9.6; 11.8	1	A
	7/13	310	1.7	2.6+ 2.6	1.3	5	5.2; 7.9; 9.4; 12.7	10	A
	8/13	80	1.7	2.0	1.3	5	4.0; 6.1; 8.0; 9.9	1	A
	9/13	60	1.3	1.3	1.3	7	2.7; 4.0; 5.3; 6.6; 8.0; 9.3	8	A
	10/13	46	/	/	/	BB	2.2-6.8	6	A
	11/13	121	/	/	/	BB	2.3-6.5	6	A
	12/13	61	3.5	3.9	3.4	2	7.6	4	A
	13/13	62	1.5	2.1	1.3	3	3.9; 6.1	3	A
170303_1-07	1/2	233	3.0	3.2+ 2.8	1.6	4	6.4; 9.6; 12	10	A
	2/2	75	2.1	2.4	1.9	3	5.1; 7.6	2	A
170303_1-36	1/1	63	/	/	/	BB	6.0-10.6	5	A
170303_1-51	1/6	79	5.5	5.8	5.2	1		7	A
	2/6	96	3.0	3.7	1.0	3	7.7; 11.4	2	A
	3/6	105	3.2	3.8	1.1	4	7.5; 11.2; 14.1	2	A
	4/6	88	2.8	2.8	1.0	4	5.3; 8.0; 10.7	1	A
	5/6	124	2.2	2.8	0.9	4	5.4; 8.2; 10.8	1	A
	6/6	133	2.1	2.8	1.3	4	5.5; 8.4; 11.2	1	A
170303_1-58	1/9	68	1.8	2.3	1.7	3	4.6; 6.8	1	A
	2/9	84	1.8	2.8	1.6	5	5.5; 8.4; 11.1; 14.0	1	A
	3/9	112	3.8	3.6+ 3.6	1.5	4	7.2+ 7.2; 10.6+ 10.6; 14.2	10	A
	4/9	92	1.8	2.6	1.3	5	5.2; 7.8; 10.4; 12.8	1	A
	5/9	76	1.6	2.1	1.4	BB	2.9-14.3	5	A

Appendix 9. Ethogram (Bouly, 2005; Desmet, 2009 from Jacobs et al., 1991; Lacey et al., 1991; Van Daele in prep.; Wallace & Bennett, 1988) – Newly added behaviours are marked in green.

Category		Behaviour	Code	Description
Resting	**R**	Dozing	RD	Animal stands motionless with its head drooped, giving the impression that it is asleep on its feet.
		Sitting	RS	From a standing position the animal curves the back of the body downward, hereby bending the hindlimbs. The head is drooped.
		Sleeping	RL	State of rest, in which the eyes close and body movement and responsiveness to external stimuli are reduced.
		Yawning	RY	Opening the mouth displaying large gape.
Nest behaviour	**N**	Disturbed while resting	ND	Animal is resting in the nest, but is suddenly awoken by another mole rat moving or entering the nest.
		Pushing gor a good nest place	NP	Individual (mostly the breeding female) pushes aside other individuals to get a better place in the middle of the nest.
Orientation	**O**	Securing	OS	The head is repeatedly moved from side to side, meanwhile the animal is moving back and forth in short bursts.
		Sniffing air	OA	The head is usually lifted slightly above the body and the animal repeatedly inhales through the nose in short bursts.
Locomotion	**L**	Walking forward	LF	Moving forward at slow speed.
		Walking backward	LW	Moving backward at slow speed.
		Running forward	LR	Moving forward at higher speed.
		Running backward	LB	Moving backward at higher speed.
		Darting forward	LD	Moving forward very fast over a short distance (1 or 2 bodylengths).
		Darting backward	LA	Moving backward very fast over a short distance (1 or 2 bodylengths).
		Splayed walking	LS	An animal walks with its legs held out to the sides of its body (resembling the stance of a salamander) and its trunk held close to the substrate.
		Crouch advancing	LC	Animal moves forward a few steps with its legs bent and its body held close to the substrate, the animal pauses, and then moves forward again.
		Rapid pawing		One animal rapidly moves its forefeet along the body of another, alternating rapidly between left an right.
		Passing over	LO	One individual passes over the other, usually preceded by rapid pawing.
		Passing sideways	LN	Two individuals pass next to each other.
		Resisting pass over	LX	An animal opposes a pass over by bracing itself or stretching its back in an upward motion.
		Purposely passing under	LU	One individual purposely passes underneath the other.
		Turning	LT	Animal turns 180°.
		Failing pass over	LM	One individual tries to pass over an other but fails because the other is resisting (or sometimes just ignoring), the individual trying to pass over will crawl on top of the other individual ans start pawing it rapidly with its forefeet.

Digging	**D**	Backshoveling	DB	The forelegs are synchronously lifted, moved forward and down onto the substrate, and then moved backward under the body, propelling the dirt underneath and slightly behind the animal.
		Chissel tooth digging	DT	The incisors are scraped along the surface, loosening chunks of substrate.
		Foreleg digging	DF	One foreleg is brought forward and the foot is scraped along the surface, removing small pieces of substrate, the foreleg is then returned to a position under the body. The action is usually repeated rapidly using alternate forelegs.
		Sweeping using hindfeet	DH	Loose substrate is swept backwards by synchronously stretching the hindlegs, hereby supporting the bodyweight on the forelegs. Often the animal itself moves backward while doing this.
Foraging	**F**	Sniffing food	FS	The nose is held close to the food and the animal inhales repeatedly through the nose in short bursts.
		Licking food	FL	Moving the tongue over the surface of the food.
		Brushing food	FB	The animal rapidly moves the forefeet up and down along the sides of a food item that is held between the upper and lower incisors.
		Gnawing	FG	The animal stands with its legs braced against the substrate, the incisors are closed across the surface of a large food item, thereby scraping off small bits of food.
		Nibbling	FN	Animal holds a (smaller) food item with both forepaws and consumes small portions of food using the tongue and incisors.
		Chewing	FC	Food is being grinded using the molars.
Transportation	**T**	Carrying food	TF	Food is being held between the upper and lower incisors and lifted off the floor, meanwhile the animal moves forward or backward.
		Carrying nesting material	TN	Nesting material is being held between the upper and lower incisors and lifted off the floor, meanwhile the animal moves forward or backward.
		Dragging	TD	Items are being held between the upper and lower incisors and dragged over the floor, usually backwards.
		Sweeping	TS	Items are swept backwards by synchronously stretching the hindlegs, hereby supporting the bodyweight on the forelegs. Often the animal itself moves backward while doing this.
		Kicking backwards	TK	Items are swept backwards by synchronously stretching the hindlegs in a powerful motion, hereby supporting the bodyweight on the forelegs. Similar to sweeping but more powerful.
Excretion	**E**	Defecating	ED	Standing in a hunched posture with the tail raised away from the substrate while excreting feces. During defecation, the animal usually scratches its rump using its hind legs, often alternating between left and right.
		Urinating	EU	The anogenital area is extended toward the surface, with the tail raised away from the substrate, hind legs spread a little bit, urine is excreted.
Coprophagy	**C**	Autocoprophagy	CA	Animal consumes its own feces, sometimes while sitting on its hindquarters with the body and head curved towards the abdomen and the mouth in contact with the anus.
		Allocoprophagy	CL	Feces of a colony member are consumed.

Grooming	**G**	Cleaning feet	GF	Cleaning the forefeet of hind feet with the incisors.
		Cleaning incisors	GI	Cleaning the incisors with the forefeet.
		Cleaning snout	GM	Wiping the face and muzzle with both forefeet.Often seen when an animal leaves the nest.
		Scratching	GC	Scratching the flank, underarm, mouth, or head regions with the hind foot.
		Grooming tail	GT	Grooming the tail with the teeth or forefeet.
		Genital grooming	GG	Grooming the genitals with the tongue or incisors.
		Incisor sharpening	GS	Repeatedly moving the upper and lower incisors along each other in such a way that the outer surface of the upper incisors grinds along the inner surface of the lower incisors, often seen following digging or gnawing.
Mating	**M**	Mate sniffing	MS	The head is drooped and turned around while sniffing the mate.
		Anogental sniffing	MA	The anogenital area is being sniffed.
		Chasing	MC	The animals chase each other, each individuals head is near the hindquarters of the other individual, so a circle is being formed by the two animals.
		Lordosis	MO	The female spreads her hind legs and pushes her trunk down, the tail is raised and the anogenital area exposed.
		Copulation	MM	The male holds the female with his forepaws and bites her neck, the female raises her head backwards en squashes her body.
		Tail pulling	MT	If the female refuses to assume lordosis posture or tries to run away the male will take the tail of the female between his incisors and pull her backwards.
		Pushing backward	MB	The female pushes the male with her hindquarters.
		Stretching	MR	The male stretches his body.
Alarm response	**H**	Startling	HS	The animals makes a small jump backwards.
		Roll up	HR	The animal lies on its back and curls up, the paws and face are pointing upward, often with open-mouth gaping and vocalisation.
		Scrambling	HC	Numerous individuals simultaneously dart forward and backward, with no single coordinated direction.
Characteristic behaviour	**B**	Rithmic pumping	BR	The trunk is being moved up and down repeatedly, creating a waving motion of the body from front to back.
Neonate tending	**N**	Carrying pups	NC	Pups are being held between the incisors and lifted off the floor while the individual moves backward or forward.
		Grooming pups	NG	Adult grasps a pup with both forepaws and either licks the pup with its tongue or nibbles at the pup with its incisors.
		Pushing	NP	Adult shoves the pup with its muzzle, the adult's head vibrates rapidly from side to side, and its body jerks forward with each push, the pup is knocked away as a result.
		Nursing	NS	Female rolls onto her back or side, making her teats accessible. Pups apparently actively seek out and approach the female, and several pups nurse simultaneously. Nursing often occurs in the nest, with the breeding female and her pups surrounded by or even lain on by nonbreeding colony members.

		Nose pressing	SN	Two individuals face each other with their heads slightly lowered and the blunt ends of their muzzles pressed together, typically lasting only one or two seconds.
		Anogenital nuzzling and sniffing	SU	An animal of one sex sniffs and uses its muzzle to nudge the genitalia of an animal of the other sex.
		Sniffing	SS	The nose is held close to the other animal and the animal inhales repeatedly through the nose in short bursts.
		Head deflecting	SH	An individual turns its head to the side and down, such that the area of the head near one ear is closest to the muzzle of the second animal, often seen while receiving allogrooming.
		Allogrooming	SA	One individual nibbles the back, neck or shoulder area of another.
		Genital allogrooming	SG	Nibbling and licking around the anogenital area.
		Tail pulling	ST	One individual takes the tail of the other between his incisors and pulls it backwards.
Social interactions	S	Resist tail pulling	SR	Animal opposes tail pulling by bracing itself.
		Rump chewing	SC	One individual takes the rump of another between the incisors and chews gently.
		Sparring	SP	Two animals stand face to face, with their mouths at right angles and their incisors locked together, the animals then shove back and forth angainst each other and rock their heads from side to side. Usually seen between juveniles or between juveniles and adult females.
		Incisor fencing	SF	Resembles sparring. In fencing however the incisors aren't locked and the animals move their heads and bodies back and forth while the incisors make contact regularly. Usually seen between juveniles or between juveniles and adult females.
		Batting	SB	Two animals swat at each other's muzzle with their forepaws, the forefeet may also be placed on the other individual's muzzle and held there, preventing the second animal from contacting the muzzle of the first. Always seen in combination with sparring.
		Vocalized hopping	SV	Individual moves its head and rump with short jerks and makes a high pitched sound, almost hopping on all fours.
		High pitched squeaking	SS	Individual makes a high pitched sounds, without a hopping movement.

		Open-mouth gaping	AG	Animal opens the mouth with upper and lower incisors seperated, air is then rapidly inhaled and exhaled through the open mouth, producing a hissing sound.
		Thrust-gaping	AH	Gape acoompanied by a lunge towards another mole-rat. The recipient will then usually respond with vocalized jerking. Usually seen when an animal is disturbed while eating or another mole-rat tries to pass over.
		Tugging	AT	Individual grasps the skin of another with its incisors and pulls backward, often while bracing against the substrate.
		Biting	AI	The jaws of one animal close on the body of another individual.
		Prolonged attack	AP	One mole-rat bites the other vigorously and sometimes positions its body slightly on top of the other. Can continue even after the recipient vocalized hopped.
Agonistic interactions	**A**	Tug-of-war	AW	Two individuals hold the same piece of food between their incisors and pull it while bracing against the substrate. Often one or both mole-rats will emit high-pitched squeaks.
		Protecting food	AF	Individual takes food between the incisors and tries to keep it away from another mole-rat by turning its head and body away.
		Power struggle	AS	Two male individuals push each other until one mole-rat will lay on its back or assume the lordosis position. Often seen between two unfamiliar males to eshablish dominance.
		Bulldozing	AL	Resembles passing over, but faster and more powerful.
		Ramming	AR	One individual chases the other and pushes against it with its head, moving the other individual forward.
		Backwards ramming	AS	Another indiviual is swept backwards by synchronously stretching the hindlegs, hereby supporting the bodyweight on the forelegs. Often the animal itself moves backward while doing this.
		Resist ramming	AE	Ramming (backward or forward) is being opposed by bracing.

Appendix 10. Comparison of vocalizations by *F. micklemi* to vocalizations by *F. anselli* (from Credner et al., 1997) and *H. glaber* (from Pepper et al., 1991).

Table 10. XX: call recorded for this species; X: physically similar call recorded for this species; H: heard but not recorded

Species	F. mickemi	F. anselli	H. glaber
adult chirp	XX		
chevron	XX		
cluck	XX	X	
cluck*	X	XX	
cry		XX	
female cluck	XX		
grunt/grunt II	XX	XX	XX
grunt *		XX	
grunt I		XX	
high call	XX		
hiss	H	XX	XX
loud call I	XX	X	XX
loud call II	XX	X	X
loud chirp	X		XX
loud squeak	XX		
loud	X	XX	X
low-pitched chirp			XX
scream *		XX	
scream			XX
shriek	XX	XX	
sneeze			XX
soft call I	XX		X
soft call II	XX		X
soft chirp	X		XX
soft squeak	XX		
squeal	XX		
tap			XX
toilet call			XX
tooth grinding	XX	XX	XX
trill		XX	
trill II		XX	
twitter		XX	
upsweep trill			XX
V-trill			XX
whistle	XX	XX	

Dark green: originally recorded for *F. micklemi*

Green: originally recorded for *F. anselli*

Light green: originally recorded for *H. glaber*