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MAMMALIAN ENERGETIC SAVINGS IN SUBTERRANEAN  
ENVIRONMENT. THE CASE OF AFRICAN MOLE-RATS

Ph.D. Thesis

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■ **Annotation:** Mole-rats are placental mammals which are perfectly adapted to subterranean life. In this thesis I present novel findings on working metabolism and thermoregulatory physiology of mole-rats. These animals cope with low availability of food and have thus employed multiple strategies how to conserve energy and/or use it more effectively. Among other adaptations this resulted in lower resting body temperature, tolerance to increase in body temperature during exercise or while at rest, surprisingly efficient cooling while digging and precise diurnal and seasonal timing of activity with regards to environmental conditions. My focus in this work is on the digging metabolic rate and thermoregulation of social *Fukomys mechowii* and solitary *Heliophobius argenteocinereus* in soft and hard substrate, thermoregulatory abilities of *Fukomys darlingi*, seasonal changes of activity in free living *Heliophobius argenteocinereus* measured as daily energy expenditure and, finally, energetic consequences of the daily activity patterns of *Fukomys anselli*.

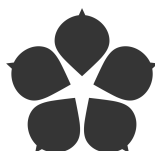
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Mgr. Jan Okrouhlík

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Jitka is the second in the row. She was the one who actually started to do some real physiology on mole-rats at our faculty and originally I was just helping her with some technical issues. Later Jitka proved to be a perfect scientific partner. The list of things she did for/with me is also very long, so I will make it brief. Jitka, thank you.

The third paper is based on endless days, which Milada spent measuring metabolic rates of pups, juveniles and adults of *Fukomys darlingi*. I would never be so patient and would never devote so much time to such a task. Although much of the results did not make it to the paper, Milada persisted and wrote it. Milada, thank you for that.

I would like also to thank Honza for inviting me to contribute to his manuscript (the fourth paper), but that is not the only thing for which I am grateful. Honza also provided valuable assistance in Africa for the second paper.

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My gratitude is also directed towards my family for their support, especially to my mother and to Monika.

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■ List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

- I. 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. *Physiology & Behavior*. 99, 54–58. (IF=3.160)  
*Jan Okrouhlík was coresponsible for the physiological part of the study, especially for the oxygen measurement experimental set-up, calculations and interpretations.*
- II. Zelová, J., Šumbera, R., **Okrouhlík, J.**, Šklíba, J., Lövy, M., Burda, H., 2011. A seasonal difference of daily energy expenditure in a free-living subterranean rodent, the silvery mole-rat (*Heliophobius argenteocinereus*; Bathyergidae). *Comparative Biochemistry and Physiology Part A. Molecular and Integrative Physiology* 158, 17–21. (IF=2.167)  
*Jan Okrouhlík introduced the DLW technique in our laboratory and participated in sample processing as well as in calculations and manuscript preparation.*
- III. Zemanová, M., Šumbera, R., **Okrouhlík, J.**, 2012. Poikilothermic traits in Mashona mole-rat (*Fukomys darlingi*). Reality or myth? *Journal of Thermal Biology* 37, 485–489. (IF=1.376)  
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- IV. Šklíba, J., Lövy, M., Hrouzková, E., Kott, O., **Okrouhlík, J.**, Šumbera, R., 2014. Social and Environmental Influences on Daily Activity Pattern in Free-Living Subterranean Rodents. The Case of a Eusocial Bathyergid. *Journal of Biological Rhythms* 29, 203–214. (IF=3.229)  
*Jan Okrouhlík participated in physiological interpretations of the data and manuscript preparation.*
- V. **Okrouhlík, J.**, Burda, H., Kunc, P., Knížková, I., Šumbera, R., 2015. Surprisingly low risk of overheating during digging in two subterranean rodents. *Physiology & Behavior* 138, 236–241, IF=3.033)  
*Jan Okrouhlík participated in the manuscript preparation, processed and evaluated the results.*

## Content

|          |                                      |           |
|----------|--------------------------------------|-----------|
| <b>1</b> | <b>Introduction</b>                  | <b>1</b>  |
| <b>2</b> | <b>Thermoregulation</b>              | <b>1</b>  |
| <b>3</b> | <b>Activity timing</b>               | <b>4</b>  |
| <b>4</b> | <b>Metabolism</b>                    | <b>4</b>  |
| <b>5</b> | <b>Conclusions</b>                   | <b>7</b>  |
| <b>6</b> | <b>References</b>                    | <b>8</b>  |
| <b>7</b> | <b>Published parts of the thesis</b> | <b>13</b> |
| 7.1      | Paper 1 . . . . .                    | 13        |
| 7.2      | Paper 2 . . . . .                    | 19        |
| 7.3      | Paper 3 . . . . .                    | 24        |
| 7.4      | Paper 4 . . . . .                    | 29        |
| 7.5      | Paper 5 . . . . .                    | 41        |

# 1 Introduction

Mammals living underground are fascinating animals and also represent some of the best examples of convergent evolution. Although I have not intended to study them deeply, they have caught my attention. The laboratory of Radim Šumbera keeps the biggest colony of mole-rats in Europe, which offers us the opportunity to study these animals. Unfortunately one cannot study everything, so we had to choose some topics, which might be of interest and see what we find. And as it could have been expected (but not by us at that moment) some of our findings are quite controversial. I am an animal physiologist in a team of zoologists, so my background is very different to theirs. We all come with questions to be answered and search for means to study various aspects of mole-rat's life. This brings a very nice synergy to almost everything we do together and because of this synergy we have published several articles so far and another is being prepared. In this thesis I focus on energetics and thermoregulation of mole-rats mainly from the energy saving viewpoint.

Mole-rats are a group of more than twenty mammalian species (Rodentia: Bathyergidae), which have adapted to life below ground. This habitat offers protection against predators as well as daily and seasonal stability in terms of temperature and humidity (Nevo, 1999; Buffenstein, 2000; Burda et al., 2007). On the other hand it also puts high demands on physical, ecophysiological, and behavioural parameters of its inhabitants. Mole-rats have almost lost their sight<sup>1</sup> and their body extremities are reduced to a minimum - they have no external ear and almost all species have shortened the tail and neck. Their legs are shaped for effective digging/soil excavation, but the main digging tool are incisors growing outside the mouth cavity. Most mole-rats live in quite arid environments, which influence food availability. The only way to find new food is to dig tunnels. But digging is energetically a very demanding activity. There are several levels of sociality found among mole-rats ranging from solitary species (genera *Bathyergus*, *Heliophobius* and *Georychus*) to eusocial ones (genus *Heterocephalus*, but other social genera are *Cryptomys* and *Fukomys*). A lowered metabolic rate, high conductance, and decreased body temperature are all important ecophysiological adaptations of mole-rats to their environment.

## 2 Thermoregulation

Thermoneutral zone (TNZ) is a range of ambient temperatures at which the metabolic rate of a species is the lowest. The lowest temperature of this range is called the lower critical temperature (LCT). At ambient temperatures below this threshold the metabolic rate increases

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<sup>1</sup>although Kott et al. (2010) found out that they do not only distinguish light and dark, but they see also some colours

usually linearly as the ambient temperature decreases. The ambient temperature (within TNZ) above which the metabolic rate increases to promote cooling is called upper critical temperature (UCT). African mole-rats have quite a broad TNZ ranging from 1 °C up to 8 °C. The LCT is the lowest (around 25 °C) in mole-rat species originating from temperate areas. Lower LCT allows the animals to conserve energy and keep a stable body temperature at low ambient temperatures. It is assumed that the wide TNZ together with low LCT in solitary species is a substitute for social thermoregulation (Šumbera et al., 2007). Mole-rats have a low resting body temperature at LCT when compared to most other placental mammals. Among mole-rat species the resting body temperature ranges from 33 °C to almost 36 °C. This decreased resting body temperature is often explained as a mechanism, which enables mole-rats to save energy (e.g. Zelová et al., 2007), by lowering the difference between body temperature and ambient temperature and thus decreasing heat losses. With increasing ambient temperature within TNZ we observe an increase of body temperature in mole-rats (e.g. McNab, 1966; Lovegrove, 1986a; Zelová et al., 2007; **Zemanová et al., 2012**). This phenomenon has been observed in other small mammals (e.g. Katzner et al., 1997; Wang et al., 2000; Mathias et al., 2003) and is considered to be a water and energy saving mechanism (Wooden and Walsberg, 2002).

Mole-rats are generally able to keep a stable body temperature at ambient temperatures below lower critical temperature. This applies to both social (*Fukomys damarensis*) as well solitary species (*Georychus capensis*, *Heliophobius argenteocinereus*, *Bathyergus suillus*) (Lovegrove, 1986a, 1987; Zelová et al., 2007). However some species deviate from this rule and show signs of heterothermy. This includes the solitary *Bathyergus janetta*, but (tendency for) heterothermy is more common in social mole-rats - *Cryptomys hottentotus pretoriae* (Viljoen et al., 2011), *Cryptomys hottentotus* (Haim and Fairall, 1986), *Fukomys anelli* (Marhold and Nagel, 1995) and even in the biggest one, *Fukomys mechowii* (Bennett et al., 1994). The extreme case of heterothermy is ectothermy. The only mammal, which is believed to be thermoconformist in active state is the social naked mole rat (Jarvis, 1978; Buffenstein and Yahav, 1991). Its body temperature follows ambient temperature at temperatures below 37 °C. There were indications that there is another social mole-rat with poikilothermic tendencies - the Mashona mole-rat *Fukomys darlingi* (Bennett et al., 1993a). The confirmation of this finding would prove that poikilothermy as an energy saving mechanism is not limited to the naked mole-rat. But we have shown that at least some populations of *Fukomys darlingi* are able to keep stable body temperature (33 °C) at ambient temperature of 10 °C (see section 7.3, **Zemanová et al., 2012**). I suppose that the animals used by Bennett et al. (1993a) were too small/young to be able to generate sufficient amount of heat<sup>2</sup> or fall into torpor. Such reaction was observed in similarly

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<sup>2</sup>We also tried to measure thermoregulatory abilities of pups and juveniles of *Fukomys darlingi*, but we were not able to obtain sufficient amount of data to publish it, but the message was that too young animals do not increase their metabolic rate at low ambient temperatures.



sized Ansell's mole-rat *Fukomys anelli* (Marhold and Nagel, 1995). The rejection of poikilothermic traits in *Fukomys darlingi* shows that poikilothermy is not a general energy saving mechanism in mole-rats.

Mole-rats are also known for relatively high conductance when compared to other mammals. High conductance helps animals to dissipate heat, whereas low conductance is preferred in terms of heat savings. Because cooling is very limited in closed humid underground burrows, the only mean of heat dissipation is its transfer through the skin. Effective heat dissipation is required especially when excavating new burrows, however no information on the energetic cost of digging in mole-rats was available. We have focused on this topic in social *Fukomys mechowii* and solitary *Heliophobius argenteocinereus* (see section 7.1., **Zelová et al., 2010**). We have found out that the efficiency of digging of the solitary *Heliophobius argenteocinereus* is much higher than in the *Fukomys mechowii*. But what is more interesting - each mole-rats species dig with the same effort (same oxygen consumption) in hard and soft substrate. There is more than 4 times more heat produced when digging than at rest. How is this amount of heat handled? We used IRT to study changes in surface temperature of these mole-rat species after digging in artificial tunnels (see section 7.5., **Okrouhlik et al., 2015**) and found that surface temperatures do not increase to facilitate heat dissipation as expected. The surface temperatures of head, feet, ventrum and trunk of both species decreased. We offer two different explanations for this observation. The first assumes unexpectedly high heat flux conveyed by the soil, while the second states that the heat is partially stored in the body, while the relatively low surface temperature is a consequence of blood flow redirection from skin to muscles. The difference between highest and lowest tolerable body temperature (caused by stored heat) saves energy reserves because it in fact increases efficiency of their utilisation - the exercise heat is used as thermoregulatory heat.

Social mole-rats are able to save energy by huddling. The idea is that social animals resting one on another effectively decrease their surface area and thus limit the heat losses. This would go even further if we were to believe Kotze et al. (2008), who claim that the weight specific resting metabolic rate is comparable to a similarly sized insect in huddling *Fukomys damarensis* even within their TNZ. This is of course an artefact, probably caused by incorrect calculations, but it is a nice example of how easy it is for an obvious nonsense to persist in literature with no doubts about its validity. The study in question has so far 13 references and none of them questions the results<sup>3</sup>. I am questioning those results, because we did a similar study on *Fukomys darlingi* with completely different outcome (Wiedenová et al., in preparation).

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<sup>3</sup>To be more precise 11 of these does not question it, I failed to obtain the remaining two.

### 3 Activity timing

High efficiency of energy utilization is very important for mole-rats, because their food resources are very limited. Apart from heat recycling there are other means of preserving valuable energy. One of them is precise timing of activity. Avoiding activity either during hottest or coldest time during the day can save significant amount of energy that would otherwise have to be used for thermoregulation (e.g. Rado et al., 1993). We have found that during cold season *Fukomys anselli* had 1 activity peak (around 2 pm) which was tightly correlated with the temperature measured at depth of foraging burrows. We believe that this pattern is probably a result of minimizing the cost of thermoregulation, because *Fukomys anselli* has high thermoregulatory requirements to maintain stable body temperature below the lower critical temperature (see section 7.4., Šklíba et al., 2014). But mole-rats do not only time their activity with regards to daily variations of temperature. We have found that *Heliophobius argenteocinereus* increases its daily metabolic rate after the rains by almost 40% (see section 7.2., Zelová et al., 2011)<sup>4</sup>. The increase of metabolic rate nicely correlates with time spent outside the nest (Šklíba et al., 2007). The soil is much easier to be dug in after rains and also contains more water, which can promote cooling of digging mole-rats (see section 7.5., Okrouhlík et al., 2015). This shows that postponing an activity even for longer periods can contribute to energy conservation.

### 4 Metabolism

Among mammals, the major part of resting metabolic rate variability can be explained by body mass. Smaller species/individuals have relatively higher mass specific metabolism compared to larger ones (Kleiber, 1961). The heat loss through the body surface is proportional to body length squared, but the heat generating mass is proportional to the length cubed. Therefore smaller individuals (species) produce more heat in order to keep stable body temperature. In the past there were doubts about validity of this rule in mole-rats (Lovegrove and Wissel, 1988). It was believed that decreased size of mole-rat workers does not result in increased energy expenditure per mass unit, effectively increasing chances of food discovery, because more workers can search for food. This very tempting hypothesis was later disproved when Zelová

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<sup>4</sup>To make this study possible we had to introduce the doubly labelled water technique in our laboratory. This included modifications of all procedures in order to be easily performed in remote areas of Africa with only a little equipment and also finding a reliable anaesthetic procedure (recently improved by Garcia Montero et al., 2014). The key was to use much lower dose of ketamine and xylazine than is the lowest recommended for rodents. The blood collection was also very tricky, but we discovered that if we heat the animal by a lamp we can do the blood sampling more easily by collecting it from dorsal hindfoot vein after its gentle puncture. We had also to tweak the cryodistillation procedure of Šantrůček et al. (2007) to work with small volumes of blood.

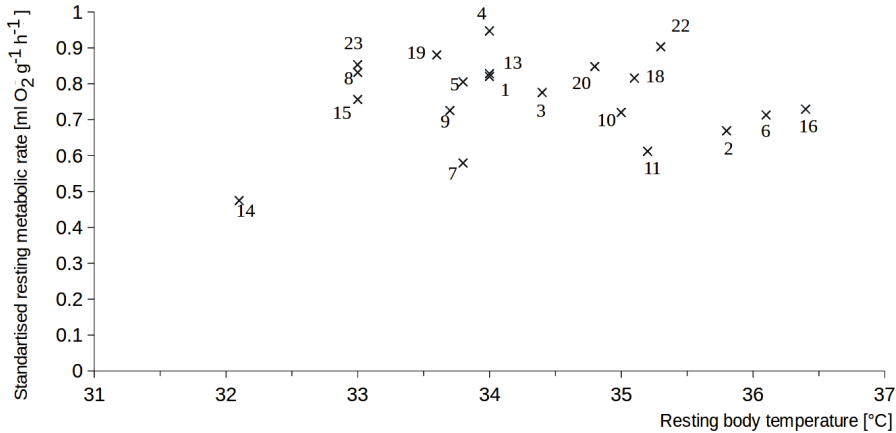
et al. (2007) showed that mole-rats are not different in energy expenditure—size relationship to other mammals.

Another energy conserving mechanism is believed to be mole-rat's lower than usual resting metabolic rate. There are three main complementary hypotheses explaining lower resting metabolic rate in subterranean rodents in general (see Zelová et al. (2007) for review). It is believed that the decreased resting metabolic rate in mole-rats is a consequence of combination of all thereby considered factors (Contreras and McNab, 1990). The respiratory stress hypothesis states that lowered resting metabolic rate together with lower heart rate help to cope with hypoxic and hypercapnic conditions in burrows (Arieli, 1979; Bennett et al., 1994; Buffenstein, 1996). Unfortunately there are no data on oxygen/carbon dioxide concentration in mole-rat burrows. We can speculate that the gas exchange may be less favourable when the soil is wet or that the gas concentration may become limiting in nests, but before some reliable data on gas concentration is obtained no definite conclusion can be made. Buffenstein (2000) assumes that gas concentration is not the primary determinant of lowered resting metabolic rate in mole-rats. The generally accepted cost of burrowing hypothesis (e.g. Lovegrove, 1986a; Brett, 1991; Spinks et al., 2000; Jarvis et al., 1998; Šumbera et al., 2008; Šklíba et al., 2011; Lövy et al., 2012; Šumbera et al., 2012) supposes that the lower resting metabolic rate decreases energetic demands and is therefore an energy saving mechanism that helps to cope with food and water scarcity. The thermal stress hypothesis claims that lowered resting metabolic rate minimises the risk of overheating in closed burrows by decreasing heat production (McNab, 1966, 1979; Buffenstein, 2000). The supporting argument for this hypothesis is that mole-rats have about 20% higher conductance than similarly sized above ground rodents. On the other hand it is most unlikely that a mole-rat would prefer to rest at temperatures above the upper critical temperature, when it has access to deeper and cooler tunnels, so the thermal stress hypothesis is more likely to be more valid for working metabolic rate rather than for the resting metabolic rate.

The decreased resting metabolic rate in mole-rats is usually put in connection with their low resting body temperature. Although this connection seems logical, because people usually think that heating a body to lower temperature cost fewer fuel than heating it to higher temperature does, and that the metabolism slows down at lower resting body temperatures. To show that this is not a correct explanation for mole-rats, I plotted mole-rat resting metabolic rate against their resting body temperature at a lower critical temperature (see Figure 1). To standardize the metabolic rate over the weight span of mole-rats, I have normalized it to 100g (assuming validity of equation  $RMR = 3.79 \times (\text{body mass})^{-0.322}$  (Lovegrove, 1986a))<sup>5</sup>. It is clear, that lower body temperature does not result in decreased resting metabolic rate among mole-rat

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<sup>5</sup>This is in fact not a good idea, because this function was derived from mole-rat data, so I may easily be getting to an argumentation circle, because bigger mole-rat species are less prone to be affected by low temperature and their body temperature can follow a different pattern.



**Figure 1.** – Normalized metabolic rate of mole-rats plotted against resting body temperature. For species key see Table 1.

species. The dependence looks very similar even if we use lower critical temperature instead of the resting body temperature. What is then the reason for lowering body temperature? I think that the basic idea is correct - it has something to do with minimizing heat losses and energy conservation. But we must not see the resting metabolic rate as a central point in the energetics. Resting metabolic rate is certainly useful when comparing MR of mammals on a large scale, it can be quite easily measured, it is reproducible, and does not require long term measurement, but it does not mean that it will help us solve all issues we encounter. The parameter which mole-rats try to keep as low as possible is the daily energy expenditure (which is to a large extent influenced by the resting metabolic rate). My hypothesis is that mole-rats decrease their lower critical temperature and tolerate better an increase in body temperature than most mammals. This enables them to store the heat for later use. Such mechanism has been identified in some animals, but it is far from being common to all mammals. We have learned to expect different physiology in birds, marsupials and monotremes, we should probably add mole-rats among these as well.

**Table 1.** – Species key, scientific name and reference used in Figure 1.

| key | species                              | Reference                    |
|-----|--------------------------------------|------------------------------|
| 1   | <i>Cryptomys hottentotus</i>         | Bennett et al. (1992)        |
| 2   | <i>Cryptomys hottentotus</i>         | Haim and Fairall (1986)      |
| 3   | <i>Cryptomys nimrodi</i>             | Bennett et al. (1996)        |
| 4   | <i>Cryptomys nimrodi</i>             | Bennett et al. (1996)        |
| 5   | <i>Cryptomys natalensis</i>          | Bennett et al. (1993b)       |
| 6   | <i>Fukomys anelli</i>                | Marhold and Nagel (1995)     |
| 7   | <i>Fukomys anelli</i>                | Bennett et al. (1994)        |
| 8   | <i>Fukomys darlingi</i>              | Bennett et al. (1993a)       |
| 9   | <i>Fukomys bocagei</i>               | Bennett et al. (1994)        |
| 10  | <i>Fukomys damarensis</i>            | Bennett et al. (1992)        |
| 11  | <i>Fukomys damarensis</i>            | Lovegrove (1986a)            |
| 12  | <i>Fukomys damarensis</i>            | Scantlebury et al. (2006b)   |
| 13  | <i>Fukomys mechowii</i>              | Bennett et al. (1994)        |
| 14  | <i>Heterocephalus glaber</i>         | McNab (1966)                 |
| 15  | <i>Heterocephalus glaber</i>         | Buffenstein and Yahav (1991) |
| 16  | <i>Georychus capensis</i>            | Lovegrove (1987)             |
| 17  | <i>Georychus capensis</i>            | Scantlebury et al. (2006a)   |
| 18  | <i>Heliophobius argenteocinereus</i> | McNab (1966)                 |
| 19  | <i>Heliophobius argenteocinereus</i> | Zelová et al. (2007)         |
| 20  | <i>Bathyergus janetta</i>            | Lovegrove (1986b)            |
| 21  | <i>Bathyergus janetta</i>            | Scantlebury et al. (2006a)   |
| 22  | <i>Bathyergus suillus</i>            | Lovegrove (1986b)            |
| 23  | <i>Fukomys darlingi</i>              | Zemanová et al. (2012)       |

## 5 Conclusions

Mole-rats are perfectly adapted to subterranean environment. In this thesis I present novel findings on the metabolism and thermoregulatory physiology of mole-rats. These animals cope with low availability of food and have thus employed multiple strategies on how to conserve energy or use it more effectively. The adaptations include lowered resting body temperature, tolerance to body temperature increase during exercise or while at rest, surprisingly efficient cooling while digging, and precise diurnal and seasonal timing of activity with regards to environmental conditions.

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## Cost of digging is determined by intrinsic factors rather than by substrate quality in two subterranean rodent species

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### ABSTRACT

Searching for food by extensive digging is one of the most important aspects of life of subterranean rodents. We studied the effect of extrinsic (substrate quality) and intrinsic factors (sex and body mass) upon the cost of burrowing, expressed as digging metabolic rate (DMR) in two African mole-rat species (Bathyergidae, Rodentia) with distinct social structures. The sexually dimorphic giant mole-rat (*Fukomys mechowii*) is a highly social species, whereas the almost monomorphic silvery mole-rat (*Heliophobius argenteocinereus*) is a solitary bathyergid. Burrowing in *F. mechowii* was more costly (DMR was greater) than in *H. argenteocinereus*, but there was no difference in burrowing speed between both species. DMR within a particular species was dependent upon body mass, but independent of sex. Different substrate quality had no effect upon DMR in either species, yet it affected burrowing speed. We conclude that less effective digging in *F. mechowii* can be compensated by the joint workforce of other family members. Alternatively, *H. argenteocinereus*, being a more effective digger, can afford a solitary way of life.

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### 1. Introduction

The subterranean way of life has become advantageous for small mammals in different phylogenetic lineages, since it provides a stable environment and shelter against predators. On the other hand, burrowing is energetically expensive activity compared to travelling the same distance aboveground [1–5]. Therefore, the cost of foraging is expected to be far greater for subterranean rodents than for their aboveground counterparts of comparable size. Consequently, several physiological characteristics of subterranean mammals, such as low resting metabolic rate (RMR) and low body temperature ( $T_b$ ), are considered adaptations to compensate for high energetic costs of burrowing [6,7].

Because of the apparent importance of burrowing for underground existence, its cost has been intensively studied in several species of subterranean (i.e. burrowing and foraging underground), and fossorial and semifossorial (i.e. burrowing but foraging more or less aboveground) rodents, such as pocket gophers (Geomysidae), African mole-rats (Bathyergidae), tuco-tucos (Ctenomyidae), degus and coruros

(Octodontidae), and spinifex hopping mice (Muridae) [1–5,8–12]. Soil conditions (soil density and cohesiveness), food availability, and body size have been repeatedly considered to be the most important factors affecting cost of digging [1,12,13].

We may expect that cooperative digging, and thus sharing the costs of excavation among more individuals, would also influence foraging performance in subterranean mammals. African mole-rats are excellent candidates to study this aspect of biology, since this rodent family includes both strictly solitary and highly social species. To date, a comparative study on burrowing energetics has been performed in only two species of bathyergids, the Damaraland mole-rat and the naked mole-rat [2]. However, since both these species are highly social, the possibilities for interpretation of the findings from the point of view of behavioural ecology are limited. Therefore, we studied cost of digging in two other mole-rat species with contrasting social organisation. The silvery mole-rat, *Heliophobius argenteocinereus*, is a solitary bathyergid with negligible sexual dimorphism, whereas *Fukomys mechowii* is a social species displaying marked sexual dimorphism. The origin from similar habitats allowed us to study the differences related to their social organisation, while keeping the confounding effect of different ecological adaptation at a minimum. Thus, the comparison between these two species provided us an opportunity to test the effect of extrinsic (substrate quality) and intrinsic factors (body mass, sex) upon digging metabolic rates (DMR) of subterranean rodents. We hypothesized, that higher soil hardness would increase digging cost in both species, due to a greater cost of soil shearing and transport of excavated soil [2,12].

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Secondly, we tested the effect of several factors on DMR. Additionally to the body mass, which had been proposed as a factor affecting DMR already previously [1,12], we supposed that distinct social organisation could be reflected also in different DMR. Two alternative hypotheses can be proposed: First, DMR in both species with a distinct social system is comparable. In such a case the cooperative burrowing in social species would enhance effectivity of foraging and could be considered an important advantage enabling social species to colonize habitats with hard workable substrate and/or scattered food resources. Or, secondly, *Heliophobius* is a more effective digger and can thus “afford” solitary subsistence (alternatively, *Fukomys* must compensate its lower digging performance by cooperative work).

## 2. Materials and methods

### 2.1. Study animals

Digging energetics was measured in six males and five females of giant mole-rat and in five males and five females of silvery mole-rat. All mole-rats originated from the same bio-geographic region (Zambezi region) where they live in a variety of habitats (grassland, savanna-woodland, and cultivated fields) [14,15]. Distribution of *F. mechowii* is in Zambia, D. R. Congo and Angola; *H. argenteocinereus* occurs from Northern Kenya, Tanzania, Malawi, Eastern D. R. Congo and Eastern Zambia to Northern Mozambique [16,17]. The giant mole-rats used for experiments were laboratory bred or wild-captured in Ndola (Zambia). The silvery mole-rats were captured in Zomba and Mulanje (Malawi). Mole-rats were housed in terrariums or systems of interconnected Perspex tunnels. The animal room was kept at  $25 \pm 1$  °C with 12 L:12D photoperiod (light on at 0700). Horticultural peat was used as bedding and laboratory filter paper as nest material. Mole-rats were fed on carrots, potatoes, apples, lettuce, and rodent pellets.

*F. mechowii* males in our study weighted  $427 \pm 127$  g and females were smaller ( $180 \pm 21$  g). Body masses in *H. argenteocinereus* were comparable in males  $241 \pm 21$  g and females  $215 \pm 46$  g. The ratio of male to female body mass (an index of sexual dimorphism) was 2.4 in *F. mechowii* and 1.1 in *H. argenteocinereus*.

### 2.2. Oxygen consumption

Oxygen consumption during burrowing was measured using open-flow respirometry system. Digging and resting respiratory chambers were made of Perspex. The digging chamber consisted of a burrowing tube attached to the soil collector (total volume 9.5 L). The resting chamber had a total volume of 1.8 L. The digging chamber received air at 1093 mL/min and the resting chamber received air at 343 mL/min from a mass flow controller (082-03 S, Aalborg Instruments, Orangeburg NY, USA), which was enough flow to ensure adequate mixing of air inside the chamber. Air with an oxygen concentration of 20.95% was received from a pressure cylinder and subsequently passed through a mass flow controller, the respiratory chamber, CO<sub>2</sub> and water absorbant calcium oxide (Merck KGaA, Germany), and finally through a Parox 1.000 (MBE Electronic AG) paramagnetic oxygen sensor within a temperate controlled chamber. The Parox was connected to the computer and each trial was monitored in real time. The electric output of Parox was digitalised by a 16b A/D converter and collected every second. The slope of the Parox signal was calibrated and corrected for current barometric pressure prior to each trial. The zero offset of the Parox signal was calibrated monthly with 99.99% nitrogen (Lindegas a.s., Czech Rep.). VO<sub>2</sub> was calculated by the program DIAdem 8.00 (GFS Aachen, Germany).

Each measurement of the resting metabolic rate (RMR) was programmed to last 90 min. The initial 60 min of the trial was considered an acclimation period and data were not analysed. VO<sub>2</sub> was analysed

during the last 30 min of each experiment. A 10 minute portion of the recording with the lowest stable oxygen consumption, when the mole-rat was resting, was calculated as RMR. The temperature inside the metabolic chamber was measured with a thermal probe (Digital thermometer-TM946) and kept at 25 °C. Tested animals were food-deprived 3 h prior to the measurement. Rectal temperatures ( $T_b$ ) were measured within 30 s after termination of each experiment with a calibrated thermometer (Thermalert TH-8, Physitemp Instrument Ins.) inserted 2 cm into the rectum.

Two substrates of different physical characteristics denoted as “hard” and “soft” were used to analyse the cost of burrowing. “Hard substrate” was heavy soil sampled in the field, whereas “soft substrate” was moistened horticultural peat. Before each trial, we determined substrate density (weight of dry soil per cm<sup>-3</sup>). Substrate density of the hard substrate was  $1.63 \pm 0.06$  g/cm<sup>3</sup>. Peat was mixed with water and substrate density was  $0.31 \pm 0.03$  g/cm<sup>3</sup>.

To determine the cost of burrowing, a 60 cm long burrowing tube was filled with soil. To avoid changes in air pressure within the system during burrowing, a rod was inserted into the tube before filling, and then pulled out, so an air canal went through the entire soil column. The burrowing tube was attached to a soil collector. Prior to each trial, the digging chamber system was flushed with air at a rate of 1093 mL/min for 15 min to achieve equilibration and to gather calibration data. The baseline of the digging chamber system was set to 20.95%, the normal oxygen content of ambient air. The mole-rats usually began to burrow within several minutes after being put into the digging chamber and burrowing behaviour was monitored continuously. Oxygen consumption was recorded every second. DMR was measured in each individual as a 5 min mean value during the plateau of oxygen consumption. Only periods of continuous burrowing were used in the subsequent analysis. Each individual was measured once to estimate DMR and once to estimate RMR. An equivalent of 20.1 J/mL O<sub>2</sub> was used to convert oxygen consumption to energy values [18].

In each animal, the burrowed distance and the period spent by digging was measured. Subsequently, the average burrowing speed (BS; m/h) and the energy of constructing a 1 m burrow segment ( $E_{seg}$ ; kJ/m) were calculated.

### 2.3. Statistical analysis

Results are presented as mean  $\pm$  SD. Data on DMR and body mass were logarithmically transformed. We analysed the data by general linear models (GLMs) using the R 2.6.2 (R development core team, 2008), assuming the Gaussian distribution (link identity) for dependent variables (DMR, BS and  $E_{seg}$ ). First we included independent variables (body mass, sex, species and substrate conditions) to the “Null Model” and tested which variables gave significant results [using the Akaike information criterion (AIC) and *F*-statistic], then used only the significant variables. GLM was used to evaluate differences in burrowing speed and  $E_{seg}$  between different substrates in each species and between species (using *F*-statistic). Finally, a Student’s *t* test was used to detect differences in body temperature before and after a burrowing trial.

## 3. Results

Body mass and species had a strong effect on DMR ( $F_{(1,40)} = 150.3$ ;  $P < 0.0001$  for body mass,  $F_{(1,40)} = 20.2$ ;  $P < 0.0001$  for species). The least-squares regression of DMR and body mass resulted in the relationship:  $DMR$  (kJ/h) =  $5.18 + 0.045 \times$  body mass (g), where the body mass explained 79% of the variation in data ( $F_{(1,40)} = 150.3$ ;  $P < 0.0001$ ; Fig. 1). When body mass was treated as a covariate, interspecific differences in DMR were still found ( $F_{(1,39)} = 42.6$ ;  $P < 0.0001$ ). *F. mechowii* had a greater DMR than *H. argenteocinereus* in both substrates (Table 1). Soil type or sex had no effect on DMR ( $F_{(1,39)} = 1.0$ ;  $P = 0.3$  and  $F_{(1,39)} = 1.3$ ;  $P = 0.3$  for soil type and sex,

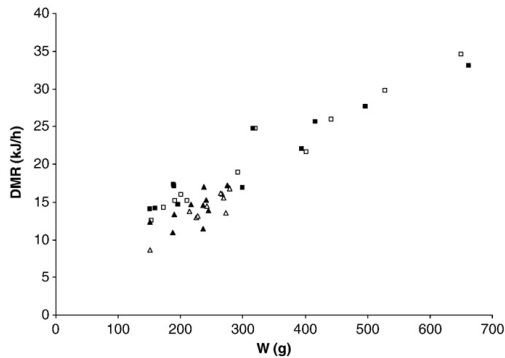


Fig. 1. Relation between digging metabolic rate (DMR) and body mass in *H. argenteocinerereus* in hard and soft substrates (solid and open triangles, respectively) and *F. mechowii* in hard and soft soils (solid and open squares, respectively).

Table 1

Body mass, resting metabolic rate (RMR) and digging metabolic rate (DMR) of *H. argenteocinerereus* and *F. mechowii* digging in soft and hard substrate.

|                         | <i>Fukomys mechowii</i> |                         | <i>Heliophobius argenteocinerereus</i> |                         |
|-------------------------|-------------------------|-------------------------|--|-------------------------|
|                         | Male                    | Female                  | Male                                   | Female                  |
| <i>n</i>                | 6                       | 5                       | 5                                      | 5                       |
| Body mass (g)           | 427 ± 127               | 180 ± 21                | 241 ± 21                               | 215 ± 46                |
| RMR (kJ/h)              | 5.4 ± 1.3               | 3.3 ± 0.7               | 3.7 ± 0.6                              | 3.1 ± 0.4               |
| DMR in hard soil (kJ/h) | 24.9 ± 5.4 <sup>a</sup> | 15.5 ± 1.6 <sup>a</sup> | 14.5 ± 2.0 <sup>b</sup>                | 13.7 ± 2.3 <sup>b</sup> |
| DMR in soft soil (kJ/h) | 25.9 ± 5.7 <sup>a</sup> | 14.6 ± 1.3 <sup>a</sup> | 14.9 ± 1.4 <sup>b</sup>                | 13.3 ± 2.9 <sup>b</sup> |

Superscript letters indicate differences in DMR between species.

respectively); body mass as a covariate. Data of RMR and DMR/RMR are summarized in Tables 1 and 2.

Burrowing speed was correlated with substrate ( $F_{(1,40)} = 200.7$ ;  $P < 0.0001$ ) and body mass ( $F_{(1,39)} = 6.4$ ;  $P < 0.02$ ) (Table 2). *H. argenteocinerereus* burrowed on average 5.8 times faster ( $F_{(1,17)} = 73.6$ ;  $P < 0.0001$ ) for soft substrate than with hard substrate, where body mass was entered as a covariate (Table 2). Similarly, *F. mechowii* burrowed an average of 6.7 times faster ( $F_{(1,19)} = 121.9$ ;  $P < 0.0001$ ) for soft soil. No difference was found in burrowing speed between species for both substrate conditions ( $F_{(1,18)} = 1.14$ ;  $P = 0.3$  for hard substrate;  $F_{(1,18)} = 0.003$ ,  $P = 0.9$  for soft substrate) when body mass was used as a covariate.  $E_{seg}$  significantly correlated with soil type ( $F_{(1,40)} = 316.9$ ,  $P < 0.0001$ ), body mass ( $F_{(1,39)} = 55.6$ ,  $P < 0.0001$ ) and species ( $F_{(1,38)} = 7.1$ ,  $P < 0.01$ ). Similar to DMR, *F. mechowii* invested more energy per burrowed segment ( $E_{seg}$ ) than *H. argenteocinerereus* in both substrates (Table 2).

Burrowing was connected with an increase in body temperature in both species (Table 2). In *H. argenteocinerereus*, the increase in  $T_b$  was

$1.0 \pm 0.7$  °C for hard soil ( $t_{(9)} = -4.4$ ;  $P < 0.01$ ) and  $1.2 \pm 0.5$  °C for soft soil ( $t_{(9)} = -7.9$ ;  $P < 0.0001$ ). The increase in  $T_b$  for *F. mechowii* was  $1.5 \pm 0.7$  °C for hard soil ( $t_{(10)} = -7.0$ ;  $P < 0.0001$ ) and  $1.7 \pm 0.7$  °C for soft soil ( $t_{(10)} = -8.0$ ;  $P < 0.0001$ ).

4. Discussion

Energetics in subterranean rodents is strongly affected by underground environment. During the search for food they must penetrate through soil and the energetic cost of extending the tunnels greatly exceeds that of searching for food aboveground (see Introduction). Burrowing by mole-rats in this study was on average 4.5 times more energetically demanding than resting (Table 2). Interestingly, mole-rats of both solitary and social species dug with the same energetic output in both substrates, and DMR was found to be independent of substrate quality (Table 1). However, social giant mole-rats exhibited greater DMR and  $E_{seg}$  than solitary silvery mole-rats, whereas the burrowing speed was the same (Table 2). This suggests that social *F. mechowii* pays a higher energy cost per unit of burrowed distance.

Contrary to our findings, the effect of soil hardness has been observed in the South American fossorial *Ctenomys talarum* [12] and semifossorial *Octodon degus* [9]. In *C. talarum*, digging in hard soil was nearly 1.5 times more energetically expensive than in soft soil. Interestingly, the burrowing speed was comparable regardless of soil type. According to cost of burrowing model which partitions the cost of burrow construction into the energy of shearing and pushing soil out of the tunnel [1], digging efficiency is not determined by DMR only, but also by burrowing speed and the amount of soil removed. Provided the same burrowing speed, the higher cost of burrowing in hard soil was probably due to a greater cost of hard soil shearing and transport [12]. This argument is also in agreement with the study of Lovegrove [2] where African mole-rats of two species, showed greater burrowing cost in damp soil than in dry soil. Damp soil incurred higher DMR due to the increased work involved in transport of heavier soil.

However, in our study, both species dug with the same effort in each substrate and only burrowing speed differed between substrates (Table 2). Similarly, substrate quality apparently did not affect the cost of burrowing in the South American coruro *Spalacopus cyanus*. Here, animals from seven populations inhabiting contrasting climatic and soil conditions exhibited similar DMR [10]. Concurrently, no effect of soil hardness on digging energetics was found in two *Ctenomys* species [5] in soils with large difference in density (5.3 times). In our study, the difference in soil density was very similar to the aforementioned study, where burrowing speed in soft soil was thus about five to six times higher in both species. Bozinovic et al. [10] has explained the lack of differences in DMR of coruros by a phylogenetically original adaptation of *Spalacopus* to live in hard soils. In *Ctenomys*, found evidence suggests that digging energetics is a plastic trait. Populations originating from soft soil environments exhibited higher DMR when digging in hard soil [12]. In another *Ctenomys* study, however, individuals originating from hard soil environments retained the adaptation to that soil condition and did not reduce DMR in soft soil [5].

Table 2

Ratio of DMR/RMR, burrowing speed,  $E_{seg}$  and body temperatures before and after digging in hard and soft substrates in *F. mechowii* and *H. argenteocinerereus*.

|  |      | DMR/RMR   | Burrowing speed         |                            | $E_{seg}$<br>(kJ/m) | $T_b$          |               |
|--|------|-----------|-------------------------|----------------------------|---------------------|----------------|---------------|
|  |      |           | (m/h)                   |                            |                     | before<br>(°C) | after<br>(°C) |
| <i>Fukomys mechowii</i>                | Hard | 4.8 ± 0.8 | 0.68 ± 0.2 <sup>a</sup> | 33.8 ± 13.7 <sup>a,1</sup> | 33.8 ± 0.7          | 35.3 ± 0.8     |               |
|  | Soft | 4.7 ± 0.7 | 4.22 ± 1.2 <sup>b</sup> | 5.5 ± 2.9 <sup>b,1</sup>   | 34.2 ± 0.7          | 35.9 ± 0.4     |               |
| <i>Heliophobius argenteocinerereus</i> | Hard | 4.2 ± 0.8 | 0.85 ± 0.3 <sup>a</sup> | 19.3 ± 8.5 <sup>a,2</sup>  | 34.3 ± 0.6          | 35.3 ± 0.6     |               |
|  | Soft | 4.2 ± 0.5 | 4.51 ± 1.3 <sup>b</sup> | 3.5 ± 1.4 <sup>b,2</sup>   | 34.4 ± 0.4          | 35.6 ± 0.5     |               |

Superscript letters indicate statistical differences in each variable within species. Superscript numbers indicate differences in  $E_{seg}$  between species.

The DMR/RMR ratio values obtained in this study are close to those in other bathyergids [2,8], when compared with known ratios in other subterranean rodents (Table 3). Bathyergid DMR/RMR ratios seem to be higher than in other studied subterranean species, however this difference is probably a consequence of lower RMR in bathyergids. Bathyergids, rodents that are highly specialised for underground life, display lower RMR compared to other rodents and other subterranean rodents of similar body sizes [19], which is considered to be an energy-saving mechanism for a low-productive underground habitat [20]. Surprisingly, some semifossorial species (burrowing for shelter but foraging aboveground) exhibit also a very high DMR/RMR ratio. However, these species, such as *O. degus* or *Notomys alexis* [9,11] are much less specialised for burrowing and therefore they accordingly show noticeably higher DMR (Table 3). It might therefore be reasonable to expect that the level of morphological and physiological specializations of subterranean rodents [21,22] have an important influence on the amount of energy invested in burrow construction and energy-saving mechanisms. In addition, burrowing activity of semifossorial rodents is only short-term, in contrast to subterranean rodents where burrowing is a crucial part of their daily program.

We found a strong correlation between body mass and DMR (Fig. 1), but not sex. This may be considered as an evidence that the major determinant of sex differences in burrowing costs is body size dimorphism. In dimorphic *F. mechowii*, absolute DMR of males significantly exceeded that of females. On the other hand, in *H. argenteocinereus* in which male and female body sizes are similar, DMR was nearly equal between sexes. It appears that larger animals sustain higher energy costs for digging simply because they are big. The linkage between body mass and long-term energetic performance has been also reported in several studies [13,23]. Interestingly, Scantlebury et al. [13] found differences in daily energy expenditure in free-living, the sexually dimorphic, African mole-rat *Bathyergus janetta* but not in the sexually monomorphic *Georchus capensis*. This confirms the “body-maintenance” hypothesis [23], where the energy cost of dimorphism is probably due to the cost of maintenance of a larger body size. Substantial influence of body mass on metabolic rates in subterranean rodents is also supported by metaanalysis of RMR in bathyergids i.e. a group where metabolic rates were previously

assumed to be body mass independent (see [19] for further details) or in *Ctenomys* [24]). In this group, only body mass (and not ecology) explained resting metabolic rates.

As previously mentioned, DMR of social *F. mechowii* was higher than that of the solitary *H. argenteocinereus* in both substrates (Table 1) with the same burrowing speed. It indicates that social *F. mechowii* pays more energy cost per burrowed distance. We propose that this difference likely results from, or is reflected in, the different social systems of both species. In social species, members of the family share the burrowing system which is a joint result of digging efforts of many individuals. Some social burrowing rodents can even dig cooperatively in chains, where family members alternate in the construction of tunnels as observed in *H. glaber* [2], in *O. degus* [25] and in *Fukomys anselli* (own observations). We assume that social *F. mechowii* can also decrease the individual cost of burrow construction in this way. The presence of more diggers and cooperative digging may ease selection pressure on digging efficacy in social species, as the animals share construction of tunnels. Therefore, we hypothesize that solitary species, being dependent on their own, are likely forced to burrow more effectively.

In conclusion, our study has shown that both bathyergid species did not adjust their digging performance relative to substrate quality. They dug with the same metabolic costs in soils of different hardness, but differed in burrowing speed. Our results also suggest that the solitary species is a more efficient digger, expending less energy to burrow a given distance. The influence of social systems and the existence of cooperative digging on digging performance should be further analysed in other subterranean rodents with different social systems to test this intriguing finding.

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**Table 3**

Available data on body mass, resting metabolic rate (RMR), digging metabolic rate (DMR), and ratio of digging to resting metabolic rate (DMR/RMR) in subterranean and fossorial rodents.

| Family and species                   | Body mass (g) | RMR (mL O <sub>2</sub> /g h) | DMR (mL O <sub>2</sub> /g h) | DMR/RMR                  | Type of soil   | Source        |
|--------------------------------------|---------------|------------------------------|------------------------------|--------------------------|----------------|---------------|
| <b>Bathyergidae</b>                  |               |                              |                              |                          |                |               |
| <i>Heterocephalus glaber</i>         | 32            | 0.64                         | 2.78                         | 4.34                     | Dry sand       | [2]           |
|                                      |               |                              | 3.36                         | 5.25                     | Damp sand      |               |
| <i>Fukomys damarensis</i>            | 152           | 0.57                         | 2.58                         | 4.52                     | Dry sand       | [2]           |
|                                      |               |                              | 2.86                         | 5.02                     | Damp sand      |               |
| <i>Georchus capensis</i>             | 197           | 1.13 (0.59) <sup>a</sup>     | 3.41                         | 3.01 (5.80) <sup>a</sup> | Loose sand     | [8]           |
| <i>Heliophobius argenteocinereus</i> | 232           | 0.77                         | 3.16                         | 4.10                     | Hard soil      | Present study |
|                                      |               |                              | 2.92                         | 3.79                     | Soft soil      |               |
| <i>Fukomys mechowii</i>              | 320           | 0.78                         | 3.60                         | 4.62                     | Hard soil      | Present study |
|                                      |               |                              | 3.43                         | 4.40                     | Soft soil      |               |
| <b>Geomyidae</b>                     |               |                              |                              |                          |                |               |
| <i>Thomomys bottae</i>               | 143           | 0.84                         | 4.10                         | 4.88                     | Damp sand      | [1]           |
| <i>Thomomys talpoides</i>            | 75            | 1.65                         | 4.08                         | 2.47                     |                | [26] in [2]   |
| <b>Ctenomyidae</b>                   |               |                              |                              |                          |                |               |
| <i>Ctenomys talarum</i>              | 126           | 0.95                         | 2.69                         | 2.85                     | Damp sand      | [3]           |
| <i>Ctenomys talarum</i>              | 131           | 0.96                         | 3.18                         | 3.31                     | Hard soil      | [12]          |
|                                      | 126           | 0.91                         | 2.14                         | 2.35                     | Soft soil      |               |
| <i>Ctenomys talarum</i>              | 126           | 0.92                         | 2.06                         | 2.23                     | Damp sand      | [4]           |
| <i>Ctenomys talarum</i>              | 125           | 0.95                         | 3.95                         | 4.15                     | Hard soil      | [5]           |
|                                      |               | 1.03                         | 3.69                         | 3.58                     | Soft soil      |               |
| <i>Ctenomys australis</i>            | 302           | 0.66                         | 2.46                         | 3.73                     | Hard soil      | [5]           |
|                                      |               | 0.7                          | 2.57                         | 3.67                     | Soft soil      |               |
| <b>Octodontidae</b>                  |               |                              |                              |                          |                |               |
| <i>Octodon degus</i>                 | 203           | 1.24                         | 6.68                         | 5.38                     | Damp sand      | [9]           |
| <b>Muridae</b>                       |               |                              |                              |                          |                |               |
| <i>Notomys alexis</i>                | 33            | 1.21                         | 6.72                         | 5.55                     | Sand-loam soil | [11]          |

<sup>a</sup> Value in parentheses is RMR estimated in [27].

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## A seasonal difference of daily energy expenditure in a free-living subterranean rodent, the silvery mole-rat (*Heliophobius argenteocinereus*; Bathyergidae)

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## ABSTRACT

In seasonal climatic regimes, animals have to deal with changing environmental conditions. It is reasonable to expect that seasonal changes are reflected in animal overall energetics. The relation between daily energy expenditure (DEE) and seasonally variable ecological determinants has been studied in many free-living small mammals; however with inconsistent results. Subterranean mammals, i.e. fossorial (burrowing) mammals which live and forage underground, live in a seasonally and diurnally thermally stable environment and represent a suitable model to test seasonality in DEE in respect to seasonal changes, particularly those in soil characteristics and access to food supply. Both factors are affected by seasonal rainfall and are supposed to fundamentally determine activity of belowground dwellers. These ecological constraints are pronounced in some tropical regions, where two distinct periods, dry and rainy seasons, regularly alternate. To explore how a tropical mammal responds to an abrupt environmental change, we determined DEE, resting metabolic rate (RMR) and sustained metabolic scope (SusMS) in a solitary subterranean rodent, the silvery mole-rat, at the end of dry season and the onset of rainy season. Whereas RMR did not differ between both periods, mole-rats had 1.4 times higher DEE and SusMS after the first heavy rains. These findings suggest that rainfall is an important environmental factor responsible for higher energy expenditure in mole-rats, probably due to increased burrowing activity. SusMS in the silvery mole-rat is comparable to values in other bathyergids and all bathyergid values rank among the lowest SusMS found in endothermic vertebrates.

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## 1. Introduction

Energy costs of animals are thought to vary seasonally in response to regular changes in environment, such as temperature and rainfall, which in turn cause substantial changes in food availability and quality. Daily energy expenditure (DEE) is the most common way to express the energy costs of free-living animals (Speakman, 2000). The relationship between DEE and seasonally variable ecological factors has been studied in many free-living small mammals. However, results of these studies are equivocal. In some mammals, including burrowing species, effect of season was found, with DEE being higher in (cold and dry) winter than in (warm and humid) summer (e.g. Degen et al., 1997; Scantlebury et al., 2004, 2006a; Künkele et al., 2005). By contrast, in some other mammals, including also burrowing species, no clear seasonal variation in DEE has been found (e.g. Holleman et al., 1982; Gettinger, 1984; Corp et al., 1999; Bozinovic

et al., 2004; Scantlebury et al., 2005). Seasonal variation of DEE in small mammals was also a subject of two comprehensive reviews (Nagy et al., 1999; Speakman, 2000). The authors initially supposed higher DEE in winter, when small mammals are expected to be under high energetic stress. Surprisingly, no difference between DEE in winter and summer was detected. In some species, there was even an increase of DEE in summer. It led Speakman (2000) to point out the importance of thermal acclimatization and effective behavioral strategies under less favorable environmental conditions.

Subterranean rodents, i.e. fossorial (=burrowing) rodents which forage underground (cf. Nevo, 1999) spend most of their lives in microclimatically stable burrows where daily and seasonal fluctuations are buffered (reviewed in Burda et al., 2007). On the other hand, the underground ecotope is also very challenging: it is less productive, with dispersed food resources (belowground parts of plants) that are energetically costly to obtain (Vleck, 1979). High costs of living belowground require special physiological adaptations that became a subject of intensive study in the past (reviewed in Nevo, 1999; Buffenstein, 2000; Sedláček, 2007).

Subterranean rodents are suitable models for examining the effects of seasonal rainfall on their biology, since fluctuation of and thus also confounding effects through most diurnal and seasonal

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microenvironmental factors are kept to a minimum. Many subterranean rodents occupy areas with highly seasonal rainfall. In dry periods, soil can be hard and not easily workable (Vleck, 1979; Luna and Antinuchi, 2006), a fact which may restrict access to food resources. With the onset of rains, environmental conditions change abruptly. Rainfall moistens soil, changes physical soil characteristics (cohesiveness and hardness), and stimulates plant growth. In respect to DEE subterranean rodents may react to the arrival of rains in several ways. First, if they do not respond to the changed conditions, then no difference in DEE in periods before and after first rains will be detected. Second, if subterranean rodents are more active and burrow more, for example in order to search for new food resources (Brett, 1991; Jarvis et al., 1998; Romañach et al., 2005) or replenish atmosphere in burrows after rains (i.e. Arieli, 1979), increased DEE will be found. Third, if they would cease burrowing activity after rains, for example in order to avoid working in wet heavy soil which is energetically more expensive (Lovegrove, 1989), their DEE will be lower.

In the present study, we quantified DEE in the free-living silvery mole-rat, *Heliophobius argenteocinereus*, a subterranean rodent of the family Bathyergidae (African mole-rats), the only solitary bathyergid from the Afrotropics. To date, most studies on energetics of bathyergids were done under laboratory conditions and provided data on resting metabolic rate and some further metabolic traits which are, however, not a reliable predictor of energy expenditure in free-living animals. Here, we attempted to assess DEE under natural conditions using the doubly labelled water (DLW) method. This method allows quantification of not only basic processes necessary to sustain life (RMR), but also the overall energy needs integrating all metabolic costs over the measured period (i.e. those of thermoregulation, growth, reproduction, and different activities). Sustained metabolic scope (SusMS) defined as the ratio of the daily energy expenditure to the resting metabolic rate (DEE/RMR) is widely used to express the energy expenditure associated with all activities in free-living animals and enables comparison between different species (for review see Hammond and Diamond, 1997; Peterson et al., 1990; Speakman, 2000).

To reveal the cost of living in a subterranean rodent and the impact of seasonal ecological changes on it, we measured DEE in the silvery mole-rat before and after the onset of rainy season. Unlike other hitherto tested bathyergids living mainly in light sandy soils in Southern Africa, *Heliophobius* lives in soils with a high content of iron and manganese oxides, which make the soil very hard to excavate during the dry season (cf. Burda et al., 2000). Additionally, this species occurs in areas with higher annual precipitation than *Bathyergus janetta*, *Georchys capensis*, and *Fukomys damarensis* – i.e. the only bathyergids for which the DEE data are available (these species occur in areas with only about 18, 64, and 51% of rainfall compared to the area of occurrence of *Heliophobius*; Burda, 2001). The buffered microenvironment in burrows and remarkable soil hardness in Afrotropics, together with high regular rainfall as well as strict seasonality of *Heliophobius* make it a suitable candidate to test the effect of season on DEE within subterranean rodents in particular and mammals in general. It is reasonable to hypothesize that activity of *Heliophobius* is much more affected by seasonal changes in soil hardness and moisture than in Southern African mole-rats. The aims of the study were as follows: 1) to measure daily energy expenditure (DEE) in *Heliophobius* under natural conditions, 2) to test the hypothesis that DEE increases after rains reflecting thus higher burrowing rates in order to get more food in better workable soils, and 3) to measure RMR to determine SusMS and examine whether it varies among seasons and what is the physiological sustainable level of activity in the silvery mole-rat. Furthermore, calculated SusMS in *Heliophobius* was compared with SusMS in other bathyergids and other mammals in order to assess the effect of ecology upon SusMS.

## 2. Materials and method

### 2.1. Study site and animals

Across *Heliophobius* distribution throughout Central and Eastern Africa, two main climatic seasons exist; a dry season with only exceptional rains and a rainy season when almost all annual rainfall is obtained (e.g. Burda, 2001). The rainy season in Malawi starts at the end of November and lasts until March/April. It is followed by a dry cold season when the temperature decreases substantially until July. From July/August, temperature increases and this part of season is denoted as the dry hot season. Months from May up to October are rainless with less than 25 mm of monthly precipitation in the study locality (Šumbera et al., 2007). It should be noted at this point that according to Jarvis et al. (1994), 25 mm of rainfall is enough to soften soil and enable mole-rats to dig effectively. Our study took place during the end of dry hot season (end of September/mid-November) and at the beginning of rainy season (end of November) in a miombo (*Brachystegia* woodland) on Mpalanganga estate, Zomba in southern Malawi (15°27'S; 35°16'E, altitude 1070 m) in 2008 (for precipitation and other details of study locality see Šumbera et al., 2007, 2008).

Hardness of soil was determined using a hand penetrometer (06.06, Eijkelkamp, Agriresearch Equipment, Giesbeek, The Netherlands) before and after the onset of rains. Cone resistance of the tool ( $\text{N cm}^{-2}$ ) was measured at nine places close to mole-rat burrow systems. Altogether 135 measurements in the depth of 15 cm (usual depth of the foraging tunnels) were taken in each season. We measured soil temperature using temperature data loggers Commet (Comet System s.r.o., Czech Republic) every 10 min in a depth of 20 cm. Rainfall was recorded every day using a rainfall gauge consisting of a funnel and a measuring tube.

Nine adult silvery mole-rats, *Heliophobius argenteocinereus* (seven females and two males), were examined before the onset of rains and four animals (all males) were examined after the onset of rains (after 57 mm of rainfall). We used a traditional indigenous method of capture by blocking the animals' retreat with a hoe when they came to seal opened sections of their burrow. Reasons for a smaller sample size at the onset of rains were two: failure of batteries in four radio-collars and the fact that three measured individuals left their respective burrow systems.

### 2.2. Daily energy expenditure (DEE)

Daily energy expenditure ( $\text{kJ day}^{-1}$ ) of the mole-rats was measured using the doubly labelled water (DLW) technique (Speakman, 1997). This method is an isotope-based procedure that allows estimation of  $\text{CO}_2$  production and hence energy expenditure. After the capture, the animals were weighed with a portable electronic balance ( $\pm 0.1$  g) and shortly anaesthetized with ketamin and xylazin for 20–30 min. Approximately a 90  $\mu\text{L}$  blood sample was obtained from the dorsal hindfoot vein to estimate the background isotope enrichments of  $^2\text{H}$  and  $^{18}\text{O}$ . The blood samples were immediately heat-sealed into  $3 \times 60 \mu\text{L}$  glass capillaries and kept refrigerated at 5 °C. Afterwards, animals were intraperitoneally injected with isotopically enriched [ $^{18}\text{O}$  water, 97% (Buchem BV, The Netherlands),  $^2\text{H}$  water 99.9% (Sigma-Aldrich, Germany)] UV sterilized distilled water (IP, 0.3 g/100 g body mass). After 2 h, allowing isotopes to equilibrate with body water, second blood samples were taken to estimate initial isotope enrichments. Then, the mole-rats were fitted with a radio-collar (BR collar; AVM Instrument Company, Colfax, CA, USA) and returned back to their burrow system at the place of capture. After 3–5 days the animals were recaptured and final blood samples were taken after whole 24 h periods to estimate isotope elimination rates. Thereafter, the radio-collars were taken off and the animals were released back to their burrow systems.

All blood samples were cryodistilled into vials (Šantrůček et al., 2007) and the distillates were used to measure isotopic ratios of  $^{18}\text{O}$ : $^{16}\text{O}$  and  $^2\text{H}$ : $^1\text{H}$  by a continuous flow stable isotope ratio mass spectrometer (DeltaPlusXL, ThermoFinnigan, Bremen, Germany). We estimated  $\text{CO}_2$  production using the single pool equation (Speakman, 1997, pp. 315):

$$r\text{CO}_2 = (N / 2.078)(k_o - k_d) - 0.0062 k_d N$$

where  $r\text{CO}_2$  (mol  $\text{day}^{-1}$ ) is the  $\text{CO}_2$  production,  $N$  (mol) is the size of body water pool,  $k_o$  and  $k_d$  ( $\text{day}^{-1}$ ) are 18-oxygen and 2-hydrogen elimination rates, respectively. The oxygen pool size ( $N$ ) was estimated by the intercept model. The rate of  $\text{CO}_2$  production was converted to DEE assuming a respiratory quotient of 0.8 and oxygen equivalent of  $20.1 \text{ kJ L}^{-1} \text{ O}_2$  (Schmidt-Nielsen, 1990). The observed DEE in the field was compared with DEE values predicted for rodents (DEE ( $\text{kJ day}^{-1}$ ) =  $5.48 \text{ body mass}^{0.712}$ ; Nagy et al., 1999).

### 2.3. Resting metabolic rate (RMR)

RMR was determined in the same individuals by measuring minimal oxygen consumption ( $\text{VO}_2$ ) by an open-flow respirometry system. We used a portable oxygen analysis instrument that included the oxygen analyzer, a variable pump and mass flow meter (FoxBox, Sable Systems International, Las Vegas, NV, USA). The respiratory chamber was made of Perspex with a total volume of 1.8 L. The pull system was used; ambient air was pumped from the respiratory chamber at a rate of  $300 \text{ mL min}^{-1}$  through a drying column with indicator (Drierite; W.A. Hammond Drierite Co. Ltd.) and  $\text{CO}_2$ -absorbent granules of calcium oxide (Merck KGaA) and then through a mass flow meter to the  $\text{O}_2$  analyzer, which was connected to the PC. Fractional  $\text{O}_2$  concentration, air pressure, ambient temperature and flow rate were recorded every second. The  $\text{O}_2$  analyzer signal was calibrated by dry air (20.95%  $\text{O}_2$ ) and was corrected for current barometric pressure prior to and after the measurement of each animal. The temperature inside the respiratory chamber was maintained at  $27 \pm 1 \text{ }^\circ\text{C}$  which is within the thermoneutral zone of this species (Zelová et al., 2007).

Each measurement of the RMR lasted at least 150 min. The initial 120 min of the trial was considered a settle-down period and data were not analyzed.  $\text{VO}_2$  was analyzed during the last 30 min of each experiment. Within this period, a 10-min segment with the lowest stable oxygen consumption was selected for a calculation of  $\text{VO}_2$  ( $\text{mL O}_2 \text{ h}^{-1}$ ) using the equation:

$$\text{VO}_2 = \text{air flow} (F_i\text{O}_2 - F_e\text{O}_2) / (1 - F_i\text{O}_2)$$

where  $F_i\text{O}_2$  is the fraction of the inlet air,  $F_e\text{O}_2$  is the fraction of the outlet air from the chamber and air flow of the dry and  $\text{CO}_2$  free air ( $\text{mL h}^{-1}$ ).  $\text{VO}_2$  was converted to RMR ( $\text{kJ day}^{-1}$ ), using an oxygen equivalent  $20.1 \text{ kJ L}^{-1}$  (Schmidt-Nielsen, 1990). In addition to DEE and RMR, SusMS was determined for each individual. Experimental protocol was approved by the Departmental Commission for Animal Protection of the Ministry of Education (No. 12921/2007-30).

### 2.4. Statistical analyses

Statistical analyses were performed in R 2.6.2 (R development core team, 2008). We used analysis of variance (ANOVA) to examine differences in mass-specific DEE ( $\text{kJ g}^{-1} \text{ day}^{-1}$ ) and SusMS (no unit) between different seasons. Analysis of covariance (ANCOVA) was used to examine differences in absolute RMR, DEE ( $\text{kJ day}^{-1}$ ), where body mass was included as a covariate. Season was included as a categorical factor. Wilcoxon Matched Pairs Test was used to compare soil hardness and moisture before and after the onset of rainy season. Results are presented as means  $\pm$  standard deviation.

## 3. Results

Mole-rats weighed on average  $161 \pm 27 \text{ g}$  upon initial capture and body masses did not differ between seasons (ANOVA,  $F_{(1,17)} = 0.002$ ,  $P = 0.96$ ; Table 1). There were almost no rains in the sampled dry period:  $0.3 \pm 1.6$  (0–10) mm per day compared to  $6.2 \pm 10.1$  (0–40) mm after first heavy rains. After the onset of rains soil moisture increased from  $1.1 \pm 0.2\%$  to  $16.1 \pm 3.0\%$  of soil mass (Wilcoxon Matched Pairs Test;  $N = 8$ ,  $P = 0.01$ ) and the soil hardness decreased from  $514 \pm 63 \text{ N cm}^{-2}$  to  $167 \pm 37 \text{ N cm}^{-2}$  (Wilcoxon Matched Pairs Test;  $N = 9$ ;  $P = 0.008$ ). Temperatures in depth of foraging tunnels were comparable in both periods  $26.3 \pm 0.88 \text{ }^\circ\text{C}$  (23.8–28.2) before rains, and  $25.5 \pm 0.89 \text{ }^\circ\text{C}$  (24.2–26.6) after rains.

After first rains mole-rats had higher absolute DEE ( $167.8 \text{ kJ day}^{-1}$ ) and mass-specific DEE ( $1.0 \text{ kJ g}^{-1} \text{ day}^{-1}$ ) compared to the values measured in dry season ( $121.8 \text{ kJ day}^{-1}$ ;  $0.76 \text{ kJ g}^{-1} \text{ day}^{-1}$ , ANOVA,  $F_{(1,11)} = 8.8$ ,  $P = 0.013$  and  $F_{(1,11)} = 9.4$ ,  $P = 0.01$ , respectively) (Table 1). In absolute terms, mole-rats had a DEE that is 38% higher after rains. In nine mole-rats measured within the dry season, DEE tended to increase with body mass (linear regression,  $R^2 = 0.43$ ,  $F_{(1,7)} = 5.3$ ,  $P = 0.054$ ; Fig. 1), so we removed effect of body mass by using it as covariate. After that, the DEE in dry season was still lower compared to the DEE after first rains (ANCOVA,  $F_{(1,10)} = 12.1$ ,  $P = 0.006$ ). Comparison of DEE with predicted values for rodents of the similar size (Nagy et al., 1999) revealed very low values, averaging only 60% (dry season) and 82% (after rains) of expected daily requirements (Table 2).

Resting metabolic rate in both periods combined was positively dependent on body mass (linear regression,  $R^2 = 0.67$ ,  $F_{(1,11)} = 22$ ,  $P < 0.001$ ; Fig. 2). When the effect of body mass was removed, RMR did not vary with season (ANCOVA,  $F_{(1,10)} = 0.0008$ ,  $P = 0.978$ ). Since RMR was independent of season, SusMS showed changes in relation to seasons. The SusMS for mole-rats after first rains was greater (ANOVA,  $F_{(1,11)} = 18.3$ ,  $P = 0.001$ ; Table 1). SusMS of silvery mole-rats was very low and comparable to that of infrequent workers in *F. damarensis* (Table 2).

## 4. Discussion

Daily energy expenditures in the silvery mole-rat in dry season and after rains were lower than predicted for rodents of the same size (Nagy et al., 1999; Table 2). Assessed DEE in other burrowing rodents follows this trend and is lower than the allometric predictions (Table 2). These findings support the idea that low DEE could be a convergent highly adaptive feature of subterranean rodents in general, because they face energy problems due to low food supply (Nevo, 1979).

Due to problems beyond our control, the sample sex ratio of the studied mole-rats was biased: only 22% of the animals (2/9)

**Table 1**

Mean and standard deviations (s.d.) of body mass, resting metabolic rate (RMR), daily energy expenditure (DEE) and sustained metabolic scope (SusMS) of silvery mole-rat at the end of dry season and after the onset of rainy season.

|   | Peak of dry season |      | Onset of rainy season |      |
|---|--------------------|------|-----------------------|------|
|   | Mean               | s.d. | Mean                  | s.d. |
| n   | 9                  |      | 4                     |      |
| Body mass (g)                               | 161                | 32   | 162                   | 13   |
| RMR ( $\text{kJ day}^{-1}$ )                | 84                 | 17.8 | 85                    | 9.1  |
| RMR ( $\text{kJ g}^{-1} \text{ day}^{-1}$ ) | 0.53               | 0.06 | 0.53                  | 0.04 |
| DEE ( $\text{kJ day}^{-1}$ )                | 121.8 <sup>a</sup> | 28.3 | 167.8 <sup>b</sup>    | 17.7 |
| DEE ( $\text{kJ g}^{-1} \text{ day}^{-1}$ ) | 0.76 <sup>a</sup>  | 0.2  | 1.0 <sup>b</sup>      | 0.1  |
| SusMS                                       | 1.5 <sup>a</sup>   | 0.2  | 2.0 <sup>b</sup>      | 0.2  |

Note: <sup>a,b</sup> indicate statistically significant difference between values for respective seasons.

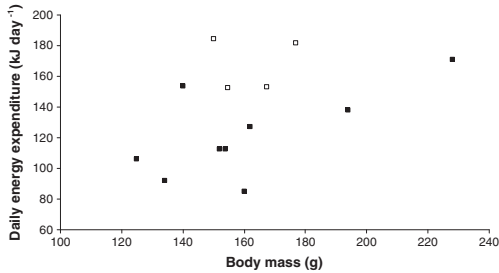


Fig. 1. Relationship between daily energy expenditure and body mass in free-living silvery mole-rats at the end of dry season (solid symbols) and after the onset of rainy season (open symbols).

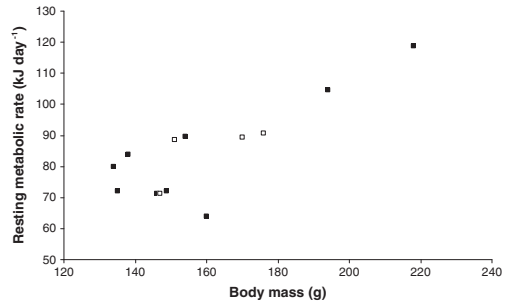


Fig. 2. Relationship between resting metabolic rate and body mass in free-living silvery mole-rats at the end of dry season (solid symbols) and after the onset of rainy season (open symbols).

measured during the dry season were male, whereas 100% (all 4) of the animals measured during the wet season were male. Still, we believe that the recorded differences in DEE between both samples reflect differences between seasons and not differences between gender. First, *Heliophobius* mole-rats are morphologically and morphometrically monomorphic (Šumbera et al., 2003a, 2008). Second, the physiological values of the two males measured during the wet season were not different from those of females in the respective sample. Third, there are no differences in the parameters of burrow systems between both sexes (Šumbera et al., 2003b, 2008), yet the features of burrow systems change seasonally (Šumbera et al., 2003b). Finally, laboratory studies also have not revealed any sex-dependent differences in energetics in *H. argenteocinerus* (Zelová et al., 2007).

The maximal limit of SusMS for endotherms was proposed to be around seven times RMR by Hammond and Diamond (1997), around five times by Peterson et al. (1990), and around four times for small mammals by Speakman (2000). The SusMS of mole-rats, ranging from 1.4 to 3.2 (Table 2), falls among the lowest values recorded for endothermic vertebrates. Similarly, African golden moles, also follow the trend of low SusMS in subterranean mammals – and they also live in regions with seasonally limited prey supply (Scantlebury et al., 2005). In fact, small mammals feeding on limited and energy-poor resources are expected to have lower SusMS, because high rates of energy expenditures probably must be compensated by high rates of energy intake and vice versa (Speakman, 2000).

Although fluctuations of temperature are buffered underground, subterranean dwellers face seasonal changes in soil hardness and moisture content and availability of food (see Introduction). Although the soil became softer and workable after onset of rains we found

higher DEE and SusMS compared to dry season. This may be explained by higher burrowing activity (excavation of new foraging tunnels or re-establishing of the old ones) and/or by higher costs of burrowing in wet (and heavier) soil (cf. Lovegrove, 1989; Romañach et al., 2005). However, our study was performed at the onset of the rainy season, when soil is getting softer and more workable but not significantly heavier. The results of our study suggest that moist soil triggers burrowing activity.

However, there are still other alternative explanations for the seasonal difference in DEE. The silvery mole-rats may have reduced energy expenses in the advanced dry season which would mean that the seemingly increased DEE at the beginning of rainy season actually represents a normal state. The question remains which energy saving mechanisms are actually employed. A simple possibility would be to reduce overall activity. Indeed, Šklíba et al. (2007) reported decreased activity of silvery mole-rats in the course of dry season. Although mole-rats are not known to utilize daily torpor as some desert rodent species (Ehrhardt et al., 2005) or golden moles do (Scantlebury et al., 2008) this mechanism cannot be excluded (cf. Marhold and Nagel, 1995). Finally, some other behavioral and physiological strategies minimizing energy expenditure, e.g. metabolic suppression without drop in body temperature and body mass during the long-lasting period of food restriction (Goldman et al., 1999), could be employed to overcome adverse environmental conditions.

We can estimate a proportion of time spent by burrowing in dry season on the base of values of energy costs of rest (RMR; Zelová et al., 2007), digging (DMR; Zelová et al., 2010), and DEE (this study), according to the formula  $DMR = DEE - RMR$ . We assume that there was no additional cost of thermoregulation; animals were non-growing and

Table 2

Comparisons of resting metabolic rates (RMR), daily energy expenditures (DEE) with values predicted by allometry (% of expected DEE) and sustained metabolic scopes (SusMS) in bathyergids with different social organisation.

| Species                                | Social system | "Caste" or sex     | Season | Body mass (g) | RMR ( $\text{kJ g}^{-1} \text{day}^{-1}$ ) | DEE ( $\text{kJ g}^{-1} \text{day}^{-1}$ ) | % Expected DEE <sup>a</sup> | SusMS |
|--|---------------|--------------------|--------|---------------|--|--|-----------------------------|-------|
| <i>Fukomys damarensis</i> <sup>1</sup> | Eusocial      | Infrequent workers | Dry    | 133           | 0.42                                       | 0.54                                       | 40                          | 1.4   |
|  |               |                    | Wet    | 139           | 0.43                                       | 0.82                                       | 62                          | 2.2   |
|  |               | Frequent workers   | Dry    | 78            | 0.57                                       | 0.90                                       | 58                          | 1.9   |
|  | Queen         | Wet                | 93     | 0.47          | 0.88                                       | 59   | 1.9                         |       |
|  |               | Dry                | 124    | 0.39          | 0.63                                       | 46   | 1.6                         |       |
|  |               | Wet                | 110    | 0.44          | 0.97                                       | 69   | 2.5                         |       |
| <i>Bathyergus janetta</i> <sup>2</sup> | Solitary      | Male               | Wet    | 423           | 0.27                                       | 0.89                                       | 92                          | 3.2   |
|  |               | Female             | Wet    | 291           | 0.34                                       | 0.88                                       | 83                          | 2.6   |
|  |               | Male               | Wet    | 137           | 0.53                                       | 1.00                                       | 75                          | 1.9   |
| <i>Georchus capensis</i> <sup>2</sup>  | Solitary      | Male               | Wet    | 127           | 0.41                                       | 1.10                                       | 81                          | 2.8   |
|  |               | Female             | Wet    | 161           | 0.53                                       | 0.76                                       | 60                          | 1.5   |
|  |               | Both sexes         | Wet    | 162           | 0.53                                       | 1.00                                       | 82                          | 2.0   |

Note: <sup>a</sup> % of expected DEE from the equation:  $DEE = 5.48 \text{ body mass}^{0.712}$  for the rodents (Nagy et al., 1999). <sup>1</sup>Scantlebury et al., 2006a; <sup>2</sup>Scantlebury et al., 2006b; <sup>3</sup>present study.

females were not lactating. Assuming that DEE amounts to 122 kJ in dry season, daily RMR = 74 kJ, the remaining daily digging expenditure (daily DMR) = 48 kJ. Furthermore, if DMR represents 14 kJ per hour of digging (Zelová et al., 2010), we can conclude that the mole-rats at the peak of dry season spend on average 3.5 h per day burrowing. Indeed, radio-collared mole-rats (studied at the same locality) at the beginning of dry hot season spent on average 9 h a day outside the nest (Šklíba et al., 2007). This time, however, was not devoted only to digging, but also to patrolling, collecting and transporting food, etc.

The non-bathyergid burrowing rodents for which seasonal data on DEE are available (*Thomomys bottae*, Gettinger, 1984; and *Octodon degus*, Bozinovic et al., 2004) do not show any clear seasonal variability in energetics. These animals forage also aboveground and are not forced to burrow extensively to get enough food (Gettinger, 1984) and are less restricted by seasonal food availability (Bozinovic et al., 2004). Unlike these species, the African mole-rats forage belowground so that seasonal variability in soil conditions and availability of food have to be reflected in their DEE and SusMS. There are only two studies measuring DEE in subterranean rodents (Scantlebury et al., 2006a,b), and the effect of rainfall was investigated only in social *F. damarensis* (Scantlebury et al., 2006a). Interestingly, a seasonal trend was found only in non-breeding “non-workers” which increased their DEE after soil moistened by rains, whereas the “workers” were active year-round with no seasonal change in DEE.

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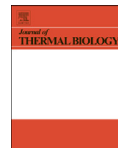
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## Poikilothermic traits in Mashona mole-rat (*Fukomys darlingi*). Reality or myth?

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Body temperature

### ABSTRACT

The African mole-rats (Bathyergidae, Rodentia) is a mammalian family well known for a variety of ecophysiological adaptations for strictly belowground life. The smallest bathyergid, the hairless naked mole-rat from arid areas in Eastern Africa, is even famous as the only truly poikilothermic mammal. Another bathyergid, the Mashona mole-rat (*Fukomys darlingi*) from Zimbabwe, is supposed to have strong poikilothermic traits, because it is not able to maintain a stable body temperature at ambient temperatures below 20 °C. This is surprising because, compared to the naked mole-rat, this species, together with all congenics, is larger, haired, and living in more seasonal environment. In addition, other *Fukomys* mole-rats show typical mammalian pattern in resting metabolic rates. In our study, we measured resting metabolic rate and body temperature of Mashona mole-rats from Malawi across a gradient of ambient temperatures to test its poikilothermic traits. We found that the adult mass specific resting metabolic rate was  $0.76 \pm 0.20 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  and body temperature  $34.8 \pm 1.1 \text{ }^\circ\text{C}$  in the thermoneutral zone (27–34 °C). Body temperature was stable ( $33.0 \pm 0.5 \text{ }^\circ\text{C}$ ) at ambient temperatures from 10 to 25 °C. We thus cannot confirm poikilothermic traits in this species, at least for its Malawian population. Factors potentially explaining the observed discrepancy in Mashona mole-rat energetics are discussed.

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### 1. Introduction

Subterranean mammals live, forage, reproduce and disperse mainly belowground (Nevo, 1999). The network of their burrows is isolated from the aboveground environment, creating a stable microclimate in terms of temperature and humidity (Burda et al., 2007). Despite these relatively stable conditions, underground life is demanding especially because dispersal and the search for food are connected with an energetically very costly activity i.e. digging of new burrows (Du Toit et al., 1985, Lovegrove, 1989, Luna and Antinuchi, 2007, Zelová et al., 2010).

Subterranean mammals share many convergent physiological specializations (Contreras and McNab, 1990, Buffenstein, 2000) such as low resting metabolic rate (RMR), low body temperature ( $T_b$ ) and high thermal conductance (C). Among the subterranean mammals, energetics has been frequently studied in the strictly belowground African mole-rats (Bathyergidae, Rodentia) especially in relation to hypotheses explaining low RMR, low  $T_b$  and high C as means for preventing thermal and respiratory stress and

adaptation for life in an energetically demanding environment (e.g. McNab, 1966, Lovegrove and Wissel, 1988, Zelová et al., 2007).

However, thermoregulatory capabilities vary a lot among particular species within this relatively small rodent family (see Zelová et al., 2007). Most mole-rats, such as the silvery mole-rat (*Heliophobius argenteocinereus*), are able to cope with a relatively wide range of ambient temperatures ( $T_a$ ). This well furred solitary mole-rat, which has the widest thermoneutral zone (TNZ) among bathyergids, maintains stable  $T_b$  at  $T_a$  in the range of 10–30 °C (Zelová et al., 2007). On the contrary,  $T_b$  of the highly social naked mole-rat (*Heterocephalus glaber*), which is virtually hairless, completely depends on  $T_a$  in the range of 12–37 °C (Jarvis, 1978, Buffenstein and Yahav, 1991). This smallest bathyergid species is thus considered to be the only poikilothermic mammal. Another social species, the Mashona mole-rat (*Fukomys darlingi*) from Zimbabwe, is considered to have a strong poikilothermic tendency, because it cannot maintain stable  $T_b$  in the range of  $T_a=5\text{--}24 \text{ }^\circ\text{C}$  ( $T_b$  decreases by about 6 °C at  $T_a=14 \text{ }^\circ\text{C}$ , Bennett et al., 1993). This is quite surprising, because, contrary to the naked mole-rat, the Mashona mole-rat resembles other species of the genus *Fukomys*, which possess ordinary mammalian energetics in its appearance and ecology. It is larger, has relatively denser fur and occurs in areas with more precipitation and a more seasonal environment (Bennett and Faulkes, 2000).

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In our study, we analyzed RMR,  $T_b$  and C of adult Mashona mole-rats from southern Malawi, the northern area of its distribution. Individuals of this population live in a hot environment at very low altitudes. The goal of our study was to test whether Malawian Mashona mole-rat's energetics is more similar to other congeners with typical mammalian energetics or to the Mashona mole-rat population from Zimbabwe having strong poikilothermic traits (sensu Bennett et al., 1993) or even to the poikilothermic naked mole-rat from Kenya occurring in a warm environment (Jarvis, 1978, Buffenstein and Yahav, 1991).

## 2. Materials and methods

### 2.1. Study animals

The Mashona mole-rat (*F. darlingi*) occurs in shrub habitats and miombo woodland in areas of predictable and relatively high rainfall (mean annual rainfall over 700 mm) (Bennett and Faulkes, 2000). This social species lives in families containing about seven animals (5–9) where reproduction is restricted to one breeding pair (Bennett et al., 1994). It is supposed that this species occurs in Eastern and Northern Zimbabwe, and is believed to occur also in Western Mozambique (c.f. Bennett and Faulkes, 2000). Based on karyology and cytochrome b analysis, a mole-rat population from southern Malawi originally assigned to *Cryptomys hottentotus* is actually *F. darlingi* (Van Daele, unpublished results).

The Mashona mole-rats were obtained in Nsanje in Southern Malawi in 2005 (16°55'S, 35°16'E, altitude 53 m a.s.l.). In the field, we captured 15 individuals from different families with mean individual weight  $118 \pm 36$  (range 56–183) g. Five pairs were founders of our breeding stock. The mole-rat families are kept in glass terrariums (80 × 60 × 50 cm) with peat as substrate, with stable ambient temperature of  $25 \pm 1$  °C and relative humidity 40–50%. The animals are fed with potatoes, sweet potatoes, carrots and commercial rodent pellets thrice a week.

Oxygen consumption ( $VO_2$ ) and body temperature ( $T_b$ ) were measured in 10 adult, non-breeding, captivity born, mole-rats (seven males and three females), age  $666 \pm 235$  (mean ± S.D.; range 359–998) days; their body mass was  $143 \pm 26$  (mean ± S.D.; range 114–198) g. Animals were food-deprived 12 h prior to measurement to ensure a postabsorptive state.

### 2.2. Experimental procedure

Each animal was closed in a hermetic metabolic chamber (total volume: 2740 mL) with controlled air input ( $343 \text{ mL min}^{-1}$ ). The PVC metabolic chamber, with a transparent Perspex upper part, was submerged in a temperature controlled water bath (ThermoHaake C10 and K15, Haake, Germany). Temperature inside the chamber was monitored by the thermal probe of a digital thermometer (Thermometer, Solid 898).

$VO_2$  was measured by an open-flow respirometer setup. This consisted of beforehand dried fresh air being pushed through flowmeter (082-03 S, Aalborg, USA), and a metabolic chamber. The excurrent air was subsampled at rate of about  $150 \text{ mL min}^{-1}$  (flowmeter 082-01 S, Aalborg, USA) and pushed through  $CO_2$  trap (NaOH+CaO), water trap (Drierite with color-indicator), and a heated paramagnetic oxygen sensor (PAROX 1000, MBE Electronic AG, Switzerland). The analog output of the sensor was digitized and recorded each second by the DIAdem 8.00 computer program (GfS Aachen, Germany).

The instrument was calibrated with 99.99%  $N_2$  (Linde) for 0% oxygen content on a monthly basis. The slope of the instrument response was calibrated prior to and after each measurement by air from air-ventilation.

Oxygen consumption was calculated by the recording software following the equation (Depocas and Hart, 1957):

$$VO_2 = \frac{f_{in}(p_{in} - p_{out})}{(1-p)w} \quad (1)$$

where  $VO_2$  is oxygen consumption in  $\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ,  $f_{in}$  is air flow at the input to the metabolic chamber (at standard conditions),  $p_{in}$  is percentage fraction of oxygen at the input to the metabolic chamber (20.95%),  $p_{out}$  is percentage fraction of oxygen in the output from the metabolic chamber and  $w$  is body mass of an animal in g.

Each measurement took at least 60 min. The animal was placed in the metabolic chamber and left for 30 min to acclimate to the environment. The animal was then measured for 30 min at extreme temperatures (10, 37 and 40 °C) to avoid harm of animals or for at least 60 min at all other temperatures. RMR was then calculated at 10 min intervals with the lowest oxygen consumption and lowest animal activity.

$VO_2$  and  $T_b$  of ten animals were measured at  $T_a = 10, 15, 20, 25, 26^*, 28, 30, 32^*, 33, 34^*, 35, 37$  and 40 °C, with the exception of temperatures marked by an asterisk (\*), when six animal were measured.

Body mass was determined by weighing each animal on digital scales (KERN & Sohn GmbH 572-45, Germany). Rectal temperatures ( $T_b$ ) were measured 10 s after termination of each experiment by a digital thermometer (Thermalert TH-8, Physitemp Instruments Inc, USA) with a temperature probe for mice.

Conductance (C) was calculated according to the equation (McNab, 1980).

$$C = \frac{VO_2}{T_b - T_a} \quad (2)$$

where C is expressed in  $\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ } ^\circ\text{C}^{-1}$ ,  $VO_2$  is oxygen consumption in  $\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ,  $T_b$  is body temperature and  $T_a$  is ambient temperature.

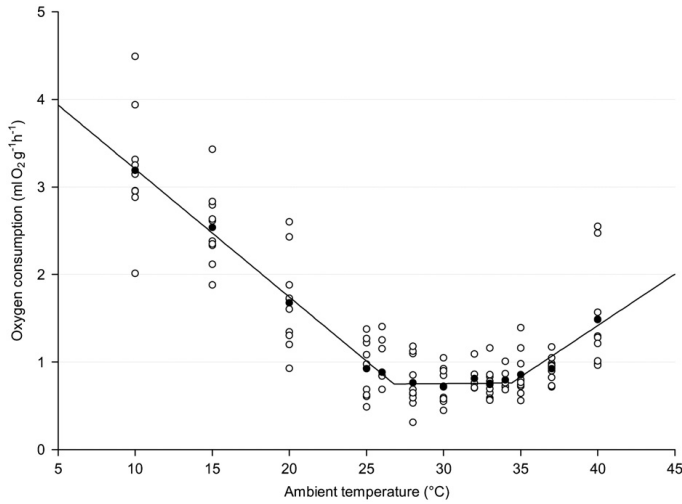
Weather data were obtained at [www.worldclimate.com](http://www.worldclimate.com) on September 2010. We compared average annual ambient temperature in Harare (17°50'S, 31°03'E, altitude 1489 m a.s.l., Zimbabwe, 1890–1989) and Nsanje (16°55'S, 35°16'E, 53 m a.s.l., Malawi, 1983–2004) for *F. darlingi* and Makindu (02°14'S, 37°49'E, altitude 993 m a.s.l., Kenya, 1961–70) for the *H. glaber*. Average annual precipitation in 1949–84, 1898–1988, 1904–88 was also calculated for these locations respectively.

All means, SD and linear regression equations were calculated by program Statistica 8.0 (Statsoft, Inc. 1984–2008). All results are presented as mean ± SD (range).

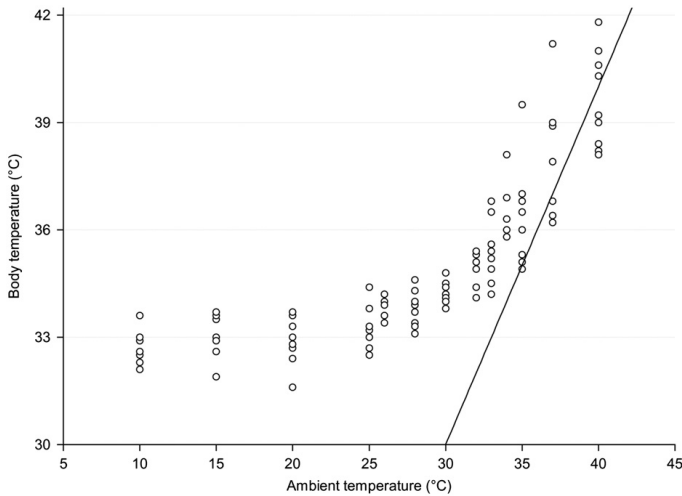
## 3. Results

The thermoneutral zone (TNZ) of Mashona mole-rats from Malawi is in the  $T_a$  range of 27–34 °C (Fig. 1). Mean  $VO_2$  in the TNZ, thus mass specific resting metabolic rate (msRMR), was  $0.76 \pm 0.20 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (0.31–1.18,  $N=42$ ). An increase in the metabolic rate below the lower limit of the TNZ was calculated by the equation:  $VO_2 = 4.67 - 0.15 T_a$  ( $F_{(1,44)} = 157.77$ ,  $p < 0.0001$ ,  $r^2 = 0.78$ ). The average  $VO_2$  at minimum  $T_a = 10$  °C was  $3.19 \pm 0.66 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (2.01–4.49,  $N=10$ ), which is more than 4 times higher than the msRMR in the TNZ (419%). The average  $VO_2$  at maximum  $T_a = 40$  °C was  $1.48 \pm 0.58 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (0.97–2.55,  $N=10$ ), which is almost double the msRMR in the TNZ (195%).

$T_b$  in the tested mole-rats was stable at  $T_a$  in the range 10–25 °C (Fig. 2) with mean value =  $33.0 \pm 0.5$  °C (31.6–34.4,  $N=48$ ). Below the TNZ, the average difference between maximal and minimal  $T_b$  of a particular individual was  $1.0 \pm 0.5$  °C (0.4–1.9,  $N=10$ ). Mole-rat average  $T_b$  in the TNZ was  $34.8 \pm 1.1$  °C (33.1–38.1,  $N=42$ ) and was positively correlated with increasing  $T_a$  within the TNZ



**Fig. 1.** Oxygen consumption ( $\text{ml O}_2 \text{g}^{-1} \text{h}^{-1}$ ) of 10 adult *Fukomys darlingi* from Malawi (open circles) as a function of ambient temperature ( $^{\circ}\text{C}$ ). Mean oxygen consumption (solid circles) is also shown. Thermoneutral zone is indicated by horizontal line.



**Fig. 2.** Body temperature of 10 adult *Fukomys darlingi* from Malawi as a function of ambient temperature ( $^{\circ}\text{C}$ ). Solid line represents equality between ambient and body temperature.

( $T_b = 21.97 + 0.41T_a$ ,  $F_{(1,40)} = 81.24$ ,  $p < 0.0001$ ,  $r^2 = 0.67$ ). Increasing  $T_b$  at  $T_a$  above the TNZ is given by the equation:  $T_b = 13.03 + 0.67T_a$  ( $F_{(1,28)} = 28.26$ ,  $p < 0.0001$ ,  $r^2 = 0.50$ ) while mean  $T_b$  was  $39.7 \pm 1.2$   $^{\circ}\text{C}$  ( $38.1\text{--}41.8$ ,  $N = 10$ ) at the highest  $T_a = 40$   $^{\circ}\text{C}$ .

The average conductance at lower limit of the TNZ ( $T_a = 27$   $^{\circ}\text{C}$ ) was  $0.12 \pm 0.06$   $\text{ml O}_2 \text{g}^{-1} \text{h}^{-1} \text{ } ^{\circ}\text{C}^{-1}$  ( $0.02\text{--}0.20$ ,  $N = 10$ ).

**4. Discussion**

Contrary to expectations based on the study of Bennett et al. (1993), the studied Mashona mole-rat from Malawi kept stable  $T_b$  in the whole range of  $T_a$  below TNZ. This means that studied

animals are able to employ a physiological mechanism to maintain stable  $T_b$  in low temperatures. Our results thus did not confirm poikilothermic traits in this species.

The TNZ of the Mashona mole-rat from Malawi is relatively broad (7  $^{\circ}\text{C}$ ) in comparison with TNZs of other species of mole-rats (see Zelová et al., 2007 for review). Its msRMR is almost equal to the RMR predicted for mole-rats and subterranean rodents in general (Table 1). However, its C at the lower limit of TNZ is more than one third higher compared to other mole-rats (Zelová et al., 2007). A higher C in subterranean mammals is considered to be an important mechanism for avoiding overheating during activity (Contreras and McNab, 1990, Luna and Antinuchi, 2007).



**Table 1**

Mean body mass ( $w$ ), mean body temperature ( $T_b$ ), mean mass-specific resting metabolic rate (msRMR), mean whole-animal resting metabolic rate (RMR), predicted RMR (%<sup>a</sup>, %<sup>b</sup>, %<sup>c</sup>, %<sup>d</sup>), conductance ( $C$ ), predicted  $C$  (%<sup>e</sup>) and range of thermoneutral zone (TNZ) in two studied populations of Mashona mole-rat (*F. darlingi*) from Zimbabwe (Bennett et al., 1993) and Malawi (this study).

| Locality | $w$ (g) | $T_b$ (°C) | RMR (ml O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> ) | RMR (ml O <sub>2</sub> h <sup>-1</sup> ) | % <sup>a</sup> | % <sup>b</sup> | % <sup>c</sup> | % <sup>d</sup> | $C$ (ml O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup> °C <sup>-1</sup> ) | % <sup>e</sup> | TNZ (°C) |
|----------|---------|------------|--|--|----------------|----------------|----------------|----------------|---|----------------|----------|
| Zimbabwe | 60      | 33.3       | 0.98   | 58.8                                     | 80             | 76             | 97             | 110            | 0.19  | 135            | 28–31.5  |
| Malawi   | 143     | 33.0       | 0.76   | 108.8                                    | 77             | 79             | 99             | 106            | 0.12  | 137            | 27–34    |

<sup>a</sup> % RMR as percentage of that predicted by curve for mammals:  $RMR=3.42w^{0.25}$  (Kleiber, 1961)

<sup>b</sup> % RMR as percentage of that predicted by curve for rodents:  $RMR=4.98w^{0.331}$  (Hayssen and Lacy, 1985)

<sup>c</sup> % RMR as percentage of that predicted by curve for subterranean rodent:  $RMR=3.79w^{0.322}$  (Lovegrove, 1986b)

<sup>d</sup> % RMR as percentage of that predicted by curve for bathyergids:  $RMR=2.4w^{0.243}$  (Zelová et al., 2007)

<sup>e</sup> %  $C$  as percentage of that predicted by curve for subterranean rodent:  $C=1.34w^{0.55}$  (Contreras and McNab, 1990)

We found that the tested animals increased their  $T_b$  in the TNZ with increasing  $T_a$ . This increase was remarkable in  $T_{a5}$  above the TNZ, e.g. at  $T_a=40$  °C the mean increase of 6 °C was observed. Elevated  $T_b$  at higher  $T_a$  is common to endothermic vertebrates and was also described in some other small mammals (e.g. Mathias et al., 2003) including some species of African mole-rats (e.g. McNab, 1966, Lovegrove, 1986b, Zelová et al., 2007). This increase is generally considered to be energy expenditure and mainly water saving adaptation (Wooden and Walsberg, 2002), because animals are not forced to fully employ energetically costly cooling mechanisms. We suppose that this phenomenon has to be very important for subterranean mammals living in the environment of closed burrow systems, where high humidity and absence of ventilation limits chances for their cooling (Burda et al., 2007).

In spite of the similarity in energetics of Mashona mole-rats under this study to other mole-rat species, our results revealed several remarkable differences compared to the population of Mashona mole-rats from Zimbabwe, especially in terms of RMR,  $C$  and  $T_b$  (Table 1). Finding of stable  $T_b$  at lower ambient temperatures in our study is in sharp contrast to Mashona mole-rats from Zimbabwe, which exhibit a strong poikilothermic tendency (Bennett et al., 1993). In fact, intraspecific differences in thermoregulatory capability are not unusual in African mole-rats. For example, Bennett et al. (1996) described that *Cryptomys hottentotus nimrodi* from higher altitude had lower body mass (80%), lower msRMR (87%) and narrow TNZ in comparison with individuals from lower altitudes. The authors supposed that the lower RMR is a consequence of lower partial pressure of oxygen in the atmosphere at higher altitudes. Nevertheless, Broekman et al. (2006) described an inverse relation in two populations of *Cryptomys hottentotus mahali*. Mole-rats from higher altitudes had higher body mass (130%), higher RMR (130%) and broader TNZ compared to mole-rats from lower altitudes. The authors claimed that good thermoregulatory capabilities are important for surviving at higher altitudes. In another mole-rat species, the Damaraland mole-rat (*Fukomys damarensis*), thermoregulatory capabilities seem to be related to the aridity of the environment. The arid population had lower body mass (77%), lower msRMR (86%) and wider range of TNZ than population from a semi-arid area (Lovegrove, 1986b, Bennett et al., 1992).

It is thus clear that different habitats and species ecology could significantly influence energetics in African mole-rats. Therefore, one could expect the same in Mashona mole-rats, because both populations live in different environments. Animals from Malawi were captured at lower altitudes (53 m a.s.l.) in an area characterized by higher average annual ambient temperature (about 24 °C). For the Zimbabwean population (altitude 1388 m a.s.l.), we expected similar temperature conditions as in nearby Harare, where average annual ambient temperature is only about 18 °C. Rainfall and the length of the rainy and dry seasons are comparable in both areas (average annual precipitation is 830 mm and

870 mm respectively). Nevertheless, we assume that differences in the energetics of both populations (Table 1) are caused by other factors than their ecology.

Differences in body mass of mole-rats play a major role, because tested individuals from Malawi were more than twice as large as those from Zimbabwe. It is thus not surprising that the Malawian population has a higher value of RMR (Table 1). But size differences between populations are in contrast to Bergmann's rule. However, body size of rodents in general is not defined only by temperature and latitude but rather by other environmental factors such as precipitation (Rezende et al., 2004) and, in subterranean rodents, also by soil hardness and quality and availability of food (Medina et al., 2007). RMR is also considered to be associated with precipitation and aridity. Lower RMR of individuals from arid areas is considered to be an adaptation to limited sources of food and water (Novoa et al., 2005). Unfortunately, there is no information available on food supply and hardness of soil in both Mashona mole-rat localities. Nevertheless, we do not expect any remarkable differences in food supply because both localities have a similar precipitation pattern which is supposed to be a main determinant of food supply in African mole-rats (Bennett and Faulkes, 2000). It seems that there is still great uncertainty concerning the relationship between body size and RMR of African mole-rats to altitude and ecological factors (see Broekman et al., 2006, Bennett et al., 1996). This needs further study.

There is still one more possible explanation related to different body size and different RMR between both populations. It is possible that individuals from Zimbabwe were still growing (although one female of that size reproduced, Bennett et al., 1994). In the energetic study of Bennett et al. (1993), the Mashona mole-rats had very small body mass when analyzed (about 60 g) and their thermoregulatory capability could not have been well developed yet. Interestingly, our results indicate that Mashona mole-rats are not able to thermoregulate for the long period of postnatal development (Zemanová unpublished results). The capability to thermoregulate and maintain stable  $T_b$  could depend rather on body mass than on age, or alternatively a combination of both. Even one young Mashona mole-rat with a weight of 90 g and age of 189 days was not able to maintain stable  $T_b$  well at lower  $T_a$  (Zemanová unpublished results). The  $T_b$  of this mole-rat was linearly dependent on  $T_a$  in the whole range of tested  $T_a$ s reaching 24 °C at the minimum  $T_b=10$  °C. Poikilothermic traits in Mashona mole-rats could thus be an artefact of testing small, probably still growing individuals.

Bennett et al. (1993) compared the energetics of the Mashona mole-rat from Zimbabwe to the naked mole-rat from Kenya and claimed that both species live in areas with similar climatic conditions and particularly ambient temperature, which could have triggered the evolution of poikilothermy. However, the Mashona mole-rat of Zimbabwe lives in colder habitats than that occupied by the naked mole-rat in Kenya (average annual

temperature about 22 °C vs. 18 °C in Makindu, Kenya vs. Harare, Zimbabwe respectively). If the suggested relationship between poikilothermy and ambient temperature is correct and simple, then the Mashona mole-rats from Southern Malawi should have even stronger poikilothermic traits than mole-rats from Zimbabwe, because annual average temperature in Nsanje (about 24 °C) is even higher to that found in the naked mole-rat habitat in Kenya. However, as mentioned above, we did not detect any poikilothermic tendencies in the Mashona mole-rats from Malawi.

We suggest that the remarkable differences between both populations of Mashona mole-rat are not necessarily caused by their different ecology, such as temperature at different altitudes and related factors such as food supply or rainfall, but most probably by different body size. Smaller individuals could be less able to maintain a stable body temperature in lower ambient temperatures. Whether the small body size is a typical trait for the Zimbabwean population, or whether the tested mole-rats were still growing, is not clear. Nevertheless, the fact that in another study on Mashona mole-rat the largest individuals in freshly captured colonies weighted more than 100 g (Gabathuler et al., 1996) indicates latter explanation. It is certain, however, that the tendency to poikilothermy in the Mashona mole-rat is not applicable for the whole species distribution and could be an artefact of analyzing small specimens.

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# Social and Environmental Influences on Daily Activity Pattern in Free-Living Subterranean Rodents: The Case of a Eusocial Bathyergid

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**Abstract** Predictable daily activity patterns have been detected repeatedly even in mammals living in stable environments, as is the case for subterranean rodents. Whereas studies on activity of these rodents under laboratory conditions almost exclusively have concerned themselves with the influence of light, many field studies have revealed signs of an association between the activity pattern and daily fluctuations of temperature under the ground. This would assume that behavioral thermoregulation is probably involved. The only exceptions to the relationship between temperature and activity are 2 eusocial mole-rats of the genus *Fukomys* (Bathyergidae, Rodentia), which indicates that activity patterns could be affected also by social cues. To better understand how social and environmental factors influence the activity pattern in a eusocial mole-rat, we monitored the outside-nest activity in another species of this genus, the Ansell's mole-rat (*Fukomys anselii*), which has a relatively small body mass, high conductance, and more superficially situated burrows. Its daily activity had 1 prominent peak (around 1400 h), and it was tightly correlated with the temperature measured at depth of foraging burrows. Since *F. anselii* has high thermoregulatory requirements to maintain stable body temperature below the lower critical temperature, we conclude that the observed pattern is probably the result of minimizing the cost of thermoregulation. There were no significant differences in the daily activity patterns of breeding males and females and nonbreeders. Members of the same family group tended to have more similar activity patterns, but consistent activity synchronization between individuals was not proven. From the comparison of available data on all subterranean rodents, we assume that social cues in communally nesting mole-rats may disrupt (mask) temperature-related daily activity rhythms but probably only if the additional cost of thermoregulation is not too high, as it likely is in the Ansell's mole-rat.

**Keywords** *Fukomys anselii*, subterranean rodent, daily activity pattern, radio-telemetry, Bathyergidae, eusociality, circadian rhythms

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Most mammals display circadian rhythms of activity and rest, which ensure that activity takes place during an optimal period of the day in terms of food availability, predation risk, and climatic conditions (Bartness and Albers, 2000). In mammals living in a stable environment with lack of external cues that would potentially reset their circadian clocks, we would expect circadian activity rhythms to be weak or absent. Examples of such mammals are subterranean rodents, that is, rodents that forage underground and rarely emerge from their sealed burrows. The subterranean ecotope is dark and thermally buffered, and neither food availability nor predation risk is thought to substantially vary there during the course of a 24-h cycle (Nevo 1999). It is therefore surprising that many field studies regarding subterranean rodents have exhibited predictable daily activity patterns (e.g., Jarvis, 1973; Gettinger, 1984; Benedix, 1994; Šklíba et al., 2007; Lövy et al., 2013). Some authors have suggested that there is a relationship between activity patterns and daily temperature cycles in the depth of the burrows, even though its amplitude is often very small (typically  $<4^{\circ}\text{C}$ ). There are few laboratory data dealing with the effect of temperature on the activity pattern in these animals (cf. Goldman et al., 1997), which contrasts with many studies testing merely the influence of the photoperiod (e.g., Oosthuizen et al., 2003, and citations therein).

The relationship between activity patterns and daily temperature fluctuations that has been detected in some free-living subterranean rodents has the character of either preference or avoidance of high or low temperatures, depending on the species, latitude, or the season, as can be illustrated by the following examples. Whereas blind mole rat *Spalax ehrenbergi*, radio-tracked during the hot Mediterranean summer, minimized its activity during the hottest period of day (Rado et al., 1993), the silvery mole-rat *Heliophobius argenteocinereus*, radio-tracked in Afrotropical woodland in the coldest period of year, showed a positive correlation between temperature and activity (Šklíba et al., 2007). Another subterranean rodent, the plains pocket gopher *Geomys bursarius*, was even found to reduce its activity in both the hottest and the coldest period of the day, resulting in a bimodal activity pattern (Benedix, 1994). The author of the latter study proposed that by displaying such an activity pattern, the animals 1) reduce additional energetic expenses of thermoregulation when the burrow temperature is low and 2) reduce the threat of hyperthermia when it is high. This statement actually represents 2 hypotheses. So far, neither of them has been addressed in any subterranean rodent under laboratory conditions, and it might be technically difficult to do so. Nevertheless, examining temperature-related daily activity patterns

across different subterranean rodents in the field and coupling them to available physiological data can help us to evaluate plausibility of such hypotheses.

African mole-rats (Bathyergidae) are a family of strictly subterranean rodents of which the majority of the species are highly social, living in multigenerational family groups. This provides us with an opportunity to study the effect of sociality in addition to environmental factors on these animals' daily activity patterns. Strong social influence on behavioral rhythms is common in social animals living in constraining environments, but it can be expressed in different ways, such as synchronization, desynchronization, or individually variable response to social cues depending, for example, on dominance status (Favreau et al., 2009). In highly cooperative mole-rat family groups, we would expect desynchronized rather than synchronized activity patterns, presumably enabling more effective cooperation in search for food and territory maintenance. This is also in accord with most laboratory observations (Bennett, 1992; Riccio and Goldman, 2000; Schielke et al., 2012; but see Davis-Walton and Sherman, 1994, for signs of activity synchronization in naked mole-rats). Field-based activity data are available from only 2 of the social species, Damara mole-rat *Fukomys damarensis* and giant mole-rat *Fukomys mechowii*, each represented by a single study dealing with a single family group (Lovegrove, 1988; Lövy et al., 2013). Both species engaged in many short activity bouts per day and showed no clear signs of activity synchronization between individuals, but none of them displayed a temperature-correlated daily activity pattern. The authors of the latter study concluded that the similarity of the activity patterns of *F. mechowii* and *F. damarensis* might be, to some extent, a consequence of similar social behaviors in these 2 species, such as use of communal nests and the need for cooperation while performing tasks, overlying the effect of environmental factors.

The 2 above-mentioned species, *F. mechowii* and *F. damarensis*, represent the largest social mole-rats (Bennett and Faulkes, 2000), inhabiting large burrow systems with deep (and therefore more temperature buffered, see Bennett et al., 1988) nests and primary burrows (Lovegrove and Painting, 1987; Šumbera et al., 2012). These factors can potentially contribute to their activity pattern not being markedly affected by the daily temperature cycle. Nevertheless, in smaller-sized social bathyergids, especially in those whose burrow systems lack deep primary burrows, some behavioral response to daily temperature might be energetically advantageous. This likely would be expressed as a predictable daily activity pattern related to the temperature cycle in the depth of the foraging tunnels, which would be similar in most individuals across different family groups.

To better understand the relative importance of social and environmental cues for the mole-rat activity pattern, we chose to study the activity of the Ansell's mole-rat *Fukomys ansellii* (Burda et al., 1999), an eusocial species (cf. Patzenhauerová et al., 2013) from Zambia, under natural conditions. This species has a relatively small body mass, and its burrow systems generally lack deeper primary burrows (see Šklíba et al., 2012). We recorded a pattern of outside-nest activity in 17 radio-collared individuals of 5 family groups in the cold dry season. Aims of the study were 1) to reveal whether there is a daily rhythm of activity in this species under natural conditions of the cold dry season; 2) to find whether activity is related to the daily temperature cycle in the depth of foraging burrows, as detected in some solitary subterranean rodents, or whether the relationship is absent, as found in larger-bodied social mole-rats; 3) to reveal how the activity pattern is affected by group affiliation, sex  $\times$  reproductive status, and body mass and what is the relative influence of these factors; 4) to test whether the activities of individuals from the same family group tend to be rather synchronized or desynchronized; and 5) to review the incidence of likely temperature-related daily activity patterns in subterranean rodent species within the context of relevant physiological parameters of the respective species.

## MATERIALS AND METHODS

### Studied Animals

The Ansell's mole-rat is a small-sized social species inhabiting a mesic area close to the Zambian capital Lusaka. In the study population, the body masses of adults ( $\geq 50$  g) were  $72 \pm 14$  g (maximum 96 g) for males and  $62 \pm 8$  g (maximum 83 g) for females. Family groups of up to 13 individuals inhabited extremely large and complicated burrow systems, with the majority of the tunnels located at a depth (from the bottom of the burrow to the soil surface) of around 11 cm (foraging tunnels). A single communal nest per family group was located  $47 \pm 17$  cm (range, 25–90 cm) underground (Šklíba et al., 2012).

### Study Locality

The study was conducted in the Lusaka East Forest Reserve in Zambia ( $15^{\circ}28'S$ ,  $28^{\circ}25'E$ , altitude 1320 m), which is covered by natural open-canopy miombo woodland. The climate in Zambia is characterized by 3 seasons: a rainy season (October/November to March/April), a dry cold season (April to July), and a dry hot season (August to October). Average annual rainfall in Lusaka is 809 mm, and on average 5.4

months per year have more than 25 mm of precipitation (<http://www.ncdc.noaa.gov/ghcnm/>, accessed May 2012).

### Fieldwork

Mole-rats were captured by use of Hickman traps. Captured individuals were weighed, their sex was determined, and they were examined for their reproductive status. We considered breeding males as those with conspicuous head muscularity, pigmented corners of the mouth, and conspicuously large testes; breeding females were recognized by perforated vagina and enlarged teats (the breeding status was later confirmed by use of genetic methods, see Patzenhauerová et al., 2013). All animals weighing more than 45 g were anesthetized for a short time by ketamine and xylazine, fitted with radio-collars (brass collar, Pip transmitter; Biotrack Ltd, Dorset, UK), and released back into the burrows where they were trapped within 48 h from capture. The weight of each radio-collar was 2.3 g ( $<5\%$  of the body mass of the smallest animals under study). Radio-tracking started 3 days after the release of the last animal.

The collared animals were radio-tracked in 2 continuous 96-h sessions. The first session (9–13 May 2010) involved 17 mole-rats from 5 family groups (named P01, P02, etc.; Table 1), which was reduced to 13 individuals in the second session (25–29 May 2010) due to the loss of some collars. Both sessions were in the cold dry season. We used the IC-R20 receiver (Icom America Inc., Kirkland, WA) and a 3-element handheld Yagi antenna to locate the animals. Position of the animals was fixed hourly starting at 0600 h. Since the animals of the same group used the same nest, these places were checked first, and then the animals that were not present here were carefully approached to a distance of 1 to 4 m and fixed. After the end of radio-tracking, all members of the examined family groups were recaptured and the complete burrow systems were excavated and mapped as a part of another study (see Šklíba et al., 2012, for complete information about group sizes, burrow system parameters, ecological parameters, etc.). Handling live animals was in accord with the Declaration of Helsinki and the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Temperatures of the soil surface and at depths of 10, 20, 30, 60 and 120 cm were recorded every 10 min over the whole period of radio-tracking using temperature loggers (Comet System s.r.o., Rožnov pod Radhoštěm, Czech Republic). The depth of 10 cm was chosen to approximate the depth of mole-rat foraging tunnels (Šklíba et al., 2012); the other depths were selected to illustrate lag and attenuation of the temperature fluctuation as a function of the distance from

the soil surface. We selected a relatively exposed site for measuring the temperatures, because most of the area of the mole-rat territories was also relatively exposed to the sun. For comparison we placed 1 additional temperature logger at a depth of 10 cm at a site shaded by dense shrubs and trees.

### Data Processing

To determine daily patterns of activity of the radio-tracked mole-rats, each radio-fix was designated as either inside or outside the nest. From our previous telemetry studies of mole-rats (Šklíba et al, 2007, 2009), we estimate the accuracy of our fixes at  $\pm 0.5$  m; thus, all fixes within a 0.5-m radius of the nest were treated as inside the nest. For each animal, an actogram of the outside nest activity was created based on the primary dataset containing the hourly presence or absence of an individual inside the nest. This dataset was also used to test the relationship of outside-nest activity with environmental factors and to test the activity synchronization of individuals within the same group. Subsequently, radio-fixes of each individual from each 96-h radio-tracking session were grouped into twelve 2-h blocks, and the proportion of fixes outside the nest was determined for each of the blocks. This dataset was used to compare activity patterns between individual mole-rats using a multivariate method.

### Statistical Analyses

Data throughout the text are provided as mean  $\pm$  SD. Generalized linear mixed models (GLMMs) were used to test the effect of various environmental factors to activity—that is, presence outside the nest. Presence outside the nest was the dependent variable with binomial distribution. The factors tested (explanatory variables) were light-dark 24-h cycle (categorical predictor: the light hours [0600-1800 h] marked as 1, dark hours [1800-0600 h] marked as 0) and temperatures at various depths (continual predictors; surface, 10, 20, 30, and 60 cm). The temperature at 120 cm underground was not included since its daily amplitude was close to the resolution of the temperature loggers. The 17 radio-tracked individuals were grouped into the following 9 subsets reflecting their sex, reproductive status, and group affiliation: (1) breeding males, (2) breeding females, (3) nonbreeding males, (4) nonbreeding females, and (5-9) members of the respective family groups. Due to the unequal sample size in the categories and their overlap, GLMMs were performed separately for each of the categories. In all GLMMs, both the individuality of a given animal and the radio-tracking

day (1-8) were treated as random factors to avoid pseudoreplications. We used Bonferroni procedure to correct the significance level of the tests since we tested the 6 factors separately ( $\alpha = 0.05 / 6$ ). All the tests were performed using R statistical software (R Development Core Team, 2013) extended with the lmer4 package (Bates et al., 2013), which is designed for models with incorporated random factors.

Partial effect of 3 variables (body mass, sex  $\times$  reproductive status, and group affiliation) on the mole-rat activity patterns (defined by proportions of outside-nest fixes in the twelve 2-h blocks of the 24-h day) was computed by variance partitioning technique (Lepš and Šmilauer, 2003) using the redundancy analysis (RDA). Sex  $\times$  reproductive status and group affiliation were expressed as sets of 4 and 5 binary (“dummy”) variables, respectively. The RDA was performed with the software package CANOCO for Windows, version 4.52 (Microcomputer Power, Ithaca, NY) (ter Braak and Šmilauer, 2002), with the proportions of outside-nest fixes arcsine transformed and all variables entering the analyses centered (to have zero mean). To avoid pseudoreplications (treating 2 sessions of the same individual as independent), the variance partitioning was performed with data from the first radio-tracking session only.

To test whether the outside-nest activity of individuals of the same group is synchronized, we developed a simple permutation test. For each group and radio-tracking session we computed score  $x$  representing total number of cases of any dyad of individuals located outside the nest at the same time. Then we randomly exchanged the order of the 4 days of the 96-h session in each individual while maintaining the daytime of the radio-fixes unchanged. Score  $x$  was then compared with the score computed for the permuted data ( $x_p$ ). The proportion of cases out of 1000 permutations where  $x_p \geq x$  was set as the significance level of the null hypothesis that the outside-nest activity of the group members is not synchronized within their overall activity pattern.

## RESULTS

Characteristics of the radio-tracked mole-rats are presented in Table 1; their actograms are depicted in Figure 1. All individuals except 1 breeding male (M967) performed a single daily peak of activity, usually between 1100 and 1600 h. There was no apparent temporal shift of the activity peak between the 2 radio-telemetry sessions. In both of them, the activity was culminating (i.e., the most animals were active) around 1400 h (Figs. 1 and 2a). Besides demonstrating a peak at about 1400 h, the breeding male M967

Table 1. Characteristics of radio-tracked mole-rats.

| Group ID | Animal ID | Sex and Reproductive Status | Body Mass, g | No. of Radio-Tracking Sessions |
|----------|-----------|-----------------------------|--------------|--------------------------------|
| P01      | M038      | Breeding M                  | 86           | 1                              |
|          | M150      | M                           | 84           | 1                              |
|          | M203      | M                           | 57           | 2                              |
|          | F217      | Breeding F                  | 69           | 1                              |
| P02      | F138      | F                           | 56           | 2                              |
|          | M967      | Breeding M                  | 90           | 2                              |
| P04      | F027      | F                           | 64           | 2                              |
|          | F465      | F                           | 68           | 2                              |
| P05      | F485      | F                           | 83           | 2                              |
|          | F419      | F                           | 57           | 2                              |
|          | F443      | F                           | 49           | 2                              |
| P10      | F532      | Breeding F                  | 54           | 2                              |
|          | F015      | F                           | 62           | 2                              |
|          | M062      | Breeding M                  | 87           | 2                              |
|          | M092      | M                           | 58           | 2                              |
|          | M352      | M                           | 47           | 2                              |
|          | F568      | F                           | 56           | 1                              |

F = female; M = male.

performed 2 additional daily activity peaks—in both radio-tracking sessions around 0100 h and 0600 h.

Mean daily temperatures at different depths are depicted in Figure 2b. At a depth of 10 cm, which corresponds with the depth of the Ansell's mole-rat foraging tunnels, mean daily temperature was 20.9 °C; mean daily minimum and maximum temperatures were 17.5 and 25.5 °C, respectively; and mean daily temperature span was 8.0 °C. In the shaded location, the mean daily temperature at a depth of 10 cm dropped to 18.2 °C and the daily temperature span decreased to 1.8 °C. The outside-nest activity in Ansell's mole-rats was best explained by the temperature cycle at a depth of 10 cm. Of all 6 explanatory variables, this variable explained the largest proportion of variability in the activity in each of the 9 subsets of individuals defined by sex, reproductive status, and group affiliation (Table 2). The effect of the light-dark cycle, although also significant, had markedly less explanatory power. In reproductive males, a markedly lower proportion of explained variability in the activity data indicates a less pronounced effect of all variables examined.

The amount of variability in the activity patterns explained by sex × reproductive status, body mass, and group affiliation was significantly higher than zero only for the dataset where both radio-tracking sessions were combined—that is, including pseudoreplications

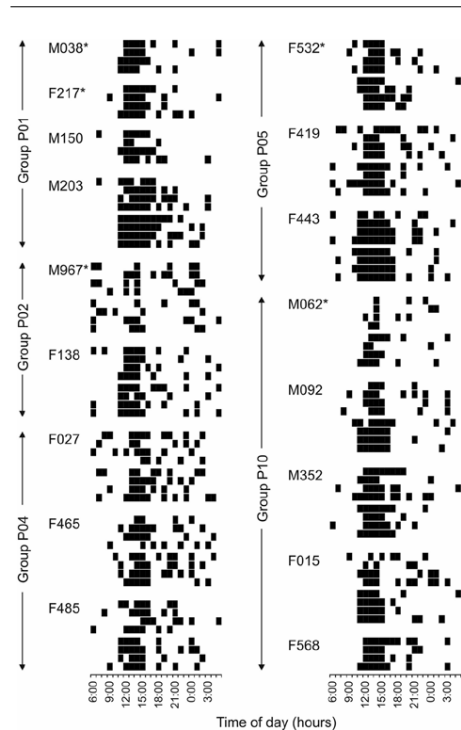


Figure 1. Actograms of 17 radio-tracked mole-rats. Dark bars represent activity (occurrence outside the nest). Every block of 4 lines represents 1 continuous 96-h radio-tracking session of a particular individual. Asterisks mark breeding individuals.

(Table 3). Group affiliation explained the largest part of the variability of the data, which again was significant only for the dataset containing both radio-tracking sessions.

Outside-nest activity of group members was significantly synchronized only in the first session of group P01 and second session of group P04 (permutation test,  $p = 0.003$  and  $0.006$ , respectively).

## DISCUSSION

The Ansell's mole-rat was previously reported to have "a weak circadian clock" based on a study of singly-housed captive individuals under artificial light regime (De Vries et al., 2008) and only a "tendency towards rhythmicity" in locomotory activity

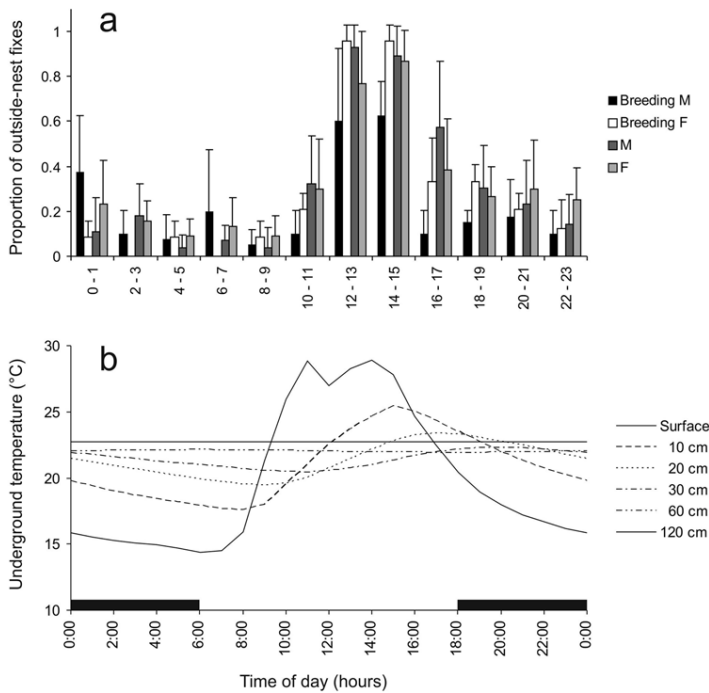


Figure 2. Outside-nest activity of the radio-tracked mole-rats (a) and underground temperature fluctuations (b) during a 24-h day. Means  $\pm$  standard deviations are presented for the proportions of outside-nest fixes. Black horizontal bars indicate the dark phase of the day. F = female; M = male.

based on monitoring of captive family groups under natural (European) light cycle (Schielke et al., 2012). On the contrary, the activity patterns recorded under natural conditions in the present study showed a clear 24-h periodicity in most individuals (Fig. 1). Since the pattern was similar in all 5 family groups studied and all individuals irrespective of body mass, sex, and reproductive status, we assume that it was determined by an external cue. As expected, of the external factors tested, the best predictor of outside-nest activity appeared to be temperature fluctuations at depth of the foraging tunnels.

An animal activity pattern under natural conditions can differ considerably from the one observed in a laboratory, as illustrated by an example of the coruro *Spalacopus cyanus*, which is nocturnal in the laboratory (Begall et al., 2002) but diurnal in the field (Urrejola et al., 2005). Nevertheless, the dissimilarity between activity patterns of captive (De Vries et al.,

2008; Schielke et al., 2012) and free-living (present study) *F. ansellii* is striking for 2 reasons. First, the cycle of light, which was also involved in the abovementioned laboratory studies, is generally thought to be a stronger and more universal zeitgeber than temperature fluctuation in vertebrates (Refinetti, 2006). Even in the strictly subterranean *S. ehrenbergi*, a larger proportion of captive individuals entrained circadian activity rhythms by the light cycle rather than by the temperature cycle (Goldman et al., 1997). Second, in a highly social species, the activity patterns can be desynchronized or disturbed by social cues (Riccio and Goldman, 2000; Lövy et al., 2013). Displaying a distinct and relatively uniform activity pattern by free-living Ansell's mole-rats across different family

groups (not performed by captive ones housed either singly or in groups) is therefore probably advantageous in terms of survival or energetic balance.

Activity of subterranean rodents includes active digging, which, especially in hard soils, requires a manifold increase in the metabolic rate. This suggests a high potential for heat production and presumably a risk of hyperthermia (Vleck, 1979). We would therefore expect that subterranean rodents would prefer to be active during parts of day when the ambient temperature is lower than average. Exactly the opposite was found in our study of the Ansell's mole-rat. What could be the reason for this behavior?

It is clear that the energetically costly activities, such as excavation of burrows, pushing soil within the burrows, and pushing soil aboveground, are probably not performed throughout the total time an individual spends outside the nest. Exploration or



**Table 2. Relationship of outside-nest activity of the radio-tracked Ansell's mole-rats to environmental variables as revealed by the generalized linear mixed model with binomial distribution.**

|                           | Temperature, °C                    |                                    |                                   |                               |                                  | Light-Dark Cycle                  |
|---------------------------|------------------------------------|------------------------------------|-----------------------------------|-------------------------------|----------------------------------|-----------------------------------|
|                           | Surface                            | 10 cm                              | 20 cm                             | 30 cm                         | 60 cm                            |                                   |
| <b>Breeding M (n = 3)</b> | 5.4 (25.9)<br><i>p</i> < 0.0001*   | 6.3 (30.7)<br><i>p</i> < 0.0001*   | 1.4 (5.9)<br><i>p</i> = 0.016     | 0.7 (1.1)<br><i>p</i> = 0.161 | 0.4 (0.6)<br><i>p</i> = 0.455    | 2.2 (9.1)<br><i>p</i> = 0.002*    |
| <b>Breeding F (n = 2)</b> | 26.5 (90.8)<br><i>p</i> < 0.0001*  | 31.8 (108.7)<br><i>p</i> < 0.0001* | 8.7 (29.7)<br><i>p</i> < 0.0001*  | 0.5 (1.8)<br><i>p</i> = 0.205 | 0.7 (2.9)<br><i>p</i> = 0.123    | 8.7 (29.9)<br><i>p</i> < 0.0001*  |
| <b>M (n = 4)</b>          | 21.6 (179.2)<br><i>p</i> < 0.0001* | 29.0 (241.2)<br><i>p</i> < 0.0001* | 8.8 (71.7)<br><i>p</i> < 0.0001*  | 0.7 (3.4)<br><i>p</i> = 0.056 | 8.2 (66.9)<br><i>p</i> < 0.0001* | 9.1 (74.3)<br><i>p</i> < 0.0001*  |
| <b>F (n = 8)</b>          | 11.7 (211.2)<br><i>p</i> < 0.0001* | 15.5 (279.4)<br><i>p</i> < 0.0001* | 5.0 (89.8)<br><i>p</i> < 0.0001*  | 0.1 (1.3)<br><i>p</i> = 0.176 | 5.2 (93.3)<br><i>p</i> < 0.0001* | 4.0 (71.8)<br><i>p</i> < 0.0001*  |
| <b>P01 (n = 4)</b>        | 22.4 (132.0)<br><i>p</i> < 0.0001* | 32.7 (193.9)<br><i>p</i> < 0.0001* | 11.0 (63.1)<br><i>p</i> < 0.0001* | 0.8 (1.6)<br><i>p</i> = 0.193 | 3.5 (17.9)<br><i>p</i> < 0.0001* | 8.2 (74.3)<br><i>p</i> < 0.0001*  |
| <b>P02 (n = 2)</b>        | 3.5 (16.0)<br><i>p</i> < 0.0001*   | 3.6 (16.6)<br><i>p</i> < 0.0001*   | 0.4 (1.4)<br><i>p</i> = 0.199     | 0.6 (2.5)<br><i>p</i> = 0.109 | 0.1 (0.24)<br><i>p</i> = 0.624   | 2.1 (9.3)<br><i>p</i> = 0.002*    |
| <b>P04 (n = 3)</b>        | 6.2 (44.2)<br><i>p</i> < 0.0001*   | 12.3 (88.3)<br><i>p</i> < 0.0001*  | 6.4 (46.1)<br><i>p</i> < 0.0001*  | 0.2 (1.2)<br><i>p</i> = 0.278 | 1.0 (7.1)<br><i>p</i> = 0.008    | 1.4 (9.6)<br><i>p</i> = 0.002*    |
| <b>P05 (n = 3)</b>        | 21.4 (155.4)<br><i>p</i> < 0.0001* | 22.0 (160.0)<br><i>p</i> < 0.0001* | 4.8 (34.2)<br><i>p</i> < 0.0001*  | 1.1 (6.8)<br><i>p</i> = 0.009 | 0.8 (5.0)<br><i>p</i> = 0.029    | 10.2 (73.6)<br><i>p</i> < 0.0001* |
| <b>P10 (n = 5)</b>        | 18.0 (174.2)<br><i>p</i> < 0.0001* | 20.9 (204.0)<br><i>p</i> < 0.0001* | 5.8 (53.3)<br><i>p</i> < 0.0001*  | 0.9 (4.4)<br><i>p</i> = 0.038 | 6.4 (59.3)<br><i>p</i> < 0.0001* | 6.1 (55.9)<br><i>p</i> < 0.0001*  |

The animals are grouped according to their sex and reproductive status and family group affiliation. For each variable, percentage of explained variability and  $\chi^2$  (in parentheses) is presented. Statistically significant results after Bonferroni correction (*p* < 0.008) are marked with asterisks; df and  $\chi^2$  df in all models are 4 and 1, respectively). F = female; M = male.

**Table 3. Quantification of the effects of the 3 variables on the temporal activity pattern of the Ansell's mole-rat as computed by the variance partitioning technique (Lepš and Šmilauer, 2003) using the redundancy analysis with the data set containing only the first radio-tracking session.**

|  | Explained Variance, % | % of Explained Variance | F         | p           |
|--|-----------------------|-------------------------|-----------|-------------|
| <b>All variables</b>                               | 56 (40)               | 100 (100)               | 1.3 (1.8) | 0.2 (0.006) |
| <b>Partial effect of group affiliation</b>         | 30 (19)               | 54 (47)                 | 1.4 (1.7) | 0.2 (0.04)  |
| <b>Partial effect of sex × reproductive status</b> | 12 (7)                | 21 (17)                 | 0.7 (0.8) | 0.8 (0.7)   |
| <b>Partial effect of body mass</b>                 | 3 (3)                 | 5 (8)                   | 0.5 (1.1) | 0.8 (0.4)   |
| <b>Shared effect</b>                               | 11 (11)               | 20 (28)                 |           |             |

Values in parentheses are computed with the data set containing both radio-tracking sessions.

patrolling the burrow systems, as well as activities such as feeding and carrying small food items into food stores, could also take considerable time. Nevertheless, most digging and soil pushing activities still occurred during a period of high daily temperature at depths of the surface tunnels (J. Šklíba, unpublished data, 2010).

Activity patterns with higher activity in the warmer part of the day and/or lower activity in the colder part are surprisingly common in so far studied rodents

with dominant subterranean activity (see Table 4 for data from the available literature). The burrow temperatures given in the corresponding studies (including the present one) are usually well below the lower critical temperature of the thermoneutral zone of the studied animals (Table 4), which means that activities at these temperatures require an increased energy expenditure, and thus the most plausible explanation for the temperature-related activity patterns seems to be energetic savings on thermoregulation (cf. Benedix, 1994). Comparable laboratory data on strictly subterranean rodents kept under artificial temperature cycles are, to our knowledge, limited to *S. ehrenbergi*, who tended to be more active in the daily period when the ambient temperature was lower (23.9 °C; Goldman et al., 1997). Pohl (1998) published interesting data on 2 less fossorial rodents kept under artificial temperature cycles. While the high temperature-adapted antelope ground squirrel *Ammospermophilus leucurus* performed wheel running activity mainly during the lower temperature part of the day, in the cold temperature-adapted Syrian hamster *Mesocricetus auratus*, the wheel running primarily coincided with the warmer part of the cycle. Another rodent, the pacific pocket mouse *Perognathus longimembris*, displayed arousal from torpor just before a period of elevated temperature (Lindberg and Hayden, 1974). These studies illustrate that entrainment of activity rhythms in rodents by a temperature

**Table 4.** Relationship of activity patterns of free-living subterranean rodents with temperature fluctuation and selected physiological characteristics of studied species connected to their thermoregulatory capabilities.

| Species                             | Source <sup>a</sup> | Physiological Parameters |   |                       | Activity-Related Observations |                             |  |                  |   |
|-------------------------------------|---------------------|--------------------------|---|-----------------------|-------------------------------|-----------------------------|--|------------------|---|
|                                     |                     | Body Mass, g             | Conductance, <sup>b</sup> $\mu\text{L O}_2 \text{ g}^{-1} \text{ h}^{-1} \text{ }^\circ\text{C}^{-1}$ | TNZ, $^\circ\text{C}$ | Source <sup>a</sup>           | Season                      | Mean Daily Range of Burrow Temperature, $^\circ\text{C}$ | n                | Activity Pattern Characteristic   |
| <i>Thomomys bottae</i>              | a                   | 143                      | 62  | 28-30                 | h                             | Summer                      | 22-26  | 8                | Peak coincided with the maximum burrow temperature.                       |
| <i>Geomys bursarius</i>             | b                   | 197                      | 100-190   | 30-32.5               | i                             | Summer                      | 21-24  | 11               | Avoiding both temperature extremes.                                       |
| <i>Spalax ehrenbergi</i>            | c                   | 124                      | 142?  | 28.4-?                | j                             | Winter                      | 13-15  | 8                | Peak between the times of maximum surface and maximum burrow temperature. |
|                                     |                     |                          |   |                       |                               | Summer                      | 31-34  | 3                | Peak coincided with the minimum burrow temperature.                       |
| <i>Spalax galili</i>                | c                   | 104                      | 124?  | 26.4-?                | k                             | Winter                      | 6-8  | 20               | Peak between the times of maximum surface and maximum burrow temperature. |
| <i>Heliophobius argenteocinerus</i> | d                   | 223                      | 77  | 25-33                 | l                             | Cold dry season             | 17-19  | 10               | Positively correlated with the burrow temperature.                        |
|                                     |                     |                          |   |                       |                               | Beginning of hot dry season | 18-22  | 9                | No correlation with the burrow temperature.                               |
|                                     |                     |                          |   |                       | m                             | Peak hot dry season         | 24-26  | 7                | Peak close to the time of the minimum burrow temperature.                 |
| <i>Fukomys damarensis</i>           | e                   | 125                      | 85  | 27-31                 | n                             | Summer                      | 34-38  | Group of 5       | No correlation with the burrow temperature.                               |
| <i>Fukomys mechowii</i>             | f                   | 272                      | 90  | 29-30                 | o                             | Cold dry season             | 18-20  | Group of 5       | No correlation with the burrow temperature.                               |
|                                     |                     |                          |   |                       |                               | Cold dry season             | 18-20  | 1 disperser      | Peak coincided with the maximum burrow temperature.                       |
| <i>Fukomys anelli</i>               | f; g                | 77; 82                   | 120; 144  | 28-32; 30-32.5        | p                             | Cold dry season             | 18-26  | 17 (of 5 groups) | Positively correlated with the burrow temperature.                        |

Burrow temperatures and soil temperatures measured roughly at depth of most foraging burrows are not differentiated.

<sup>a</sup>Sources: a = Vleck, 1979; b = Bradley and Yousef, 1975; c = Nevo and Shkolnik, 1974, and Haim and Izhaki, 1993; d = Zelová et al., 2007; e = Lovegrove, 1986; f = Bennett et al., 1994; g = Marhold and Nagel, 1995; h = Gettinger, 1984; i = Benedix, 1994; j = Rado et al., 1993; k = Hadid et al., 2013, and J. Šklíba and M. Lövy, unpublished data, 2012; l = Šklíba et al., 2007; m = J. Šklíba and M. Lövy, unpublished data; n = Lovegrove, 1988; o = Lövy et al., 2013; p = present study.

<sup>b</sup>Conductance (measure of heat losses) below the lower critical temperature.

cycle may involve both high and low temperature preference/avoidance and may not be limited to only heterothermic rodents.

*Fukomys anelli* has very high thermoregulatory requirements to maintain stable body temperature

below the lower critical temperature, as can be derived from relatively high values of minimal conductance when compared with larger bathyergids and especially with subterranean rodents from other families (see Table 4 and corresponding citations).

This is mostly a consequence of its low body mass and relatively large body surface (similar values of conductance were detected for some other small-bodied mole-rats; see Zelová et al., 2007). Because the mean range of daily burrow temperatures during our radio-tracking study was 18 to 26 °C, the mole-rats had to deal with temperatures below their lower critical temperature (28 °C; Bennett et al., 1994) with the result that the lower the burrow temperatures were, the more energy the animals spent on thermoregulation. This means that at 26 °C and 18 °C, the mole-rats have to increase their energy expenditure by 38% and 190%, respectively, compared with their resting metabolic rate (which is 0.63 ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>; Bennett et al., 1994), just to maintain thermoregulation. We will attain even higher metabolic increases if we consider the lower critical temperature to be 30 °C as reported by Marhold and Nagel (1995). Observed outside-nest activities synchronized with high burrow temperatures are thus favorable from the energetic point of view.

Since the high relative humidity in the burrows of subterranean rodents complicates evaporative cooling, it might be necessary for these animals to be better physiologically adapted to cope with high burrow temperatures than with low temperatures, as they need to spend some time per day digging and pushing soil in surface tunnels. (Subterranean rodents were rarely reported to build food caches large enough to span extended periods of time such as the hot dry season in tropical Africa; Šklíba et al., 2011, and references therein.) The lack of an effective cooling mechanism makes higher temperatures potentially more fatal than lower ones, which can be coped with by torpor (a mechanism also known in *F. anselli*, Marhold and Nagel, 1995), thermogenesis, and various behavioral mechanisms such as building nests with insulating nest bedding and, in social species, huddling in a communal nest. Behavioral mechanisms that reduce overheating, such as resting breaks during digging, cooling in deeper tunnels, and heat dissipation by increasing physical contact with the substrate (cf. Marhold and Nagel, 1995; Zelová et al., 2007), may not be sufficient if not coupled with high conductance above the upper critical temperature. Because conductances below the lower critical temperature and above the upper critical temperature are interconnected (both are influenced, e.g., by fur quality), better physiological adaptation to low temperatures would result in worse adaptation to high temperatures, which are more likely to be fatal in subterranean ecotope. We may therefore expect that daily activity patterns in small bathyergids will probably be more adjusted to avoid the coldest daily temperatures. Published data support this assumption (Table 4), although activity data from the hottest periods of the year are lacking.

The herein described activity patterns in Ansell's mole-rats come from the cold period of the year. Whereas the temperature inside the nests is probably constant year-round, because of depth, communal nesting, and a large amount of bedding (Šklíba et al., 2012), seasonal variation of temperature at shallower depths can be prominent (e.g., Šumbera et al., 2004). Seasonal changes in daily activity pattern of mole-rats would thus be a very effective tool for saving energy on thermoregulation while reducing the risk of overheating. This kind of seasonal change in the activity pattern was described in *S. ehrenbergi* (Rado et al., 1993), and indications of it were also found in the solitary bathyergid *H. argenteocinereus* (Šklíba et al., 2007; J. Šklíba and M. Lövy, unpublished data in Table 4). It is also relatively common in other rodents, such as fossorial *S. cyanus* (Rezende et al., 2003) and less fossorial hairy-tailed Bolo mouse *Necromys lasiurus* (Vieira et al., 2010), Cabrera vole *Microtus cabrerai* (Pita et al., 2011), and the ice rat *Otomys sloggetti* (Hinze and Pillay, 2006). There is a possibility that the activity pattern herein described in *Fukomys anselli* is also transient, confined to the colder part of the year.

Daily temperature fluctuations are substantially buffered, even in top layers of the soil (Bennett et al., 1988), so it might be surprising that even small temperature differences would still affect activity patterns of subterranean rodents. Gettinger (1984) found that peak activity of the pocket gopher *Thomomys bottae* occurred when mean burrow temperature was the highest (≈26 °C), but he considered it unlikely that rest periods were timed to avoid lower temperatures since burrow temperatures during these time periods were "not substantially different (≈22 °C)." Nevertheless, a similarly small daily range of burrow temperatures (4 °C) was later detected in another species of the pocket gopher, *Geomys bursarius*, whose daily activity was even bimodal, avoiding both temperature extremes (Benedix, 1994). Many other field studies of subterranean rodents revealed signs of temperature-affected daily activity patterns under very small daily temperature changes (Table 4). During our study, the mean amplitude of temperature changes at depth of foraging tunnels was relatively large (8 °C) where the surface soil was exposed to the sun but was reduced to only 1.8 °C under dense vegetation (nevertheless, more exposed places dominated in the study area). To estimate the minimal daily temperature difference that would elicit behavioral response, further laboratory experiments are necessary. Captive and usually ad libitum fed laboratory animals are not likely to display as fine-tuned energy-saving behavioral strategies as might be expected under natural conditions, but useful information can be found even in studies of circadian rhythms and their maintenance on account of temperature changes. Goldman et al. (1997)

described a case of 1 captive blind mole-rat who coordinated locomotory activity with a temperature phase even after the temperature differential was decreased to 1.1 °C. Similar temperature amplitude (1.5 °C) was reported to entrain circadian rhythms in *P. longimembris* (Lindberg and Hayden, 1974). In the light of these anecdotal observations, the temperature amplitude between 1.8 and 8 °C detected in the present study seems to be most probably large enough to affect the behavior of the Ansell's mole-rat.

A question remains as to whether the daily activity pattern detected in this species under natural conditions indicates entrainment to a temperature change as a nonphotic zeitgeber or represents masking. This is a challenge for future laboratory studies, but one point should be taken into account—the communal nest of social mole-rats is probably temperature-stable (Šklíba et al., 2012; Šumbera et al., 2012). This means that a change of ambient temperature would hardly trigger activity of an individual that is resting inside. The entrainment of circadian rhythms by temperature changes in the tunnels near the surface thus seems to be more plausible explanation for the observed pattern than masking. Impact of temperature changes on resetting circadian clock was also proposed in *Spalax* (Oster et al., 2002).

In the present study, the group affiliation explained a larger part of the interindividual variability in the activity pattern than did sex × reproductive status and body mass. Members of the same family group thus tended to be more similar in their activity patterns, as visible even in their actograms: For example, group P01 started to be active slightly earlier than group P04 (Fig. 1). Therefore, we would assume that individuals within a group are rather synchronized in their outside-nest activity. Surprisingly, a significant synchronization of the individuals' outside-nest activity was detected only in 2 of the 5 family groups. We would need more frequent activity sampling to address this problem in greater detail. Only an indistinctive synchrony of sleep and wakefulness was detected in captive family groups of *Heterocephalus glaber*, which as a whole did not perform any activity rhythms under either of the applied light regimes (Davis-Walton and Sherman 1994). Members of captive family group of *Cryptomys hottentotus* under constant dark performed asynchronously in their activity cycles (Bennett, 1992).

The activity pattern of *F. anselii* differs markedly from that described in *F. damarensis* (Lovegrove 1988) and *F. mechowii* (Lövy et al., 2013). The latter 2 species were active in multiple short bouts per day, which were only slightly more concentrated into the night hours in *F. mechowii*. Lövy et al. (2013) proposed that the high number of short activity bouts per day could be a byproduct of the use of the communal nest by

many individuals, and therefore more frequent disturbance by other family members, or a consequence of cooperation in work tasks. Similar influence of social contact with other group members on the individuals' activity patterns, which could be viewed as social masking, was illustrated in captive *H. glaber* by Riccio and Goldman (2000). Whereas most of the singly-housed individuals performed clear light-entrained or free-running activity rhythms, individuals monitored while they were housed in a group were mostly arrhythmic. In our study the distinct daily activity pattern performed by Ansell's mole-rat, despite using communal nest, would signify that the effect of the external determinant of activity (i.e., most probably the daily temperature fluctuations) was stronger than in previous radio-telemetry studies of social African mole-rats (Lovegrove, 1988; Lövy et al., 2013). This is not surprising since this species has low thermoregulatory capabilities at low temperatures (see Table 4) and a burrow system mostly void of deep primary burrows (Šklíba et al., 2012), where the temperature fluctuations would be less prominent. We can therefore conclude that social cues in communally nesting mole-rats can disrupt temperature-related daily activity patterns, but probably only if the additional cost of thermoregulation is not too high. Anecdotal support for this is provided by the activity pattern of a dispersing female *F. mechowii* radio-tracked by Lövy et al. (2013). This solitarily living individual displayed a clear activity peak at a time of maximum temperature at depth of the foraging tunnels, unlike the rest of individuals under study, which lived in a family group.

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#### CONFLICT OF INTEREST STATEMENT

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Surprisingly low risk of overheating during digging in two subterranean rodents



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### HIGHLIGHTS

- Two mole-rat species increased body core temperature after digging.
- Surprisingly, both species decreased surface temperature ( $T_s$ ) remarkably.
- The decrease of  $T_s$  was especially distinct on head and trunk regions.

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### ABSTRACT

Capacities for and constraints of heat dissipation are considered to be important factors governing maximum intensity and duration of physical activity. Subterranean mammals are endurance diggers, but because of lack of air currents in their burrows, high relative humidity and other physical constraints, the capacity of common mammalian cooling mechanisms underground is very limited. We analyzed surface and body core temperature changes after digging in soft and hard substrates in two species of African mole-rats (Bathyerigidae, Rodentia); the social giant mole-rat *Fukomys mechowii* and the solitary silvery mole-rat *Heliophobius argentocinereus*. As expected, we observed an increase of body core temperature in both species after digging in both substrates. Surprisingly, and contrary to our expectations, we observed remarkable decrease of mole-rats' surface temperature immediately after the end of the digging trials. This decrease was greater in soft and moister soil than that in hard and drier soil. Our results suggest that mole-rats may effectively avoid overheating in burrows by effective cooling while digging, especially in wet soil. This indicates that burrowing in soils moistened by rains could be easier than previously thought contributing thus to mole-rats success in challenging environment of subterranean burrows.

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### 1. Introduction

Physical activity of animals leads to production of metabolic heat and increase of body temperature. Due to high thermal capacity of water and its high content in tissues, heat can be partly buffered without significant increase of body temperature. Because the increase of body temperature beyond certain critical limit eventually results in irreversible tissue damage and successive death, it is crucial that the heat production and the heat dissipation are in balance. Apart from cardiovascular scope, pulmonary gas-exchange capacity or muscle performance, it is also the heat dissipation capacity that imposes upper

boundary for maximal metabolic rate [1]. Diverse high energy consuming activities such as thermoregulation at low ambient temperatures and locomotion increase metabolic rate substantially. For example, flight might increase metabolic rates up to ten or twelve times in birds or bats respectively (e.g., [2,3]), burrowing through solid substrate about five times in the degu (*Octodon degu*) [4], and cold stress up to almost 13 times in the kowari (*Dasyuroides byrnei*) [5]. One of the largest energetic increases in mammals was found in high speed running horses with almost 32 fold metabolic increase [6]. However, smaller mammals are not able to increase their metabolic rate to more multiples of their resting metabolic rate because the extent of metabolic rate increase is positively correlated with the animal weight (reviewed in [7]).

Among mammals, subterranean mammals live and forage in self-constructed underground burrows facing very challenging living conditions. Similar selection pressures on subterranean mammals of different phylogenetically unrelated taxa have induced convergent evolution of

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morphological, behavioral, and physiological adaptations [8–11]. Important eco-physiological adaptations for life underground include low resting metabolic rate, high conductance, and low body temperature [8,10]. The underground ecotope is characterized by low food availability, high relative humidity, and necessity to dig through mechanically resistant substrate (cf. [11]). In subterranean rodents, digging of new burrows in order to find food and sexual partner and to disperse is probably the most energy consuming activity because the ratio of the digging metabolic rate to resting metabolic rate ranges up to 5.0 [see [12] for a review]. Remarkably increased heat production probably requires effective heat dissipation to avoid body overheating and risk of death.

However, sealed burrows of subterranean mammals are remarkable for high relative humidity, absence of ventilation, and stable temperature [13]. These factors together with usually good insulating pelage bring subterranean dwellers severe problems regarding heat dissipation and body cooling [8,9,14]. Common cooling mechanisms typical for aboveground mammals thus do not work effectively here. Heat loss by water evaporation is limited by high relative humidity in burrows and heat loss by radiation is limited by reduced or missing body appendages in subterranean mammals. The only efficient heat loss pathway is supposed to be conduction by soil. Conductive heat loss is enabled by high conductance of most subterranean mammals (e.g., [15,16]). It is expected that vasodilatation in some bared body parts such as feet or rhinarium could enable heat dissipation [8]. Interestingly, it was shown that thin and short pelage on the ventral side of the body probably play a key role in exchanging heat in two species of African mole-rats [17]. This body part was found to be important for heat dissipation also in other mammals [18–21]. However, the question arises how effective dissipation of metabolic heat might be just after energetically demanding activity such as excavation of new burrows in thermally challenging environment such as in closed humid burrows.

The aim of this study was to analyze temperature changes in two similarly sized subterranean rodent species with different social systems, the solitary silvery mole-rat (*Heliophobius argenteocinereus*) and the social giant mole-rat (*Fukomys mechowii*). In particular, we focus on differences in body temperature and surface temperature after burrowing in two different substrates, hard and soft. We predicted that (a) body temperature will increase substantially in both species during digging; (b) surface temperature will increase during digging on less haired body regions, especially on the ventral side of the body and on feet, in order to facilitate heat loss; (c) increase of surface temperature will be greater during digging in hard substrate than that in soft one because of higher mechanical resistance of the former; and (d) increase of surface temperature will be smaller in the solitary *H. argenteocinereus* than that in the social *F. mechowii* because its denser and longer pelage prevents effective cooling (cf. [17]).

## 2. Materials and methods

### 2.1. Tested animals

Seven adult giant mole-rats *F. mechowii* (five males and two females; mean weight  $399 \pm 93$  g) and seven adult silvery mole-rats *H. argenteocinereus* (four males and three females;  $239 \pm 56$  g) were analyzed. Both species originate from the Zambezi region in central and eastern Africa, and they live in similar habitats (grassland, woodland, and agricultural fields). Mole-rats were born or kept for at least 4 years in captivity and were accustomed to handling. Parental giant mole-rats were captured in Ndola (Zambia) and the wild-born silvery mole-rats in Blantyre-Limbe and Mulanje (Malawi). Giant mole-rat families were housed in large terrariums with a home box and plastic tubes. Silvery mole-rats lived in systems of interconnected Perspex tunnels with two home boxes. Horticultural peat was used as bedding and tissue paper as nest material. Mole-rats were fed on carrots, potatoes,

apples, lettuce, and rodent pellets. All experiments on animals were approved by the Ethical Committee of the University of South Bohemia.

### 2.2. Measurements of temperature

The body temperature was measured by a digital thermometer (Thermalert TH-8, Physitemp Instruments, Inc.) inserted at least 2 cm into the rectum. The surface temperature ( $T_s$ ) of four defined body parts (head, trunk = dorsum + flanks, ventrum and feet) was determined from infrared images (see below) of the body surface from dorsal, lateral, and ventral side, captured perpendicularly to avoid possible errors due to angle distortion [see [17]]. For the evaluation, the mean temperature of the respective body parts was taken. Eyes, ears, and nose were excluded from the head temperature measurements.

Infrared images (thermograms) of the body surface were obtained by a thermographic camera AGA 570 (Agema infrared systems AB, Danderyd, Sweden, long wave,  $7.5\text{--}12\ \mu\text{m}$ , 24 FOV lens). To obtain correct values of surface temperatures by compensating for the effects of different radiation sources, the following parameters were supplied for the camera: emissivity of the subject, reflected temperature, distance between the subject and the camera, and relative humidity. Air temperature, relative humidity, reflected temperature, and air flow rate were recorded by thermometers TESTO 415, 615, and pyrometer TPT 64+. All thermograms were evaluated using analytical software (Irwin 5.3.1, Agema infrared systems AB, Danderyd, Sweden).

### 2.3. Experimental design

The Perspex tunnel system consisted of a starting chamber ( $75 \times 75 \times 300$  mm), separated from the digging tunnel ( $75 \times 75$  mm square cross-section) by a partition. The tunnel was filled with one of the two tested types of substrate: compressed peat (water content  $29 \pm 3.5\%$ ) simulating soft soil or dried clay (water content  $9.5 \pm 1.8\%$ ) simulating hard soil. The tunnel length was 60 cm (for hard substrate) or 90 cm (for soft substrate).

Tested animals were removed from their housing setting and let to acclimatize individually in boxes ( $40 \times 23$  cm) with food and bedding for 1 day before the experiment. Then the body temperature and the surface temperature of mole-rats in the rest were measured, and the animal was put into the starting chamber of the digging system and let to calm down for another 30 min. After this period, the partition between the starting chamber and the digging tunnel was removed and the animal was allowed to dig for 15 min or alternatively until it reached the end of the tunnel. The animals usually started to dig immediately. At the end of the trial, the animal was removed from the tunnel and post-digging body, and surface temperatures were measured immediately. All temperatures were measured three times, during resting and after digging in soft and hard soil.

The ambient temperature and humidity during the whole experiment was  $23.7 \pm 0.5$  °C and  $43.6 \pm 2.0\%$ . The temperature of hard and soft soils was  $22.3 \pm 0.3$  °C and  $22.2 \pm 0.8$  °C, respectively.

### 2.4. Statistical analysis

Because there was no effect of mole-rat weight or sex on resting body temperature in both species (ANCOVA; Wald statistics = 0.286;  $df = 1, p = 0.59$ ; and Wald statistics = 0.344;  $df = 1, p = 0.56$ , respectively), the effect of these predictors was not considered in the following test. Body temperatures while resting, after digging in soft and hard substrate were analyzed in both species by repeated measures two-way ANOVA. Within-factor differences were identified by the Tukey HSD test (Statistica 10).

We analyzed differences in surface temperatures prior to and after digging (dependent variable) by mixed-effect mode using the R 2.14.1 (R development core team, package lme4.), assuming the Gaussian distribution (link identity). First, we extended the null model to include



one of independent variables (body mass, sex, species, substrate type, ambient temperature, digging time, digging distance, resting body temperature, and post-digging body temperature) and tested which of them improved the model in the best way—using the Akaike information criterion and *F*-statistic. Subsequently, we extended this model by inclusion of another variable in the same way as described above and we continued until there was no variable left which would significantly improve the model. Each individual was treated as a random factor. Data are presented as mean  $\pm$  SD.

### 3. Results

Mean body temperature of resting *H. argenteocinereus* was 35.1 °C, i.e., 1.1 °C higher than that of *F. mechowii* (34.0 °C, ANOVA;  $F = 10.76$ ;  $df = 1,37$ ;  $p = 0.002$ ; Fig. 1). After digging trials, body temperature increased in both species (repeated measures ANOVA;  $F = 21.48$ ;  $df = 2$ ;  $p < 10^{-5}$ ), depending on the substrate type. The relative increase was 0.3 °C (Tukey HSD test;  $p = 0.44$ ) and 0.7 °C (Tukey HSD test;  $p = 0.058$ ) for *H. argenteocinereus* and 1.0 °C (Tukey HSD test;  $p = 0.005$ ) and 1.7 °C (Tukey HSD test;  $p < 0.0001$ ) for *F. mechowii* in the soft and hard substrate, respectively (Fig. 1).

In contrast to body temperature, the mean post-digging surface temperature decreased after digging in both substrates in all analyzed body regions in both species (Table 1). The decrease was greater in soft substrate than that in hard one and varied by the body part; it was greater on trunk and head than on ventrum. About 13% of observed variability of changes in surface temperature was explained together by following three factors: body part, type of substrate, and resting body temperature (see Table 2 for digging times, distances and speeds and Tables 3 and 4 for model parameters and estimates). Inclusion of factors such as species, body mass, sex, digging time, digging distance, post-digging body temperature, and ambient temperature did not improve the model significantly in terms of the Akaike information criterion.

### 4. Discussion

In our study, we found that body temperature of mole-rats of both species increased after digging. Contrary to our expectation, surface temperature of all observed body parts sank remarkably during digging in spite of the increase of body temperature. This is really a surprising finding, suggesting that mole rats employ an effective means of avoiding overheating by surplus of metabolic heat.

The increase in body temperature during intensive physical activity is a common phenomenon in mammals (e.g., [12,22,23]). Aerobic heat production is a function of oxygen consumption; for each liter of oxygen, about 20 kJ of heat is produced [24]. In our previous study on both species, oxygen consumption (and associated heat production) increased during digging in soft and hard substrates about five times

compared to resting values [12]. Similarly to results of the present study, both species reached almost the same body temperatures after digging in the hard substrate in spite of the fact that resting body temperature of *H. argenteocinereus* was higher (cf. [12]). Greater increase of body temperature in *F. mechowii* after digging can be explained by its lower body temperature before trial. Lower body temperature at rest is probably related to its inability to keep stable body temperature at decreasing ambient temperatures. It is known that its body temperature decreases when exposed to temperatures below lower critical temperature compared to silvery mole-rat which keeps the same body temperature even in temperatures deep below LCT (see [16,25]). In addition, in ambient temperatures similar to those in which we have carried out our experiments (23.7 °C), *F. mechowii* has higher mass specific oxygen consumption than *H. argenteocinereus* (and thus increased heat production) (cf. [16,26]). After digging in hard substrate, the mean post-digging body temperature was around 35.7 °C in both species. Interestingly, this temperature is close to the upper critical temperature of *H. argenteocinereus* (35.3 °C; [16]). This indicates that this temperature could be a threshold above which the body temperature is actively regulated to avoid high body temperatures.

It is possible that part of the heat produced during activity of mole-rats (exercise heat) can substitute part of the heat produced to maintain stable body temperature (thermoregulatory heat). This substitution may effectively decrease the energetic cost of locomotion and activity in temperatures below their thermoneutral zone. Chappel et al. [27] observed almost 50% energy savings on locomotion in the running deer mouse at 10 °C. Similar effect was observed in the laboratory rat [28] or a bird, the verdin, *Auriparus flaviceps* [29]. On the other hand, substitution of thermoregulatory heat by exercise heat was not found in the Merriam's chipmunk *Neotamias merriami* [30] or in the fossorial Talas Tuco-tuco *Ctenomys talarum* [31]. Mole-rats and other strictly subterranean mammals with generally limited food resources [11] spend most of their life in temperatures below lower critical temperature [32]. For example, in both species under our study, the difference between lower critical temperature and temperature of soil in depth of the foraging burrows was more than 10 °C [33,34]. Any thermoregulatory heat that can be substituted by exercise heat will reduce the energetic cost and thus enable more effective digging and foraging.

Our current knowledge of surface temperatures in mammals after intensive physical activity is based mainly on sport and veterinary medical research [e.g., [35–37]]. Generally, the surface temperature of homoiothermic mammals is very tightly related to subcutaneous blood flow and pelage properties. Body surface acts as a heat exchanger between the body and the environment. In mammals, the body surface is usually covered by pelage, which has an important thermoregulatory function determined by length and density of hairs. Exposing of the less insulated and well-vascularized body parts, which serve as thermal windows, enables heat dissipation, especially if those areas are relatively large [38]. Blood flow directed into these parts usually results in heat loss and thus serves as an effective cooling mechanism. In subterranean mammals, the ventral part of the body may serve as the main heat window [17,39], but in burrowing mammals also some smaller areas such as feet may play role as it has been shown in the Woodchuck *Marmota monax* [40]. It was predicted that feet and some other bare body areas are important for heat dissipation in subterranean mammals (cf. [8, 41]). Among these areas, feet could be very important in haired mole-rats especially if we consider they could be in direct contact with the soil while digging. However, it is known that the regulation of surface temperature in small mammalian species (<1 kg) is limited and its contribution to changes of heat flux in these mammals is considered to be low [42]. On the other hand, mole-rats' feet are relatively large and fine regulation of blood flow through the foot skin can be expected (cf. [8]). Nevertheless, the role of feet in thermoregulation in subterranean mammals and African mole-rats particularly should still be tested.

It is assumed that subterranean mammals after intensive digging activity, move to colder (usually deeper) tunnels to dissipate metabolic

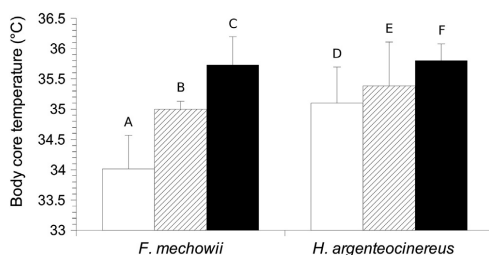


Fig. 1. Body temperature (mean  $\pm$  SD) of *Fukomys mechowii* and *Heliophobius argenteocinereus* during the rest (white bars) and after digging trials in soft (gray bars) or in hard substrate (black bars). Following differences were found significant at probability levels: C > B, B > A, C > A at  $p < 0.05$ ; F > D at  $p < 0.06$ ; D > A at  $p < 0.01$  (single sided Tukey HSD post hoc test).

**Table 1**

Mean resting surface temperatures ( $T_s$ ; in °C) of analyzed body areas and their relative changes (°C) after digging in soft and hard substrate in *Heliophobius argenteocinereus* and *Fukomys mechowii*. Data are presented as mean ± SD.

| Body part | <i>Heliophobius argenteocinereus</i> |                               |                   | <i>Fukomys mechowii</i> |                               |                   |
|-----------|--------------------------------------|-------------------------------|-------------------|-------------------------|-------------------------------|-------------------|
|           | Resting ( $T_s$ )                    | Change of $T_s$ after digging |                   | Resting ( $T_s$ )       | Change of $T_s$ after digging |                   |
|           |                                      | In soft substrate             | In hard substrate |                         | In soft substrate             | In hard substrate |
| Trunk     | 26.1 ± 0.9                           | -5.0 ± 0.7                    | -2.9 ± 1.6        | 28.4 ± 1.2              | -3.8 ± 1.4                    | -1.7 ± 1.4        |
| Ventrum   | 30.5 ± 1.3                           | -2.3 ± 1.3                    | -1.8 ± 1.5        | 30.2 ± 1.5              | -1.8 ± 1.3                    | -0.8 ± 1.3        |
| Head      | 27.1 ± 1.0                           | -4.4 ± 1.9                    | -3.1 ± 1.3        | 28.3 ± 1.1              | -4.0 ± 1.4                    | -1.8 ± 1.2        |
| Feet      | 26.0 ± 2.8                           | -3.2 ± 2.3                    | -2.3 ± 4.3        | 26.5 ± 2.0              | -3.4 ± 2.2                    | -1.6 ± 1.4        |

**Table 2**

Digging time, distance and speed in soft and hard substrate of *Fukomys mechowii* and *Heliophobius argenteocinereus*. Data are presented as mean ± SD.

|                                      | N | Soft substrate     |                       |                                       | Hard substrate     |                       |                                       |
|--------------------------------------|---|--------------------|-----------------------|---------------------------------------|--------------------|-----------------------|---------------------------------------|
|                                      |   | Digging time (min) | Digging distance (cm) | Digging speed (cm·min <sup>-1</sup> ) | Digging time (min) | Digging distance (cm) | Digging speed (cm·min <sup>-1</sup> ) |
| <i>Fukomys mechowii</i>              | 7 | 13.4 ± 2.4         | 72.1 ± 20.4           | 5.4 ± 1.5                             | 14.2 ± 1.3         | 22.9 ± 9.1            | 1.6 ± 0.7                             |
| <i>Heliophobius argenteocinereus</i> | 7 | 13.4 ± 2.7         | 77.1 ± 18.9           | 6.2 ± 2.7                             | 13.8 ± 1.5         | 31.1 ± 17.1           | 2.6 ± 0.9                             |

heat by pressing their body to burrow ground [8,39]. During experiments, we did not observe animals to stop digging or to press the ventrum to the ground for remarkable period. Nevertheless, while digging animals are in direct contact with colder soil and after digging, they have colder surface than at the beginning of digging. Considering that other thermoregulatory options (water evaporation or heat radiation) are very limited underground, the substantial decrease of surface temperature (instead of its increase) is really surprising and unexpected. This suggests that some very effective mechanism of cooling is employed during digging.

Pronounced decrease in surface temperature on all parts of the body may be explained in two different ways and/or their combination. First, it is known that giant and silvery mole-rats do not adjust their digging efforts to substrate quality but rather dig with the same oxygen consumption in both substrates [12]. In order to supply working muscles with oxygen during digging, the blood flow can be directed into muscles by arteriolar vasodilatation which is induced by sympathetically released norepinephrine and is mediated by  $\beta$ -adrenergic receptor. Simultaneously, norepinephrine induces peripheral vasoconstriction mediated by  $\alpha_1$ - and  $\alpha_2$ -adrenergic in the skin [43]. This vasoconstriction results in decreased blood flow through the skin and thus in decrease of the surface temperature [22,44]. Decrease in skin temperature not associated with perspiration after starting physical activity has been recorded also in the rat [45], the human [36], and the horse [37], and it was explained by restriction of blood flow into skin after start of physical activity.

Second, the substrate through which the mole-rat is digging can effectively and directly remove most of the excess heat from the animal by thermal conduction or by convection by passing colder soil against their bodies. The smallest decrease of surface temperature (i.e., the smallest difference between the starting and final surface temperatures) was measured on the ventral part of the body which is in direct contact with the floor of tunnels or with transported soil. Note that mole-rats dig with their incisors, move loosened soil under body, and kick it with their hind feet backwards. Since abdominal pelage is rather thin and feet are almost naked, and since these body parts are most exposed to the direct contact with soil, we may expect that most body heat gets lost through these body regions. The situation is somehow similar to that in diving mammals, where the excess of heat is quickly removed by ubiquitous water with high thermal conduction and capacity [46]. Digging in softer soil resulted in a more pronounced decrease of surface temperature in both species than digging in harder substrate. Regarding the fact that there is no difference in metabolic costs of digging in different substrates [12], we attribute this difference to higher content of water in softer substrate because water and soil have higher thermal

capacity and can better absorb heat [47]. In mole-rats, high heat losses during digging in wet substrate compared to dry substrate were observed in *Heterocephalus glaber* [48]. Unfortunately, our experimental design did not allow differentiating between effects of soil hardness and soil humidity.

When we tested the effect of species and substrate type on decrease of surface temperature on trunk only, both factors were evaluated as additively significant with greater decrease in *H. argenteocinereus* and soft substrate (ANOVA,  $F(1,24) = 5.419, p = 0.028$ , and  $F(1,24) = 17.465, p = 0.00033$ , respectively). Although the GLM model, which was used in this study, did not identify the effect of species as significant in all four tested body areas, there were greater decreases of surface temperatures in well-haired body areas in both species after digging (see Table 1). The most peripheral layer of pelage, i.e., area which is actually measured by the infrared camera, is in the direct contact with the colder substrate resulting in its immediate cooling. During digging and also during rest the heat moves from the skin in the peripheral direction heating thus the pelage. We may assume that rate of heat penetration and the surface temperature increase are negatively correlated with the pelage insulating properties. This mechanism may not only explain the observed greater decrease of surface temperature in the relatively more haired trunk and head in both species, but also the tendency to greater surface temperature decreases in the *H. argenteocinereus*, i.e., a species with longer and thicker pelage.

The finding that burrowing mole-rats are able to cool down very efficiently or at least avoid overheating and that this capability is governed by the substrate properties (mainly by its water content and associated thermal capacity) is very intriguing. It exemplifies that mole-rats—and probably subterranean rodents in general—are well adapted to underground existence also with regard to overheating during digging, and that overheating is apparently a less substantial stress

**Table 3**

Linear mixed models (and their Akaike information criterion, AIC) used to explain variability in observed surface temperature.  $\chi^2$ , df, and  $p$  denote values of these parameters of the specified model compared to its simpler predecessor. The last model was used to explain variability in observed surface temperatures.

| Model variables                           | AIC   | $\chi^2$ | df  | $p$               |
|---|-------|----------|-----|-------------------|
| Null model                                | 468.9 |          |     |                   |
| Substrate type                            | 448.7 | 22.2     | 1,4 | <10 <sup>-7</sup> |
| Substrate type + body part                | 429.9 | 24.7     | 3,7 | <10 <sup>-4</sup> |
| Substrate type + body part + $T_{B,rest}$ | 426.8 | 5.1      | 1,8 | <0.023            |

**Table 4**

Estimates of model variable effects in the final linear mixed model (see Table 3), which explain the variability in observed changes of post-digging surface temperature relatively to resting surface temperature. Surface temperature of the head part, digging in hard substrate and resting body temperature ( $T_B = 0$  °C are included in the intercept of the model.

|                               |               | Estimate | SE    | t-value |
|-------------------------------|---------------|----------|-------|---------|
| Intercept—head/hard substrate |               | 27.90    | 13.06 | −2.14   |
| Body part                     | Trunk         | −0.025   | 0.38  | 0.07    |
|                               | Ventrum       | 1.68     | 0.38  | −4.44   |
|                               | Feet          | 0.73     | 0.38  | −1.94   |
| Substrate type                | Soft          | −1.48    | 0.27  | 5.55    |
|                               | Resting $T_B$ | −0.88    | 0.38  | 2.34    |

factor than hitherto assumed. It is well known that mole-rats and subterranean rodents in general increase digging activity after the end of drought, at the beginning of rainy season [49–52]. This used to be attributed to higher digging efficiency in soil softened by rains, increased food supply after rains, or need to replenish oxygen in their tunnels. Our findings suggest that extensive digging in that period might also be enabled by increased efficiency of cooling by soil due to higher water content. Further experiments should be designed to test the effect of different soil water content upon cooling and to estimate the heat flux through selected body parts to the environment

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