# 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.

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#### 1. Introduction

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

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

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

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

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

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

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

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

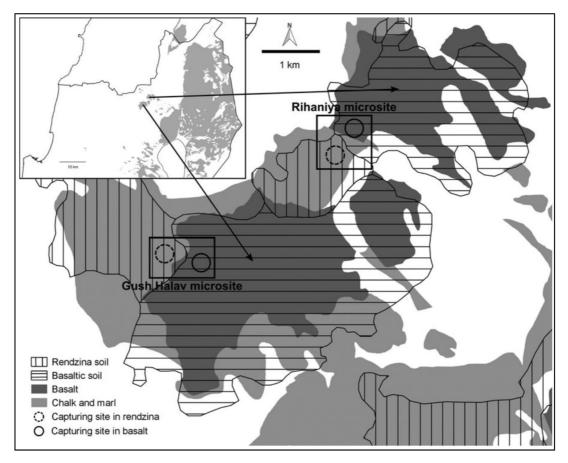
The work of this master's thesis is divided into two parts. The first part involves testing the mole rats' preferences towards various natural and crop plants with the help of cafeteria trails. The main objectives are: 1) to characterize the geophytes collected from the study microsite Rihaniya, and several crop plants for comparison in terms of their nutritional value; 2) to test whether the mole rats of the two different soil types show any variation in the decrease in body mass during starvation period. We hypothesised that since rendzina soil represents more stressful living conditions for mole rats due to scarce food supply (Lövy et al., 2015a, 2017), individuals residing in rendzina soil may spend relatively longer durations in search of food compared to basaltic-soil animals and therefore the decrease in their body mass during the starvation period before the trails, would be less compared to basaltic-soil animals.; 3) to test whether the mole rates have a preference for various natural and crop plants and if so, whether the preference differ between animals from the two soil types. The second part comprises of series of T-maze experiments to study the kairomone perception ability of these mole rats. The objectives include: 1) to test whether the captive individuals of *N. galili* can discriminate between control soil and kairomone soil. If so, 2) to test from which distance they can successfully locate kairomones in the soil. Finally, 3) to test if the ability to perceive kairomone differs in individuals based on their soil of origin.

#### 2. Materials and Methods

#### 2.1 Cafeteria trials

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

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



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

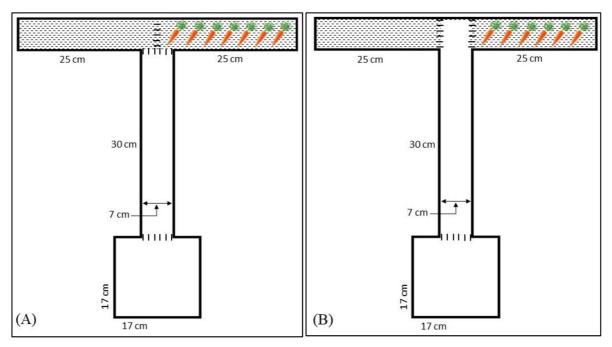
Before the trials began, the animals were starved for nine hours. Each animal was placed in a testing container and presented with one set of cafeteria test which contained approximately 15 g of each tested plant. One set of pieces of the same food plants were set aside to measure weight loss due to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

#### 2.2 Kairomone in hydroponics

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

#### **Experiment:**

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



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

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

digging in peat. During starvation period, a small piece of apple was provided for hydration.

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

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

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

For the first two sets of experiments, kairomone water was obtained by keeping 3 large yellow onions in a bucket of water (~9 1). Two of such buckets were kept at the ambient temperature and the condition of onions was observed every day to check for any possible spoilage. For the first test, onions were removed from one bucket at the end of 3<sup>rd</sup> day and water was filtered using filter paper. Since no spoilage was observed in the remaining onions for up to 8 days, second set of experiments were run with the water filtered at the end of 8th day. The control water (pure water without any onions) was kept in similar condition. After filtering, both the kairomone and control water were stored in refrigerator and utilized within three to five days. The water was brought to ambient temperature prior to using it in experiments. For the third set of experiments, kairomone water was obtained in a similar way by keeping a bunch of carrots in the similar quantity of water for 8 days. For the fourth set, we experimented with a common wild plant dandelion (Taraxacum officinale). Small dandelion plants with roots intact, were excavated from the field (Stromovka park, České Budějovice) during June, 2023 and brought to the lab. The roots were carefully washed to remove as much soil particles as possible. The plants were then kept at ambient temperature in sunlit area in the bucket filled with water in such a way that only roots remained submerged in water (Appendix Picture 3). At the end of 12<sup>th</sup> day, plants were removed and water was filtered to be used in the experiment. In the fifth set of experiments, kairomone water was obtained using sweet potatoes in the following way: five commercially available sweet potatoes (three of which were organically grown/Bio) were kept under sunlight in separate jars with pointy end of the tubers submerged in water until they started growing roots and slips. Water in the jars was changed frequently. It took two months for non-organic and one month for organic sweet potatoes to grow appropriate amounts of roots (Appendix Picture 4), after which all five of the sweet potatoes were kept in the bucket of water (~7.5 l) with roots submerged, at ambient temperature in sunlight for 15 days. On the seventh day, additional 11 of water was added as the water was being absorbed very quickly. At the end of 15<sup>th</sup> day, sweet potatoes were removed and the water was filtered to be used in the experiments. Based on the observations and unsatisfactory results obtained through five tests of kairomone in hydroponics (see the Results), last test was carried out using soil in which plants had been growing. Dandelion (Taraxacum offficinale) were excavated from the same location as previously mentioned, with

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

#### 2.3 Statistical analysis

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

#### 3. Results

#### 3.1 Cafeteria trials

Two sets of cafeteria trials were carried out with 22 individuals of *N. galili* – 10 from basaltic soil 1 and 12 from rendzina soil (8 females and 2 males from basaltic soil, 6 females and 6 males from rendzina). The average weight for basaltic and rendzina animals was  $135\pm39$  g and  $143\pm35$  g, respectively. There was no significant difference in the body mass of animals from different soil types (ANOVA:  $F_{(1,20)} = 0.268$ , p=0.61). However, the body mass differed by sex ( $F_{(1,20)} = 4.503$ , p=0.04), with males being heavier than females ( $160\pm45$  g and  $128\pm25$  g for males and females, respectively) regardless of their soil of origin (ANOVA - soil of origin \* sex :  $F_{(1,18)} = 3.624$  p= 0.0731).

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

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

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

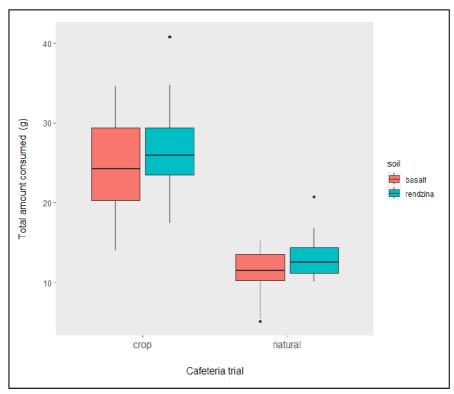


Figure 3. Box plot showing total amount of food consumed by *N. galili* of different soil origin in both cafeteria trails

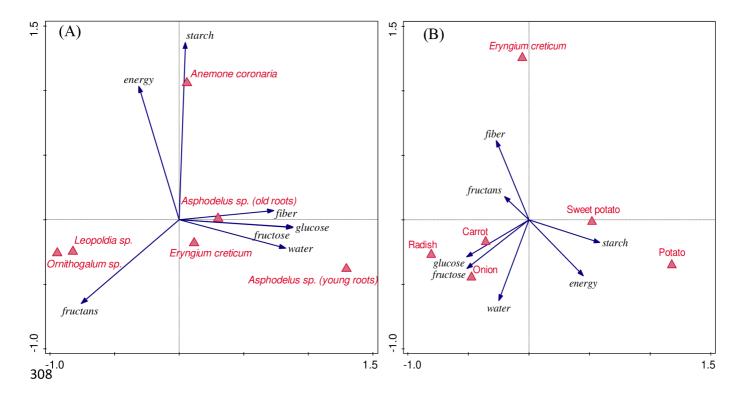
All the natural and crop plants used in cafeteria trials were characterised for their energetic and nutritional value (carbohydrates, fibre and water content), which is presented in Table 2.

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

Table 2. Nutritional characteristics of plant species in cafeteria trial (TNC: Total Nonstructural Carbohydrates)

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



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

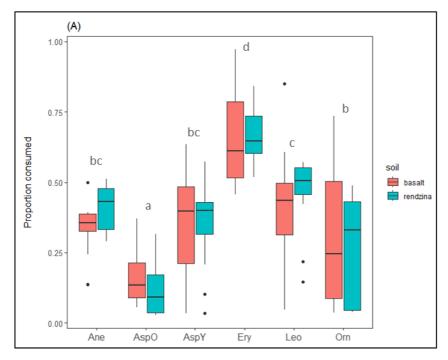
The results of the linear mixed effect models indicated a clear preference towards particular plant species in both the cafeteria sets, whereas neither the soil of origin of animals nor sex had a significant effect on the preference in either of the cafeteria trail (Figure 4, the model statistics are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

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

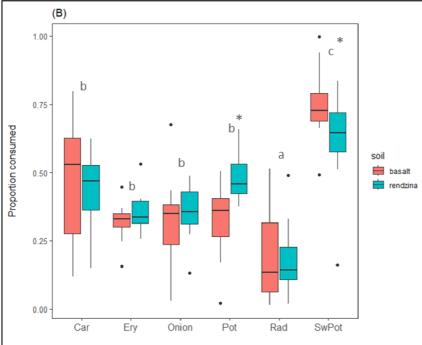
Plant species	26.75	<0.0001
Soil origin	0.12	0.74
Sex	0.00	0.97
Plant species * Soil origin	2.322	0.049
Plant species * Soil sex	1.36	0.24

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

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







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

#### 3.2 Kairomone in hydroponics

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

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

Test	L	atency (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 ± 37.1
3 (carrot – 8 days)	$24.3 \pm 33.9$	11.3 ± 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)	$7.2 \pm 4.9$	$3.6 \pm 1.5$

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

The average latency for test four was  $7.7 \pm 3.8$  s and it didn't differ either with the soil 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 five, the average latency was  $5.3 \pm 2.6$  s, which was the lowest among all the tests and did not 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 = 0.17). In the last test, test six, average latency was  $5.5 \pm 4.0$  s and basaltic animals showed a significantly higher latency compared to rendzina animals ( $F_{(1,20)} = 4.59$ , p = 0.04) and it did not differ between males and females ( $F_{(1,20)} = 1.49$ , p = 0.24).

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

Test	No. of animal s tested	Preference for Kairomone soil	Preferenc e for Control soil	χ2	р	GLM (family=binomial, line) (Response variable – preference)  predicto   Percentage   of   explained   variation		0 ,
1 (onion –	23	10	13	0.35	0.55	soil	0.15	0.83
4 days)  2 (onion –	23	13	10	0.35	0.55	sex soil	1.10 18.37	0.55 <b>0.01</b>
8 days)	23	13	10	0.55	0.55	sex	2.97	0.33
3 (carrot –	22	11	11	0	1	soil	5.43	0.20
8 days)				O		sex	6.33	0.16
4 (dandelion – 12 days)	22	14	8	2.27	0.13	soil sex	<b>20.57</b> 5.78	<b>0.02</b> 0.21
5 (sweet potato	22	8	14	2.27	0.13	soil	0	1
– 15 days)			11		0.13	sex	0.95	0.60
6 (dandelion	22	12	10	0.09	0.76	soil	9.91	0.08
soil – 30 days)						sex	1.86	0.45

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

369 These preferences did not vary significantly between males and females in all six tests.

However, a significant variation in preference was observed in tests two and four based on the

soil of origin of individuals (GLM, Table 5).

In both tests (two and four), a significantly higher number of rendzina animals preferred to

burrow through the arm filled with kairomone soil over control soil compared to the basaltic-

soil individuals (Table 6). A similar trend was present in test six as well, but the results were

not statistically significant. However, after correcting the results for multiple testing using The

Benjamini-Hochberg procedure (False Discovery Rate), none of the abovementioned results

were found significant ( $\alpha$ =0.05)

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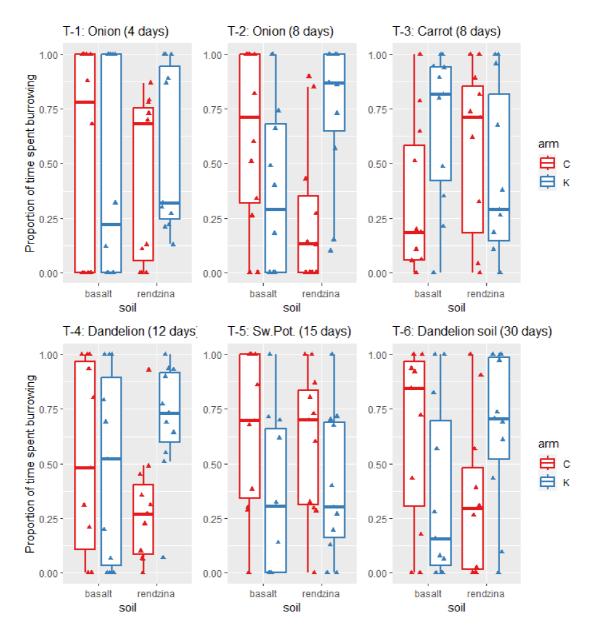
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Test		Time spent burrowing (s)		p	p
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	Soil of origin	Arm filled with	Arm filled with	χ2		adj.
	of animal	Karimone soil	Control Soil			
1	Basaltic	$11.9 \pm 15.2 (6)$	$17.6 \pm 18.5 (6)$	0	1	1
(onion – 4 days)	Rendzina	$33.1 \pm 22.0 (5)$	$38.1 \pm 33.8 (6)$	0	1	1
2	Basaltic	$31.5 \pm 39.3 $ (4)	$31.2 \pm 21.8 $ (8)	1.50	0.2	0.52
(onion – 8 days)	Rendzina	58.4 ± 43.5 (9)	$31.2 \pm 44.2$ (2)	6.54	0.01	0.06
3	Basaltic	$24.5 \pm 14.6 (7)$	$11.0 \pm 12.1$ (4)	0.73	0.39	0.52
(carrot – 8 days)	Rendzina	16.1 ± 14.3 (4)	$25.3 \pm 28.4$ (7)	0.73	0.39	0.52
4	Basaltic	$13.2 \pm 13.4$ (5)	19.4 ± 18.7 (6)	0	1	1
(dandelion –						
12 days)	Rendzina	$46.4 \pm 27.6 $ (9)	$18.7 \pm 15.5$ (2)	6.54	0.01	0.06
5	Basaltic	$26.4 \pm 42.2 $ (4)	$28.4 \pm 20.8 (7)$	0.73	0.39	0.52
(sweet potato –	Rendzina	$16.6 \pm 11.3 $ (4)	$30.1 \pm 22.6 (7)$	0.73	0.39	0.52
15 days)				0.73	0.37	
6	Basaltic	$14 \pm 15.8$ (4)	$21.6 \pm 18.0 (7)$	0.73	0.39	0.52
(dandelion soil – 30 days)	Rendzina	$23.0 \pm 14.7$ (8)	12.1 ± 11.5 (3)	2.91	0.08	0.32

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

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



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

#### 4. Discussion

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

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

#### 4.1 Cafeteria Trails

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Cafeteria trial has been a standard method to understand the dietary preferences in animals (e.g. Drożdż, 19 66; Górecki & Gębczyńska, 1962; Kwieciński et al., 2017; Renner et al., 2012). In the trial with natural plants, Eryngium creticum was the most preferred plant followed by Leopoldium sp. and least preferred was old roots of Asphodelus sp. by all the animals despite their soil origin. E. creticum is also the most abundant in both basaltic and rendzina soil (Lövy et al., 2015b) compared to other natural plants include in the trails. Despite relatively less content of starch compared to A. coronaria and the least of energy compared to all other natural plants, such highly significant preference for E. creticum indicates that the mole rats preferred in these trails the most common food plant over the most energetic or nutrient rich ones. This finding is in agreement with food generalism found in subterranean rodents (Heth et al., 1989). To increase their foraging success, the mole rats consume and collect every food items they encounter while foraging instead of being selective about particular plant sp. which is nutrient rich, sometime even collecting poisonous bulbs as well (Heth et al., 2000). In the trails with crop plants, the most preferred one by all the animals was sweet potato followed by onion, carrot and potato, while the least preferred was radish. The preference for sweet potato exceeded over the most preferred natural plant E. creticum, Thus, when given a choice such as in crop cafeteria trial, sweet potato which had higher starch and energy content (although less than potato) along with simple sugars was preferred more than the common natural plant. Moreover, the consumption for potato and sweet potato also significantly differed between basaltic and rendzina individuals, rendzina animals consumed potatoes more than the basaltic animals whereas sweet potatoes were consumed less by rendzina animals compered to basaltic ones. Given that rendzina-mole rats are required to spend more energy towards foraging due to much lower density and diversity of geophytes (Hadid et al., 2013; Lövy et al., 2015b), we can expect that they would prefer high energetic plants over others in crop cafeteria trial however, this cannot be concluded based on only one instance. Foraging success below ground depends on soil and climatic conditions, food supply and population density of the animals which can vary seasonally. Seasonal variations can also cause variation in the choice of preferred food (e.g. (Gasperini et al., 2018; Górecki & Gębczyńska, 1962). (Gasperini et al., 2018; Górecki & Gębczyńska, 1962). Cafeteria trials can be carried out during different seasons with more diverse plants from both basaltic and rendzina soil including the plants which differ in their nutritional values on comparatively

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

#### 4.2 Kairomone in Hydroponics

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

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

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

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

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

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

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

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

#### 5. Conclusion

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

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### **Appendix**



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





Picture 2. T-maze experimental set-up



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



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





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

**Table 1.** Statistical summary of the contrasts generated during post hoc pairwise comparison which is comparing the proportion of given plant species consumed by individuals of *N. galili* originating from basaltic and rendzina soil

Contrast	Plant Species	Estimate	SE	df	t.ratio	p
	Anemone coronaria	-0.079	0.07	19	-1.11	0.28
	Asphodelus	0.051	0.07	19	0.72	0.48
na ies)	(old root)					
ndzii spec	Asphodelus	-0.023	0.07	19	-0.33	0.75
- Rer	(young root)					
Basalt – Rendzina (Natural plant species)	Eryngium creticum	-0.009	0.07	19	-0.13	0.89
Ba (Natu	Leopoldium sp.	-0.079	0.07	19	-1.12	0.28
	Ornithogalum	0.107	0.07	19	1.50	0.15
	lanceolatun					
	Carrot	0.042	0.06	20	0.67	0.51
Basalt – Rendzina (Crop plants)	Eryngium creticum	-0.034	0.06	20	-0.54	0.59
Renc	Onion	-0.044	0.06	20	-0.71	0.49
salt – Rendzi (Crop plants)	Potato	-0.157	0.06	20	-2.51	0.02
Basa (C	Radish	0.013	0.06	20	0.20	0.84
	Sweet potato	0.127	0.06	20	2.03	0.05