Kairomone-guided food location in subterranean Zambian mole-rats (*Cryptomys* spp., Bathyergidae)

Simone LANGE*, Birgit NEUMANN, Petra HAGEMEYER and Hynek BURDA

University of Duisburg-Essen, Department of General Zoology, Faculty of Biology and Geography, Universitätsstraße 5, 45117 Essen, Germany; e-mail: lange_simone@web.de

Received 31 January 2005; Accepted 2 September 2005

A b s t r a c t . A recent study (H e t h et al. 2002) challenged the idea of “blind” foraging in herbivorous underground dwellers by showing that subterranean rodents of several species use olfaction to discriminate between soils in which plants had or had not been growing. Here we address additional questions about odour-based foraging underground. We tested responses of Zambian mole-rats (*Cryptomys anselli* and *C. kafuensis*) to putative carrot kairomones using tunnel T-mazes. Mole-rats distinguished peat moistened with hydroponic as well as filtered hydroponic “carrot water” from peat moistened with distilled water. Furthermore, mole-rats detected carrot kairomones that percolated over the course of a week through the soil to a distance of 30 cm. These results demonstrate that 1) Attractiveness of soil is given by contents of primary root kairomones not caused by microbial activity in planted soil. 2) Carrot-kairomones are water-soluble molecules of less than 0.6 μm diameter. 3) Carrot-kairomones diffuse around the plant, making plants detectable from a distance.

Key words: subterranean rodents, kairomones, food odours, olfaction, foraging, *Cryptomys*

Introduction

Subterranean rodents spend their life underground and feed exclusively on roots, bulbs and tubers found during burrowing. Foraging requires digging long distances through solid soil, a process with high energetic costs (V l e c k 1979, L o v e g r o v e 1989). Even so, underground dwellers have commonly been thought to search for food randomly and “blindly”, i.e. without using any sensory cues, a hypothesis which strongly contradicts the optimality principle. Blind foraging seemed likely because hearing and vision are ineffective in foraging for bulbs underground, and taste and touch are only effective in direct contact. Furthermore, smell was not considered to be effective for detecting plants at distances of more than 10 cm (in the case of naked mole-rats, B r e t t 1991), although professional foraging with pigs or dogs for truffles growing under the ground at depths of about 30 cm has been documented.

A recent study revealed that subterranean rodents of several species were able to discriminate by smell between plain soil and soil in which plants such as carrots had been growing (H e t h et al. 2002). The animals dug in the soil that contained substances (exudates) released by roots of growing plants. These chemical cues can thus be called “kairomones”, i.e. allelochemicals (emitter and receiver belonging to different species) mediating an interaction that only benefits the receiver (cf., D i c k e & S a b e l i s 1988).

The study by H e t h et al. (2002) demonstrated that soil in which plants had been growing, but from which the roots had been removed, contained biologically active kairomones. The soil was a complex naturally occurring mixture containing many microorganisms and thus the plant exudates (i.e. primary kairomones) could have been affected by unspecific microbial activity that created secondary kairomones.

The current study was conducted to determine whether mole-rats respond to odorous molecules that had not been affected by microorganisms (i. e. primary kairomones) and whether kairomones diffuse around the root to distances greater than 10 cm, enabling detection of kairomones farther from the plant. We used an experimental design that would facilitate chemical-analytical detection of kairomones in later studies.

Materials and Methods

Animals

Mole-rats of two sibling species (Ansel's mole-rat, *Cryptomys anselli,* and Kafue mole-rat, *Cryptomys kafuensis*) and their hybrids (family Bathyergidae) served as subjects in the experiments. Individuals of both sexes were captured in the field (central Zambia) and brought to the laboratory or were born in captivity. Families consisting of 2–15 animals were housed in a windowed animal room in the natural dark-light cycle, at room temperature of about 22°C in glass terrariums of varying size, depending on the size of the family, on a thick layer of horticultural peat. Additionally, structures like bricks and stones were provided. Under these conditions they were able to burrow regularly. Captivity-born animals were fed with potatoes and carrots *ad libitum*. Lettuce, apples and cereals were provided on a weekly basis. Wild-captured animals were fed with potatoes and sweet potatoes *ad libitum*. Mole-rats do not drink free water. Tested adult animals of both sexes were either familiar with carrots (i.e. captive-born were "carrot-experienced" animals) or unfamiliar with carrots (i.e. wild-captured were "carrot-naïve" animals). In each condition, every animal was tested only once. Animals had a break of at least one week between different tests. The representation of particular species and sexes in each of the four experiments (experimental versus control trials) was comparable. For the numbers of animals tested see Table 1.

Experimental design

Two types of experiments were conducted, designated here as "kairomones in hydroponics" and "kairomones in peat".

"kairomones in hydroponics": Tests were conducted in a transparent Perspex T-maze of tunnels with square cross-section 7 cm x 7 cm. It consisted of an open runway (20 cm long) starting from a start box (20 cm x 20 cm) and leading to a 40 cm crossbar (Fig. 1). Each arm of the crossbar (20 cm long) was filled with 12 cm of peat taking care to avoid mixing it (cf., Heth et al. 2000). Before filling, the peat was moistened – in each arm with the same volume of liquid – on one side with distilled water and on the other side with either a) hydroponic "carrot water" or b) filtered "carrot water". The substrate was evenly blended with the carrot water, so that there was no concentration gradient in the horizontal plane of digging. The carrot water was obtained by letting a bunch of 5–7 carrots (with a total mass of 600 g) grow in 3.5 l water for three days; the filtered carrot water additionally was filtered with membrane filters of the pore size 0.6 μm. The carrot water was stored at 8 °C and no longer than one week; it was warmed to room temperature before testing. The test or control arms as well as the compass orientation of the whole maze were selected randomly. The open runway allowed the animals to sniff at the peat prior to digging. The tests were performed as follows: an animal was put into the start box for 2 minutes of adaptation to the apparatus. Then the door to the runway was opened and the animal moved – mostly immediately – to the crossbar. Latency time and total time were recorded. The trial was finished when the animal dug into one arm of the T-maze with the length of its entire body. After each trial, the start box and the maze were thoroughly washed with hot water, cleaned with MELISEPTOL and dried with tissue paper. During trials the experimenter wore disposable rubber gloves to avoid any odorous contamination.

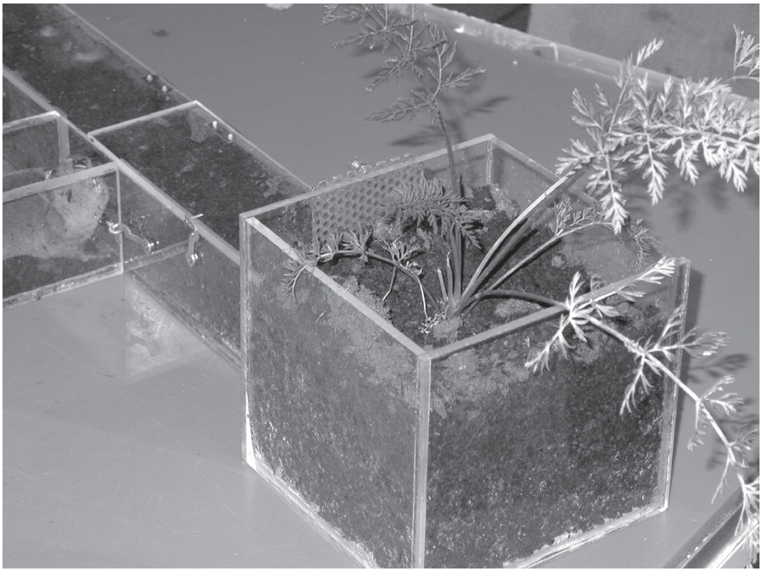

Fig. 1. T-maze with growing carrot during experiment (photo: S. Lange).

"kairomones in peat": The same T-maze was used. Additionally, at the end of one arm, a box (20 cm x 20 cm) was attached. One week before testing, a single carrot was planted in the box filled with peat (assuming that root exudates would spread away from the root) (Fig. 1 and 2). The other arm was filled with peat moistened with distilled water to have a comparable moisture. The distance between the carrot and the middle of the T-maze, i.e. the "choice" point, was either a) 30 cm or b) 50 cm (when each arm was extended by a 20 cm Perspex tunnel). The test itself was performed in the same way as described above.

We recorded whether the animals dug through the peat with the carrot odour or the peat moistened with distilled water. Experiments were performed throughout the year. All the trials for a particular condition were conducted by only one person.

Ethical note

All efforts were taken to minimize stress of the animals. Animal husbandry and all experimental procedures complied with Zambian and European Community regulations on the care and use of experimental animals. The behavioural experiments were consistent with situations encountered by animals during housing and associated handling and did not require special permission. Export and capture permits were issued by the Zambian Wildlife Authority (No. 014508 & 009534). Permits for import and keeping of mole-rats at the University of Duisburg-Essen were issued by the Bezirksregierung Düsseldorf.

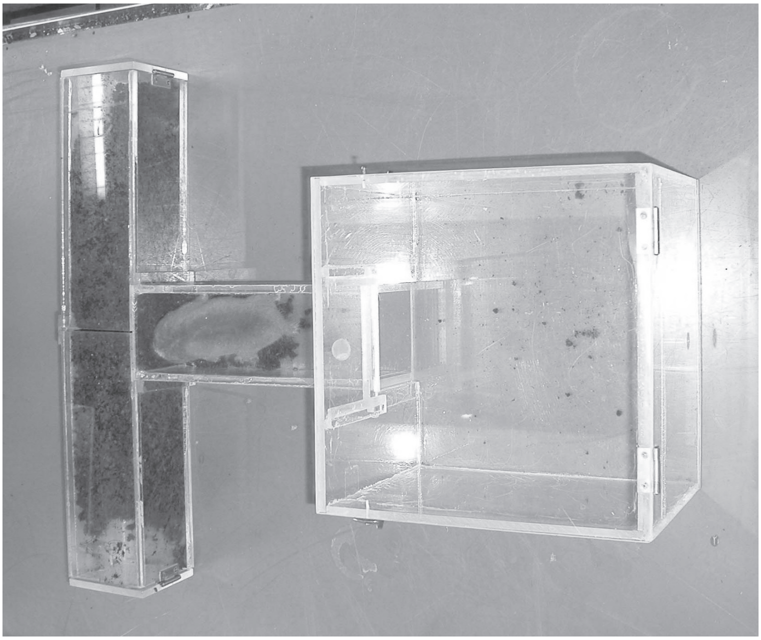

Fig. 2. Mole-rat in the test apparatus encountering the peat (photo: S. Lange).

D a t a   a n a l y s i s

The one-tailed χ^2-tests were performed to evaluate the null hypotheses that mole-rats chose their direction in the T-mazes randomly (50 : 50) against the alternative hypothesis that they would dig in the direction of the carrots´ exudates. The results from the two sibling species and their hybrids were pooled to increase the sample size. This is justified because: Both sibling species have been identified on genetic and geographic grounds. Morphologically, behaviourally and ecologically they are indistinguishable and were previously considered to belong to a single species (B u r d a et al. 1999). Being identical in their ecologies, particularly in foraging strategies and diet choice, and sharing the same challenges and problems of the subterranean way of life, there is no reason to assume that there would be species-specific differences in motivation or sensory capacities. Additionally, a χ^2-test (Pearson) was used to test whether captive-born "carrot-experienced" mole-rats behaved differently from wild-captured "carrot-naïve" animals towards hydroponic water. Statistical software SPSS 11.0 was used.

Results

In most cases, as soon the door of the start box was opened, the animal proceeded directly down the runway to the peat (Fig. 2). The mean latency time was 7.1 s +/- 9.5 s (n = 168), between 0 s and 50 s. They sniffed intensively at both sides of the exposed peat (fast breathing through moving nostrils). Then they started to dig straight away pausing regularly and sniffing at both sides again. As soon as they began digging in one arm of the T-maze, most animals dug till its end. The mean trial duration was 261.2 s +/- 141.6 s (65 s-735 s; n = 168). In a few rare cases, animals first dug in one and then changed to dig in the other arm. The trial ended when the animal dug through the 12 cm of peat of one arm (so that they entered it with their whole body length). Animals did not appear to take soil into their mouth during the trials.

Both “carrot-experienced” and “carrot-naïve” mole-rats preferred to dig in peat moistened with hydroponic carrot water as opposed to peat moistened with water only (one-tailed χ^2-test, experienced: $\chi^2 = 14.969$, $p < 0.05$, naïve: $\chi^2 = 3,903$, $p < 0.05$, cf., Table 1). There was no significant difference in the responses of “carrot-experienced” and “carrot-naïve” animals (χ^2-test (Pearson), $\chi^2 = 1.066$, NS).

Mole-rats dug in peat moistened with filtrated carrot water significantly more than in neutral peat (one-tailed $\chi^2 = 3.33$, $p < 0.05$, cf., Table 1).

In the “hydroponics in peat” experiment, the animals dug significantly more in the arm that had the carrot growing in the attached box when the carrot grew at a distance of 30 cm (one-tailed $\chi^2 = 8.533$, $p < 0.05$, cf., Table 1). They did not show differential digging when the distance was 50 cm (one-tailed $\chi^2 = 1.58$, NS, cf., Table 1).

Table 1. Overview of the results of all performed experiments (df = 1, exper. = carrot-experienced, naïve = carrot-naïve, asterisk = statistically significant p-values < 0.05).

Type of experiment	Animals	n	Proportion „pro-carrot” vs. „contra-carrot” choices	Asymptotical χ^2-probability for p = 0.05 (one-tailed)
„kairomones in hydroponics”				
a) hydroponic water	naïve	31	21 : 10	0.024*
	exper.	46	36 : 10	0.000*
b) filtered water	exper.	30	20 : 10	0.034*
„kairomones in peat”				
a) 30 cm	exper.	30	23 : 7	0.002*
b) 50 cm	exper.	31	19 : 12	0.105

Discussion

The purpose of the study was to determine whether mole-rats respond to kairomones presented in different conditions than in the previous study (H e t h et al. 2002). Two different mole-rat species and their hybrids were tested in two types of experiments:

The “kairomones in hydroponics” experiment showed that kairomone molecules are water soluble (as they pass into the water phases) and have a certain maximum size. Because the peat was moistened immediately before experimental trials, the positive response showed that mole-rats detect kairomone molecules that had not been subject previously to activity of soil microorganisms. Hydroponic water may be further chemically analyzed in the future, and this kind of experiment may be used as a bioassay to test diverse fractions at diverse dilutions.

The “kairomones in peat” experiment simulated a slightly more natural situation in that it enabled a build-up of a natural gradient in a horizontal plane. Nevertheless, the particular absolute values of the result (a carrot root planted for one week in peat produced detectable concentration at the distance of 30 cm) cannot be extrapolated directly to natural situations, as different plants in different soil types of different density and moisture produce root exudates in different amounts and thus the maximum distance at which a certain concentration could be detected may be very different. This experiment demonstrates, however, that a carrot produces a detectable concentration at a distance of 30 cm after a week. In the same time, the carrot given comparable water did not produce a sufficient kairomone concentration to be detected by mole-rats consistently at a distance of 50 cm. However, the exact proportions demonstrate that the difference between the significant proportions the hydroponic water experiment (naïve animals: 21 : 10, p = 0.024) and the insignificant proportions of the 50 cm experiment (19 : 12, p = 0.105) is quite small (cf. Table 1).

In Zambian mole-rats the detection of kairomones released as root exudates by carrots into water does not depend on familiarity with the root. The results indicate that either exudates of food plants are chemically similar and there are common root kairomone or that the "smell of carrots" is detectable to any root eater irrespective of previous familiarity with it.

These findings extend the previous findings on odour-guided foraging underground by various mole-rat genera (Heth et al. 2002) by investigating and demonstrating responses to root exudates or kairomones apart from microbial activity in the soil and at different more naturally occurring distances from the source plant. Additional studies will be necessary to determine the effects of different soil types and differential humidity of the soil on kairomone detection and kairomone-guided food location.

Acknowledgements

We thank G. Hamann and R. Hohmeister for technical assistance, and D. Netzer for assistance in performing behavioural tests.

LITERATURE

BRETT R. A. 1991: The ecology of naked mole-rat colonies: burrowing, food and limiting factors. In: Sherman P. W., Jarvis J. U. M. & Alexander R. D. (eds), The biology of the naked mole-rat. *Princeton University Press, Princeton, NJ: 137–184.*

BURDA H., ZIMA J., SCHARFF A., MACHOLÁN M. & KAWALIKA M. 1999: The karyotypes of *Cryptomys anselli* sp. nova and *Cryptomys kafuensis* sp. nova: new species of the common mole-rat from Zambia (Rodentia, Bathyergidae). *Z. Säugetierkd. 64: 36–50.*

DICKE M. & SABELIS M. W. 1988: Infochemical terminology: based on cost-benefit analysis rather than origin of compounds? *Funct. Ecol. 2: 131–139.*

HETH G., TODRANK J. & NEVO E. 2000: Do *Spalax ehrenbergi* blind mole-rats use food odours in searching for and selecting food? *Ethol., Ecol. Evol. 12: 75–82.*

HETH G., TODRANK J., BEGALL S., KOCH R., ZILGIGER Y., NEVO E., BRAUDE S. H. & BURDA H. 2002: Odours underground: subterranean rodents may not forage "blindly". *Behav. Ecol. Sociobiol. 52, 53–58.*

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

VLECK D. 1979: The energy cost of burrowing by the pocket gopher *Thamomys bottae. Physiol. Zool. 52: 122–136.*