

**The University of South Bohemia in České Budějovice**

**Faculty of Science**

**Foraging Ecology of subterranean Blind Mole Rat**

***(Nannospalax galili)***

Master's thesis

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**Annotation:**

*Nannospalax galili* is a species of subterranean blind mole rats originating from the Upper Galilee mountains from northern Israel. The species resides in a habitat which is sharply subdivided edaphically into abutting basaltic and rendzina soil. An adaptive ecological speciation has been proposed in this species as the individuals living in these two different soil types have shown several distinct genetic, physiological and behavioural differences. The species has also demonstrated the ability to forage using the chemical cues released from plant roots (kairomone). This master thesis is divided into two parts: the first part investigates whether the basaltic-soil and rendzina-soil individuals have differential preference for food plant species using cafeteria trials. The second part involves experiments with captive individuals of *N. galili* to test whether they can distinguish between soils with and without kairomones and does the kairomone perception ability vary among individuals based on their soil of origin.

**Declaration:**

I declare that I am the author of this qualification thesis and that in writing it I have used the sources and literature displayed in the list of used sources only.

Date: 08-12-2023

České Budějovice

  
Prapti Gohil

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## 1 **1. Introduction**

2 *Nanospalax galili* is a species of East-Mediterranean blind mole rats (Spalacinae, Rodentia)  
3 distributed in the Upper Galilee Mountains in the northern part of Israel. The species is  
4 solitary, territorial, aggressive and highly adapted to subterranean life foraging in underground  
5 burrow system (Heth et al., 2000). Spalacines represents an actively speciating taxon with four  
6 parapatric chromosomal species of *Spalax ehrenbergi* superspecies identified ((Nevo, 1985;  
7 Nevo et al., 2001) and *N. galili* ( $2n=52$ ) is the only species among them in which incipient  
8 adaptive ecological speciation has been proposed (Hadid et al., 2013) based on two distinct  
9 populations detected in two abutting different types of soil.

10 The majority part of the Upper Galilee Mountains is formed by Terra Rossa (Singer,  
11 2007) which is predominated by Mediterranean ‘batha’ vegetation (Danin A., 2015) and  
12 rendzina soil along with several ‘islands’ of basaltic soil (Lövy et al., 2015). There is notable  
13 difference between these soil types. Pale rendzina soil is formed from chalk/marl bedrock  
14 during late Cretaceous 89-65 Mya while the basaltic soil is of volcanic origin and is a part of  
15 the Dalton basalt plateau from Pleistocene 3.6-2.6 Mya (Singer, 2007). At a recent study  
16 microsite which is sharply subdivided into abutting rendzina and basaltic soil, the dominant  
17 vegetation on chalk is *Sarcopterium spinosum* while basaltic soil is dominated by *Carlina*  
18 *hispanica* (Hadid et al., 2013). *Sarcopterium sp* is avoided by the mole rats and *Sarcopterium*  
19 free patches covered by other grassy or herbaceous vegetation provide suitable site for mole  
20 rats living in rendzina soil (Lövy et al., 2015a). Basaltic soil is markedly devoid of this and  
21 other such shrubs and trees, while being plentiful in grasses and hemicryptophytes (Singer,  
22 2007). Basaltic soil also has higher proportion of clay making it is stickier and harder when  
23 moist compared to rendzina soil (Smith et al., 1985) and therefore it is more difficult to burrow  
24 for mole rats. Moreover, there was found to be dramatically lower level of O<sub>2</sub> and higher level  
25 of CO<sub>2</sub> in basaltic burrows than burrows in rendzina at a previously studied microsite (Shams  
26 et al., 2005). Despite several shortcomings, since basaltic soil has higher biomass of grasses  
27 and herbs, including underground storage organs, thus larger food supply, it harbours higher  
28 population density of mole rats (Lövy et al., 2015a). Based on the differences in these  
29 parameters, there may exist different selection pressures in these two soils which can pave a  
30 path for ecological speciation in *N. galili*.

31 Several studies have been conducted over the past two decades which indicated distinct  
32 genetic, physiological and behavioural differences in basaltic and rendzina individuals.  
33 Genomic diversity study based on AFLP marker genetic polymorphism was higher in



34 individual living in more stressful rendzina soil compared to basalt (Polyakov et al., 2004).  
35 Sequence analysis of mtDNA and control region showed all major haplotype clusters (HC) to  
36 be highly soil-biased. Up to 40% of the mtDNA diversity was edaphically dependent,  
37 suggesting possibly constrained gene flow (Hadid et al., 2013). Multiple other studies carried  
38 out at genomic, transcriptomic, repeatomic and epigenomic levels also suggested strong  
39 possibility of ecological speciation (Li et al., 2015, 2016, 2020). Resting metabolic rate  
40 (oxygen consumption) was found notably higher in basalt-dwelling animals (Hadid et al.,  
41 2013) and basaltic animals were found significantly more often active outside the nest than  
42 those living on chalk (Šklíba et al., 2016). Burrow system architecture was found to be  
43 different according to ecological and edaphic parameters as well where rendzina animals tend  
44 to form longer, larger and more linear burrow with higher proportion of stored food compared  
45 to basaltic individuals who displayed shorter and more complex branched burrowing patterns  
46 likely due to high population density in basalt (Lövy et al., 2015a). Mole rats displayed  
47 significant preference for the familiar soil of their origin in a T-maze experiments carried out  
48 with moist soil collected from their habitats (Lövy et al., 2017). However, reproductive  
49 isolation seems unlikely due to continues gene flow, another trials of T-maze experiments  
50 demonstrated the absence of assortative female mate choice based on olfactory cues and  
51 inconsistent male preferences (Lövy et al., 2020). Besides these differences in individuals of  
52 *N. galili*, a few other studies have also found soil bacterial community significantly diverging  
53 between two soils and corresponding differentiation in their function to adapt to ecological  
54 stresses of the respective local soil (Kuang et al., 2022; Mukherjee et al., 2022). Although  
55 Kuang and colleagues (2022) did not find clear differentiation of gut microbiome composition  
56 between two populations of mole rats, the functional compositions were sharply distinct which  
57 was correlated with the divergent host diet.

58 This study focuses on foraging ecology of *N. galili*. As a rodent species highly adapted to  
59 subterranean niche, burrowing through solid earth in search of food is an energetically  
60 demanding process for these animals which, depending on the type of soil (Luna & Antinuchi,  
61 2006), can result in expending energy 360-3400 times higher than that required to move the  
62 same distance across the soil surface (Vleck, 1979). Therefore, the decision about such  
63 expensive locomotion for foraging is usually in line with optimal foraging theory i.e. less  
64 selective and nondirected (Heth et al., 1989; Pyke et al., 1977), and to increase their foraging  
65 success in a low-productive underground environment, the mole rats are food generalist (Heth  
66 et al., 2000). They feed on various underground parts (tubers, bulbs, corms, rhizomes etc.) of  
67 geophytes and perennial herbs (Nevo, 1961) and hoard them in their food-storage chambers.

68 To counter the periods of limited food supply or patchy distribution of geophytes with long  
69 distance between patches, many species of subterranean rodent also adapt to ‘geophyte  
70 farming’, in which partially consumed large bulbs are plugged into soil again to allow it to  
71 regenerate for later consumption (Brett, 1991; Spinks et al., 1999).

72 Since the sensory cues of vision, hearing, taste and vibration are of very limited use for  
73 navigation and foraging belowground, for long time it was assumed that subterranean rodents  
74 find their food through chance encounters (Jarvis et al., 1998; Spinks et al., 1999). Although,  
75 these rodents are considered ‘blind’, they do not forage completely randomly and have been  
76 observed to burrow strategically, i.e. digging in straight line instead of circle or ‘area restricted  
77 search’ where they dig in a branching manner in the vicinity of a located food item (Benedix,  
78 1993; Jarvis et al., 1998). Many later studies have also shown that these rodents can use  
79 olfactory cues to locate the food plants from some distance and can orient their direction of  
80 burrowing accordingly (Heth et al., 2000; Lange et al., 2005; Schleich & Zenuto, 2007; Šklíba  
81 et al., 2011; Vitámvás, 2013). Through olfactory senses, these animals detect chemical  
82 exudates released from the roots of growing plants, also known as kairomones.

83 It’s been long known from laboratory experiments that *N. galili* can use kairomones while  
84 foraging and selecting for food and do not forage blindly ((Heth et al., 1989, 2000, 2002). The  
85 individuals showed significant preference for odour for edible bulbs (*Narcissus tazetta*) than  
86 poisonous one (*Urginea maritima*) (Heth et al., 2000). They could also discriminate between  
87 soils in which plant had or had not been growing (i.e. kairomone and control soil, respectively),  
88 and soils in which edible as opposed to poisonous plant had been growing (Heth et al., 2002).  
89 The study also showed that the blind mole rats could identify the gradient of kairomone to  
90 some extent and would select the soil with ‘more kairomone’. However, since the air  
91 movement underground is very limited and food odours cannot travel very far, it’s not been  
92 clear from which distance *N. galili* can locate the food source and accordingly orient their  
93 direction for burrowing. Furthermore, it has not been examined whether there is any variance  
94 in the ability to perceive kairomone in the individuals originating in two different soil types.  
95 Moreover, since the plant species found in basalt and rendzina soil vary greatly and as shown  
96 in one of the studies that only 28% of the plant species are present in both soil types in differing  
97 abundance (Hadid et al., 2013), there is a possibility for differential preferences for food plant  
98 species in individuals living in different soils which hasn’t been tested yet.

99 The work of this master’s thesis is divided into two parts. The first part involves testing  
100 the mole rats’ preferences towards various natural and crop plants with the help of cafeteria

101 trails. The main objectives are: 1) to characterize the geophytes collected from the study  
102 microsite Rihaniya, and several crop plants for comparison in terms of their nutritional value;  
103 2) to test whether the mole rats of the two different soil types show any variation in the  
104 decrease in body mass during starvation period. We hypothesised that since rendzina soil  
105 represents more stressful living conditions for mole rats due to scarce food supply (Lövy et  
106 al., 2015a, 2017), individuals residing in rendzina soil may spend relatively longer durations  
107 in search of food compared to basaltic-soil animals and therefore the decrease in their body  
108 mass during the starvation period before the trails, would be less compared to basaltic-soil  
109 animals. ; 3) to test whether the mole rates have a preference for various natural and crop  
110 plants and if so, whether the preference differ between animals from the two soil types. The  
111 second part comprises of series of T-maze experiments to study the kairomone perception  
112 ability of these mole rats. The objectives include: 1) to test whether the captive individuals of  
113 *N. galili* can discriminate between control soil and kairomone soil. If so, 2) to test from which  
114 distance they can successfully locate kairomones in the soil. Finally, 3) to test if the ability to  
115 perceive kairomone differs in individuals based on their soil of origin.

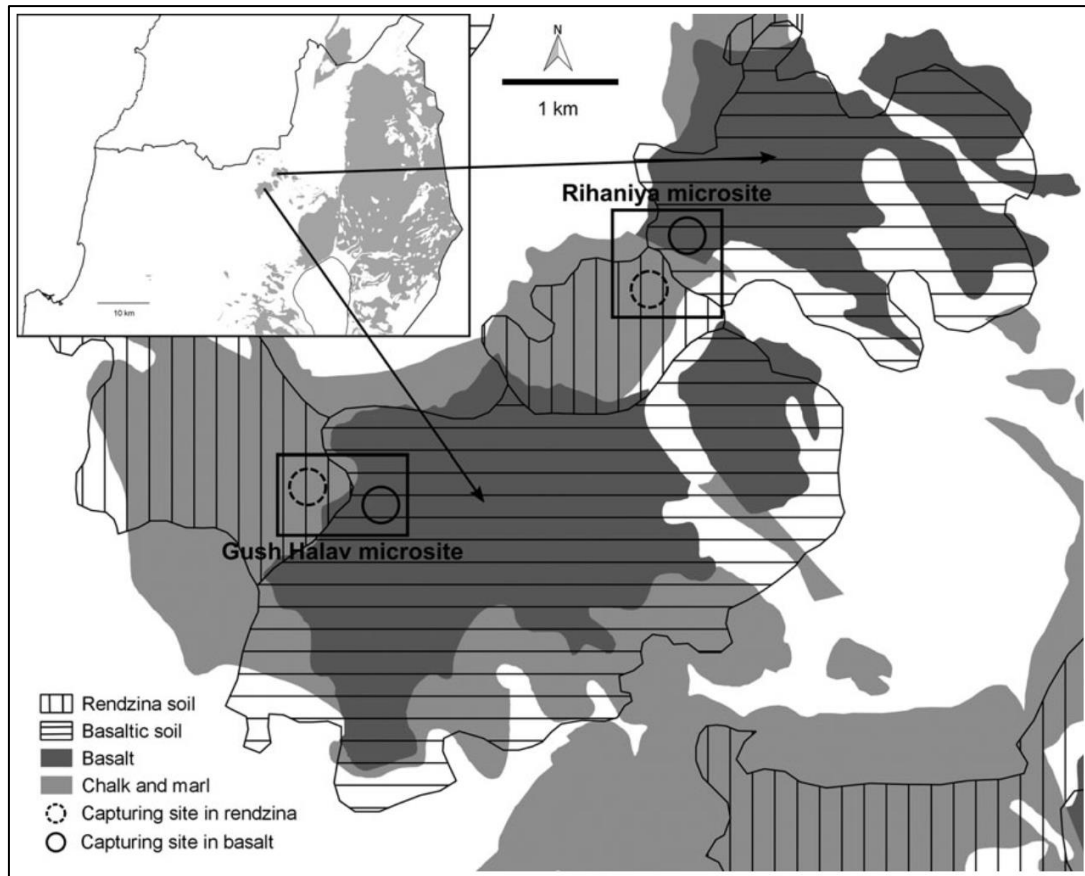
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## 117 **2. Materials and Methods**

### 118 **2.1 Cafeteria trials**

119 22 individuals of *N. galili* (12 from basaltic and 10 from rendzina soil) were captured using  
120 modified Hickman's traps near Rihaniya in the eastern Upper Galilee Mountains (33°02.5'N,  
121 35°29.2'E, altitude 760 m) in northern Israel in 2012 (Figure 1). The climate in Upper Galilee  
122 is Mediterranean, with 714±163 mm of annual precipitation most of which (79±10%) falls in  
123 December–March (<http://www.ncdc.noaa.gov/ghcnm/>, accessed September 2014, (Lövy et  
124 al., 2015a). Predominating soil in this region is terra rosa, with two islands of basaltic soil  
125 abutting with the surrounding rendzina soil. The body mass and sex of the animals were  
126 recorded upon capturing. Two sets of cafeteria experiments were prepared: first, with natural  
127 plant species occurring in the mole rats' habitat (*Eryngium creticum* and *Anemone coronaria*  
128 present in both rendzina and basaltic soils, *Asphodelus ramosus* (old and young roots) and  
129 *Leopoldia sp.* occur more in rendzina soil, and *Ornithogalum lanceolatum* found mostly in  
130 basaltic soil) and second, with crop plants (Sweet potato, Potato, Carrot, White Radish and  
131 Onion along with the most preferred natural plant *Eryngium creticum*). All the food plants  
132 were characterised in terms of their nutritional characteristic (glucose, fructose, fructans,

133 saccharose, starch, along with fibre and water content and available energy). (For details on  
134 methods to obtain nutritional values, see: Chlumská et al., 2014).



135

136 **Figure 1.** Geological and soil map of the two study microsites from where the individuals of  
137 *N. galili* were captured. (Image source: Lövy et al., 2017)

138

139 Before the trials began, the animals were starved for nine hours. Each animal was placed in a  
140 testing container and presented with one set of cafeteria test which contained approximately  
141 15 g of each tested plant. One set of pieces of the same food plants were set aside to measure  
142 weight loss due to evaporation. After 2 hours, the weight of the remaining pieces was measured  
143 allowing to assess the proportion of the plant consumed. The body mass of each animal was  
144 noted at the beginning and at the end of starvation period as well as at the end of cafeteria trial.  
145 All the trials were performed within a few days after the individuals were captured from their  
146 burrow systems in 2012 and the natural plants used during the trails were excavated just before  
147 the beginning of the experiment.

148

149

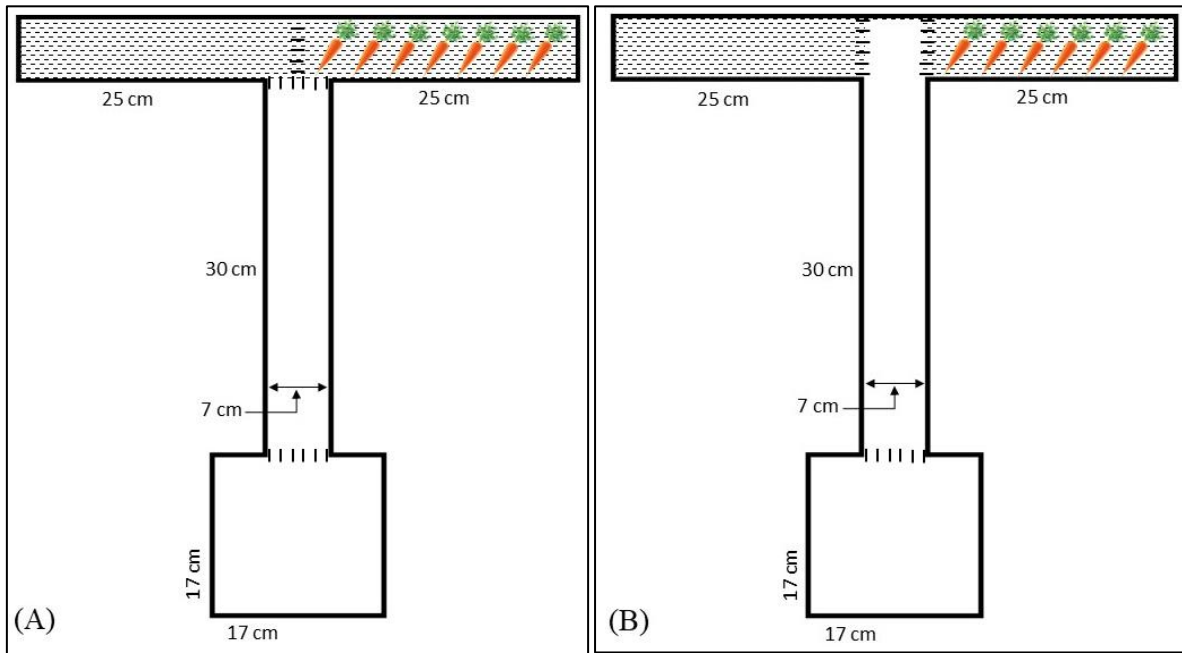
150

## 151 2.2 Kairomone in hydroponics

152 This study was carried out in a series of six experiments with 23 adult individuals of *N. galili*  
153 for test one and two, and 22 individuals for test three to six during February to August 2023.  
154 All of these individuals were captured from the field in March 2022 near Gush Halav, northern  
155 Israel (Figure 1) and transported to the animal facility at the Department of Zoology, Faculty  
156 of Science, University of South Bohemia. The animal room is maintained at  $24\pm 1^\circ\text{C}$  with  
157 photoperiod of 12L/12D and relative ambient humidity ranged from 40% to 60%. All the  
158 individuals are kept in their own terrarium with sawdust bedding and are fed with potatoes,  
159 sweet potatoes, carrots, onions, apples and commercially available dry rodent food *ad libitum*.  
160 The body mass of each animal, along with overall health conditions is recorded weekly as part  
161 of maintenance of the animals in the facility.

### 162 **Experiment:**

163 To investigate whether the mole-rats are able to differentiate between kairomone soil and  
164 control soil using chemical cues produced by plants, a transparent Perspex T-maze tunnel (7x7  
165 cm square cross section) was used in all experiments which consisted of a starting box (17x17  
166 cm) leading to 30 cm long crossbar (Figure 2). Each arm of the crossbar (25 cm each) was  
167 filled with peat. Prior to filling, peat was moistened in one arm with control water and in the  
168 other arm with the same volume of kairomone water and blended evenly to avoid formation  
169 of any horizontal concentration gradient. During test one and two, the soils were filled adjacent  
170 to each other keeping a removable partition in between so as to avoid mixing of control and  
171 kairomone soils (Figure 2A; Appendix I, Picture 2). During tests three to six, the set up was  
172 changed to leave a space of ~ 8 cm between two soils (Figure 2B; Appendix picture 2) so it  
173 allowed to better see towards which side the animal turns/sniffs to make sure that the animal  
174 is making a choice between two soils and not rushing randomly towards one side.



175

176 **Figure 2.** T-maze set up with removable partition and habituation box, for test one and two  
 177 (A) and test three to six (B); position of carrots represents the arm filled with kairomone soil

178

179

180 Each animal was tested once in each set of experiment and prior to each test, the  
 181 respective food item (i.e. plant used to prepare kairomone water) was removed from its diet  
 182 for one week. In the first two tests, the animals were not starved prior to running the  
 183 experiments due to their health condition. For test three to six, each animal was starved  
 184 overnight (~24 hours) and was placed in a Perspex system (Appendix I, Picture 1) to let them  
 185 be familiarised with peat and maze, which could also help to wear off their enthusiasm towards  
 186 digging in peat. During starvation period, a small piece of apple was provided for hydration.

187 At the beginning of each test, the test animal was placed into the start box, separated  
 188 from the runway and cross bar with a removable partition. After 5 minutes of habituation, all  
 189 the partitions were removed and the animal was allowed to choose and dig freely in the  
 190 crossbar. The time from opening the gate to start digging was denoted as latency. The time  
 191 spent in each of the arm was noted as well. The test finished when the animal burrowed  
 192 completely through one arm. The testing apparatus was randomly rotated before each animal  
 193 was tested to eliminate the possible influence of electromagnetic field. Which arm of the  
 194 crossbar would be filled with kairomone soil was chosen randomly with a flip of a coin to  
 195 avoid any potential preference for direction in the test animals. At the end of each trial, the  
 196 soil was discarded, the whole apparatus was cleaned thoroughly with water, sprayed with 35%  
 197 ethanol and dried with paper tissue. Laboratory gloves were worn all the time while filling the

198 T-maze with soil to eliminate any type of odorous contamination. All the tests were video  
199 recorded with a camera placed above the test apparatus.

200

#### 201 **Preparation of Kairomone water:**

202 For the first two sets of experiments, kairomone water was obtained by keeping 3 large yellow  
203 onions in a bucket of water (~9 l). Two of such buckets were kept at the ambient temperature  
204 and the condition of onions was observed every day to check for any possible spoilage. For  
205 the first test, onions were removed from one bucket at the end of 3<sup>rd</sup> day and water was filtered  
206 using filter paper. Since no spoilage was observed in the remaining onions for up to 8 days,  
207 second set of experiments were run with the water filtered at the end of 8<sup>th</sup> day. The control  
208 water (pure water without any onions) was kept in similar condition. After filtering, both the  
209 kairomone and control water were stored in refrigerator and utilized within three to five days.  
210 The water was brought to ambient temperature prior to using it in experiments. For the third  
211 set of experiments, kairomone water was obtained in a similar way by keeping a bunch of  
212 carrots in the similar quantity of water for 8 days. For the fourth set, we experimented with a  
213 common wild plant dandelion (*Taraxacum officinale*). Small dandelion plants with roots  
214 intact, were excavated from the field (Stromovka park, České Budějovice) during June, 2023  
215 and brought to the lab. The roots were carefully washed to remove as much soil particles as  
216 possible. The plants were then kept at ambient temperature in sunlit area in the bucket filled  
217 with water in such a way that only roots remained submerged in water (Appendix Picture 3).  
218 At the end of 12<sup>th</sup> day, plants were removed and water was filtered to be used in the  
219 experiment. In the fifth set of experiments, kairomone water was obtained using sweet potatoes  
220 in the following way: five commercially available sweet potatoes (three of which were  
221 organically grown/Bio) were kept under sunlight in separate jars with pointy end of the tubers  
222 submerged in water until they started growing roots and slips. Water in the jars was changed  
223 frequently. It took two months for non-organic and one month for organic sweet potatoes to  
224 grow appropriate amounts of roots (Appendix Picture 4), after which all five of the sweet  
225 potatoes were kept in the bucket of water (~7.5 l) with roots submerged, at ambient  
226 temperature in sunlight for 15 days. On the seventh day, additional 1 l of water was added as  
227 the water was being absorbed very quickly. At the end of 15<sup>th</sup> day, sweet potatoes were  
228 removed and the water was filtered to be used in the experiments. Based on the observations  
229 and unsatisfactory results obtained through five tests of *kairomone in hydroponics* (see the  
230 Results), last test was carried out using soil in which plants had been growing. Dandelion  
231 (*Taraxacum officinale*) were excavated from the same location as previously mentioned, with

232 roots intact. After carefully washing the roots, the plants were replanted in small pots in  
233 commercially sold garden soil, 4 plants per pot (Appendix Picture 5) and were left to grow in  
234 the pots for 30 days. The pots were kept under sunlight at ambient temperature and watered at  
235 regular intervals. At the end of 30 days, the plants were removed from the pots and soil was  
236 obtained by carefully separating any remaining small roots. This soil was directly used in the  
237 experiment. For control soil, plain garden soil was moistened with pure water to bring it similar  
238 texture as kairomone soil.

239

### 240 **2.3 Statistical analysis**

241

242 To assess the data of cafeteria trials, linear mixed effect models were estimated using  
243 maximum likelihood method from NLME package. The model included the identity of  
244 animals as a random effect (formula:  $\sim 1 | \text{animal}$ ), while the predictors soil, sex, body mass  
245 and type of cafeteria trial (Natural or Crop) were used as fixed effects to estimate the  
246 preferences for different food plant species in terms of the proportion consumed (arcsine  
247 transformed) and decrease in body mass occurring during starvation (response variables). A  
248 backward stepwise elimination process was used to identify possible predictors out of the  
249 candidate variables mentioned above. At each step, variables were dropped based on p-values,  
250 until the final model with statistically significant predictor/s was obtained. Tukey test for  
251 multiple pairwise comparisons was calculated using the emmean package. To evaluate  
252 kairomones in hydroponics experiment, a Chi-Square Goodness of Fit Test was performed to  
253 determine whether the mole rats demonstrate a preference for control or kairomone soil.  
254 Furthermore, generalized linear models (GLM) with binomial distribution were used to  
255 estimate if these preferences differed based on animal's sex and soil of origin (i.e. basalt and  
256 rendzina). The graphs are plotted using ggplot2 package. Throughout the text, means are given  
257 as mean  $\pm$  SD. PCA (Principle Component Analysis) to visualise the nutritional characteristics  
258 of natural and crop plants, was performed in CANOCO 5 (Šmilauer, P., & Lepš, J., 2014) and  
259 all the other statistical tests in R statistical software (R Core Team 2022).

260

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262

263

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265



266 **3. Results**

267 **3.1 Cafeteria trials**

268 Two sets of cafeteria trials were carried out with 22 individuals of *N. galili* – 10 from basaltic  
 269 soil 1 and 12 from rendzina soil (8 females and 2 males from basaltic soil, 6 females and 6  
 270 males from rendzina). The average weight for basaltic and rendzina animals was  $135\pm 39$  g  
 271 and  $143\pm 35$  g, respectively. There was no significant difference in the body mass of animals  
 272 from different soil types (ANOVA:  $F_{(1,20)} = 0.268$ ,  $p = 0.61$ ). However, the body mass differed  
 273 by sex ( $F_{(1,20)} = 4.503$ ,  $p = 0.04$ ), with males being heavier than females ( $160\pm 45$  g and  $128\pm 25$   
 274 g for males and females, respectively) regardless of their soil of origin (ANOVA - soil of  
 275 origin \* sex :  $F_{(1,18)} = 3.624$   $p = 0.0731$ ).

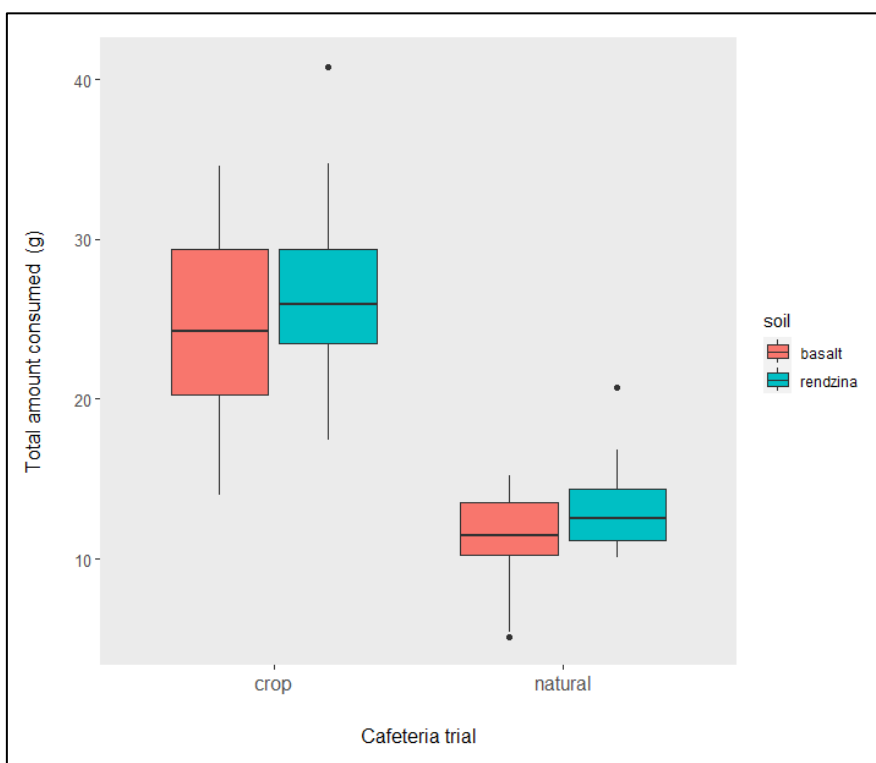
276 The body mass of the mole rats decreased during 9- h starvation period before the start  
 277 of the cafeteria trails. This loss of body mass did not differ between individuals from the  
 278 basaltic soil and rendzina soil or males and females (Table 1). The results were similar with  
 279 regard to the amount of food consumed, which was not affected by the soil of origin, sex of  
 280 the individuals, or decrease in body mass during the starvation period in both the cafeteria  
 281 trials (Table 1). However, the proportions of total food consumed varied significantly between  
 282 the two sets of cafeteria trials (Table 1), with the average consumption of natural plant species  
 283 being 13.4 g less compared to crop plant species (Figure 3).

284

Response variable	Predictor	F value	p value
Decrease in body mass during starvation before the trail	soil	$F_{(1,19)} = 1.49$	0.23
	sex	$F_{(1,19)} = 0.46$	0.51
Proportion of food consumed during the trail	<b>cafeteria trail</b>	<b><math>F_{(1,21)} = 179.43</math></b>	<b>&lt;0.0001</b>
	soil	$F_{(1,19)} = 0.96$	0.33
	sex	$F_{(1,20)} = 3.60$	0.07
	decrease in body mass during starvation	$F_{(1,21)} = 0.21$	0.65

285 **Table 1.** Statistical summary of linear mixed effect model explaining variation in body mass  
 286 and proportion of food consumed in given cafeteria trails.

287



288

289 **Figure 3.** Box plot showing total amount of food consumed by *N. galili* of different soil origin  
 290 in both cafeteria trails

291

292 All the natural and crop plants used in cafeteria trials were characterised for their energetic  
 293 and nutritional value (carbohydrates, fibre and water content), which is presented in Table 2.

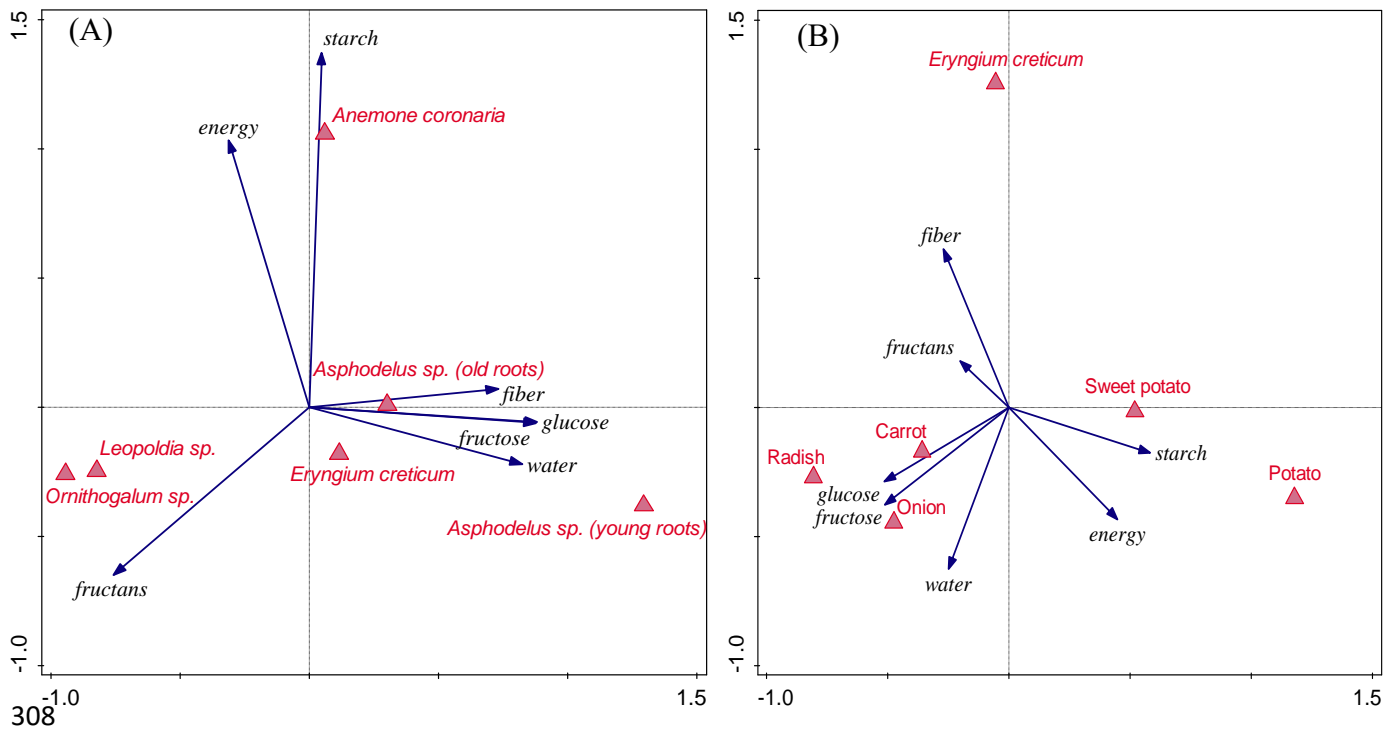
	Plant	cal/g	Water %	Fiber %	Glucose %	Fructose %	Sucrose %	Starch %	Fructans %	TNC
Natural	<i>Anemone coronaria</i>	3950	0.79	10.7	1.78	2.44	6.29	10.28	0.00	20.78
	<i>Leopoldium</i> sp.	3690	0.76	2.7	0.39	1.18	0.79	0.00	70.11	72.47
	<i>Eryngium creticum</i>	3340	0.76	19.0	1.05	1.35	3.20	2.08	8.13	15.81
	<i>Ornithogalum lanceolatum</i>	3710	0.77	3.1	0.16	0.28	0.23	0.00	78.28	78.95
	<i>Asphodelus</i> (old root)	3760	0.84	13.4	1.55	3.21	2.72	0.00	10.85	18.33
	<i>Asphodelus</i> (young root)	3480	0.92	14.9	4.56	5.85	3.85	0.00	8.87	23.13

	Plant	cal/g	Water %	Fiber %	Glucose %	Fructose %	Sucrose %	Starch %	Fructans %	TNC
Crop	Potato	3820	0.87	4.9	0.44	0.49	0.37	51.35	0.00	52.64
	Sweet potato	3630	0.81	4.4	2.02	3.08	7.64	16.43	0.00	29.17
	Carrot	3420	0.91	8.5	4.24	6.45	3.69	0.00	0.00	14.37
	Onion	3660	0.91	6.7	4.09	10.85	1.91	0.00	13.05	29.90
	Radish	3410	0.91	11.5	8.79	8.62	1.61	0.00	0.00	19.02

294 **Table 2.** Nutritional characteristics of plant species in cafeteria trial (TNC: Total Non-  
295 structural Carbohydrates)

296

297 Figure 3 shows visual representation of the nutritional characteristics in all plant species in a  
298 PCA plot. For the natural plant species (Figure 3A), the first axis is represented by simple  
299 carbohydrates, water, and fibre content. Among them, *Asphodelus* young roots had the highest  
300 amount of water and glucose content, whereas *Leopoldium sp.* and *Ornithogalum sp.* had  
301 relatively higher content of fructans. *Anemone coronaria* lacked simple sugars but had the  
302 highest amount of starch and energy as represented by the second axis in the plot. For the crop  
303 plants (Figure 3B), the first axis was dominated by starch and simple sugars. Among them,  
304 potato, followed by sweet potato, had the highest starch and energy content, while carrot,  
305 onion, and radish lacked starch but had a higher percentage of simple carbohydrates and water.  
306 Compared to all the crop plants, *Eryngium creticum* had a higher amount of fibre content and  
307 a lower amount of starch and sugars, as indicated by the second axis.



309 **Figure 4.** PCA plot representing plant species and their nutritional characteristics used in the  
 310 two sets of cafeteria trials (A: Natural plants, variation explained by 1st axis is 55.88% and  
 311 2nd axis is 23.82%; B: Crop plants, variation explained by 1st axis is 44.42% and 2nd axis is  
 312 33.33%).

313

314 The results of the linear mixed effect models indicated a clear preference towards particular  
 315 plant species in both the cafeteria sets, whereas neither the soil of origin of animals nor sex  
 316 had a significant effect on the preference in either of the cafeteria trail (Figure 4, the model  
 317 statistics are summarised in Table 3).

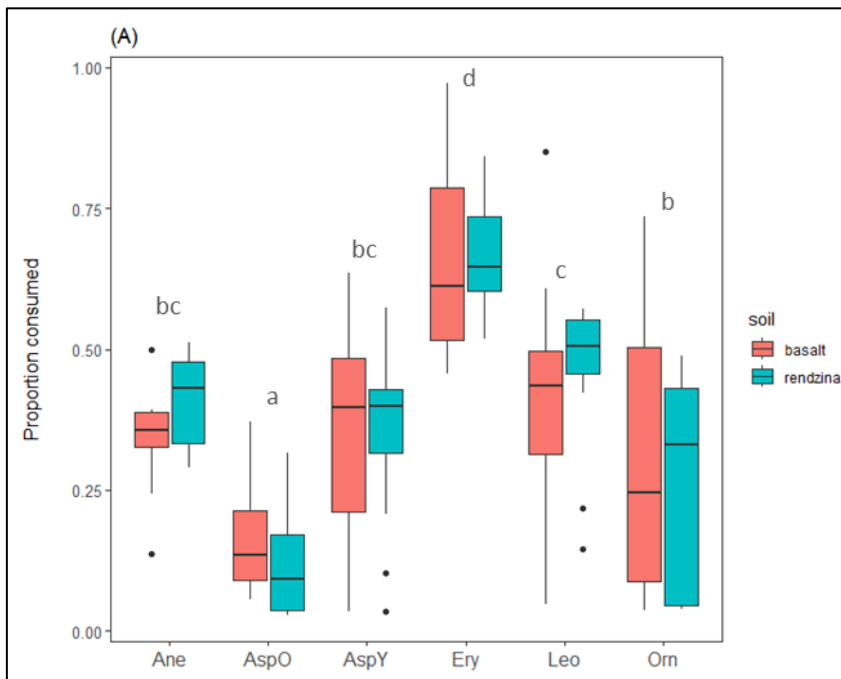
Cafeteria set	Predictor	F value	p value
Natural plants	<b>Intercept</b>	<b>734.95</b>	<b>&lt;0.0001</b>
	<b>Plant species</b>	<b>26.49</b>	<b>&lt;0.0001</b>
	<i>Soil origin</i>	<i>0.06</i>	<i>0.81</i>
	<i>Sex</i>	<i>0.02</i>	<i>0.88</i>
	<i>Plant species * Sex</i>	<i>1.81</i>	<i>0.12</i>
	<i>Plant species * Soil origin</i>	<i>0.47</i>	<i>0.80</i>
Cafeteria set	Predictor	F value	p value
Crop plants	<b>Intercept</b>	<b>954.12</b>	<b>&lt;0.0001</b>

	<b>Plant species</b>	<b>26.75</b>	<b>&lt;0.0001</b>
	<i>Soil origin</i>	0.12	0.74
	<i>Sex</i>	0.00	0.97
	<i>Plant species * Soil origin</i>	2.322	0.049
	<i>Plant species * Soil sex</i>	1.36	0.24

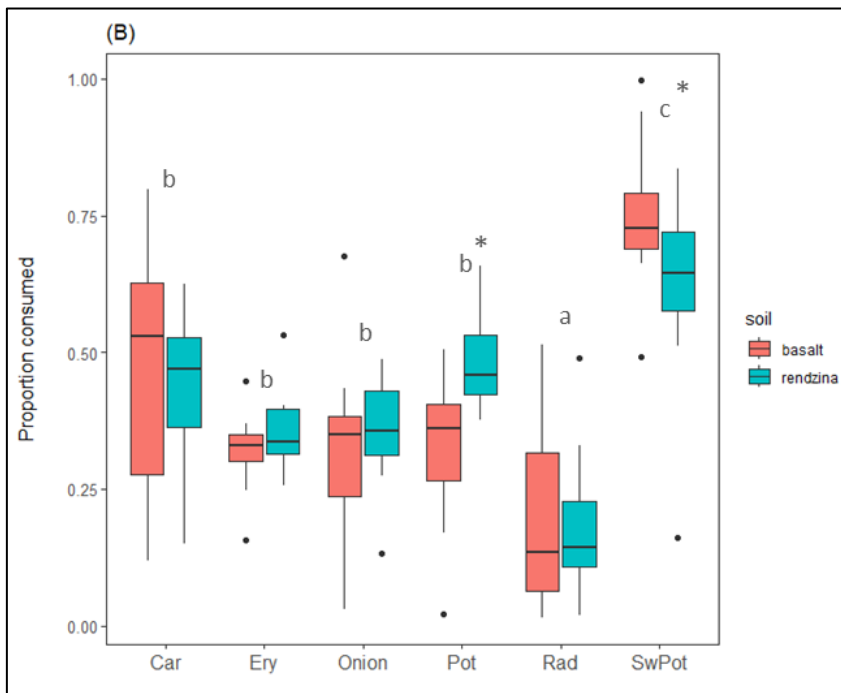
318 **Table 3.** Statistical summary of linear mixed effect model explaining variability in proportion  
319 of plant consumed in given cafeteria trails (significant predictors are in bold, predictors in  
320 italics were dropped from the final model during model simplification and are displayed with  
321 probabilities when last included in the model).

322

323 In the trials with natural plants, *Eryngium creticum* was the most preferred one followed by  
324 *Leopoldium sp.* while the old roots of *Asphodelus sp.* were preferred the least (Fig. 4A).  
325 Although, the interaction of Plant species \* Soil origin for crop plants was found to be close  
326 to being significant (Table 3), further examination using the post hoc pairwise comparisons  
327 revealed that the meaningful contrasts (comparing consumption of one plant between basaltic  
328 and rendzina animals) were only significant for potato and sweet potato (Table 1, Appendix).  
329 All the individuals, regardless of their soil of origin, collectively showed the same preferences  
330 for the crop plants i.e. sweet potatoes were preferred the most over *Eryngium creticum* and  
331 other crop plants whereas radish was preferred the least of all and these preferences did not  
332 vary significantly between basaltic and rendzina animals as it can be seen in Figure 4B.



333



334

335 **Figure 5.** Box plot showing variation in preferences for plant species in *N.galili* of different  
 336 soil origin; (A: Natural plants, B: Crop plants). Letters represent pairwise comparison of Tukey  
 337 test comparing the collective preferences of all the mole rats for different plant species, species  
 338 sharing the same letter/letter combination are not significantly different, letter with an asterisk  
 339 represents plant species for which the proportion consumed was significantly different  
 340 between mole rats from basaltic and rendzina soil ( $p \leq 0.05$ ) (Ane= *Anemone coronaria*, AspO  
 341 and AspY= *Asphodelus* Old and Young roots, Ery= *Eryngium creticum*, Leo= *Leopoldium sp.*, Orn=  
 342 *Ornithogalum lanceolatum*, Car= Carrot, Pot= Potato, Rad= Radish, SwPot= Sweet Potato)

343

344 **3.2 Kairomone in hydroponics**

345 In series of six different tests, total 134 T-maze trials were performed on 23 individuals in test  
 346 one and two, and 22 individuals in test four to six, to test whether the mole rats showed  
 347 preference towards control soil or kairomone soil.

348 For test one, average latency was  $17.9 \pm 33.1$  s. It did not differ between animals  
 349 originating from basaltic soil compared to rendzina soil (ANOVA:  $F_{(1,21)} = 0.68$ ,  $p = 0.42$ ;  
 350 Table 4) and it did not vary with sex of the animals (ANOVA:  $F_{(1,21)} = 0.006$ ,  $p = 0.94$ ). For  
 351 the second test, average latency was  $15.2 \pm 27.2$  s, which did not vary either with animals' sex  
 352 ( $F_{(1,21)} = 0.99$ ,  $p = 0.33$ ) or the soil of origin ( $F_{(1,21)} = 0.03$ ,  $p = 0.86$ ). In test three, average  
 353 latency was found to be  $17.8 \pm 25$  s, which again did not vary either with individuals' soil of  
 354 origin ( $F_{(1,20)} = 0.18$ ,  $p = 0.67$ ) and or sex ( $F_{(1,20)} = 1.01$ ,  $p = 0.33$ ).

Test	Latency (s)	
	Basaltic animals	Rendzina animals
1 (onion – 4 days)	$22.2 \pm 40.8$	$13.4 \pm 23.3$
2 (onion – 8 days)	$13.2 \pm 14.9$	$17.5 \pm 37.1$
3 (carrot – 8 days)	$24.3 \pm 33.9$	$11.3 \pm 8.2$
4 (dandelion – 12 days)	$8.3 \pm 4.9$	$7.2 \pm 2.6$
5 (sweet potato – 15 days)	$5.8 \pm 2.3$	$4.7 \pm 2.8$
6 (dandelion soil – 30 days)	$7.2 \pm 4.9$	$3.6 \pm 1.5$

355 **Table 4.** Latency (mean  $\pm$  SD)

356

357 The average latency for test four was  $7.7 \pm 3.8$  s and it didn't differ either with the soil  
 358 of origin ( $F_{(1,20)} = 0.18$ ,  $p = 0.67$ ; Table 4) or sex ( $F_{(1,20)} = 1.25$ ,  $p = 0.27$ ). Similarly, for test  
 359 five, the average latency was  $5.3 \pm 2.6$  s, which was the lowest among all the tests and did not  
 360 differ with the soil of origin ( $F_{(1,20)} = 1.54$ ,  $p = 0.23$ ) or the sex of the animals ( $F_{(1,20)} = 2.03$ ,  $p =$   
 361  $0.17$ ). In the last test, test six, average latency was  $5.5 \pm 4.0$  s and basaltic animals showed a  
 362 significantly higher latency compared to rendzina animals ( $F_{(1,20)} = 4.59$ ,  $p = 0.04$ ) and it did  
 363 not differ between males and females ( $F_{(1,20)} = 1.49$ ,  $p = 0.24$ ).

364 In none of the six tests, significant preference was found towards either control or  
 365 kairomone soil, the results of which are summarised in Table 5.

Test	No. of animals tested	Preference for Kairomone soil	Preference for Control soil	$\chi^2$	p	GLM (family=binomial, link=logit) (Response variable – preference)		
						predictor	Percentage of explained variation	p
1 (onion – 4 days)	23	10	13	0.35	0.55	soil	0.15	0.83
						sex	1.10	0.55
2 (onion – 8 days)	23	13	10	0.35	0.55	<b>soil</b>	<b>18.37</b>	<b>0.01</b>
						sex	2.97	0.33
3 (carrot – 8 days)	22	11	11	0	1	soil	5.43	0.20
						sex	6.33	0.16
4 (dandelion – 12 days)	22	14	8	2.27	0.13	<b>soil</b>	<b>20.57</b>	<b>0.02</b>
						sex	5.78	0.21
5 (sweet potato – 15 days)	22	8	14	2.27	0.13	soil	0	1
						sex	0.95	0.60
6 (dandelion soil – 30 days)	22	12	10	0.09	0.76	soil	9.91	0.08
						sex	1.86	0.45

366 **Table 5.** *N. galili* preferences for digging in control and kairomone soil and whether the  
367 preference varies based on individual sex and soil of origin (i.e. basalt or rendzina).

368

369 These preferences did not vary significantly between males and females in all six tests.  
370 However, a significant variation in preference was observed in tests two and four based on the  
371 soil of origin of individuals (GLM, Table 5).

372 In both tests (two and four), a significantly higher number of rendzina animals preferred to  
373 burrow through the arm filled with kairomone soil over control soil compared to the basaltic-  
374 soil individuals (Table 6). A similar trend was present in test six as well, but the results were  
375 not statistically significant. However, after correcting the results for multiple testing using The  
376 Benjamini-Hochberg procedure (False Discovery Rate), none of the abovementioned results  
377 were found significant ( $\alpha=0.05$ )

378

Test		Time spent burrowing (s)		p	p
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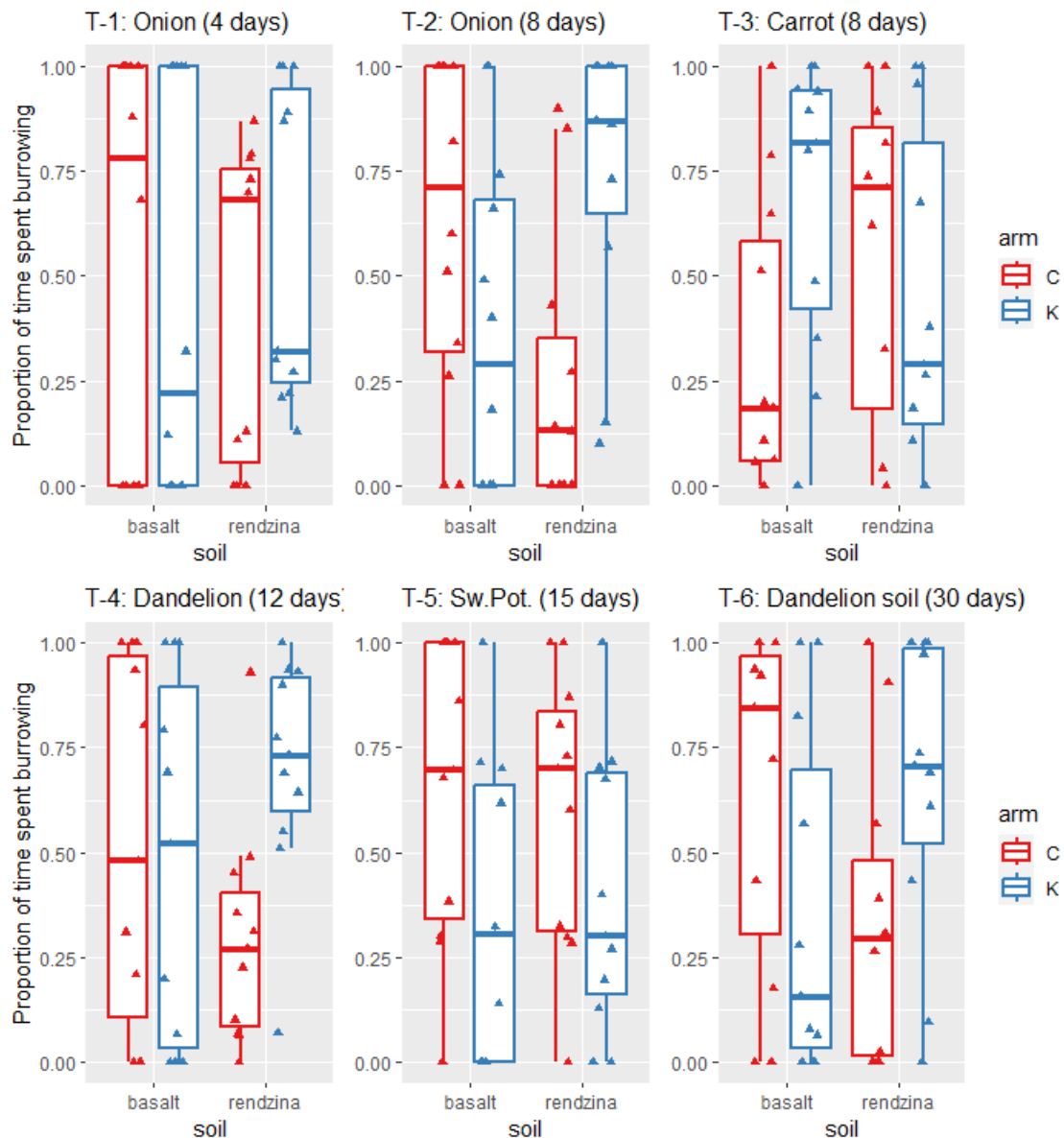


	Soil of origin of animal	Arm filled with Karimone soil	Arm filled with Control Soil	$\chi^2$		adj.
1 (onion – 4 days)	Basaltic	11.9 ± 15.2 (6)	17.6 ± 18.5 (6)	0	1	1
	Rendzina	33.1 ± 22.0 (5)	38.1 ± 33.8 (6)	0	1	1
2 (onion – 8 days)	Basaltic	31.5 ± 39.3 (4)	31.2 ± 21.8 (8)	1.50	0.2	0.52
	<b>Rendzina</b>	<b>58.4 ± 43.5 (9)</b>	<b>31.2 ± 44.2 (2)</b>	<b>6.54</b>	<b>0.01</b>	<b>0.06</b>
3 (carrot – 8 days)	Basaltic	24.5 ± 14.6 (7)	11.0 ± 12.1 (4)	0.73	0.39	0.52
	Rendzina	16.1 ± 14.3 (4)	25.3 ± 28.4 (7)	0.73	0.39	0.52
4 (dandelion – 12 days)	Basaltic	13.2 ± 13.4 (5)	19.4 ± 18.7 (6)	0	1	1
	<b>Rendzina</b>	<b>46.4 ± 27.6 (9)</b>	<b>18.7 ± 15.5 (2)</b>	<b>6.54</b>	<b>0.01</b>	<b>0.06</b>
5 (sweet potato – 15 days)	Basaltic	26.4 ± 42.2 (4)	28.4 ± 20.8 (7)	0.73	0.39	0.52
	Rendzina	16.6 ± 11.3 (4)	30.1 ± 22.6 (7)	0.73	0.39	0.52
6 (dandelion soil – 30 days)	Basaltic	14 ± 15.8 (4)	21.6 ± 18.0 (7)	0.73	0.39	0.52
	Rendzina	23.0 ± 14.7 (8)	12.1 ± 11.5 (3)	2.91	0.08	0.32

379 **Table 6.** Time spent burrowing in different arms of T maze (mean ± SD) and the preferences  
380 demonstrated by basaltic and rendzina individuals for the respective substrate; the number in  
381 parenthesis indicates number of individuals completed burrowing in arms filled with  
382 respective soil in the given test; p adj. = adjusted p values using The Benjamini-Hochberg  
383 procedure (False Discovery Rate)

384

385 Similar to the preferences towards kairomone soil in test two, four and six (as seen in Table  
386 6), the proportion of time spent burrowing in kairomone soil was also higher in rendzina  
387 animals compared to basaltic ones in the mentioned tests (Figure 5). In rest of the tests (one,  
388 three and five), rendzina animals spent similar proportion of time burrowing in control soil.  
389 As for the basaltic animals, there was no difference between time spent burrowing in control  
390 soil and kairomone soil (Figure 5).



391

392 **Figure 6.** Box plot showing the proportion of time spent burrowing in control and kairomone  
 393 soil in T-maze experiments by basaltic-soil and rendzina-soil individuals of *N. galili*

394

395

396 **4. Discussion**

397 In the current study, all the individuals of *N. galili* collectively showed significant preference  
 398 for particular plant species in the cafeteria trials with both natural and agricultural plants,  
 399 however this preference did not differ between individuals originating from basaltic and  
 400 rendzina soil with an exception of two crop plants – potato and sweet potato. With regards to  
 401 T-maze experiments to understand the ability to perceive kairomones, no significant  
 402 preference for the kairomone soil was found in any of the six experiments. Although, whether  
 403 the mole rats preferred control or kairomone soil, did differ based on the individual's soil of

404 origin in test two and four in which, rendzina individuals showed significantly higher  
405 preference for kairomone soil compared to basaltic ones.

#### 406 **4.1 Cafeteria Trails**

407 Cafeteria trial has been a standard method to understand the dietary preferences in  
408 animals (e.g. Drożdż, 1966; Górecki & Gębczyńska, 1962; Kwieciński et al., 2017; Renner  
409 et al., 2012). In the trial with natural plants, *Eryngium creticum* was the most preferred plant  
410 followed by *Leopoldium* sp. and least preferred was old roots of *Asphodelus* sp. by all the  
411 animals despite their soil origin. *E. creticum* is also the most abundant in both basaltic and  
412 rendzina soil (Lövy et al., 2015b) compared to other natural plants include in the trails. Despite  
413 relatively less content of starch compared to *A. coronaria* and the least of energy compared to  
414 all other natural plants, such highly significant preference for *E. creticum* indicates that the  
415 mole rats preferred in these trails the most common food plant over the most energetic or  
416 nutrient rich ones. This finding is in agreement with food generalism found in subterranean  
417 rodents (Heth et al., 1989). To increase their foraging success, the mole rats consume and  
418 collect every food items they encounter while foraging instead of being selective about  
419 particular plant sp. which is nutrient rich, sometime even collecting poisonous bulbs as well  
420 (Heth et al., 2000). In the trails with crop plants, the most preferred one by all the animals  
421 was sweet potato followed by onion, carrot and potato, while the least preferred was radish.  
422 The preference for sweet potato exceeded over the most preferred natural plant *E. creticum*,  
423 Thus, when given a choice such as in crop cafeteria trial, sweet potato which had higher starch  
424 and energy content (although less than potato) along with simple sugars was preferred more  
425 than the common natural plant. Moreover, the consumption for potato and sweet potato also  
426 significantly differed between basaltic and rendzina individuals, rendzina animals consumed  
427 potatoes more than the basaltic animals whereas sweet potatoes were consumed less by  
428 rendzina animals compared to basaltic ones. Given that rendzina-mole rats are required to  
429 spend more energy towards foraging due to much lower density and diversity of geophytes  
430 (Hadid et al., 2013; Lövy et al., 2015b), we can expect that they would prefer high energetic  
431 plants over others in crop cafeteria trial however, this cannot be concluded based on only one  
432 instance. Foraging success below ground depends on soil and climatic conditions, food supply  
433 and population density of the animals which can vary seasonally. Seasonal variations can also  
434 cause variation in the choice of preferred food (e.g. (Gasperini et al., 2018; Górecki &  
435 Gębczyńska, 1962). (Gasperini et al., 2018; Górecki & Gębczyńska, 1962). Cafeteria trials  
436 can be carried out during different seasons with more diverse plants from both basaltic and  
437 rendzina soil including the plants which differ in their nutritional values on comparatively

438 wider scale than those involved in current study might provide better insight if the two  
439 populations may have diverging preferences

#### 440 **4.2 Kairomone in Hydroponics**

441 The original idea of this study was to determine from which distance *N. galili* are able to detect  
442 the plant exudates (kairomones) released from the growing roots. To achieve that it was first  
443 required to test whether the captive individuals could discriminate between control and  
444 kairomone soil. However, we did not find any consistent significant preference kairomone soil  
445 in all the six sets of T maze experiments. The ability to perceive kairomones by many species  
446 of subterranean rodents has long been established and T-maze experiments have routinely  
447 been used to test the same. In a similar set up, Zambian mole-rats (*FukomysI anseli* and *F.*  
448 *kafuensis*) were able to distinguish between peat moistened with control water and carrot water  
449 in and they could also detect carrot root kairomones percolated through the soil from the plant  
450 which had been growing for a week in the T-maze over the distance of 30 cm (Lange et al.,  
451 (2005). Various African mole-rats species (*Heliophobius argenteocinereu*, *F. mechowii*, *F.*  
452 *darlingi*, *F. anseli*) also showed positive detection of kairomone through experiments both  
453 with soils in which carrot plant had or had not been growing and with carrot water obtained  
454 through hydroponics (Vitámvás, 2013). This study also concluded 30 cm to be the maximum  
455 distance for all the species to locate the kairomone soil (even 40 cm for *H. argenteocinereu*  
456 and *F. mechowii*). Similar results were found in another studies with *Ctenomys talarum*  
457 (Schleich & Zenuto, 2007, 2010) in which the animals could detect and discriminate between  
458 soils where their natural food plant species had or had not been growing. Besides these other  
459 species, *N. galili* also showed significant preference for kairomone soil, even discriminating  
460 to some extent, different concentrations of kairomones (Heth et al., 2002) including  
461 discriminating between odours of edible and non-edible plants and soils in which edible as  
462 opposed to poisonous plant had been growing (Heth et al., 2000), but we were not able to  
463 obtain similar results in the present study.

464 Moreover, majority of these studies used carrots to obtain kairomone water. In this study,  
465 in addition to carrots, we tried three other plants. Based on the results of the cafeteria trials  
466 with the crop plants, it was evident that these mole rats considerably preferred sweet potato  
467 over all other plants present in the trail. However, using sweet potato for this experiment (test  
468 five) did not yield any significant results. Another possibility was to use a natural plant to  
469 obtain kairomone water and since growing *Eryngium creticum* at this study location was  
470 difficult we tried locally available dandelion plants. When these captive animals were

471 presented with leaves and roots of dandelion plants, they consumed them readily, but during  
472 the experiments both with dandelion water and soil (test four and six respectively) no positive  
473 detection of kairomones was indicated. The only significant results, although not consistent  
474 across all the tests, were found in test two (onion water – 8 days) and four (dandelion water –  
475 12 days) in which the individuals originating from rendzina soil preferred to burrow through  
476 kairomone soil significantly more than those of basaltic individuals. As mentioned before,  
477 rendzina soil being scarce in food supply (Lövy et al., 2015b) is relatively more stressful  
478 environment where the inhabiting mole rats are required to burrow longer distances in search  
479 of food. Therefore, it would be expected that the rendzina animals may develop heightened  
480 sense to perceive kairomones to aid in successfully locating the food source from some  
481 distance compared to their basaltic counterparts. It'd be interesting to see if such patterns  
482 persist in similar further experiments, although from the experience during this study, the mole  
483 rats were rather more enthusiastic about digging in peat in most of the tests. Based on this  
484 behaviour, the first presumption was that since the animals were kept in sawdust in their  
485 respective terrarium, they showed keen enthusiasm for digging when they encountered peat in  
486 the T-maze during the experiments. To counter this, from test three to six, each individual was  
487 kept in Perspex tunnel system (Appendix Picture 1) overnight to weaken their keenness for  
488 burrowing in peat and let them be familiarised with the apparatus. However, this change made  
489 little to no difference. In only 40% of the trials, the mole-rats showed clear preference i.e. they  
490 selected one arm of the T-maze and burrowed through it entirely at once without digging back  
491 and forth between both the arms. In rest of the trials, the preference seemed more random  
492 yielding no consistent patterns. These individuals have lived in captivity for over a year before  
493 the experiments were conducted and they are fed ad libitum with various food plants and dry  
494 rodent food which eliminates the need to forage for the food and to use olfactory senses to  
495 sense kairomones which could be the reason the animals were not motivated despite being  
496 starved for ~ 24 hours. Keeping the animals food deprived prior to running experiments is a  
497 standard practice to increase their activity level (Oosthuizen & Lutermann, 2023; Schleich &  
498 Zenuto, 2010; Schwarting & Borta, 2005; Vitámvás, n.d.) and *N. galili* have shown positive  
499 detection of vegetation cues in previous studies without starvation (Heth et al., 2000, 2002).  
500 However, whether the animals were food deprived or not made no difference during this study.  
501 Moreover dietary changes in captivity and unnatural captive environment have potential to  
502 alter the gut microbiome of the mole rats (Alberdi et al., 2021, 2021; Bensch et al., 2023;  
503 Clayton et al., 2016; Schmidt et al., 2019) unless the time in captivity is short for wild-captured  
504 animals (Kohl & Dearing, 2014; Liu et al., 2021). Gut microbiota have been known to

505 influence change in olfactory behaviour in invertebrates (e.g. Akami et al., 2019; Qiao et al.,  
506 2019; Yuval, 2017) but can such captivity induced microbiome changes affect the kairomone  
507 perception in the mole rats is a question for further investigation.

508 Besides these factors, inter individual variation, i.e. animal personality, could also be  
509 responsible for inconsistent patterns observed in the results. During the experiments, it was  
510 frequently observed that some individuals were more active/aggressive in almost all the tests,  
511 they would not settle during the habituation period (rather might be more aggravated) and  
512 would rush as soon as the barrier opened and these individuals usually dug very randomly.  
513 Understanding the personality and choosing the individuals who could be more cooperative  
514 during such laboratory trials may enable us to achieve the original objective of the study. Also,  
515 in many rodent species, behavioural lateralization (i.e. differential use of one side of body) is  
516 common which can be detected in turning biases at individual level ((Jacobs & Oosthuizen,  
517 2023, 2021; Oosthuizen & Lutermann, 2023). There may be possibility that these mole rats  
518 would prefer one side of the T-maze due to such inherent bias. Recent study with wild-  
519 captured and captive individuals of *N. galili* indicated a collective preference by all individuals  
520 for right-side. The wild-caught group showed an overall stronger lateralization bias regardless  
521 of the side compared to captive group and the basaltic-soil mole rats showed a right-side  
522 preference while rendzina ones showed no preference (Rendulić 2023). Corelating this  
523 lateralization behaviour with the kairomone perception experiments may give novel insights  
524 into the behavioural asymmetry observed during this study.

#### 525 **4.3 Implications for Ecological Speciation in *N. galili***

526 The two populations of *N. galili*, i.e. from the basaltic and rendzina soils, have been found  
527 genetically different (Hadid et al., 2013; Li et al., 2015; Polyakov et al., 2004) based on which,  
528 ecological speciation has been proposed in this species (Hadid et al., 2013). This study  
529 attempted to understand if there were any differences in the foraging behaviour in terms of the  
530 preferred food plant species and the ability of the mole rats to perceive kairomones. Although  
531 the results were not consistent, in two out six of the tests, higher preference towards kairomone  
532 soil was found in mole rats from rendzina soil compared to the ones from basaltic soil. If one  
533 can find a way to overcome the shortcomings encountered during the T-maze experiments as  
534 mentioned in the discussion above, it'd be interesting to replicate similar experiment to see if  
535 such pattern for rendzina animals really persists. The results of the cafeteria trial with natural  
536 plants indicate no diverging preference between individuals of basaltic and rendzina origin for  
537 any of the included plant species. Moreover, both the population of mole rats showed

538 collective preference for *E. creticum*, which is abundant in both the soil types. This result  
539 strongly supports that both basaltic and rendzina mole rats are food generalist. High  
540 specialization on food resource coupled with high preference for a particular habitat can cause  
541 intraspecific niche divergence which has possibility to lead to reproductive interference which  
542 can drive ecological speciation, however since the most preferred food plant species in this  
543 study is also the most common one, such proposition cannot be supported in case of *N. galili*.

544

## 545 **5. Conclusion**

546 The work of this master's thesis attempted to understand two aspects of foraging ecology in  
547 Israeli blind mole rats *Nannospalax galili* and whether these aspects differ between two  
548 populations of the mole rats originating in two different soil types i.e. basaltic soil and rendzina  
549 soil. Firstly, with the help of cafeteria trials with various natural and crop plants, preferences  
550 for the food plant species were evaluated. All the mole rats showed preference for particular  
551 plant species in both the trials and these preferences did not vary between basaltic and rendzina  
552 individuals except for potato and sweet potato in cafeteria trails with crop plants. Secondly,  
553 the ability to detect kairomones was tested in series of six T-maze experiments. No significant  
554 preference for kairomone soil was found in any of the tests, although in two of the tests,  
555 individuals from rendzina soil showed significantly higher preference for kairomone soil  
556 compared to their basaltic counterparts. There have been number of genetic, physiological and  
557 ecological differences documented between the basaltic and rendzina populations of *N. galili*  
558 which indicate possibility for ecological speciation, however, the results of the current study  
559 did not find strong support for such proposition in the mentioned aspects of foraging ecology.

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571 **References**

- 572 Akami, M., Andongma, A. A., Zhengzhong, C., Nan, J., Khaeso, K., Jurkevitch, E., Niu, C.-  
573 Y., & Yuval, B. (2019). Intestinal bacteria modulate the foraging behavior of the  
574 oriental fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae). *PLOS ONE*, *14*(1),  
575 e0210109. <https://doi.org/10.1371/journal.pone.0210109>
- 576 Alberdi, A., Martin Bideguren, G., & Aizpurua, O. (2021). Diversity and compositional  
577 changes in the gut microbiota of wild and captive vertebrates: A meta-analysis.  
578 *Scientific Reports*, *11*(1), 22660. <https://doi.org/10.1038/s41598-021-02015-6>
- 579 Bensch, H. M., Tolf, C., Waldenström, J., Lundin, D., & Zöttl, M. (2023). Bacteroidetes to  
580 Firmicutes: Captivity changes the gut microbiota composition and diversity in a social  
581 subterranean rodent. *Animal Microbiome*, *5*(1), 9. [https://doi.org/10.1186/s42523-023-](https://doi.org/10.1186/s42523-023-00231-1)  
582 [00231-1](https://doi.org/10.1186/s42523-023-00231-1)
- 583 Chlumská, Z., Janeček, Š., & Doležal, J. (2014). How to Preserve Plant Samples for  
584 Carbohydrate Analysis? Test of Suitable Methods Applicable in Remote Areas. *Folia*  
585 *Geobotanica*, *49*(1), 1–15. <https://doi.org/10.1007/s12224-013-9153-5>
- 586 Clayton, J. B., Vangay, P., Huang, H., Ward, T., Hillmann, B. M., Al-Ghalith, G. A., Travis,  
587 D. A., Long, H. T., Tuan, B. V., Minh, V. V., Cabana, F., Nadler, T., Toddes, B.,  
588 Murphy, T., Glander, K. E., Johnson, T. J., & Knights, D. (2016). Captivity humanizes  
589 the primate microbiome. *Proceedings of the National Academy of Sciences*, *113*(37),  
590 10376–10381. <https://doi.org/10.1073/pnas.1521835113>
- 591 Drożdż, A. (1966). Food habits and food supply of rodents in the beech forest. *Acta*  
592 *Theriologicala*, *11*, 363–384. <https://doi.org/10.4098/AT.arch.66-15>
- 593 Gasperini, S., Bonacchi, A., Bartolommei, P., Manzo, E., & Cozzolino, R. (2018). Seasonal  
594 cravings: Plant food preferences of syntopic small mammals. *Ethology Ecology &*  
595 *Evolution*, *30*(1), 12–25. <https://doi.org/10.1080/03949370.2017.1310141>
- 596 Górecki, A., & Gębczyńska, Z. (1962). Food conditions for small rodents in a deciduous forest.  
597 *Acta Theriologicala*, *6*, 275–295. <https://doi.org/10.4098/AT.arch.62-10>
- 598 Hadid, Y., Tzur, S., Pavlíček, T., Šumbera, R., Šklíba, J., Lövy, M., Fragman-Sapir, O., Beiles,  
599 A., Arieli, R., Raz, S., & Nevo, E. (2013). Possible incipient sympatric ecological  
600 speciation in blind mole rats ( *Spalax* ). *Proceedings of the National Academy of*  
601 *Sciences*, *110*(7), 2587–2592. <https://doi.org/10.1073/pnas.1222588110>
- 602 Heth, G., Golenberg, E. M., & Nevo, E. (1989). Foraging strategy in a subterranean rodent,  
603 *Spalax ehrenbergi*: A test case for optimal foraging theory. *Oecologia*, *79*(4), 496–505.  
604 <https://doi.org/10.1007/BF00378667>



- 605 Heth, G., Todrank, J., Begall, S., Koch, R., Zilbiger, Y., Nevo, E., Braude, S., & Burda, H.  
606 (2002). Odours underground: Subterranean rodents may not forage “blindly.”  
607 *Behavioral Ecology and Sociobiology*, 52(1), 53–58. [https://doi.org/10.1007/s00265-](https://doi.org/10.1007/s00265-002-0476-0)  
608 002-0476-0
- 609 Heth, G., Todrank, J., & Nevo, E. (2000). Do *Spalax ehrenbergi* blind mole rats use food  
610 odours in searching for and selecting food? *Ethology Ecology & Evolution*, 12(1), 75–  
611 82. <https://doi.org/10.1080/03949370.2000.9728324>
- 612 Jacobs, P. J., & Oosthuizen, M. K. (2023). Laterality in the Damaraland Mole-Rat: Insights  
613 from a Eusocial Mammal. *Animals*, 13(4), 627. <https://doi.org/10.3390/ani13040627>
- 614 Jacobs, Paul. J., & Oosthuizen, M. K. (2021). Laterality in the Cape mole-rat, *Georchus*  
615 *capensis*. *Behavioural Processes*, 185, 104346.  
616 <https://doi.org/10.1016/j.beproc.2021.104346>
- 617 Kohl, K. D., & Dearing, M. D. (2014). Wild-caught rodents retain a majority of their natural  
618 gut microbiota upon entrance into captivity. *Environmental Microbiology Reports*,  
619 6(2), 191–195. <https://doi.org/10.1111/1758-2229.12118>
- 620 Kuang, Z., Li, F., Duan, Q., Tian, C., Nevo, E., & Li, K. (2022). Host diet shapes functionally  
621 differentiated gut microbiomes in sympatric speciation of blind mole rats in Upper  
622 Galilee, Israel. *Frontiers in Microbiology*, 13, 1062763.  
623 <https://doi.org/10.3389/fmicb.2022.1062763>
- 624 Kwieciński, Z., Rosin, Z. M., Dylewski, Ł., & Skórka, P. (2017). Sexual differences in food  
625 preferences in the white stork: An experimental study. *The Science of Nature*, 104(5–  
626 6), 39. <https://doi.org/10.1007/s00114-017-1457-5>
- 627 Lange, S., Neumann, B., Hagemeyer, P., & Burda, H. (n.d.). *Kairomone-guided food location*  
628 *in subterranean Zambian mole-rats (Cryptomys spp., Bathyergidae)*.
- 629 Li, K., Hong, W., Jiao, H., Wang, G.-D., Rodriguez, K. A., Buffenstein, R., Zhao, Y., Nevo,  
630 E., & Zhao, H. (2015). Sympatric speciation revealed by genome-wide divergence in  
631 the blind mole rat *Spalax*. *Proceedings of the National Academy of Sciences*, 112(38),  
632 11905–11910. <https://doi.org/10.1073/pnas.1514896112>
- 633 Li, K., Wang, H., Cai, Z., Wang, L., Xu, Q., Lövy, M., Wang, Z., & Nevo, E. (2016).  
634 Sympatric speciation of spiny mice, *Acomys*, unfolded transcriptomically at Evolution  
635 Canyon, Israel. *Proceedings of the National Academy of Sciences*, 113(29), 8254–  
636 8259. <https://doi.org/10.1073/pnas.1608743113>
- 637 Li, K., Zhang, S., Song, X., Weyrich, A., Wang, Y., Liu, X., Wan, N., Liu, J., Lövy, M., Cui,  
638 H., Frenkel, V., Titievsky, A., Panov, J., Brodsky, L., & Nevo, E. (2020). Genome

639 evolution of blind subterranean mole rats: Adaptive peripatric versus sympatric  
640 speciation. *Proceedings of the National Academy of Sciences*, 117(51), 32499–32508.  
641 <https://doi.org/10.1073/pnas.2018123117>

642 Liu, D., Song, P., Yan, J., Wang, H., Cai, Z., Xie, J., & Zhang, T. (2021). Gut Microbiome  
643 Changes in Captive Plateau Zokors (*Eospalax baileyi*). *Evolutionary Bioinformatics*,  
644 17, 117693432199635. <https://doi.org/10.1177/1176934321996353>

645 Lövy, M., Šklíba, J., Hrouzková, E., Dvořáková, V., Nevo, E., & Šumbera, R. (2015a). Habitat  
646 and Burrow System Characteristics of the Blind Mole Rat *Spalax galili* in an Area of  
647 Supposed Sympatric Speciation. *PLOS ONE*, 10(7), e0133157.  
648 <https://doi.org/10.1371/journal.pone.0133157>

649 Lövy, M., Šklíba, J., Hrouzková, E., Dvořáková, V., Nevo, E., & Šumbera, R. (2015b). Habitat  
650 and Burrow System Characteristics of the Blind Mole Rat *Spalax galili* in an Area of  
651 Supposed Sympatric Speciation. *PLOS ONE*, 10(7), e0133157.  
652 <https://doi.org/10.1371/journal.pone.0133157>

653 Lövy, M., Šklíba, J., Šumbera, R., & Nevo, E. (2017). Soil preference in blind mole rats in an  
654 area of supposed sympatric speciation: Do they choose the fertile or the familiar?  
655 *Journal of Zoology*, 303(4), 291–300. <https://doi.org/10.1111/jzo.12489>

656 Lövy, M., Šumbera, R., Heth, G., & Nevo, E. (2020). Presumed ecological speciation in blind  
657 mole rats: Does soil type influence mate preferences? *Ethology Ecology & Evolution*,  
658 32(1), 46–59. <https://doi.org/10.1080/03949370.2019.1646809>

659 Mukherjee, S., Kuang, Z., Ghosh, S., Detroja, R., Carmi, G., Tripathy, S., Barash, D., Frenkel-  
660 Morgenstern, M., Nevo, E., & Li, K. (2022). Incipient Sympatric Speciation and  
661 Evolution of Soil Bacteria Revealed by Metagenomic and Structured Non-Coding  
662 RNAs Analysis. *Biology*, 11(8), 1110. <https://doi.org/10.3390/biology11081110>

663 Nevo, E. (1985). Speciation in action and adaptation in subterranean mole rats: Patterns and  
664 theory. *Bolletino Di Zoologia*, 52(1–2), 65–95.  
665 <https://doi.org/10.1080/11250008509440344>

666 Oosthuizen, T., & Lutermann, H. (2023). Learning and laterality in wild-caught Namaqua rock  
667 mice (*Micaelamys namaquensis*). *Behavioural Processes*, 209, 104890.  
668 <https://doi.org/10.1016/j.beproc.2023.104890>

669 Polyakov, A., Beharav, A., Avivi, A., & Nevo, E. (2004). Mammalian microevolution in  
670 action: Adaptive edaphic genomic divergence in blind subterranean mole-rats.  
671 *Proceedings of the Royal Society of London. Series B: Biological Sciences*,  
672 271(suppl\_4). <https://doi.org/10.1098/rsbl.2003.0112>

- 673 Qiao, H., Keeseey, I. W., Hansson, B. S., & Knaden, M. (2019). Gut microbiota affects  
674 development and olfactory behavior in *Drosophila melanogaster*. *Journal of*  
675 *Experimental Biology*, jeb.192500. <https://doi.org/10.1242/jeb.192500>
- 676 Renner, S. C., Baur, S., Possler, A., Winkler, J., Kalko, E. K. V., Bates, P. J. J., & Mello, M.  
677 A. R. (2012). Food Preferences of Winter Bird Communities in Different Forest Types.  
678 *PLoS ONE*, 7(12), e53121. <https://doi.org/10.1371/journal.pone.0053121>
- 679 Schleich, C. E., & Zenuto, R. (2007). Use of vegetation chemical signals for digging  
680 orientation in the subterranean rodent *Ctenomys talarum* (Rodentia: Ctenomyidae).  
681 *Ethology*, 113(6), 573–578. <https://doi.org/10.1111/j.1439-0310.2007.01352.x>
- 682 Schleich, C. E., & Zenuto, R. (2010). Testing detection and discrimination of vegetation  
683 chemical cues in the subterranean rodent *Ctenomys talarum*. *Ethology Ecology &*  
684 *Evolution*, 22(3), 257–264. <https://doi.org/10.1080/03949370.2010.502320>
- 685 Schmidt, E., Mykytczuk, N., & Schulte-Hostedde, A. I. (2019). Effects of the captive and wild  
686 environment on diversity of the gut microbiome of deer mice (*Peromyscus*  
687 *maniculatus*). *The ISME Journal*, 13(5), 1293–1305. [https://doi.org/10.1038/s41396-](https://doi.org/10.1038/s41396-019-0345-8)  
688 [019-0345-8](https://doi.org/10.1038/s41396-019-0345-8)
- 689 Schwarting, R. K. W., & Borta, A. (2005). Analysis of behavioral asymmetries in the elevated  
690 plus-maze and in the T-maze. *Journal of Neuroscience Methods*, 141(2), 251–260.  
691 <https://doi.org/10.1016/j.jneumeth.2004.06.013>
- 692 Shams, I., Avivi, A., & Nevo, E. (2005). Oxygen and carbon dioxide fluctuations in burrows  
693 of subterranean blind mole rats indicate tolerance to hypoxic–hypercapnic stresses.  
694 *Comparative Biochemistry and Physiology Part A: Molecular & Integrative*  
695 *Physiology*, 142(3), 376–382. <https://doi.org/10.1016/j.cbpa.2005.09.003>
- 696 Singer, A. (2007). *The soils of Israel*. Springer Berlin.
- 697 Šklíba, J., Lövy, M., Koeppen, S. C. W., Pleštilová, L., Vitámvás, M., Nevo, E., & Šumbera,  
698 R. (2016). Activity of free-living subterranean blind mole rats *Spalax galili* (Rodentia:  
699 Spalacidae) in an area of supposed sympatric speciation. *Biological Journal of the*  
700 *Linnean Society*, 118(2), 280–291. <https://doi.org/10.1111/bij.12741>
- 701 Smith, C. W., Hadas, A., Dan, J., & Koyumdjisky, H. (1985). Shrinkage and Atterberg limits  
702 in relation to other properties of principal soil types in Israel. *Geoderma*, 35(1), 47–65.  
703 [https://doi.org/10.1016/0016-7061\(85\)90055-2](https://doi.org/10.1016/0016-7061(85)90055-2)
- 704 Vitámvás, B. M. (n.d.). *Sensory abilities and food localization in four species of African mole-*  
705 *rats with diverse social organization*.

706 Yuval, B. (2017). Symbiosis: Gut Bacteria Manipulate Host Behaviour. *Current Biology*,  
707 27(15), R746–R747. <https://doi.org/10.1016/j.cub.2017.06.050>

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740 **Appendix**



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742 **Picture 1.** Perspex tunnel system in which mole rats were kept overnight prior to T-maze  
743 experiments (Test three to six)

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752 **Picture 2.** T-maze experimental set-up

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754 **Picture 3.** Excavated dandelion plants kept in a bucket of water for test four

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757 **Picture 4.** Sweet potatoes with roots and slips (A), five of these were kept in a bucket of water  
758 for test five (B)

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761 **Picture 5.** Dandelion plants five days (A) and 30 days (B) after replanting

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774 **Table 1.** Statistical summary of the contrasts generated during post hoc pairwise comparison  
 775 which is comparing the proportion of given plant species consumed by individuals of *N. galili*  
 776 originating from basaltic and rendzina soil  
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Contrast	Plant Species	Estimate	SE	df	t.ratio	p
Basalt – Rendzina (Natural plant species)	<i>Anemone coronaria</i>	-0.079	0.07	19	-1.11	0.28
	<i>Asphodelus</i> (old root)	0.051	0.07	19	0.72	0.48
	<i>Asphodelus</i> (young root)	-0.023	0.07	19	-0.33	0.75
	<i>Eryngium creticum</i>	-0.009	0.07	19	-0.13	0.89
	<i>Leopoldium</i> sp.	-0.079	0.07	19	-1.12	0.28
	<i>Ornithogalum lanceolatum</i>	0.107	0.07	19	1.50	0.15
Basalt – Rendzina (Crop plants)	Carrot	0.042	0.06	20	0.67	0.51
	<i>Eryngium creticum</i>	-0.034	0.06	20	-0.54	0.59
	Onion	-0.044	0.06	20	-0.71	0.49
	<b>Potato</b>	<b>-0.157</b>	<b>0.06</b>	<b>20</b>	<b>-2.51</b>	<b>0.02</b>
	Radish	0.013	0.06	20	0.20	0.84
	<b>Sweet potato</b>	<b>0.127</b>	<b>0.06</b>	<b>20</b>	<b>2.03</b>	<b>0.05</b>

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