University of South Bohemia in České Budějovice Faculty of Science

Comparison of exploratory behavior of three subterranean rodent species

Master thesis

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Annotation:
The ability to explore is essential for subterranean mammals, especially due to very high energetic cost of digging. The goal of this study was to examine whether three strictly subterranean rodents with different social system have different pattern of building spatial representation and behaviors in a novel environment represented by open field test. Results showed that the process of building spatial representation in solitary *Heliophobius argenteocinereus* seems to be similar to the pattern of solitary spalacid, the blind mole rat, from other studies, whereas it clearly differs from both social species. *Heliophobius argenteocinereus* travelled longer distance than *Fukomys mechowii* and *Fukomys darlingi*. Comparison of time spent in different zones of the arena showed more cautious behavior of solitary species as they spent less time in the centre in comparison with both social species. Also the behavioral parameters during the 30 minutes trial showed differences. Parameters connected with loss of interest in pure locomotion like pause and grooming. were more prevalent in social species. No significant sex difference was found, this result is not surprising when compared to other studies.

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Lukáš Plánka

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Content:

1. Introduction

Exploratory behavior refers to the tendency of an animal to investigate a novel environment or new objects (e.g. Barnett and Cowan 1976, Berlyne 1950, Welker 1957, Heyser and Ferris 2013). Once an individual enters a novel environment it needs to familiarize itself with the new space in order to establish home ranges, efficiently utilize resources or to effectively avoid predators, etc. (Russell et al. 2010). Exploratory behavior is thus the process of gathering information about the surroundings in order to obtain an appropriate spatial representation of the environment. It was suggested by O'Keefe and Nadel (1978) that exploration is also a prerequisite for successful navigation. When collecting new spatial information, animals are faced with two opposing pressures: to investigate the surroundings for potential benefits such as food, but at the same time to remain inconspicuous so as to avoid predation.

The balance between risk-taking and potential profit (food, mate, shelter etc.) varies in different situations and has an impact on the animal's motivation and emotional state (Lima and Dill 1990). For example, a predator's presence may induce a set of physiological and behavioral changes in the potential prey that allows them to maximize their chances of short-term survival such as shyness, aggression, increased activity, effort to find a shelter etc. (Boonstra 2005, Fletcher and Boonstra 2006). This was demonstrated in many studies (e.g. de Paula et al. 2005, Zangrossi and File 1992, Dielenberg and McGregor 2001). In a study on laboratory rats de Paula et al. (2005) revealed that after the odor of a predator was detected the rats spent more time displaying risk assessment behaviors (stretched approach and sniffing specifically the odor source). They also defecated intensively, which is widely accepted as an indicator of anxiety and fear in rats. Mastrangelo and colleagues (2009) found that individuals of fossorial Talas tuko-tuko (*Ctenomys talarum*) performed the spatial memory test differently when predator cues were present. Latency in finding food the food reward and the number of errors increased after the predatory cue was apparent. This could mean that under the threat of predation, Talas tuko-tukos are more cautious.

There are many other factors that influence activity during exploration such as gender, age of individuals and motivation (Costanzo et al. 2009, Harris et al. 2006, Shillington and Verrell 1997, Spritzer et al. 2005). Several studies have investigated differences between the

sexes in the spatial behavior of rodents. The common outcome was that males outperformed females in their navigational abilities (see review Jones et al. 2003). Few hypotheses have been suggested to explain possible differences between males and females during spatial tasks. One of the most pronounced hypothesis, the so-called polygyny-range size hypothesis, suggests that sex divergence in spatial ability depends on the size of the home range between both sexes in polygynous species (Gaulin 1992; Gaulin and Fitzgerald 1986). For example Lebowitz and Brown (1999) showed that male laboratory rats performed the tasks that required spatially organized representations more accurately than females. Also in a study on deer mice (*Peromyscus maniculatus*), males performed better than females in the Morrison water maze (animals had to acquire and retain the location of a submerged hidden platform) (Kavaliers et al. 1996). Kavaliers et al. (1998) showed a difference between male and female meadow voles (*Microtus pennsylvanicus*). Males successfully completed the trial in the Morrison water maze qicker than females. This could support the polygyny-range size hypothesis. Interestingly, the polygynous breeding system does not necessarily indicate a difference in spatial abilities. For example, in polygynous Talas tuko-tuko with sexual differences in home-range (Antinuchi and Busch 1992), a difference in spatial ability was not demonstrated (Mastrangelo et al. 2010). Another factor influencing exploratory behavior is the age of the individual. This was tested in a study by Harris and colleagues (2006) on prairie voles (*Microtus ochrogaster*). In this study, older individuals explored more compared to the younger conspecifics. The authors suggested that with increasing age of individuals the prairie voles became less averse to explore novel environments.

Most of the research on exploration and spatial navigation was carried out on surface-dwelling animals (e.g. Able 1980, Healy 1998, Wehner et al. 1996, Yaski and Eilam 2007). In the context of research into exploratory behavior and spatial navigation, subterranean rodents are an interesting model group due to their life in specific environment. The subterranean ecotope is dark, monotonous and deprived of most sensory cues available aboveground (Nevo 1999, Begall et al. 2007). The importance of effective spatial orientation is crucial for subterranean species. It is exaggerated by energetically costly activities such as digging necessary to obtain food, sexual partners, and to disperse (see Zelová et al. 2010 for review). In this type of environment subterranean species may use olfactory and somatosensory stimuli, as well as the

earth's magnetic field and path integration to create a cognitive map of their surroundings and successfully navigate (Heth et al. 2002, Burda et al. 1990, Kimchi and Terkel 2004a).

It has been suggested that subterranean mammals possess remarkable abilities concerning spatial learning and memory in the complex maze (Costanzo et al. 2009, Kimchi and Terkel 2002, 2003a, b). For example, the blind mole rat (*Spalax ehrenbergi*) is able to efficiently detour a disconnected tunnel in the field (Kimchi and Terkel 2003a, b). Even when the authors placed an obstacle or dug a ditch across the blind mole rat's burrow, the mole rats were able to successfully reconnect the broken parts. In addition, when the ditch or obstacle was placed asymmetrically across the tunnel, the mole rats detoured around the shorter side. The authors suggested that mole-rats use self-produced seismic vibrations as a mechanism to determine the size, nature and location of the obstacle and internal self-generated references to determine their location relative to the disconnected tunnel section (Kimchi and Terkel 2003a, b).

During the tests of spatial learning ability and memory in the maze, subterranean mammals achieved better results compared to surface dwelling species (blind mole rat vs. laboratory rat and Levant vole). Nevertheless, the subterranean blind mole rat needed disproportionally more time to finish the task during the first exploratory trial than surface dwelling rats (Kimchi and Terkel 2001) (Fig. 2S). This result implies that there may not be a difference in spatial learning abilities, but only a difference in initial exploration behavior. In other studies there was a difference between subterranean species where solitary silvery mole-rat (*Heliophobius argenteocinereus*) and solitary Cape mole-rats (*Georychus capensis*) spent more time on completing a task, travelled longer distances, and made more errors in comparison to social giant mole-rats (*Fukomys mechowii*) and social Damaraland mole-rats (*Fukomys damarensis*). This difference was remarkable, particularly in the first trial (Costanzo et al. 2009, Mazoch 2008, see Figure 1S). The author suggested that the observed difference could have been caused by differences in the complexity of burrow systems between solitary and social species. There may be stronger selective pressures regarding exploration behavior on species with a more complex burrow structure and thus the difference in sociality could be another factor influencing exploration (Costanzo et al. 2009).

As mentioned above, exploration is fundamental in examining a new environment. An important insight into how subterranean rodents explore and construct a spatial representation of their environment was observed by Avni and colleagues (2008). The authors tested blind mole rats in an unfamiliar square arena. At the beginning of the trial, the mole rats displayed a build-up phase consisting of frequent pauses, retraces and slow walking along the perimeter. This was followed by them gradually traversing the entire arena perimeter as they exhibited continuous and faster locomotion along the perimeter, with fewer stops and retraces. The centre of the arena was entered during a later phase of observation. Familiarity with the arena was also reflected by local (at the arena corners) and global shortcuts (through the arena centre). Scanning the perimeter during the build-up phase constituted a process of spatial calibration for forming an initial representation of the size or even space layout of the environment (a sort of basic global map). This spatial representation could be used later for navigation, which is in agreement with the fact that the mole rats started to take global shortcuts only after the build-up phase.

One of the simplest ways of analyzing animal exploration in captivity is an open field test (OFT), which was introduced for the first time by Hall in 1932 (for recent review on using OFT see Gould et al. 2009). One of the advantages is the possibility to measure both the quality and amount of the activity. The open field is usually an enclosure, generally square, rectangular, or circular in shape with surrounding walls that prevent escape. The most usual outcome of this test is movement, which can be influenced by motor output, exploratory drive, freezing or other fear-related behaviors. There are also many studies which use OFT to examine the influence of medication (Szechtman et al. 1994, Choleris et al. 2001, for review see Prut and Belzung 2003). Measured parameters usually are: travelled distance, time spent in motion, rearing and a change in activity over the time, etc. Some outcomes, particularly defecation, time spent in the centre/perimeter of the arena, and activity within first minutes, is being connected to aspects of emotionality including anxiety (Bailey and Crawley 2009, Bourin and Hascoët 2003, Prut and Belzung 2003).

The aim of this study was to uncover whether the three mole-rat species use the same patterns of exploration and construction of spatial representation of environment as was shown in study on the blind mole rat (*Spalax ehrenbergi*), i.e. species from a phylogenetically unrelated

family (Spalacidae) (see Avni et al. 2008). Additionally, I would like to examine the possible differences in the process of exploration in a novel environment (OFT) in three strictly subterranean African mole-rats which could be related to their different social organization. Solitary silvery mole-rats (*Heliophobius argenteocinereus*) live alone in a self-constructed burrow systems, therefore they could have more anxious reactions to new stimuli, because it could be connected with intruder/predator activity. Social giant mole-rats (*Fukomys mechowii*) and Mashona mole-rats (*Fukomys darlingi*) are probably more accustomed to the modification of their burrow environment (i.e. new tunnels built by other family members), which could lead to less anxious behavior when they encounter novel environments (c.f. Mazoch 2008). Therefore, I assume that social species could be more willing to explore the centre of an arena and could sooner change behavioral patterns from exploratory locomotion to comfort behavior (grooming) or other rest related activities. I also wanted to find out if there are differences between the sexes in locomotion activity. According to the polygyny-range size hypothesis, there should not be any difference because males and females have the same home ranges in all three mole rat species. Despite the fact that using OFT for examining exploration of such species could seem to be unnatural, there are indications that even strictly subterranean mole-rats use aboveground locomotion for breeding dispersal (e.g. Patzenhauerová et al. 2010), natal dispersal (Braude 2000) or searching for new territories (Šklíba et al. 2009). We may assume that such activity could be more frequent than expected.

2. Methods

2. 1 Studied species

The giant mole-rat (*Fukomys mechowii*) is a social bathyergid, previously known as *Cryptomys mechowi* (Kock et al. 2006). *Fukomys mechowii* is distributed among mesic areas of subequatorial central Africa in tropical woodlands and savannas with relatively high annual rainfall (Bennett and Faulkes 2000). Sexual dimorphism is apparent especially in body mass. The weight of a wild caught individual is 345 ± 95g ♂ and 252 ± 34g ♀ (Scharff et al. 2001; Sichilima et al. 2008). The burrow system of a single family could spread over 0.2 − 2.5 ha (Šumbera et al. 2012, Sichilima et al. 2008, see Figure 3S). Giant mole-rats were trapped in Zambie (Ndola and Chichele) between the years 1995-1999, others were born in captivity.

The Mashona mole-rat (*Fukomys darlingi*) is also a social bathyergid, previously known as *Cryptomys darlingi* (Kock et al. 2006), which occurs in shrub habitats and Miombo woodland in areas of predictable and relatively high annual rainfall (Bennett and Faulkes 2000). This species lives in families containing about seven members (up to nine) (Bennett and Faulkes 2000). Distribution of the species covers eastern and northern Zimbabwe, western Mozambique and southern Malawi. The average body size for both sexes is 45-90g in mole-rats from Zimbabwe (c.f. Bennett and Faulkes 2000). Mole-rats from the Malawi population in our stock are considerably larger 110-193g with a slight gender difference in body mass (159 ± 18g ♂ and 125 ± 12g ♀). Some Mashona mole-rats were trapped in Southern Malawi (Nsanje) in 2005 and others were born in captivity.

The silvery mole-rat (*Heliophobius argenteocinereus*) is a solitary bathyergid. It occurs in a variety of habitats such as grassland, savanna-woodland and cultivated fields. The silvery mole-rat occurs from northern Kenya, Tanzania, Malawi, eastern D. R. Congo and eastern Zambia to northern Mozambique where the annual rainfall is high (Bennett and Faulkes 2000, Burda 2001). They exhibit sexual dimorphism, with adult animals weighting 190 ± 58g ♂ and 162 ± 47g ♀, however the difference is small (Šumbera et al. 2003). The home range of their burrow system is approximately 0.07 (Fig. 4S). All tested animals were captured between 2000 and 2005 in Malawi (Mulanje and Zomba Plateau) and in 2010 in Zambia (Petauke and Kacholola).

2.2 Animal maintainance

Tested animals are kept in a breeding room kept at stable temperature 24 ± 1 °C and 12L: 12D light regime (light from 7:30 AM to 7:30 PM). Food (carrots, potatoes and commercial rodent pellets) was provided ad libitum. Peat is used as bedding and filter paper as nesting material. Social mole-rats are kept in large terrariums with flower-pots and plastic tubes for shelter. Solitary *H. argenteocinereus* is kept in Plexiglas mazes (4 m long).

2.3 Apparatus and testing conditions

In the experiment twenty adult individuals (10 ♀ and 10 ♂) of *F. mechowi* and twenty adult individuals (10 ♀ and 10 ♂) of *F. darlingi* and nineteen individuals (12 ♀ and 7 ♂) of *H. argenteocinereus* were used. All silvery mole-rats were removed from their mazes and placed in the glass terrariums one week before testing to accustom them to the same condition as the social species. Testing was undertaken in a square open field test (arena 2 × 2 m) enclosed within 0.3 m – high opaque Plexiglas, PP-MRF with smooth surface and light grey color (Figure 1) (Avni et al. 2008). The testing apparatus was placed in an air-conditioned room (24 ± 1 °C) and during testing the room was illuminated by 2 florescent tubes which were overshadowed because of diffused illumination of the arena. A video camera (TAYAMA C4702-01A1) was placed 2.5 m above the centre of the arena, providing a top view that was recorded onto a computer by means of a Chateau RT4 video grabber as a VGZ digital file.

2.4 Experiment

The procedure of the experiment followed the study of Avni and colleagues (2008). Before the start of each trial, an individual was placed into a habituation box (12 l bucket) for 30 min to minimize the influence of manipulation stress. At the beginning of each test session, each individual was placed in a randomly selected corner of the arena and covered with the bucket. After two minutes the bucket was removed and the behavior of the subject was video-recorded for 30 minutes. After each trial, the animal was returned to its breeding cage/maze and the arena was cleaned with soapy water, followed by ethanol (70%) and dried. The experiments were carried out between 08:00 – 18:00. Any necessary handling was carried out carefully to minimize manipulation stress. All tested animals were used only once. All individuals were adult non-

breeders. During the 30 minute trial, various factors were recorded. Some of them can be both frequency and/or durational (see below).

1) Frequency (Counts):

- Freezing – interruption of movement for less than three seconds.
- Pause – interruption of locomotion during exploration for more than three seconds.
- Facing centre– turning head toward the centre of the arena.
- U turn– turn of the whole body by 180°.
- Reversing – backward movement (more than one body length).
- Centre crossing – change of path from the perimeter to the centre of the arena.
- Digging – attempt to dig.
- Loops – performed round trips back to different place in which they had recently traveled.
- Grooming
- Climbing – climbing the walls of the arena.

2) Duration:

- Latency – time needed to travel a distance longer than the body size of the individual at the beginning of the trial.
- Locomotion – uninterrupted locomotion within provided environment.
- Pause – interruption of locomotion during exploration for more than 3 seconds.
- Climbing – climbing the walls of the arena.
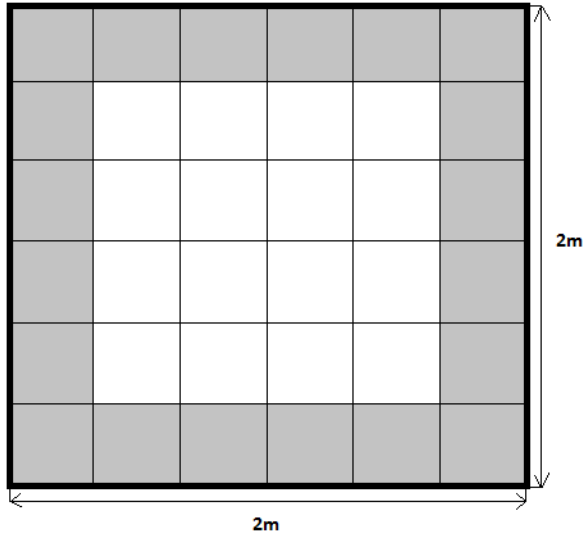- Digging – attempt to dig under the perimeter walls.

Figure 1. A scheme of the used arena. Grey squares are termed as perimeter, white squares as centre.

2.5 Data Analyses

The video files were analyzed in two steps. At first I used the trackManAvi application for obtaining the entire trail travelled by each individual and the way they explored the novel environment. The arena was divided into 36 identical squares in order to compare where and how much time they spent in different parts of the arena. The second step was observing their behavior during the 30 minute period via arquivos-EthoWatcherRev0161RC. Selected behavior was recorded (see Procedure) and used for Statistical analysis. I divided the 30 minute trial into five minute intervals to see whether individuals behaved differently during different periods of the experiment. Total scores across the full test session were statistically analysed using Two-Way ANOVA (species and sex defined as factor) on unmodified data, followed by a post-hoc test. For assessing possible differences in the distance travelled within corresponding time intervals we used Repeated Measures ANOVA with Individual set as a random factor and interval as within factor for species. All results are presented as mean ($\pm$ SD). Data for total path travelled (m), duration of exploration (s), time spent in perimeter vs. in centre zone (%) have homogenous variances and normal distribution, I used Shapiro-Wilk normality test and Bartlett test of homogeneity of variances for confirmation. Logarithmic transformation for latency (s), comparison of travelled path between sexes (m), time spent in home-base against any other zone

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(min), frequency of: **reversing**, **digging**, **climbing**, **loops**, **grooming**, **centre facing**, **U turns** (turn by 180°), **centre crossing**, **freezing** (stop < 3s) and **pause** (stop > 3s) was applied to attain a homogenous variance and normal distribution. Interrelations between exploratory parameters in the three different mole-rat species were explored by principal component analysis (PCA). I used linear discrimination analysis (LDA) to select a group of variables best discriminating the exploratory behavior in the three mole-rat species. I used the following set of exploratory parameters as explanatory variables: **total path**, **frequency of stops < 3s (freezing)**, **locomotion**, **latency**, **pause** – stops > 3s, **frequency of shortcuts**, **frequency of head turns** – towards the centre of the arena, U **turns**. The best model was selected using stepwise selection of the variables, which significantly contributed to the variability explained. Since this analysis revealed that the variable locomotion was correlated with other variables in the data set (it contains some of the same information as other parameters), I ran a separate analysis without this variable. The LDA was computed using the canonical correspondence analysis (CCA) with Hill's scaling. The PCA and LDA analyses were performed with the software package CANOCO for Windows, version 4.52 (terBraak and Šmilauer, 2002).

3. Results

3.1 Time spent in centre vs. perimeter

There was a significant difference in the times spent in different parts of the arena (centre vs. perimeter). All three species spent a significant amount of time around the perimeter in comparison with the centre of the arena (Factorial ANOVA, $F_{(2;112)} = 28.7$, MSE= 3.67, $p < 0.0001$). Post-hoc tests revealed the differences between the three mole-rat species, where *F. mechowii* spent significantly more time in the centre of the arena (16.6 %) than *H. argenteocinereus* (4.2 %) and *F. darlingi* (9.6 %) (Tab. 2S). There was no difference in the time spent in the centre of the arena between *F. darlingi* and *H. argenteocinereus* (see Tab. 2S).

There was one square (usually a corner) in which the tested animals spent significantly more time than in any other square (one-way ANOVA, $F_{(36;2036)} = 101.71$, MSE = 0.7, $p < 0.0001$). Only in *Fukomys mechowii* two individuals spent significantly more time in different than corner square (Fig. 2).
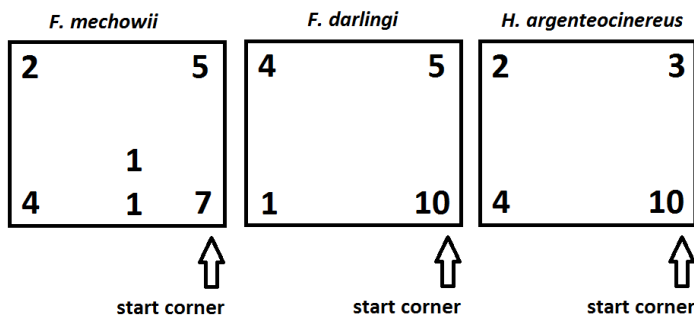

Figure 2. Distribution of the home bases (=square where an animal spent significantly largest amount of time) within the three mole-rat species. The numbers inside the squares represent how many individuals chose particular place as the home base.
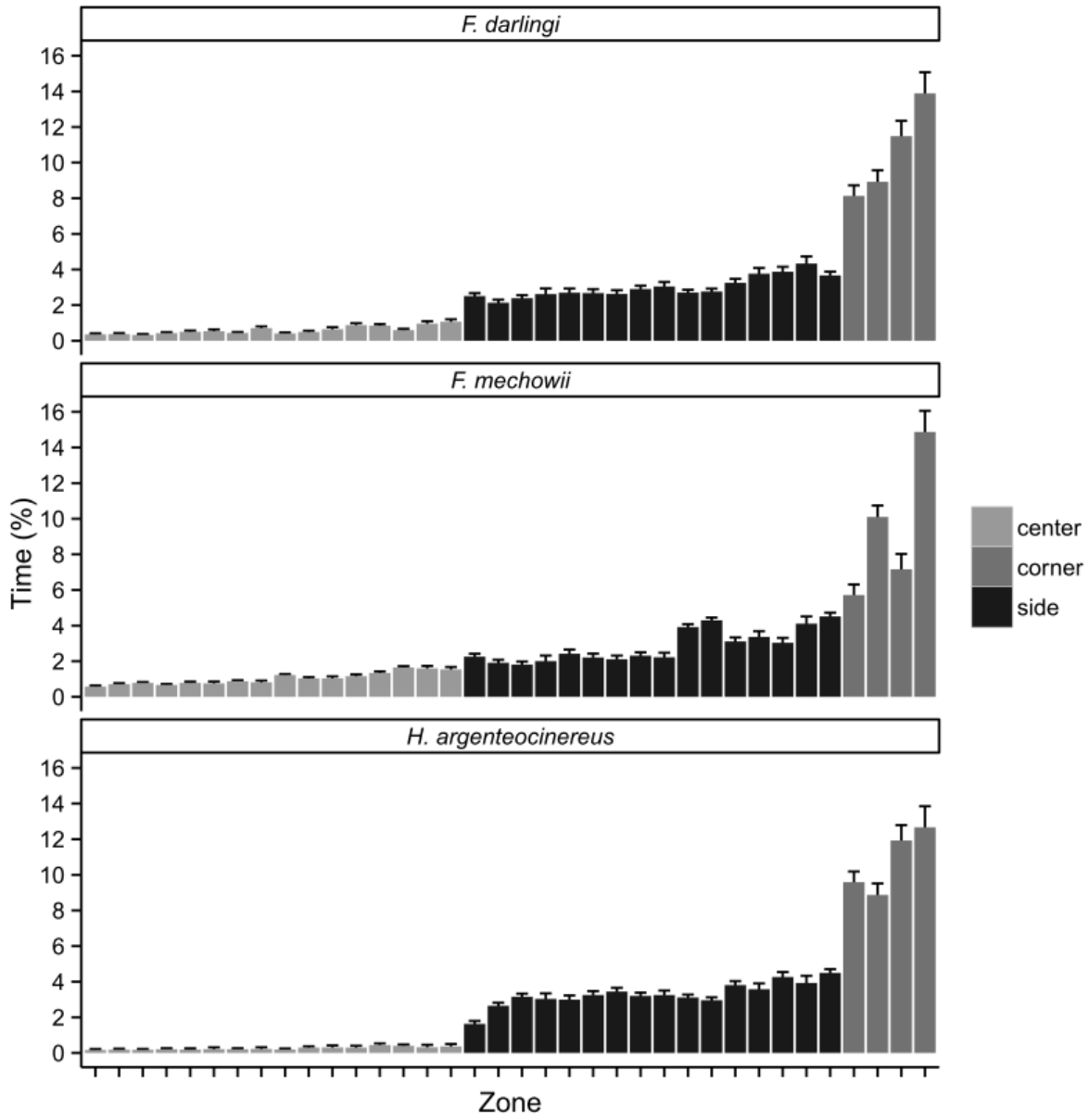
Figure 3. Mean time (in %) spent in different squares of the arena during the 30 minute trial in the three mole-rat species. Error bars show SE.

3.2 Travelled distance in five minute intervals

Travelled distances were dependent on species and the particular five minute interval in question during 30 minutes of testing (RM ANOVA, $F_{(10;280)} = 10.95$, $p < 0.0001$). Influence of interval and species were highly significant (RM ANOVA, $F_{(5;280)} = 10.05$, $p < 0.0001$ and RM ANOVA, $F_{(2;56)} = 19.27$, $p < 0.0001$, respectively). The average travelled distance by *H. argenteocinereus* was significantly greater during almost all intervals except the first one (the first five minutes of the trial) in comparison with both social species *F. darlingi* and *F. mechowii* during the 30 minute trial (Fig. 4).
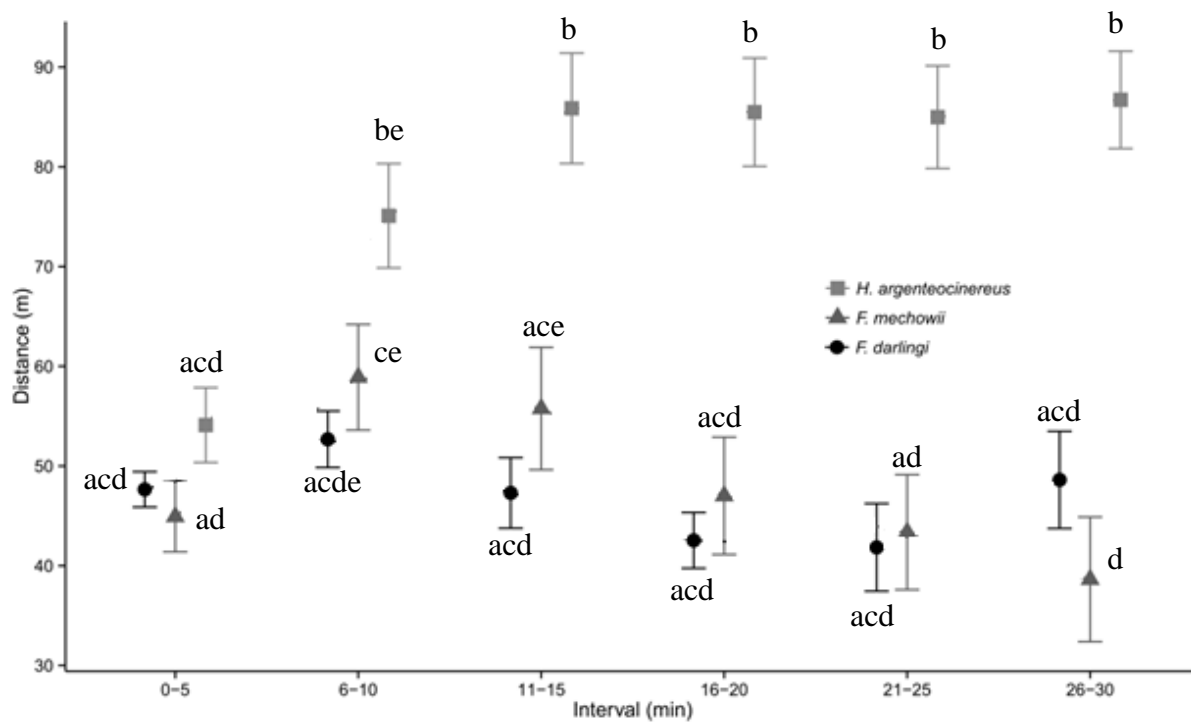

Figure 4. Travelled distance within each five minute interval during the 30 minutes trial for all three species. Lines are connecting Means (different symbols for each species) and error bars showing standard errors. Means with the same letter are not significantly different showing differences among species and intervals as well.

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3.3 Total distance travelled

There was a significant difference in the total distance travelled among the three mole-rat species (Two way ANOVA, $F_{(2;56)} = 13.72$, $p < 0.0001$). In both social species the distances were lower in comparison to the solitary *H. argenteocinereus* (Tukey HSD, $p < 0.001$ for both cases), whereas no difference was observed between the two social species (Tukey HSD, $p = 0.97$) (Fig. 5 and 7S. Learning performance for mole-rats – number of wrong turns. Damaraland mole-rats, white diamonds: male Damaraland mole-rats, black triangles: female Cape mole-rats, white triangle: male Cape mole-rats (c.f. Costanzo et al. 2009).

Table 1S).

There was no significant influence of gender on the total distance travelled ($F_{(2;53)} = 2.019$, $p = 0.143$) (Fig. 6).
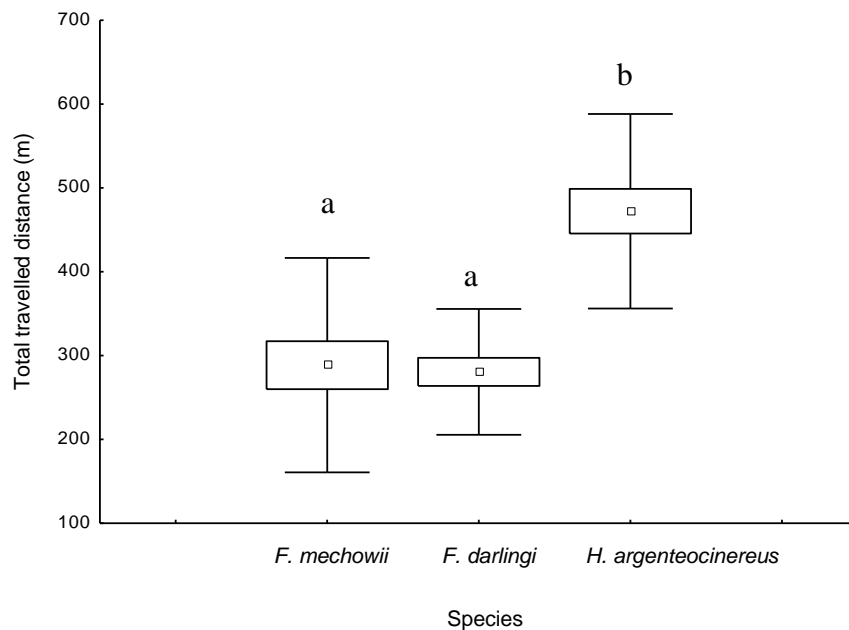

Figure 5. Total travelled distance (m) in the three mole-rat species. Boxes with the same letter are not significantly different. ▫ Mean, □ SE, Ɪ SD.
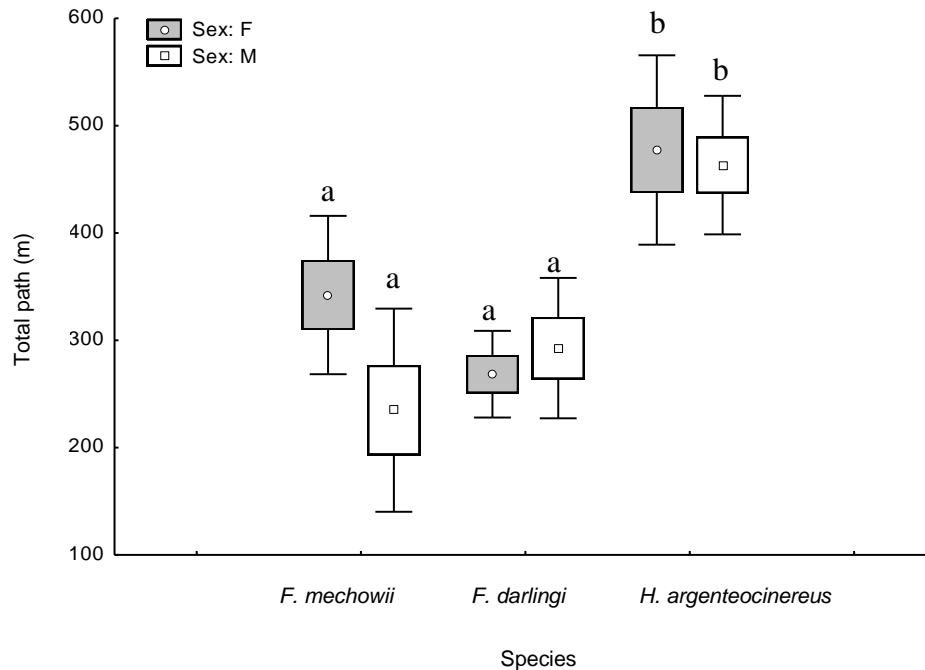
Figure 6. Comparison of the total travelled path (m) in both sexes in the three species of mole-rat. Grey boxes = females and white boxes = males. Boxes with the same letter are not significantly different. ▫ Mean, □ SE, ⊥ SD.

3.4 Duration of an activity during the 30 min exploratory trial

There was a difference between the three mole-rat species in latency of exploratory activity (one-way ANOVA, $F_{(2;56)} = 7.11$, MSE = 0.49, p = 0.0018) (see Fig. 7). There was a significant difference between social *F. darlingi* and solitary *H. argenteocinereus* (ANOVA Tukey HSD, p = 0.0012), but not between *F. mechowii* and *H. argenteocinereus* (ANOVA Tukey HSD, p = 0.1), or *F. mechowii* and *F. darlingi* (ANOVA Tukey HSD, p = 0.21).

The time spent in movement throughout the test was different among the three mole-rat species (one-way ANOVA, $F_{(2;56)} = 39.37$, MSE = 26669, p < 0.0001). The largest difference was found between the social and the solitary species (see Fig. 8). Both social species spent less time by locomotion than *H. argenteocinereus* (1313.2 ± 109.5 s; 73% of tested time) (ANOVA Tukey HSD, *H. argenteocinereus* − *F. darlingi*: p = 0.00012; *H. argenteocinereus* − *F. mechowii*: p = 0.00012). There was also difference between *F. darlingi* (997.9 ± 124.6 s; 55%

of tested time) and *F. mechowii* (858.9 ± 218.6 s; 48% of tested time) (ANOVA Tukey HSD, p = 0.025).
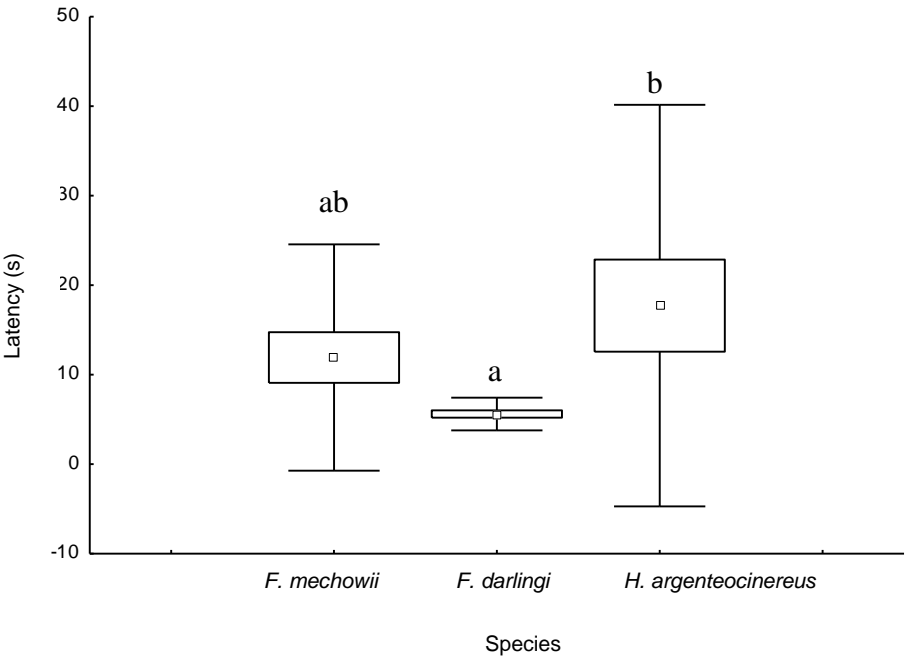

Figure 7. Latency (s) of beginning the exploration between three mole-rat species. Boxes with the same letter are not significantly different. ▫ Mean, □ SE, I SD.
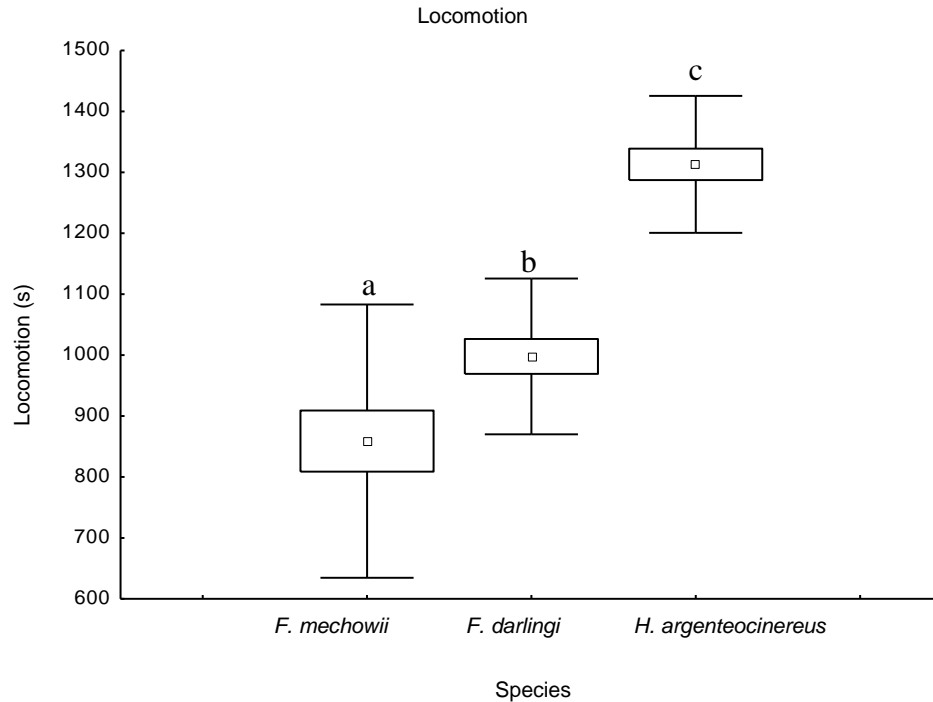
Figure 8. Difference in time spent by locomotion (s) during 30 minute trial. Boxes with the same letter are not significantly different. ▫ Mean, □ SE, I SD.

3.5 Frequency of behavioral parameters during the 30 minute trial

There was a significant interspecific difference in the frequency of **CENTRE CROSSING** (one-way ANOVA, $F_{(2;56)}$ = 12.36, MSE = 0.60, p = 0.001). Both social species travelled more frequently through the centre of the arena than *H. argenteocinereus* (ANOVA Tukey HSD, *F. darlingi* vs. *H. argenteocinereus* p = 0.0014; *F. mechowi* vs. *H. argenteocinereus* p = 0.00016). There were also interspecific differences in the frequency of **PAUSES** (one-way ANOVA, $F_{(2;56)}$ = 33.024, MSE = 0.37, p = 0.001). Both social species made **PAUSES** more frequently than the *H. argenteocinereus* (ANOVA Tukey HSD, *F. darlingi* vs. *H. argenteocinereus* p = 0.00012; *F. mechowi* vs. *H. argenteocinereus* p = 0.00012). In the motion of turning the head to face the centre (**FACE TO CENTRE**) there were also interspecific differences (one-way ANOVA, $F_{(2;56)}$ = 18.84, MSE = 0.45, p = 0.001). Again the solitary species differed from social species as both social species turned their heads towards the centre more frequently (ANOVA Tukey HSD, *F. darlingi* vs. *H. argenteocinereus* p = 0.00012; *F. mechowi*

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vs. *H. argenteocinereus* p = 0.00036). The next significant result was observed in frequency of **GROOMING** (one-way ANOVA, $F_{(2;56)}$ = 10.44, MSE = 0.54, p = 0.001), where both social species groomed themselves more frequently than *H. argenteocinereus* (ANOVA Tukey HSD, *F. darlingi* vs. *H. argenteocinereus* p = 0.00013; *F. mechowi* vs. *H. argenteocinereus* p = 0.00043) (Fig. 8). In all the parameters listed above, were no observed differences found between *F. mechowii* and *F. darlingi* (Fig. 9 and Tab. 1). The rest of the parameters are presented in Table 1.

Table 1. Comparison of the frequency parameters within the three mole-rat species given as Mean(SD). P-values are Bonferroni corrected.

Behavior	Frequency			
	F. mechowii	*F. darlingi*	*H. argenteocinereus*	F statistic, P value
Reversing	4.65 (6.26)[a]	7.3 (4.08)[b]	2.26 (2.99)[a]	$F=8.63_{(2;56)}$, 0.005
Digging	7.95 (9.78)[a]	18.6 (12.4)[b]	4.05 (4.77)[a]	$F=15.26_{(2;56)}$, 0.001
Climbing	3 (2.86)[a]	4.65 (5.22)[a]	1.95 (5.42)[a]	$F=4.45_{(2;56)}$, 0.16
Loops	14.45 (17.95)[a]	15.15 (45.04)[a]	7.26 (6.15)[a]	$F=1.8_{(2;56)}$, 1.7
Grooming	3.85 (4.05)[a]	3.35 (2.95)[a]	0.05 (0.22)[b]	$F=10.44_{(2;56)}$, 0.001
Facing centre	38.65 (24.98)[a]	47.6 (15.81)[a]	15.68 (8.78)[b]	$F=18.84_{(2;56)}$, 0.001
U turns	63.6 (34.41)[a]	100.95 (49.88)[b]	141.26 (69.61)[b]	$F=12.85_{(2;56)}$, 0.001
Centre crossing	33.85 (18.86)[a]	21.15 (7.31)[a]	10.42 (7.46)[b]	$F=12.36_{(2;56)}$, 0.001
Freezing	234.25 (91.9)[a]	285.55 (40.24)[a]	246.47 (55.11)[a]	$F=4.23_{(2;56)}$, 0.19
Pause	48.9 (17.05)[a]	40.05 (18.54)[a]	14.42 (11.82)[b]	$F=12.12_{(2;56)}$, 0.001

Frequency of performed behavior
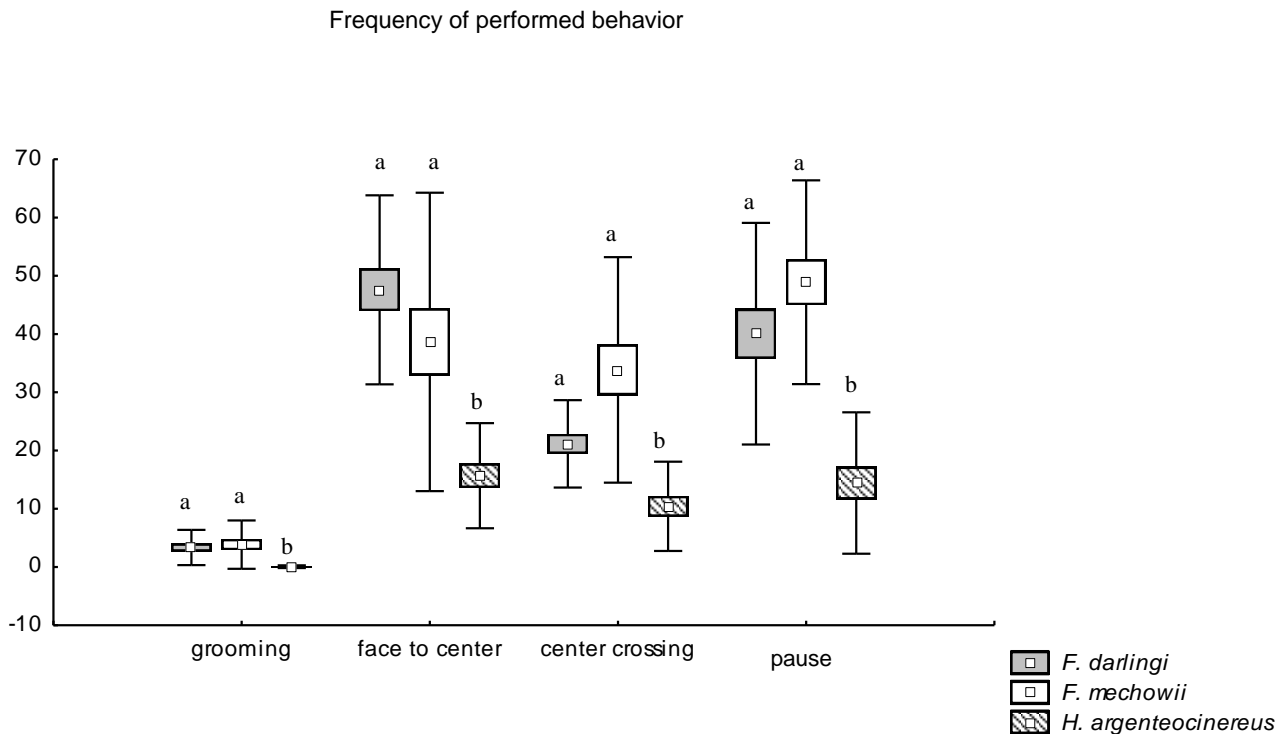
Figure 9. Frequency of selected behavior during 30 minute trial. Boxes with the same letter are not significantly different. ▫ Mean, □ SE, I SD.

3.6 Results of linear discrimination analysis

The relationships among the different components of behavior displayed during the 30 minute trial in the three mole-rat species are shown in figure 10. The behavior of the solitary *Heliophobius* was characterized by a longer time spent by locomotion and greater overall distance travelled with fewer pauses and a lower frequency of head turns and shortcuts across the centre compared to both social *Fukomys* species (Fig. 10). The two first ordination axes of the PCA plot were closely associated mainly with locomotion and latency, respectively and together both axes explained 68.6 % of the variability. Differences between the three mole-rat species were best explained by the set of exploratory variables combining the effects of **locomotion**, **frequency of shortcuts**, **frequency of head turns** (towards the centre), **pauses** and **total path travelled** (Tab. 2, CCA with Hill's scaling). The exploratory-behavioral model including **locomotion** explained 61.1% of the variability in our dataset. The model with locomotion excluded (for details see Methods) explained 57.4% of the variability (Tab. 3). Adding other variables did not significantly increase the amount of variability explained.
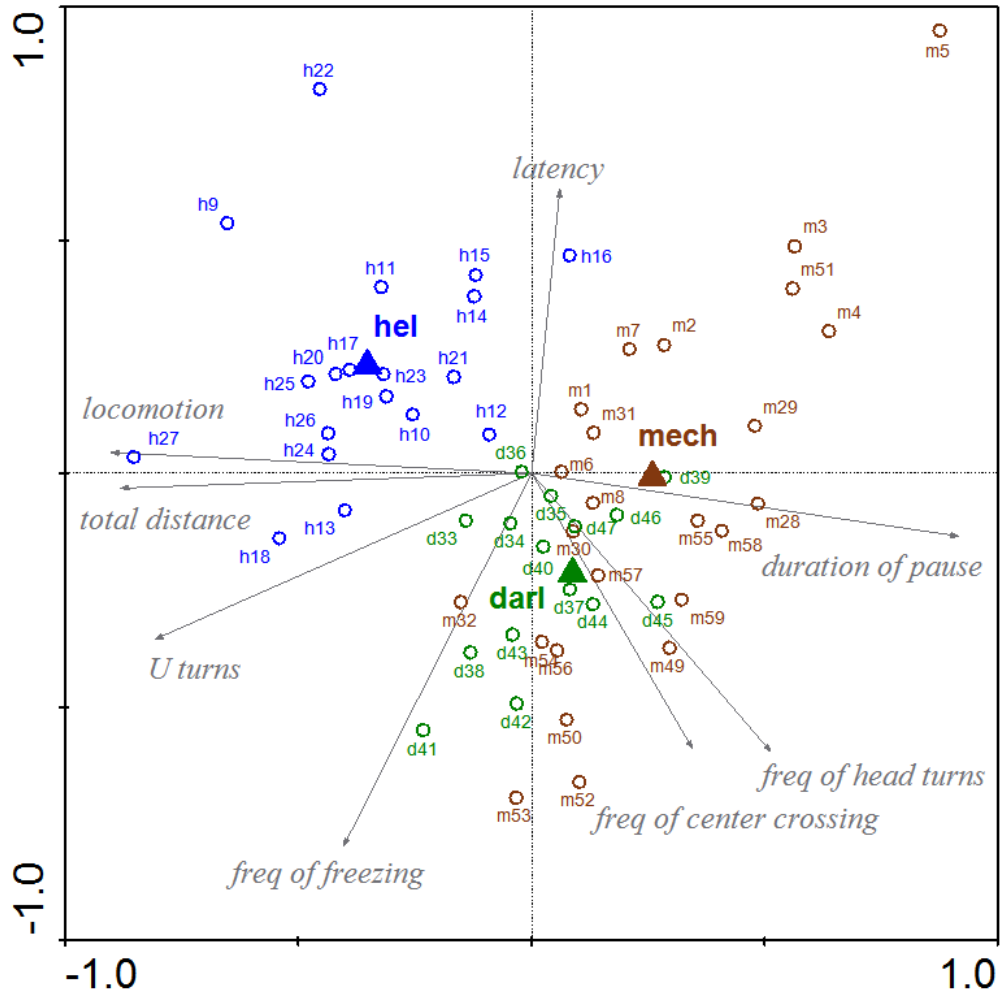
Figure 10. Principal component analysis (PCA) of the performed behavior of the three mole-rat species. Open symbols (circles) represent individuals and solid symbols (triangles) represent centroids of the three mole-rat species. Social species: Darl – *F. darlingi* and mech – *F. mechowii*, solitary species: hel – *H. argenteocinereus*. **Latency** – duration of the latency, **duration of pause** – duration of the stops > 3s, **freq of head turns** – frequency of the turning head towards the centre, **freq of centre crossing** – frequency of short-cuts through centre of the arena, **freq of freezing** – frequency of stops < 3s, **U turns** – frequency of the turns around of whole body by 180°, **total distance** – distance travelled during 30 minute trial, **locomotion** – time spent on exploring provided arena.

Table 2. Results of the linear discrimination analysis (LDA) where a complete set of explanatory variables were tested.

	F	p
Locomotion	26.084	0.002
F_shortcut	8.984	0.002
F_head turns	9.328	0.002
Pause	8.047	0.008
Total path	6.045	0.012

Table 3. Results of the linear discrimination analysis (LDA) without locomotion.

	F	p
Pause	16.776	0.002
F_shortcut	12.049	0.002
Total path	14.472	0.002
F_freezing	8.839	0.006

4. Discussion

My results revealed that the behavior during OFT (development of locomotory activity and ratio of the time spent in centre vs. perimeter) of the solitary *H. argenteocinereus* is more similar to the solitary blind mole rat species than to the social mole-rat species *F. mechowii* and *F. darlingi*. It was found that during the first five minutes there was no difference in distance covered between mole-rat species. The activity of all three mole-rat species increased during the second time period. During the third period there was substantial change in pattern between the social and solitary species, because the solitary species continued to increase their locomotor activity, whereas both social species decreased their activity. Further results revealed other remarkable differences between the three bathyergids during 30 min test, especially between solitary and social species. For example, solitary *H. argenteocinereus* used 73% of the testing time for locomotion compared to about 50% in both social species (*F. mechowii* – 48% and *F. darlingi* – 55% respectively). More time spent in motion resulted in almost double the total distance travelled in solitary species. In spite of the fact that solitary species travelled longer distances and spent more time in locomotion, it spent less time in the centre of the arena. Behavior during the whole test also revealed some other significant differences. Several parameters (grooming, face to centre, centre crossing and pause) also revealed differences between the social species and *H. argenteocinereus*. According to linear discrimination analysis these parameters explain most of the variability between the social and solitary species: locomotion, frequency of shortcuts through centre, frequency of turning head towards the centre, pause and total path travelled.

The majority of the tested individuals spent a significant amount of time in one of the corner squares, which could be denoted as home-base (Eilam and Golani 1989). This phenomenon is in agreement with Avni et al. (2008) where individuals of the blind mole rat also preferred to stay in one of the corner squares. Only two individuals of *F. mechowii* spent more time in a non-corner square (one of them on the length of the perimeter and the second even in more central position) (see Fig. 2). All individuals spent most of the 30 minutes along the perimeter in comparison to the centre zone of the arena (Fig. 3 and Tab. 2S). This result is not surprising and is in agreement with Avni et al. (2008) where blind mole rats spent 98% of experimental time in the perimeter zone during OFT. This behavior of blind mole rat is more

similar to behavior of *H. argenteocinereus* which spent 96% of time in perimeter in comparison to social *F. mechowii* 83%. *Fukomys darlingi* spent 90% of the time on perimeter which was significantly different from *F. mechowii*, but not from *H. argenteocinereus* (Tab. 2S). Nevertheless, the difference between *F. darlingi* and *H. argenteocinereus* was distinctive. Subterranean rodents rely on tactile contact with walls where it is supposed to be safer when exploring new environments. It is thus understandable that open space is not preferred. This is also supported by results from a study by Kimchi and Terkel (2004b), where blind mole rats navigated better in a maze with alleys the same widths as their bodies compared to a maze with wider alleys (Kimchi and Terkel 2004b). A comparison of time spent in the centre and the frequency of shortcuts through the centre (centre crossing) of the arena revealed some interesting interspecific differences (Tab. 2S, Fig. 9). Even though solitary silvery mole-rats travelled almost twice as far as the social species (Fig. 5 and Tab. 1S), they spent less time in the centre of the arena and they visited this area less often than them (Tab. 2S). Similarly, the frequency of head turns towards the centre zone indicates that social species were more willing to examine the centre zone than solitary species (Fig. 9). The animals usually turned their heads towards the centre zone before they entered it (own personal observation). This willingness in the social species to explore open spaces could be compared to the behavior of terrestrial rodents tested in dark conditions during exploration in OFT (Eilam 2004, Zadicarion 2005). When the authors tested Tristam's jird (*Meriones tristrami*) and spiny mice (*Acomys cahirinus*) in dark conditions, they tended to explore the centre of the OF more often, suggesting an increase in boldness. This behavior is more similar to the investigation of the OF by social species *F. mechowii* and *F. darlingi* than by solitary species. It should be noted that the Mashona mole-rat's difference in time spent in the centre zone in comparison with *H. argenteocinereus* was remarkable but not significant. It could be assumed that differences during the test in OFT may be a consequence of the different social systems of the three species. Solitary mole-rats are highly xenophobic, and they are well familiarized with their self-constructed burrow system, so it is likely that any new stimulus/environment will cause cautious reactions in solitary species. It is thus not surprising that they spent less time in the centre of the arena (potentially more dangerous areas) than both social species. Social species seem to be less cautious. They could be more accustomed to the presence of a novel stimulus/environment (i.e. burrows built by another family member).

There were significant differences between the lengths of distances travelled among different 5-minute periods. Solitary *H. argenteocinereus* increased its activity during the first three periods of the trial and maintained the same level of activity for the remaining 5-minute intervals, whereas both social species increased their activity only during the first two 5-minute periods, followed by remarkable reduction for the last four intervals (Fig. 4). The first phase of the exploration where the animals increased their activities is comparable to the build-up phase found in the blind mole rat (Avni et al. 2008). After this period the second phase called "free-locomotion" should follow. This phase is characterized by walking along the perimeter of the maze with few stops or retracing of previous paths. This "free-locomotion" phase was not distinct in the social species. After increasing of their activity (for the first 10 minutes of the trial), the frequency and length of pauses increased for later phases of the trial (see Tab. 2S) and therefore were not fluently travelling along the perimeter but decreased their activity. This reduction of activity after the initial ten minutes in social species is concurs with the study of Avni et al. (2006), where Tristram's jird (*Meriones tristrami*) also decreased the travelled path after ten minutes of the trial. This observation strengthens the behavioral similarity during OFT between our social subterranean mole-rats and aboveground dwelling rodents, whereas *H. argenteocinereus* diverges at the point where it stabilizes its activity and in development after this point as well. The solitary silvery mole-rat followed the same pattern as described for blind mole rats (Avni et al. 2008). After the build-up phase which lasted about 15 minutes, a phase of free-locomotion with fewer pauses followed (Tab. 3S). This result implies an alternative explanation that the social subterranean species successfully obtained a basic spatial representation of the environment sooner (during first ten minutes) and then lost interest in further examination of the arena but rather engaged themselves in comfort activities as i.e. grooming (Tab. 3S). However, tested mole-rats belonging to the same family (Bathyergidae), solitary *H. argenteocinereus* seems to have more similar behavior pattern in OFT to solitary blind mole rat (Spalacidae) than to both social species. It suggests that the social organization may have an influence on spatial performance.

Our results regarding the differences in latency between solitary and social mole-rat species partially confirm the results of previous studies by Costanzo et al. (2009) and Suchanová (2011). In these studies the solitary Cape mole-rat (*Georychus capensis*) and the silvery mole-rat

started to leave the starting area later than the social species, Damaraland mole-rat and giant mole-rat (Fig. 2S). In my study the solitary *H. argenteocinereus* had a longer latency of beginning the exploratory activity than *F. darlingi*. *Fukomys mechowii* had also shorter latency period than *H. argenteocinereus* but this result was not significant. This result might be influenced by high variability in latency within some individuals in *F. mechowii* and *H. argenteocinereus* (Fig. 7).

The total distance travelled during the experiment differed between social and solitary species (Fig. 5). *Heliophobius argenteocinereus* travelled almost twice longer distance compared to the both social species. The distance travelled seems to be influenced by more frequent pauses and grooming in the two social species (Fig. 9, 10). These results strongly agree with previous comparative studies where solitary species travelled for longer distances and made more errors (they examined more dead ends of the maze) during the exploration phase of navigation tests in comparison to social species (Costanzo et al. 2009, Mazoch 2008). In the study by Costanzo et al. (2009), the solitary Cape mole-rat needed more time to finish the task and made more errors (see Fig 6S, 7S). Together with a reluctance to abandon relatively safe space of the arena perimeter, the longer travelled distance in the solitary species could have led to, at first sight, paradoxical results. Longer total distance travelled of the solitary species is caused by enhanced overall locomotor activity especially during the second half of the OFT (15 to 30 minutes). This behavior suggests an urge to continually explore the novel environment, and search for potential escape.

These differences in exploratory behavior between social and solitary species can possibly influence results in studies dealing with spatial learning and memory abilities. For example, the obvious difference between social and solitary mole-rats during the first trial of the spatial learning experiment and similarly during the subsequent trials, does not have to mean remarkable difference in learning abilities but as our study shows, could be an artifact of more cautious behavior and enhanced locomotor activity of the solitary species (see Fig. 1S and compare to Fig. 4). Analogous unwanted artifact can be present in studies comparing spatial learning abilities in unrelated species. For example Kimchi and Terkel (2001) compared blind mole rats, laboratory rats and Levant voles and concluded that the blind mole rat demonstrate better spatial learning and memory compared to surface dwelling species (Fig. 2S). This again could be influenced by different exploratory behavior between species. The blind mole rat took considerably longer to

finish the first trial than both surface dwelling species as individuals wasted the time by running back and forth along the same dead end paths. According to my results I suggest that the behavior of the solitary blind mole rat during the exploratory trial could be comparable to the behavior of the silvery mole-rat in our study. In addition, the fast completion of the task by surface dwellers (made fewer unnecessary movements – fewer errors) seems to be comparable to the behavior of social species in our study.

The time spent in locomotion in OFT showed almost same pattern as distance travelled (Fig. 8). Solitary *H. argenteocinereus* spent 73% of the 30 minutes as opposed to 48% and 55% for *F. mechowii* and *F. darlingi*, respectively. However, the difference between the two social species was also significant. This result could have been influenced by body size. The giant mole-rat is generally larger than the Mashona mole-rat. It could mean that larger individuals of bigger species could lose motivation faster than the smaller ones because it is easier for them to explore experimental space. However, the size of individuals does not seem to have such strong impact on time spent in locomotion. Even though *F. darlingi* is the smallest of the three species, the medium-sized *H.argenteocinereus* spent significantly more time in motion.

Principal component analysis provides overall picture of mole-rats' activity and behavioral patterns. The three mole-rat species were best discriminated by the effect of locomotion, frequency of shortcuts, frequency of head turns (towards the centre), pauses and total path travelled. The PCA clearly separated cluster of the individuals of solitary species from looser cluster of both social species (Fig. 10). Activities such as locomotion, distance travelled, and bold behaviors such as pauses and centre crossing discriminate between solitary and social species. Whereas the solitary species was characterized mainly by the activity behaviors both social species were determined more by bold behavior. The behavioral pattern showed in the PCA plot could also imply the more diversified behavior during OFT displayed by social species in comparison with the solitary one. This analysis therefore supports the evidence of different exploratory behavior between the social and solitary mole-rats in this study.

No sexual difference was found in any of three mole-rat species (Fig. 6). Sex difference within many species (e.g. polygynous species because of difference in the size of home ranges) can influence spatial abilities, where males usually outperform females (Jonasson 2005, review in Jones et al. 2003). In the case of the studied species, the home range size in subterranean animals

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is equal to the size of their burrow system. Males and females of the social *F. mechowii* and *F. darlingi* live together in one burrow system, which means that there should not be a difference in spatial test performance. In addition, such difference between sexes was found neither in the Kimchi and Terkel's (2001 – among the first trial) study, nor in thesis of Suchanová (2011) and Mazoch (2008) focused on subterranean species.

To conclude, I found some substantial differences in exploratory behavior between the three African mole-rats. Among others, both social species spent more time in the centre of the arena than did solitary ones. After the build-up phase the social species reduced their activity which could have been caused by faster gathering of the necessary spatial information or by less cautious behavior. Interestingly, the behavior of solitary silvery mole-rats in open arena were similar to the solitary blind mole rat in the same experimental setting. It indicates that the level of social organization could have an influence on spatial performance.

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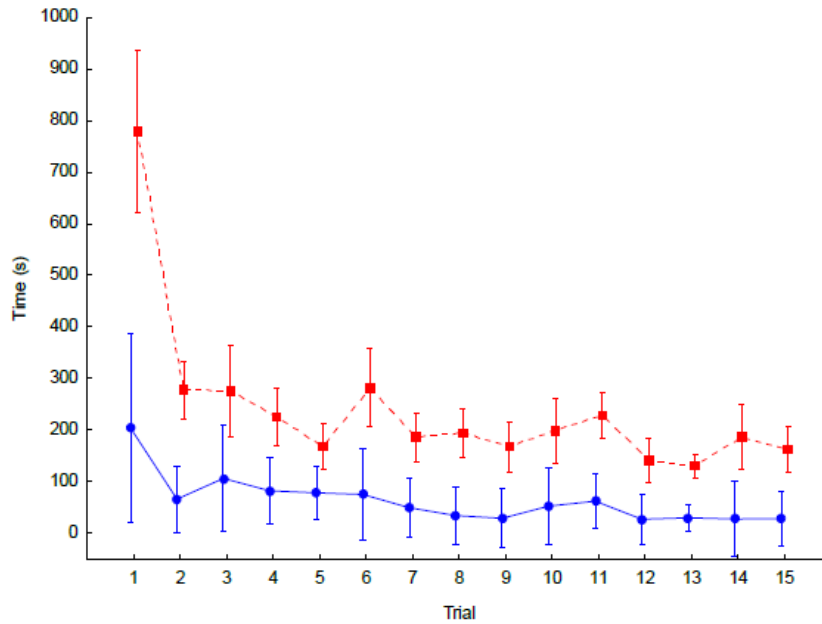
6. Supplementary material

Figure 1S. Time (mean ± 0.95 confidence int.) required to complete spatial learning task in 15 consecutive trials for solitary H. argenteocinereus (dotted line) and social F. mechowii (solid line) (master thesis Mazoch 2008).
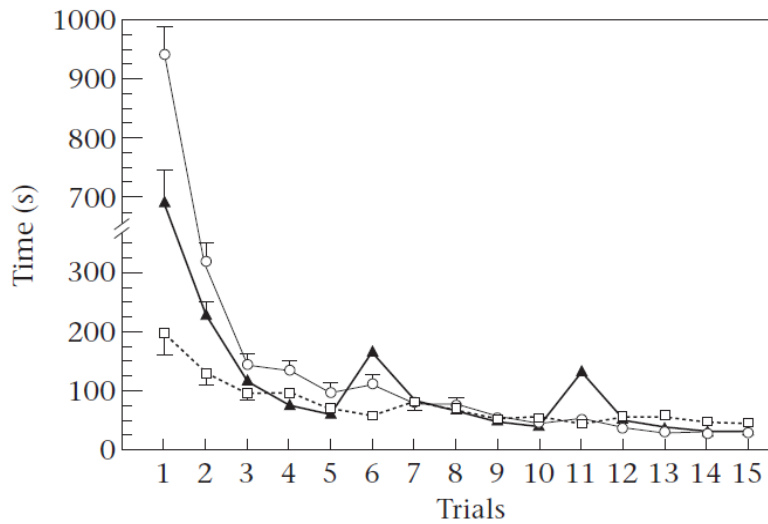

Figure 2S. Time (s) ±SE required by the mole rats (empty circles), rats (black triangles) and voles (empty squares) to reach the end of the complex maze in 15 trials (c.f. Kimchi and Terkel 2001).
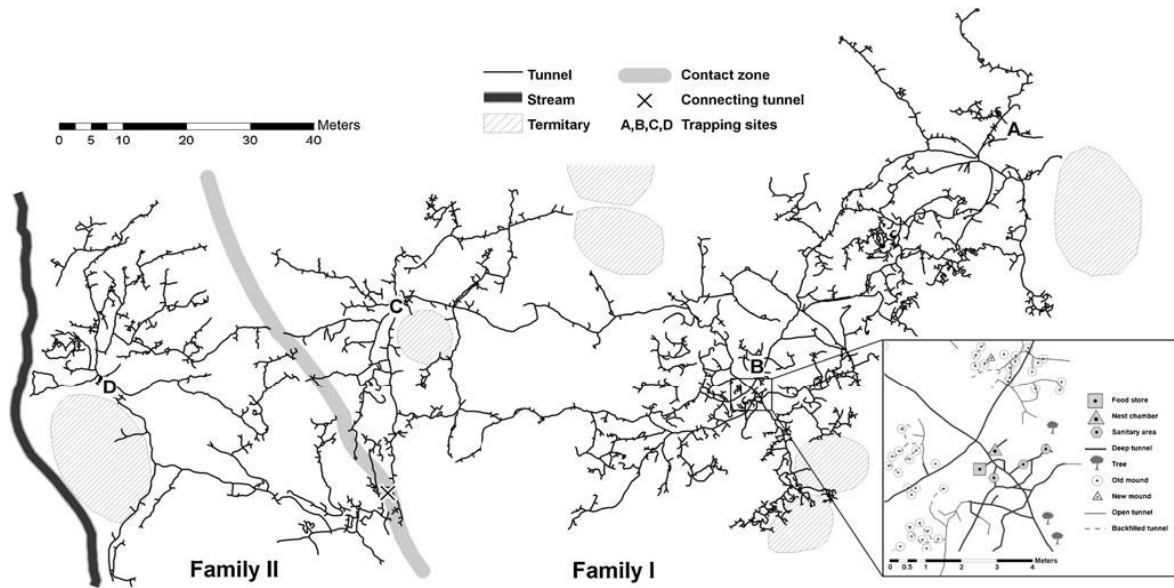
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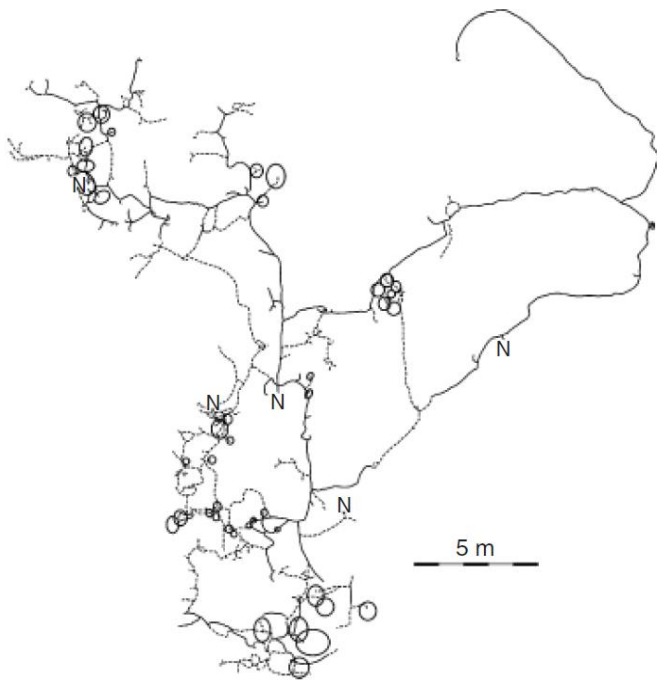Figure 3S. Burrow systems of giant mole-rat (*Fukomys mechowii*) added from Šumbera et al. 2012.

Figure 4S. The shape of burrow system of an adult female silvery mole-rat (Heliophobius argenteocinereus) excavated in the middle of the dry season. N = the nest chambers, dashed lines symbolize backfilled tunnels, circles and eclipses = mounds (c.f. Šumbera et al. 2008)

Figure 2.3a: CZG67
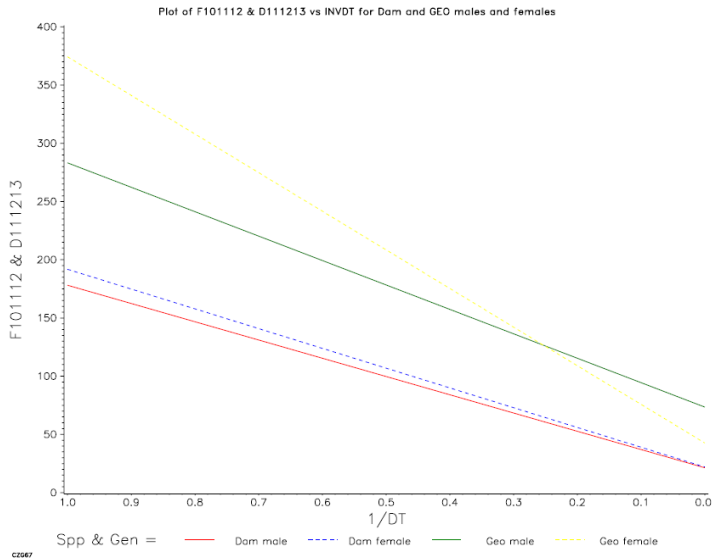Y: Mean Time (Tunnel Entry, Goal, & Food) (s) against X: 1/ Day-Trial

Figure 5S. Time needed before animals participated in the experiment. Dam male/female – social Damaraland mole-rat, solitary Geo male/female – Cape mole-rat (c.f. Costanzo 2005).

Figure 2.3d: CZG70
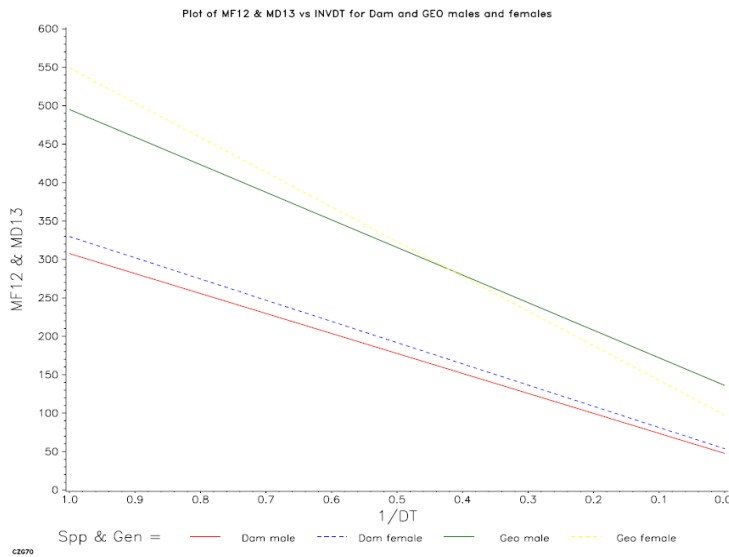Y: Mean Time to Food (s) against X: 1/ Day-Trial

Figure 6S. Time needed to finish the task (successful finish = food reward). Dam male/female – social Damaraland mole-rat, solitary Geo male/female – Cape mole-rat (c.f. Costanzo 2005).
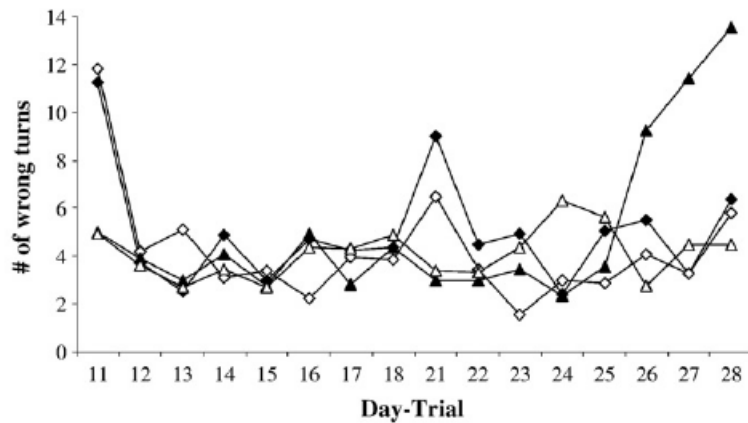
Figure 7S. Learning performance for mole-rats – number of wrong turns. Damaraland mole-rats, white diamonds: male Damaraland mole-rats, black triangles: female Cape mole-rats, white triangle: male Cape mole-rats (c.f. Costanzo et al. 2009).

Table 1S. Descriptive statistics for total travelled distance (m) in all three mole-rat species and also for each sex.

	F. mechowii		*F. darlingi*		*H. argenteocinereus*	
	Mean (±SD)	N	Mean (±SD)	N	Mean (±SD)	N
All	288.6 (±127.9)	20	280.6 (±75)	20	472.2 (±116)	19
Males	234.9 (±132.3)	10	292.8 (±91.5)	10	463.3 (±69.8)	7
Females	342.2 (±103.1)	10	268.5 (±56.5)	10	477.4 (±138.9)	12

Table 2S. Time spent in different parts of the arena, significant results are given in bold.

	Spent time (%)		
	Fukomys mechowii	*Fukomys darlingi*	*Heliophobius argenteocinereus*
perimeter	83.44 %	90.42 %	95.82 %
centre	16.56 %	9.58 %	4.18 %

		Fukomys mechowii		*Fukomys darlingi*		*Heliophobius argenteocinereus*	
		perimeter	centre	perimeter	centre	perimeter	centre
Fukomys mechowii	perimeter		**0.0001**	**0.0083**	**0.0001**	**0.0005**	**0.0001**
	centre	**0.0001**		**0.0001**	**0.0095**	**0.0001**	**0.0001**
Fukomys darlingi	perimeter	**0.0083**	**0.0001**		**0.0001**	0.9507	**0.0001**
	centre	**0.0001**	**0.0095**	**0.0001**		**0.0001**	0.0762
Heliophobius argenteocinereus	perimeter	**0.0005**	**0.0001**	0.9507	**0.0001**		**0.0001**
	centre	**0.0001**	**0.0001**	**0.0001**	0.0762	**0.0001**	

Table 3S. Development of behavioral activity during six 5-minute intervals (mean duration of **Pause** and **Grooming** (min) and frequency of **Pause**, **U turn** and **Centre crossing**).

Interval	*Fukomys darlingi*						*Fukomys mechowi*						*Heliophobius argenteocinereus*					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Pause (s)	22	31	40	41	58	42	36	44	62	80	83	102	28	16	14	11	9	15
Grooming (s)	0	1	11	5	13	21	13	37	13	21	13	34	0	0	0	0	0	5
U turn (freq.)	6	17	19	17	20	22	3	13	11	13	13	13	10	18	25	27	33	29
Centre cross. (freq.)	4	3	3	3	3	4	6	7	6	6	6	4	2	1	2	2	2	2
Pause (freq.)	4	6	7	7	9	8	5	6	7	9	11	10	4	3	2	3	2	2