University of South Bohemia in České Budějovice
Faculty of Science

Sensory abilities and food localization in four species of African mole-rats with diverse social organization

Master thesis

Bc. Miloš Vitámvás

Supervisor: Doc. Mgr. Radim Šumbera, PhD.

České Budějovice 2013

Vitámvás, M., 2013: Sensory abilities and food localization in four species of African mole-rats with diverse social organization. Mgr. Thesis, in English - 23 p., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Annotation:

Until recently, it was assumed that African mole-rats search for food randomly, as it is in concordance with Aridity food distribution hypothesis. However, recent studies indicate that some subterranean rodent species including African mole-rats could be able to use plant chemicals (kairomones) for food localization. In my master thesis I conducted a battery of experiments on four mole-rat species to prove that these species also posses the ability of kairomone guided foraging independently of their social organization.

V Českých Budějovicích dne 23.4.2013

....
Miloš Vitámvás

Acknowledgement

Content

Abstract

Food localizing in African mole-rats (Bathyergidae, Rodentia) was expected to be random for a long time, supporting thus the widely accepted hypothesis on evolution of sociality as a means of surviving in harsh arid environment (Aridity Food Distribution Hypothesis). However, there was a lack of empirical data supporting this assumption. I tested the ability to discriminate between soil where food had and had not been growing in three social and one solitary mole-rat species. The capability of localizing food resources without any physical contact and the distance at which mole-rats can register food was also examined. Results of simple tests demonstrated that all tested mole-rats are capable of a nonrandom search as well as locating food source at greater distances and that they could use olfactory during searching for food. Results of my study suggest that mole-rats probably use some cues to locate food and they are also able to detect it at some distance (probably plant metabolites). This contradicts the precondition of the Aridity food distribution hypothesis about blind foraging.

Introduction

Subterranean ecotope provides its inhabitants with various advantages. Among them, lower predation risk and stable microclimate are among those frequently mentioned (Nevo 1999). Temperature fluctuations underground are lower than above ground and in tunnels there is almost constant microenvironment during the day and also seasonally. High humidity in burrows enables water saving (reviewed in Burda *et al*. 2007). On the other hand, life in the underground environment is also very stressful. Mammals spending whole life in this specific niche construct large burrow systems in soil and virtually every aspect of their life is dependent on their digging skills. Burrowing is energetically very costly activity (Vleck 1979; Lovegrove, 1989; Luna and Antinuchi 2006; Zelová *et al*. 2010). Energetically demanding digging can result in difficulties in searching for mates and dispersion. Mating and dispersion occur mainly during the rainy season when soil is softer, moreover some species were reported to move aboveground to avoid costly digging and save energy (Nevo 1999; Bennett *et al*. 2000; Bennett and Faulkes 2000). Subterranean ecotope influences also sensory biology of burrow inhabitants. Constant darkness excludes vision as a means of communication and orientation, hearing is shifted into lower frequencies, and absence of air flow in tunnels eliminates the olfactory sense for greater distances (Francescoli 2000).

Localization of food in burrow inhabitants is thus deprived of most sensory cues available aboveground. Food resources here can be located randomly or by using some sensory adaptations. In insectivorous subterranean mammals, the food localizing has been solved by evolving some sensory improvements as hearing and olfactory modalities (Burda *et al.* 1990). The ability to perceive vibrations emitted by respective prey has also been documented (Narins *et al*. 1997). In subterranean rodents, neither of these sensory modalities besides the olfactory sense can be use because their food sources do not move or emit any sound.

Subterranean rodents feed almost exclusively on plants with storage organs such as roots, bulbs and tubers. These underground organs provide them not only with energy but also with water as they do not drink free water. Food items are reached by burrowing (Nevo 1999). Subterranean rodents are usually food generalists (Nevo 1999). It fits into the prediction of Optimal foraging theory (OFT; Pyke *et al.* 1977; Stephens and Krebs 1986) which assumes that high energy demands for foraging force animals to be less selective in food preference. To maximize energy intake, subterranean mammals feed even on poisonous plants (Heth *et al*. 2000). There are also some observations about carnivorous behavior in subterranean rodents, and mole-rats specifically (Burda and Kawalika 1993). The giant mole-rats (*Fukomys mechowii*) were observed to consume earthworms, insects, mice and even various kind of meat (Burda and Kawalika 1993). Brett (1991) reported naked mole-rats (*Heterocephalus glaber*) gnawing a bone. Consumption of bones and other animal parts can supplement minerals such as calcium necessary for rapid tooth growth (Pitcher *et al*. 1994).

African mole-rats (Bathyergidae, Rodentia) are strictly subterranean rodents because they spend most of their life in self constructed underground burrow systems (Bennett and Faulkes 2000). They inhabit areas in Sub-Saharan Africa where precipitations are seasonal with periods of dry season. Under these conditions food source is limited because of the clumped geophyte distribution with great distances between patches (reviewed in Lövy *et al.* 2012). To overcome the period when food supply is limited and difficult to access, some behavioral adaptations evolved. Patchy distribution of geophytes led into evolution of so called "geophyte farming" described only in mole-rats so far. Mole-rats partially consumed large bulbs which they plug with soil to avoid bulb desiccation and left it *in situ* to regenerate. Later they feed on the same, regenerated bulb (Spinks *et al.* 1999; Šklíba *et al.* 2011). Another behavioral adaptation which allows maximum intake from the patch of

clumped geophytes is area restricted search (intensive searching for food in the vicinity of food item enables locating more food items in areas with clumped food sources; Stephens and Krebs 1986). Area restricted search was also documented in subterranean rodent *Geomys bursarius* (Benedix 1993). Among Bathyergids, it was reported in *Heterocephalus glaber* (Jarvis and Sale, 1971; Brett 1991) and *Cryptomys hottentotus* (Spinks *et al.* 1999). Food searching mole-rats dig a foraging tunnel and when they approach a bulb they dig several smaller side tunnels in its vicinity in order to maximize the food gain from the patch (reviewed in Bennett and Faulkes 2000; Šumbera *et al.* 2008). Food storing is well known phenomenon in African mole-rats (e.g. Bennett and Faulkes 2000; Šumbera *et al.* 2008; Šklíba *et al.* 2011). For sure, these abovementioned food localization and food storing strategies greatly reduce the risk of starvation during the dry period of the year. However, before they can be used, the food source has to be found.

An important theory which includes food localizing in mole-rats is the Aridity food distribution hypothesis (AFDH, (Jarvis *et al.* 1994, Bennett and Faulkes 2000). According to the AFDH, sociality in mole-rats evolved as a result of arid environment where food source is supposed to be unequally and patchily distributed and soil is difficult to work. AFDH was firstly highlighted in Jarvis (1978) where it is stated that the naked mole-rat (*Heterocephalus glaber*) is social because of the harsh semi-desert environment where mole-rats have higher survival rate if they live in greater numbers of family members. Later, Lovegrove and Painting (1987) and Jarvis *et al*. (1994) assumed that mole-rats find their food sources by random searching i.e. blindly. Blind foraging together with spatial distribution of food resources (Spinks and Plaganyi 1999), where food patches with geophytes are far from each other (Brett 1991; Jarvis *et al*. 1998) and hardly workable soil (Bennett and Faulkes 2000), presumes arid environments to be inhabited only by social mole-rat species. In later studies, it was discovered that even solitary mole-rats inhabit areas which contained even less food sources than areas inhabited by most social species (Šumbera *et al*. 2003). Because areas with patchily distributed food resources can also be inhabited by solitary mole-rats (Šklíba *et al*. 2011), it would be a benefit to possess abilities to locate food source at some distance. Although random foraging (Lovegrove and Painting 1987; Jarvis *et al*. 1994) has been accepted for a long time, later studies (Heth *et al*. 2002; Lange *et al*. 2005; Schleich and Zenuto 2007) indicated the ability to locate food actively using plant chemical signals.

It is known that plants release metabolites through their root system which can be detected by many herbivorous insects and nematodes (reviewed in Johnson and Nielsen

3

2012). These findings indicate the presence of some chemical substances which can be detected by herbivorous animals. So far, there is practically no evidence of specific substances that can provide such clues for subterranean rodents. It is widely accepted that sensory cues are provided by kairomones (Heth *et al.* 2002; Lange *et al.* 2005; Schleich and Zenuto 2007). In general, kairomones are any chemical signals released by emitter and detected by receiver who benefits from them (Dicke and Sabelis 1988). In case of subterranean rodents, geophyte provides chemical signals as cues for a herbivorous mole-rat which is able to navigate to this particular plant or clump of plants.

Heth *et al.* (2002) discovered the ability to discriminate between the soil where cultivated plants had been growing and the control soil. Four tested species of different rodent lineages namely blind mole-rats (*Spalax ehrenbergii*), Ansell's mole-rats (*Fukomys anselli*), naked mole-rats (*Heterocephalus glaber*) and cururos (*Spalacopus cyanus*) were able to guide their food locating into the soil where food had been growing and where kairomones were expected. Similar preference was discovered in tuco-tucos (*Ctenomys talarum*) (Schleich and Zenuto 2007). Another study demonstrated that the Ansell's mole-rat and the Kafue mole-rat (*Fukomys kafuensis*) positively responded to kairomone molecules (dissolved in water), that had not been subject of previous activity of soil microorganisms. Both species also detected food source at the distance of 30 centimeters (Lange *et al.* 2005).

To get better overview whether the guided foraging adaptation is a general phenomenon in subterranean rodents we need to investigate more closely related species. For comparative purposes, it is desirable to carry battery of tests on these species than testing only a few species with limited number of tests. In African mole-rat, it is also useful to test differences between species with different social organization. In this study, I tested four different species of African mole-rats with various social systems in testing situations published in different studies together with new tests and addressed the following questions:

1. Are these mole-rats able to discriminate between soil where food had and had not been growing?

2. What is the distance at which tested mole-rats successfully locate their food source without any contact with it?

3. Design of the first two experiments does not exclude that mole-rats searching for food come in contact with root hairs physically. In this experiment, I ask if mole-rats will locate food when contact with root hairs it is prevented.

4. In another experiment, I ask if mole-rats use also olfactory cues to guide their foraging.

5. In the last experiment, I tested if mole-rats are able to associate the smell of food with reward of food and remember it. This approach could be useful, especially if we have limited number of specimens to analyze it in traditional preference tests.

Materials and methods

Tested animals

In this study adults of both sexes of four mole-rat species were used: the solitary silvery mole-rat *Heliophobius argenteocinereus* n=28 (21) and three social species: the giant mole-rat *Fukomys mechowii* n=45, the mashona mole-rat *Fukomys darlingi* n=24 and the Ansell's mole-rat *Fukomys anselli* n=15.

Animal husbandry

All animals are kept in animal facility at the University of South Bohemia. Tested animals were both captured in the field or born in captivity. All animals were kept at least for two years in the animal facility. Animals are fed with carrots, potatoes, sweet potatoes, apples and dry rodent food mixture (Tukan). The animal room is maintained at 24±1°C and photoperiod of 12L/12D. Relative ambient humidity ranges from 40% to 60%.

Preparation of experiments

To investigate whether the mole-rats are able to locate food source by using chemical cues produced by plants, I conducted series of trials. A transparent Perspex T-maze (7 cm in diameter, 50 cm long preparation chamber and 30 cm each arm) was used in all experiments with some modifications (sensu Heth *et al*. 2002). Each arm of the T-maze was filled with carrot soil and control soil. Carrot soil preparation is described for each experiment separately. Each animal was tested once in each experiment and prior to each test it was held one week on a potato diet. 24 hours before the test, the animal was food deprived to keep it motivated for trial. During the deprivation period, only a small piece of potato was supplied to keep the animal hydrated. At the beginning of each test, tested animal was placed into the

open runway of the T-maze for 10 minutes in order to adapt. The animal was kept away from the soil by a removable partition prior to the testing. Another removable partition was placed between carrot and control soil to avoid mixing together (Fig. 1). After habituation all partitions were removed and the animal could freely choose from the two soil types. Time from opening of gates to start digging (animals usually sniff in that time) was denoted as latency. The trial finished after the animal dug throughout one arm. The testing apparatus was randomly rotated before each animal was tested to eliminate the possible influence of electromagnetic field. Whether the left or right arm of the testing apparatus was filled with carrot soil was determined randomly to avoid potential preference of the testing animals to one side. After the end of the trial the soil was removed, the whole apparatus was cleaned with water and 20% ethanol and dried with paper tissue. T-maze was filled with soil using laboratory gloves to eliminate any type of odorous contamination.

Kairomones in soil (test 1)

The first trial was designed to test whether the mole-rats are able to discriminate soil where carrots (food) had or had not been growing (sensu Heth *et al*. 2002; Schleich and Zenuto 2007).

One arm of the T-maze was filled with "Carrot" soil. The opposite arm was filled with control soil. Carrot soil was obtained by planting three carrot seedlings (8 cm in length) into one pot filled with soil. Carrots were kept in a partial shade, under the temperature varying from 15°C to 20°C and watered every second day. Control soil was prepared by putting soil into a pot and keeping it at the same place and under the same conditions as the carrot soil. After one month carrots were removed and the soil was sieved.
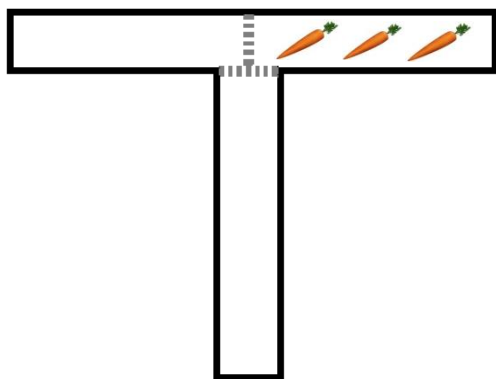

Figure 1 T-maze with removable partitions between both arms and habituation chamber. Carrots illustrate position of carrot soil in one arm of the maze.

Distant food localizing (test 2)

The second test was performed in order to estimate the distance at which the mole-rats are able to locate their food without any contact with it (sensu Lange *et al.* 2005).

I used a transparent Perspex T-maze with attachable arms of three different lengths (30, 40, 50 cm) with a box connected to one end of the arm. Each arm with a box was filled with soil and three carrot seedlings were planted into the box and growing there for two weeks. Between the box and the arm was a sieve attached, to avoid carrot roots to grow into the arm. The same arm and box set was filled with control soil without a carrot and was kept under the same conditions. After two weeks of growth the whole set was attached to form a
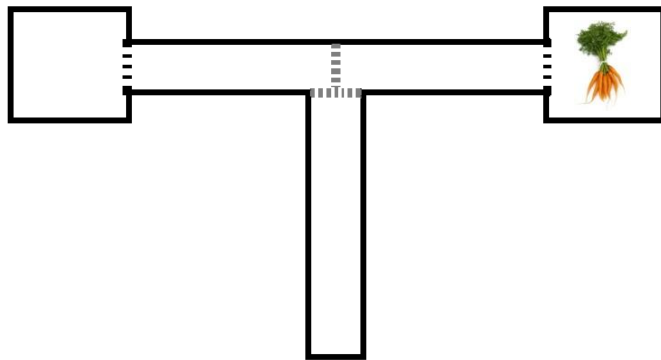
T-maze with carrot left in the box and trials were conducted (Fig. 2). Each animal was tested once at each distance (30, 40, and 50 cm). Order of the arm lengths during the test was selected randomly. Test was ended after the animal dug 30 cm into one arm.

Figure 2 T-maze with the attached boxes, removable partitions between arms and habituation chamber and sieves between arms and boxes. Position of planted carrot is also illustrated.

Kairomones in "hydroponics" (test 3)

Because of the inability to sieve out all the carrot roots in previous experiments, I performed trial with hydroponically grown carrot where no roots occurred in experiment (sensu Lange *et al*. 2005).

I performed preferential test in a transparent Perspex T-maze (Fig. 1) with soil moistened with carrot water and control soil moistened with pure water. Carrot water was obtained from hydroponically grown carrots which had been growing in a bucket of water for 14 days under the temperature of 15°C. Control water was kept under the same conditions. After two weeks carrots were removed and the water was filtered through filter paper to remove all root hairs. Both carrot and control water were left in the testing room

before trial to gain the ambient temperature. Prior to the trial, one part of soil was evenly moistened with carrot water and one part with control water and arms of the T-maze were randomly filled with both types of soil.

Olfactory cues (test 4)

During digging, soil could enter an oral cavity thus providing gustatory cues to the mole-rat. I used method without soil to test the ability of food locating using olfactory sense (sensu Schleich and Zenuto 2010).

At the end of one T-maze arm Petri-dish with grated carrot (15 g) was placed. At the end of the opposite arm, there was a Petri-dish filled with pure water to maintain the same humidity in both arms. Test ended after the animal reached one of the Petri-dishes. Latency was not measured in this experiment.

Conditioning (test 5)

Last trial was conducted in order to test whether the mole-rats are capable to associate smell of carrot food with reward (different food) and whether they are able to improve their performance in time. Conditioning experiment could be useful for analyzing low number of specimens which cannot be tested using simple preference tests.

The same method as in the test 1 has been used (Fig. 1). The only difference was a dry rodent food Tukan (5 g) as a reward placed at the end of arm filled with carrot soil. I used animals repeatedly in this trial. There were five tests in one day per each individual; each test separated by a 60 min pause. The same set of tests was performed on three consecutive days. When the animal chose to dig in soil where carrot had been growing it was left to eat one particle of reward.

Statistical analysis

To calculate digging preference for the two types of substrate in all tested animals, I used chi-square test. A null hypothesis that mole-rats in a T-maze dig randomly was evaluated against an alternative hypothesis that mole-rats will prefer to dig in carrot soil. The conditioning experiment was evaluated with repeated measures ANOVA and Tukey HSD

test. All of the tests were performed in STATISTICA 10 (StatSoft, Inc.). Means are given ± SD throughout the text.

Results

Kairomones in soil

Mole-rats started burrowing immediately when the test started. Latency was 2.8 ± 1.3s in *H. argenteocinereus,* 2.5 ± 1.2s in *F. mechowii*, 2.5 ± 2.1s in *F. darlingi* and 1.2 ± 0.5s in *F. anselli*. All species except for *F. anselli* chose soil where carrots were growing compared to the control soil (Table 1).

Table 1 Mole-rats digging preferences for the two types of substrates. Statistically significant values are marked (<0.05*, <0.01**).

Species	N of tested animals	Carrot soil (n)	Control soil (n)	χ^2	p
H.argenteocinereus	28	20	8	5.1	0.023*
F.mechowii	45	32	13	8.0	0.005**
F.darlingi	24	18	6	6.0	0.014*
F. anselli	15	11	4	3.3	0.071

Distant food localizing

Latency in *H. argenteocinereus* was 3.1 ± 1.0s, *F. mechowii* 2.0 ± 1.8s, *F. darlingi* 1.9 ± 1.1s and for *F. anselli* 1.0 ± 1.0s. In this experiment with arms of different length, all species dug significantly more in the arm with carrot soil at a distance of 30 cm. At the distance of 40 cm *H. argenteocinereus* showed significant preference for carrot soil as well as *F. mechowii* but not *F. darlingi and F. anselli*. All species showed no preference when the distance was 50 cm (Table 2).

Table 2 Mole-rats digging preferences for the two types of substrate at different lengths. Statistically significant values are marked (<0.05*, <0.01**).

Species / arm length	N of tested animals	Carrot soil (n)	Control soil (n)	χ^2	p
H. argenteocinereus	21				
a) 30 cm		16	5	5.8	0.016*
b) 40 cm		15	6	3.9	0.0495*
c) 50 cm		12	9	0.4	0.513
F. mechowii	45				
a) 30 cm		30	15	5.0	0.025*
b) 40 cm		32	13	8.0	0.005**
c) 50 cm		25	20	0.6	0.456
F. darlingi	24				
a) 30 cm		20	4	10.7	0.001**
b) 40 cm		16	8	2.7	0.103
c) 50 cm		11	13	0.2	0.683
F. anselli	15				
a) 30 cm		12	3	5.4	0.020*
b) 40 cm		9	6	0.6	0.439
c) 50 cm		9	6	0.6	0.439

Kairomones in "hydroponics"

Latency measured for *H. argenteocinereus* was 2.5 ± 1.2s, *F. mechowii* 1.9 ± 1.0s, *F. darlingi* 2.8 ± 2.0s and for *F. anselli* 2.3 ± 1.1s. All species except of *F. anselli* preferred to dig in carrot soil than in the control soil (Table 3).

Table 3 Mole-rats digging preferences for substrate moistened with carrot water and substrate moistened with control water. Statistically significant values are marked ($<0.05^*$, $<0.01^{**}$).

Species	N of tested animals	Carrot soil (n)	Control soil (n)	χ^2	p
H.argenteocinereus	21	18	3	10.7	0.001**
F.mechowii	45	31	14	6.4	0.011*
F.darlingi	24	18	6	6.0	0.014*
F. anselli	15	10	5	1.7	0.197

Olfactory cues

All tested species preferred to visit the arm with grated carrot compared to the control arm (Table 4).

Table 4 Mole-rats direction preference to one side of the T-maze. Statistically significant values are marked (<0.05*, <0.01**).

Species	N of tested animals	Carrot (n)	Control (n)	χ^2	p
H.argenteocinereus	21	17	4	8.048	0.005**
F.mechowii	45	35	10	13.889	0.0001**
F.darlingi	24	19	5	8.167	0.004**
F. anselli	15	13	2	8.067	0.005**

Conditioning

Latency in conditioning experiment was measured each day for every species separately (Table 5). All tested species showed increase of successful reward locating between day 1 and day 2 (*H. argenteocinereus* - p = 0.003; *F. mechowii* - p = 0.0003; *F. darlingi* - p = 0.001; *F. anselli* - p = 0.004). Between the second and third day, there was no significant difference in *H. argenteocinereus, F mechowii and F. darlingi* (Fig. 3)*. F anselli* showed also significant difference between the second and third day (p = 0.03) (Fig. 3). Latency decreased significantly during the three testing days. In all four species, there was a significant difference between day 1 and day 3 (Table 5)

Table 5 Measured latency in four mole-rat species during the three consecutive days of experiments. Statistically significant decrease in latency between day 1 and day 3 is also presented (<0.05*, <0.01**).

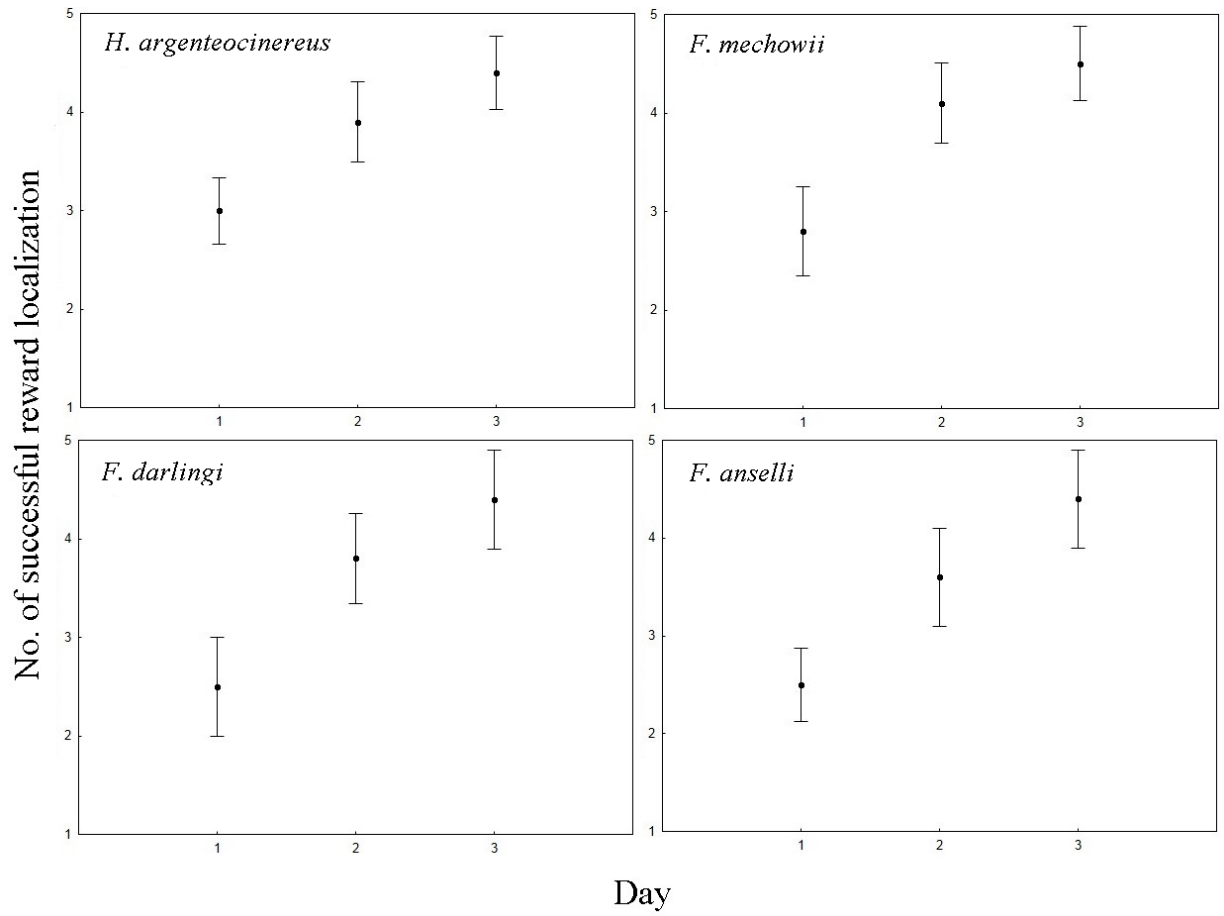| Species	Day 1 (s)	Day 2 (s)	Day 3 (s)	p
H.argenteocinereus	3.0 ± 1.9	2.4 ± 1.3	1.9 ± 1.3	0.012*
F.mechowii	2.2 ± 1.2	2.0 ± 1.0	1.4 ± 1.0	0.004**
F.darlingi	2.6 ± 1.8	1.9 ± 1.2	1.4 ± 1.1	0.04*
F. anselli	1.5 ± 1.0	1.5 ± 0.8	1.0 ± 0.6	0.049*

Figure 3 Number of successful reward localization in *H. argenteocinereus*, *F. mechowii*, *F. darlingi* and *F. anselli* during five trials during three consecutive days (mean ± S.E.).

Discussion

Results of this study showed that mole-rats have good sensory abilities to find food. All tested mole-rats were able to discriminate between soil where food had or had not been growing and they were also able to locate food source up to a distance of 30 cm (and 40 cm in two species) without any contact with it. In addition, I found that mole-rats can locate food source by using chemical plant signals that are soluble in water and also that mole-rats can guide their food localizing with help of airborne substances. Finally, conditioning experiment demonstrated that tested mole-rats can connect smell of food with a reward.

Kairomones in soil

My results indicate that tested mole-rat species are able to discriminate between soil where food had been growing and had not been growing. These results are in accordance with similar studies conducted on subterranean rodent species from different phylogenetic lineages. Blind mole-rats (*Spalax ehrenbergi*), cururos (*Spalacopus cyanus*), Ansell's mole-rats (*Fukomys anselli*) and naked mole-rats (*Heterocephalus glaber*) are known to orient their digging in direction of soil where food had been growing (Heth *et al*. 2002). Blind mole-rats in Heth *et al*. (2002) study were also able to detect kairomones at concentrations as low as 25%. Similarly, a fossorial rodent tuco-tuco (*Ctenomys talarum*) from Southern America is able to orient its digging into the soil where food had been growing also at kairomone of 50% (Schleich and Zenuto 2007). In my study I confirmed the ability to detect food source in three other mole-rat species (*H. argenteocinereus, F. mechowii* and *F. darlingi*). The results for *F. anselli* are not significant probably because of low number of tested individuals. In a similar study of Lange *et al.* (2005) it was found that on larger sample size of the Ansell's mole-rat and sibling species the Kafue mole-rat (*F. kafuensis*) the test proved the ability to detect food source. *F. anselli* is used mainly as a control species in my study. In addition, there could be also a problem of low motivation in animals. Some individuals from all tested species dug enthusiastically in random direction after opening gates, which could contribute to insignificant results especially when sample size is low.

Kairomones in "hydroponics"

Previous experiment was conducted with substrate where carrots had been growing; it is possible that some root hairs left in soil could present undesirable physical cues (instead of kairomones) to tested animals. Even in the experiment with prolonged arms, where a sieve was attached between arm and box to prevent carrot roots to spread is impossible to claim that no roots were involved. In the experiment with kairomones in hydroponics, tested mole-rat species showed that no physical cues (root hairs) are needed in order to discriminate between two types of soil. Significant results were obtained for three species (*H. argenteocinereus, F. mechowii* and F. *darlingi*). Insignificant result in *F. anselli* is probably caused by effect of low number of tested animals, because proportion of positive and negative responses is the same as in species with significant results (see Table 3). These results further support the work of Lange *et al.* (2005) where *F. anselli* and *F. kafuensis* also showed ability to detect kairomones diluted in water. As stated in Lange *et al.* (2005) kairomones appear to be water-soluble. We may suppose that during the dry season with limited water, kairomones probably cannot be dissolved and spread away from plant roots. It explains finding of Jarvis *et al.* (1998) where mole-rats left a bulb near the tunnel without "noticing" it during the dry season. Findings of unnoticed geophytes near the tunnel can be explained as an energy saving modification of "geophyte farming" (Spinks *et al.* 1999; Šklíba *et al.* 2011). Because mole-rats have an excellent spatial memory (Costanzo *et al.* 2009) it is possible that this geophyte was noticed at some distance without any contact but left *in situ* on purpose to harvest it later when food in food stores is depleted. This ability to smell food source at some distance detecting volatile cues was also tested in my study (Test 4).

How far could food source be localized?

The ability of localizing food source at some distance would be an indisputable advantage for underground foraging mole-rats. My results show that all tested species were able to detect food source at the distance of 30 centimeters and orient their digging in this direction. This result is the same for another two mole-rat species such as Kafua mole-rat (*F. kafuensis*) and Ansell's mole-rat (*F. anselli*) (Lange *et al.* 2005). In my study I tested also the distance of 40 and 50 centimeters. Only two species (silvery mole-rat and giant mole-rat) were able to detect kairomones at 40 centimeters. Results assessed for other two species

were not significant for these distances. This can again be a result of low number of tested animals as the ratio of Ansell's mole-rat and silvery mole-rat individuals digging in carrot soil versus control soil was similar as the ratio of other two tested species in which result was significant. Insignificant results at 50 centimeters arm length for all species can indicate that mole-rats are probably unable to sense food source at this distance. Nevertheless, it can also mean that carrot kairomones were unable to reach this distance after two weeks. The distance which kairomones can reach under these laboratory conditions can also differ with amount of watering and soil permeability. Of course, these results do not necessarily represent foraging in natural conditions. The fact that tested mole-rats were able to locate food source at 30 respectively 40 centimeters indicates that carrot kairomones are able to reach the distance of 30 respectively 40 centimeters after two weeks of carrot growth under specific watering conditions in concentrations detectable by mole-rats under laboratory conditions. It is reasonable to expect that different plant species release different amount and concentration of kairomones (Lange *et al.* 2005). Also soil density, humidity and temperature are probably very important factors. The distance at which kairomones can be detected by mole-rats in their natural habitat could differ greatly especially between rainy and dry season. Some reports indicate the inability to localize distant food source (Heth *et al.* 1989, Brett 1991, Jarvis *et al*. 1998), but these observations were made during the dry season when soil permeability for kairomones is probably low thus distribution of chemical cues is limited. In addition, during harsh conditions such as during long drought geophytes probably enter a dormant phase in order to save water and produce limited (if any) amount of exudates (Dafni *et al.* 1981). Results provided in my study indicate that mole-rats posses the ability of detecting chemical signal released by roots (carrot in this case) at specific distance. More experiments especially in natural habitat are needed to estimate how far this ability goes under specific humidity conditions.

Use of olfactory sense

In this experiment, all tested mole-rat species showed ability to distinguish and guide their foraging in direction of food by olfactory cues. It means that these mole-rat species are able to detect not only kairomones dissolved in water as showed in previous experiments but also some volatile substances spreading through air. The ability to discriminate between unpalatable or even poisonous and palatable food items by using smell was proved for

example in blind mole-rat (Heth *et al.* 2000). This study also suggests that blind mole-rats are unable to locate bulbs under laboratory conditions by smell at the distance of 20 centimeters. Authors explain it as a result of thick layer of husk preventing odors to be released at greater distances. In my study I used grated carrot so the airborne scent was intense and probably easily detectable. In mole-rat natural habitat this ability could be useful mainly during the dry season but it still needs to be tested. It is known that plants produce airborne substances into the soil (reviewed in Johnson and Nielsen 2012). This is connected with plant respiration and carbon dioxide release into the soil which remains almost stable under different water content in soil (Bouma *et al.* 1997). This could provide some scent detectable by mole-rats at short distances and improve their ability to locate food even under the harsh conditions of dry season.

Conditioning

My results indicate that tested mole-rat species are able to locate food reward with a help of plant metabolites released in soil. My experiment shows that mole-rats dig in carrot soil in order to retrieve reward. Learning the ability to connect the scent of food with actual food reward further supports my result in previous soil discrimination experiments. Similar conditioning experiment has never been done on mole-rats so far. The ability to associate olfactory perception with food was proved for example in blue tits (*Parus caeruleus*) (Mennerat *et al.* 2005) or in honey bees (Menzel and Erber 1978). All tested mole-rat species in my study showed significant difference in number of positive responses (digging in carrot soil) between day one and day two. It indicates that positive reward conditioning strengthens the mole-rat motivation to dig into soil with kairomones just after single day of learning. Three species (*H. argenteocinereus, F. mechowii* and *F. darlingi*) did not show significant difference in number of positive responses between second and third day, in spite of the fact, that there was still some increase (see Fig. 3). *Fukomys anselli*, on the other hand, showed significant difference between the second and third day. Slower learning ability in this species can be a possible explanation of this result. Nevertheless, within three consecutive trial days all tested species were able to learn to dig in carrot soil with a help of positive motivation. Measured latency further supports the ability to learn because mole-rats spent less time sniffing the soil on the second and third day of experiments (Table 5). This method can be used especially when tested animals are in low numbers and simple preference

experiment would give not significant results. Through conditioning in mole-rat it is possible to prove the ability to recognize kairomones in soil which could be used for food searching.

Conclusion

Results of my study show that several species of African mole-rats are able to locate food source by using chemical plant signals. Only difference is *F. anselli* in which not significant results in kairomones in soil test and kairomones in "hydroponics" test were probably caused by number of tested animals. In addition, both water soluble and airborne substances can be perceived to locate geophyte storage organs. I suppose that in mole-rats natural habitat water soluble substances are mainly usable during the rainy season (or during the time of geophyte metabolic activity). On the other hand, airborne substances could be probably used during dry periods as mole-rats search for food also under these conditions. Detection of airborne substances and their amount during dry periods still needs to be tested in natural habitat. It will be also interesting to know, how long the kairomones remain in form detectable for mole-rats. The ability to locate food source at greater distance was also proved in my study in all four tested species. It is very difficult to extrapolate my results into natural conditions, because the distance at which food can be localized in natural habitats depends for sure on humidity of soil and activity of geophytes. Nevertheless, the olfactory perception of food sources is present in both social and solitary African mole-rats. Every centimeter at which food resources can be detected provides great benefit to foraging mole-rats. These results are thus in contradiction with the Aridity food distribution hypothesis and its precondition of blind foraging.

References:

Benedix, J.H. (1993): Area-restricted search by the plains pocket gopher (*Geomys bursarius*) in tallgrass prairie habitat. Behav. Ecol. 4: 318–324.

Bennett, N.C., Faulkes, C.G. (2000): African Mole-Rats: Ecology and Eusociality. University Press, Cambridge, Cambridge.

Bennett, N.C., Faulkes, C.G., Molteno, A.J. (2000): Reproduction in Subterranean Rodents., in: Life Underground: The Biology of Subterrannean Rodents. The University of Chicago Press, Chicago and London, pp. 145–177.

Bouma, T.J., Nielsen, K.L., Eissenstat, D.M., Lynch, J.P. (1997): Estimating respiration of roots in soil: Interactions with soil CO_2, soil temperature and soil water content. Plant Soil 195: 221–232.

Brett, R.A. (1991): The ecology of naked-mole-rat colonies: Burrowing, food, and limiting factors., in: The Biology of the Naked Mole-Rat. Princeton University Press, Princeton, New Jersey, pp. 137–148.

Burda, H., Bruns, V., Müller, M. (1990): Sensory adaptations in subterranean mammals., in: Evolution of Subterranean Mammals at the Organismal and Molecular Levels, Progr. Clin. Biol. Res.: 335. Alan R. Liss, Inc., New York, pp. 269–294.

Burda, H., Kawalika, M. (1993): Evolution of eusociality in the Bathyergidae. The case of the giant mole-rats (*Cryptomys mechowii*). Naturwissenschaften 80: 235–237.

Burda, H., Šumbera, R., Begall, S. (2007): Microclimate in Burrows of Subterranean Rodents — Revisited, in: Begall, D.S., Burda, P.D.H., Schleich, D.C.E. (Eds.), Subterranean Rodents. Springer Berlin Heidelberg, pp. 21–33.

Costanzo, M.S., Bennett, N.C., Lutermann, H. (2009): Spatial learning and memory in African mole-rats: The role of sociality and sex. Physiol. Behav. 96: 128-134.

Dafni, A., Cohen, D., Noy-Mier, I. (1981): Life-Cycle Variation in Geophytes. Ann. Mo. Bot. Gard. 68: 652.

Dicke, M., Sabelis, M.W. (1988): Infochemical Terminology: Based on Cost-Benefit Analysis Rather than Origin of Compounds? Funct. Ecol. 2: 131-139.

Francescoli, G. (2000): Sensory capabilities and communication in subterranean rodents., in: Life Underground. The University of Chicago Press, Chicago and London., pp. 111 – 144.

Heth, G., Golenberg, E.M., Nevo, E. (1989): Foraging strategy in a subterranean rodent *Spalax ehrenbergi*: a test case for optimal foraging theory. Oecologia 79: 496–505.

Heth, G., Todrank, J., Begall, S., Koch, R., Zilbiger, Y., Nevo, E., Braude, S.H., Burda, H. (2002): Odours underground: subterranean rodents may not forage "blindly". Behav. Ecol. Sociobiol. 52: 53–58.

Heth, G., Todrank, J., Nevo, E. (2000): Do *Spalax ehrenbergi* blind mole-rats use food odours in searching for and selecting food? Ethol. Ecol. Evol. 12: 75–82.

Jarvis, J.U.M. (1978): Energetics of survival in *Heterocephalus glaber* (Ruppell), the naked mole-rat (Rodentia: Bathyergidae). Bull. Carnegie Mus. Nat. Hist. 6: 81–87.

Jarvis, J.U.M., Bennett, N.C., Spinks, A.C. (1998): Food availability and foraging by wild colonies of Damaraland mole-rats (*Cryptomys damarensis*): implications for sociality. Oecologia 113: 290–298.

Jarvis, J.U.M., O'Riain, M.J., Bennett, N.C., Sherman, P.W. (1994): Mammalian eusociality: a family affair. Trends Ecol. Evol. 9: 47–51.

Jarvis, J.U.M., Sale, J.B. (1971): Burrowing and burrow patterns of East African mole-rats *Tachyoryctes, Heliophobius* and *Heterocephalus*. J. Zool. Lond. 163: 451–479.

Johnson, S.N., Nielsen, U.N. (2012) Foraging in the Dark – Chemically Mediated Host Plant Location by Belowground Insect Herbivores. J. Chem. Ecol. 38: 604–614.

Lange, S., Neumann, B., Hagemeyer, P., Burda, H. (2005): Kairomone-guided food location in subterranean Zambian mole-rats (*Cryptomys spp.*, Bathyergidae). Folia Zool. 54: 263–268.

Lovegrove, B.G. (1989): The cost of burrowing by the social mole rats (Bathyergidae) *Cryptomys damarensis* and *Heterocephalus glaber*: The role of soil moisture. Physiol. Zool. 62: 449–469.

Lovegrove, B.G., Painting S. (1987): Variation in the Foraging Behavior and Burrow Structures of the Damara Mole-rat *Cryptomys damarensis* in the Kalahari Gemsbok National Park. Koedoe 30: 149–163.

Lövy, M., Škliba, J., Burda, H., Chitaukali, W.N., Šumbera, R. (2012): Ecological characteristics in habitats of two African mole-rat species with different social systems in an area of sympatry: implications for the mole-rat social evolution. J. Zool. 286: 145–153.

Luna, F., Antinuchi, C.D. (2006): Cost of foraging in the subterranean rodent *Ctenomys talarum*: effect of soil hardness. Can. J. Zool.-Rev. Can. Zool. 84: 661–667.

Mennerat, A., Bonadonna, F., Perret, P., Lambrechts, M.M. (2005): Olfactory conditioning experiments in a food-searching passerine bird in semi-natural conditions. Behav. Processes 70: 264–270.

Menzel, R., Erber, J. (1978): Learning and memory in bees. Sci. Am. 239: 102–110.

Narins, P.M., Lewis, E.R., Jarvis, J.U.M., ORiain, J. (1997): The use of seismic signals by fossorial southern African mammals: A neuroethological gold mine. Brain Res. Bull. 44: 641–646.

Nevo E. (1999): Mosaic Evolution of Subterranean Mammals: Regression, Progression and Global Convergence. Oxford University Press Inc., New York.

Pitcher, T., Pettifor, J.M., Buffenstein, R. (1994): The effect of dietary calcium content and oral vitamin D3 supplementation on mineral homeostasis in a subterranean mole-rat *Cryptomys damarensis*. Bone Miner. 27: 145–157.

Pyke, G.H., Pulliam, H.R., Charnov, E.L. (1977): Optimal foraging: a selective review of theory and tests. Q. Rev. Biol. 137–154.

Schleich, C.E., Zenuto, R. (2007): Use of vegetation chemical signals for digging orientation in the subterranean rodent *Ctenomys talarum* (Rodentia : Ctenomyidae). Ethology 113: 573–578.

Schleich, C.E., Zenuto, R. (2010): Testing detection and discrimination of vegetation chemical cues in the subterranean rodent *Ctenomys talarum*. Ethol. Ecol. Evol. 22: 257–264.

Spinks, A., Branch, T., Croeser, S., Bennett, N., Jarvis, J. (1999): Foraging in wild and captive colonies of the common mole-rat *Cryptomys hottentotus hottentotus* (Rodentia: Bathyergidae). J. Zool. 249: 143–152.

Spinks, A.C., Plaganyi, E.E. (1999): Reduced starvation risks and habitat constraints promote cooperation in the common mole-rat, *Cryptomys hottentotus hottentotus*: a computer-simulated foraging model. Oikos 85: 435–444.

StatSoft, Inc. (2011): STATISTICA (data analysis software system), version 10. www.statsoft.com

Stephens, M., Krebs, J.R. (1986): Foraging theory. Princeton University Press.

Šklíba, J., Šumbera, R., Vitámvás, M. (2011): Resource characteristics and foraging adaptations in the silvery mole-rat (*Heliophobius argenteocinereus*), a solitary Afrotropical bathyergid. Ecol. Res. 26: 1081–1088.

Šumbera, R., Burda, H., Chitaukali, W., Kubová, J. (2003): Silvery mole-rats (*Heliophobius argenteocinereus*, Bathyergidae) change their burrow architecture seasonally. Naturwissenschaften 90: 370–373.

Šumbera, R., Šklíba, J., Elichová, M., Chitaukali, W.N., Burda, H. (2008): Natural history and burrow system architecture of the silvery mole-rat from Brachystegia woodland. J. Zool. 274: 77–84.

Vleck, D. (1979): The energy costs of burrowing by the pocket gopher *Thommomys bottae*. Physiol. Zool. 52: 122–136.

Zelová, J., Šumbera, R., Okrouhlík, J., Burda, H. (2010): Cost of digging is determined by intrinsic factors rather than by substrate quality in two subterranean rodent species. Physiol. Behav. 99: 54–58.