

Food selection of tree seeds under three-week laboratory conditions in the bank vole and yellow-necked mouse

Authors: Mühlböck, Peter, Šerá, Božena, and Sedláček¹, František

Source: Journal of Vertebrate Biology, 73(24040)

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: <https://doi.org/10.25225/jvb.24040>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Food selection of tree seeds under three-week laboratory conditions in the bank vole and yellow-necked mouse

Peter MÜHLBÖCK¹ , Božena ŠERÁ²  and František SEDLÁČEK^{1*} 

¹ Department of Zoology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic; e-mail: fsedlac@prf.jcu.cz, peter.muehlboeck@drei.at

² Department of Environmental Ecology and Landscape Management, Faculty of Natural Sciences, Comenius University in Bratislava, Slovak Republic; e-mail: bozena.sera@uniba.sk

► Received 22 March 2024; Accepted 18 June 2024; Published online 19 July 2024

Abstract. The study of personality traits could reveal new links between behaviour and population ecology and evolutionary and developmental biology. Our study aimed to broaden the existing research by investigating the impact of personality traits on food preferences for forest tree seeds in two rodent species: the bank vole (*Myodes glareolus*) and the yellow-necked mouse (*Apodemus flavicollis*). Under laboratory conditions, behavioural parameters were evaluated in 33 individuals. Using LMM, we calculated the intercepts (considered to represent personality trait) for each individual in freezing and exploration duration (latency reaching the farthest point of the test box). For the preference test, seeds of several typical forest tree species were selected: common hornbeam, European beech, Norway spruce, Scots pine, wild cherry, common oak and small-leaved lime. Ivlev's electivity index (IEI) was calculated for each individual and seed species. The testing day was a significant variable in all GLMMs (seven tree seeds). In spruce, pine and lime seeds, the clear positive selection at the beginning decreased during the test; in hornbeam and oak, the selection was the opposite. In five models, the personality trait (exploration duration), and in two models for oak and lime, rodent species, were significant variables. Personality traits can create structured food niches.

Key words: small terrestrial mammals, animal personality, Ivlev's electivity index, seed nutritional composition

Introduction

Behavioural activities of small mammals in forests, such as moving soil (ventilation), gnawing vegetation (supporting or preventing particular tree species), and disturbing plant seeds, are well known (Bäumler 1989, Kollmann et al. 1998, Li & Zhang 2003). Seeds, especially tree seeds, are a large part of the diet of small terrestrial forest rodents (Drozd 1966, Hansson 1971, Niethammer 1978). Several researchers have

studied tree seeds as food for small ground rodents (Wereszczyńska & Nowakowski 2004, Wereszczyńska et al. 2007, Lobo & Millar 2011). Current studies include food consumed in terms of substances and energy received and the creation of stores (larder hoarding) in various spatial arrangements (Lu & Zhang 2008, Sunyer et al. 2013, Zwolak & Sih 2020). Populations of small terrestrial mammals fluctuate in time and space, and thus, the influence mentioned above on the ecosystem is due to their behaviour.

* Corresponding Author

This is an open access article under the terms of the Creative Commons Attribution Licence (CC BY 4.0), which permits use, distribution and reproduction in any medium provided the original work is properly cited.



In the forests of Central Europe, the bank vole (*Myodes glareolus*) and the yellow-necked mouse (*Apodemus flavicollis*) are the most abundant rodent species (Niethammer 1978, Viro & Niethammer 1982). Jędrzejewska et al. (2004) measured low densities of rodents in the spring of years following small and failed autumn crops (especially oak, lime and hornbeam seeds) and high densities in the summer and autumn (up to 160 individuals/ha) in years where oak and hornbeam trees had mast years. These facts emphasise that tree seeds are an important part of the diet (especially in summer and autumn) and that the mentioned species (granivores/omnivores) must quickly change their feeding habits, suggesting that there must be high behavioural plasticity in foraging. This observation has been supported by several studies investigating diet through stomach analyses (Drozd 1966, Watts 1968, Gębczyńska 1976). Drozd (1966) also did feeding experiments with the abovementioned species under laboratory conditions. For six days, cafeteria tests were carried out on three to five seasonal plant foods (herbs, bark of shrubs, seeds, and fruits). Both rodents preferred tree seeds; however, seed preference was slightly higher in the yellow-necked mouse. Beech seeds dominated seed food; however, intraspecific variation was not thoroughly considered.

The relatively high intraspecific variability we observed in a pilot test gave us the idea of using the concept of personality traits to interpret our findings (Lantová et al. 2011, Zwolak & Sih 2020, Brehm & Mortellitia 2022). Animal personality traits are understood as individual behaviour differences consistent across time and ecological contexts (Réale et al. 2007, 2010). These traits have been well-documented in a wide array of species, including both vertebrates (Gosling 2001) and invertebrates (Mather & Logue 2013), and several models explaining how they are maintained have been suggested. These models emphasise a variety of mechanisms; for example, density dependence, life-history trade-offs, sexual selection, and social niche specialisation (Tomkins & Brown 2004, Biro & Stamps 2008, Bergmüller & Taborsky 2010). Ongoing exploration of personality traits continues to unveil fresh connections between behaviour and the fields of population ecology, evolutionary biology, and developmental biology (Stamps & Krishan 2014, 2017, Carere et al. 2015, Dingemanse 2017). We focused on the seed preference of forest trees, especially on its change during a several-week laboratory experiment. In two species of forest rodents, we also investigated

how their intraspecific variation influences this selection, which must be considered a part of the cognitive processes controlled by taste (Muñoz & Bonal 2008, Lobo & Millar 2011).

Based on published data, we began the presented study with the following hypothesis: selectivity will not be firmly fixed but will change over time according to the species' food strategy and seed supply, as well as according to personality traits associated with search or movement activity. We tested the following assumptions: i) At the beginning of the experiments, seed consumption mirrors experience with the food on offer at the trapping location. During the experiment, seed preferences will change according to the best-tasting food (basic nutrients) because the animals will not suffer from a lack of food. ii) The yellow-necked mouse is a more pronounced seed specialist; therefore, in this species, the differences between the start and end of the experiment will be lower (more conservative) than in the omnivorous bank vole. iii) This will also correspond to a greater intraspecific variation and influence of personality traits in the bank vole.

Material and Methods

Material

The animals were caught in a mixed broad-leaved forest at the edge of České Budějovice, Czech Republic (N 48°58'41", E 14°25'42") in September and October 2011-2012 (see Table S1). In the forest, Norway spruce (*Picea abies*) was most abundant, pedunculate oak (*Quercus robur*) was common, Scots pine (*Pinus sylvestris*) and European beech (*Fagus sylvatica*) were infrequent. In the mentioned years, the population density ranged between 5-10 animals per 100 trap nights. Nineteen individuals (eight M and 11 F) of the yellow-necked mouse and 14 individuals (seven M, seven F) of the bank vole were used for the food tests. The animals were housed individually in wooden cages of 100 × 60 × 80 cm (L, W, H) covered with a wire grid at the top. The floor of the cage was filled with fine sand. The layer was about 2 cm in depth. In the period between catching and starting the experiments as well as between the tests (from day two up to day 21), the animals were fed pellets for mice and rats and for guinea pigs and rabbits (Velaz Prague Ltd., Czech Republic). In addition, all tested seeds were also provided, and carrots and potatoes were added once a week. All food and water were provided *ad libitum*. Hay and a plastic pipe were provided for building a nest.

Methods

Behavioural parameters in the test arena

One week elapsed between the capture of each animal and the first behavioural observation, along with the first offering of seeds for the test. To determine personality traits, we observed the behaviour of both rodent species for the first five minutes when they were first placed in the wooden cage. Further monitoring took place after each complete removal of the sand for the purpose of determining uneaten remains of seeds and subsequent pouring of completely new sand, i.e., after the first and second day and then at the end of the test on the 21st and 22nd day. During individual test days, the testing order of the rodents was randomised. The duration of the following parameters was determined: locomotion – duration of locomotor activity; scanning – exploratory activity involving head orientation and sniffing or gnawing on something; grooming – self-grooming of the fur; freezing – animal immobile; shelter – hidden in the shelter; exploration – activity/ability (latency) in reaching the most distant point in the test box (diagonally opposite corner to the insertion point); jumping – trying to test the test-box walls (in yellow-necked mice only).

Seed preference

On day 0 of the experiment, a ‘Cafeteria’ test was prepared for the individuals of both species, with food placed on Petri dishes. We used an indirect approach, like measuring the giving-up density

(GUD), which is the amount of food left when individuals stop foraging (Pickett et al. 2005). The remaining seeds were detected each morning, and a new seed set was subsequently offered. For this experiment, seeds of seven tree species, typical for forests in Central Europe and the trapping site, were offered. The mix contained four grams of oak seeds (acorns) and one gram each from the other seed species: common beech, Norway spruce, Scots pine, small-leaved lime (*Tilia cordata*), common hornbeam (*Carpinus betulus*), and wild cherry (*Prunus avium*). The seeds were collected near the experiment site or bought from the Czech State Forests (spruce seeds) and Lower Saxony State Forests, Germany (beech nuts). Seed consumption was calculated from food remains removed by thoroughly sifting the sand. This consumption evaluation was done from day zero to day one and from day one to day two. The same analysis was done from day 20 to day 21 and from 21 to day 22 (end of the test). Based on seed residues from the thoroughly sifted sand, the Ivlev’s electivity index was calculated according to the following relationship: $E_i = (r_i - p_i)/(r_i + p_i)$ (1) where ‘*r*’ is the amount consumed and ‘*p*’ is the food offered.

Analysis of the seed nutrients

All tested seeds were completely dried (30 °C, 48 h), decorticated (testa removal) and crushed to powder biomass. For each type of seed, 100 g of biomass was used for the chemical analysis. Determination

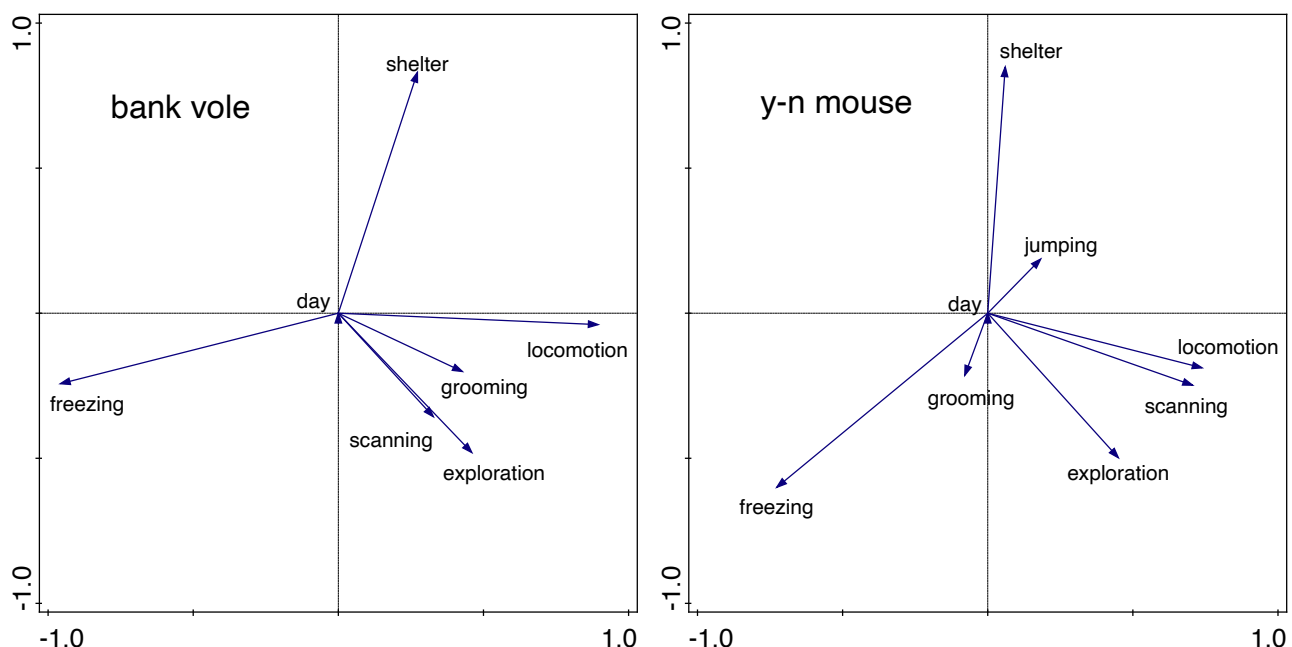


Fig. 1. PCA ordination graphs for the behavioural parameters in the bank vole and yellow-necked mouse. Explained cumulative variation according to axes 1 and 2 were in bank vole 40 and 60%, and in yellow-necked mouse 27 and 50%, respectively. To determine personality traits using LMM, the behavioural parameter freezing duration and exploration – activity/ability (latency) in reaching the most distant point in the test box were selected.

Table 1. Description of LMM for the selected behavioural parameters for estimation of the random effects of intercepts as personality traits.

Response var.	model	df	AIC	BIC	logLik	deviance	Chisq	df	Pr(>Chisq)
bank vole									
freezing	~ day	3	1,195.77	1,203.46	-594.88	1,189.77			
freezing	~ day + (1 id)	4	1,139.77	1,150.03	-565.89	1,131.77	57.997	1	< 0.0001
exploration	~ day	3	1,128.69	1,136.38	-561.34	1,122.69			
exploration	~ day + (1 id)	4	1,118.47	1,128.72	-555.23	1,110.47	12.219	1	0.0005
yellow-necked mouse									
freezing	~ day	3	1,182.75	1,190.44	-588.38	1,176.75			
freezing	~ day + (1 id)	4	1,164.19	1,174.45	-578.10	1,156.19	20.559	1	< 0.0001
exploration	~ day	3	1,128.83	1,136.52	-561.41	1,122.83			
exploration	~ day + (1 id)	4	1,109.51	1,119.76	-550.75	1,101.51	21.323	1	< 0.0001

of basic nutrients, i.e. proteins (nitrogen content – N), starch, glucose, lipids and cellulose, was carried out by AGRO-LA Comp. Ltd. (Jindřichův Hradec, Czech Republic <http://eagri.cz/public/web/ukzuz/portal>). The following methods were used: nitrogen – Kjeldahl method and coulometric determination; starch – Ewers polarimetric method; glucose – extraction with ethanol, titration by the Luff-Schoorl method; lipids – extraction with hydrochloric acid; cellulose – oxidative hydrolysis and gravimetry.

Statistical evaluation

Selection of the best parameter for personality assessment

A principal component analysis (PCA) was performed as an initial evaluation to obtain an overview of the relationships among behavioural traits (Budaev 2010). Behavioural traits were analysed using Canoco v.5.0 software for the multivariate analysis (Ter Braak & Šmilauer 2018). Based on this analysis, we chose two behavioural parameters: freezing duration and exploration duration (distant point latency), see Fig. 1. These two non-correlated parameters were selected for personality trait assessment using a linear mixed-effects model (LMM) (Table 1).

Personality trait assessment

The definition of personality traits implies that these are elements of behaviour that repeat over time and under different conditions (Réale et al. 2007 2010). When repeated tests are available, then LMM processing just makes it possible to isolate the stable individual part of behaviour in repeated tests, i.e. random intercept (considered personality trait). In addition, the slope (behavioural plasticity) associated with time is also generated by the model. In our study, LMMs were used to estimate the variation in the random intercepts for the two behavioural parameters (freezing, exploration) using four

measurements (i.e. the four different time points) for each individual (see Nicolaus et al. 2013). We used the *lmer* function in the statistical software R v.3.5.2 (R Core Team 2018) using the *lme4* package (Bates et al. 2015) to build mixed effect models for the two mentioned behavioural parameters (e.g. Schaeffer 2004, Bates et al. 2015). Statistical significance for fixed and random (individual – id) effects was obtained using a likelihood-ratio test, which compared a particular model with the null model (Table 1). Random intercepts have already been used as a proxy for personality traits, e.g. in the common vole (Urbánková et al. 2020).

Determination of Ivlev's electivity index in chosen seed species

For the mixture of seven seed species, Ivlev's electivity index (Ivlev 1961) was calculated using the ratio of each consumed seed species to the total amount in the mixture (1). This index ranges from –1 (total avoidance of food) to +1 (highest preference of food) (Krebs 1989). Ivlev's indices were calculated for all seven tree seed species on days one and two, and days 21 and 22 (end of the test). Values from the first two days and the last two days were averaged for further analyses and graphs. Ivlev's electivity index of the tested seed species and their chemical composition (nitrogen, starch, glucose, lipids and cellulose) were evaluated by redundancy analysis (RDA). Calculations for RDA were performed using the above-mentioned Canoco v.5.0 program. Some calculations were performed using a test implemented within STATISTICA v.13.2 (Dell Inc. 2016).

Influence of selected independent variables on Ivlev's electivity index

The influence of selected independent variables on Ivlev's electivity index was analysed using a

Table 2. Excerpt from Table S2 for the case of spruce seeds. Selection of the best GLMM for Ivlev's electivity index response variables with different fixed factors (day, species, sex, personality trait, locomotor activity, body weight). All models used (day|id) specification as their random effects. Presented likelihood-ratio tests compare the particular model with a reduced model of the \sim +day + (day|id).

Response variable	Model	AIC	BIC	logLik	P
spruce	\sim +1 + (1 id)	72.17	80.82	-33.09	
	\sim +day + (day id)	65.21	82.51	-26.60	0.005
	\sim +day + species + (day id)	66.23	86.41	-26.12	0.322
	\sim +day + sex + (day id)	64.81	84.99	-25.40	0.121
	\sim +day + freezing + (day id)	66.79	86.97	-26.40	0.520
	\sim +day + exploration + (day id)	58.42	78.60	-22.21	0.003
	\sim +day + weight + (day id) + (weight id)	66.22	95.04	-23.11	0.136

Table 3. Fixed effect estimates of the best GLMM for individual Ivlev's index of electivity extracted from R calculation of models shown in Table S2.

Response variable	LMM estimated fixed effects					
	Intercept (electivity index)	\pm SE	Slope (day ⁻¹)	\pm SE	Species in O, L Personality in S, P, B, C, H	\pm SE
Oak (O)	-0.0868	0.0647	0.0068	0.0037	-0.5045	0.0623
Lime (L)	0.2814	0.0441	-0.0041	0.0024	0.1480	0.0432
Spruce (S)	0.3495	0.0440	-0.0050	0.0034	-0.0009	0.0003
Pine (P)	0.3180	0.0460	-0.0035	0.0035	-0.0008	0.0003
Beech (B)	0.0708	0.0840	0.0063	0.0044	0.0007	0.0004
Cherry (C)	-0.4297	0.0923	-0.0051	0.0040	-0.0020	0.0009
Hornbeam (H)	-0.8968	0.0472	0.0053	0.0025	0.0011	0.0005

SE – standard error, slope – increase or decrease over the course of a day.

generalised linear mixed-effect model (GLMM). Statistical significance for day, species, sex, personality traits, body weight, and random (individual – id) effects were obtained using a likelihood-ratio test, which compared a particular model with the null model. We used the statistical software R v.3.5.2 (R Core Team 2018) and the software package *lme4* (Bates et al. 2015) for the calculations. Results are shown also graphically using ANOVA and Tukey HSD *post-hoc* test.

Results

Selection of behavioural parameters and personality traits

PCA was used to obtain an overview of the relationships among the observed behavioural parameters. The parameters freezing duration and exploration duration (latency in reaching the most distant point (Fig. 1)) were selected for bank voles and yellow-necked mice because of their relationship

to each other and to PCA axes. In both species, the intercepts were highly significant as determined by the random effects of the LMMs (Table 1). The freezing and exploration behaviours of the bank vole and the yellow-necked mouse were highly significant ($P < 0.001$). In further calculations, the intercepts of these random effects were used as personality traits.

Selection of individual seed species

Table 2, an excerpt from Table S2 from the supplementary material for the case of spruce seeds, gives an example of the tested models and their level of significance. For the seeds of hornbeam, beech, spruce, pine, and cherry, the best models included, besides the test day predictor, the exploration personality trait. For the oak and lime seeds, besides the test day predictor, the predictor species was included amongst the best models (see Fig. S1). This inclusion indicates that the selectivity of oak and lime seeds was species-specific. Table 3 lists the fixed effects of the GLMMs based on the best models; the

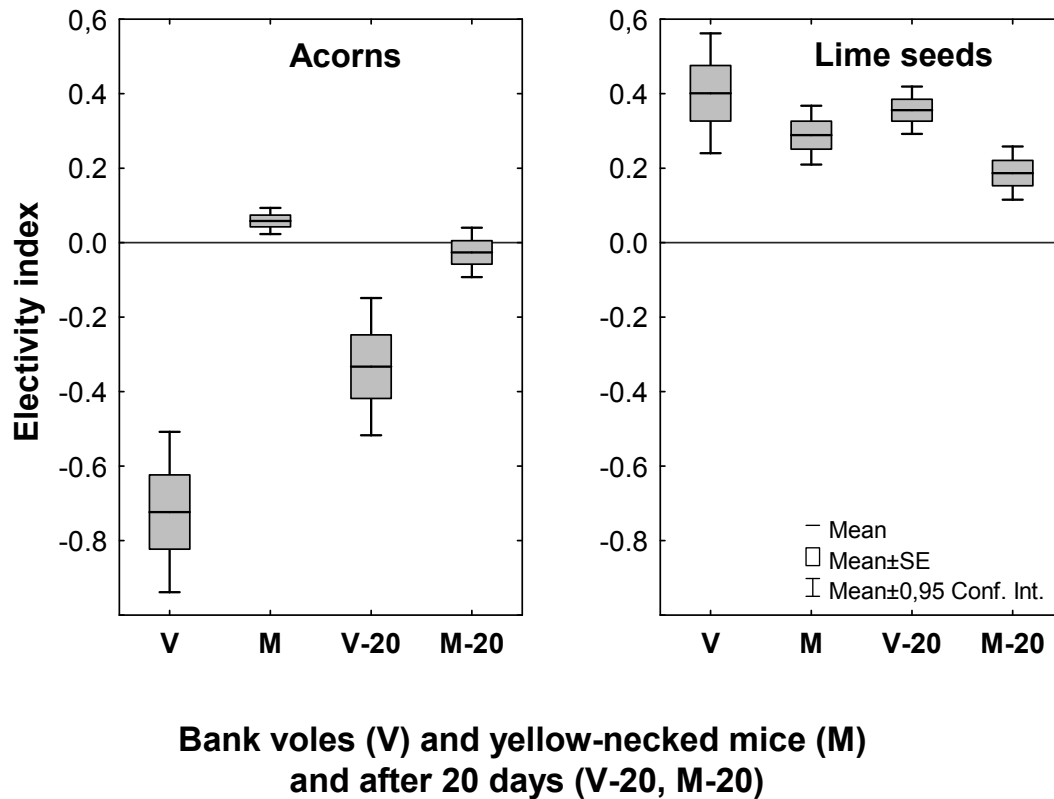


Fig. 2. Comparison of Ivlev's electivity index of acorns according to the tested species at the beginning and after 20 days under laboratory conditions ($F_{(3,54)} = 26.866, P < 0.001$; *post-hoc* test V-M: $P < 0.001$, V-V20: $P = 0.001$, M-M20: $P = 0.819$, V20-M20: $P = 0.006$). Concerning the lime seeds ($F_{(3,62)} = 4.369, P = 0.007$; *post-hoc* test V-M: $P = 0.303$, V-V20: $P = 0.911$, M-M20: $P = 0.318$, V20-M20: $P = 0.050$).

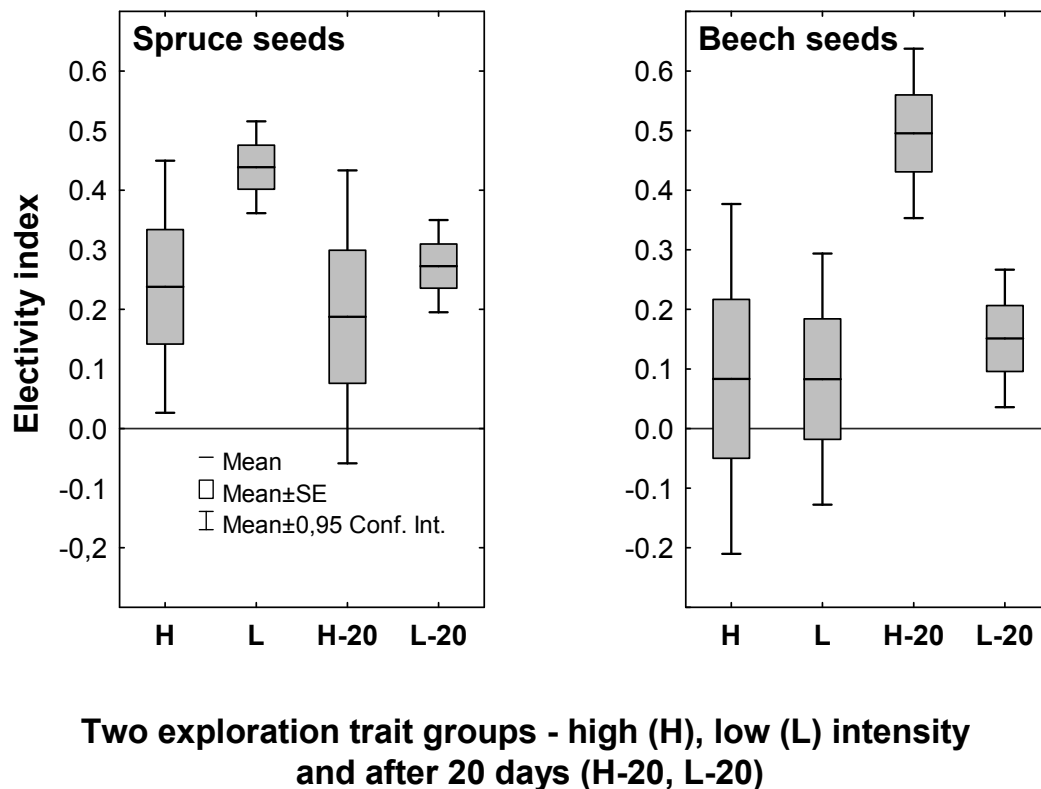


Fig. 3. Comparison of Ivlev's electivity index of spruce seeds according to the higher (H) and lower (L) exploratory personality traits on the beginning and after 20 days under laboratory conditions ($F_{(3,62)} = 3.171, P = 0.030$; *post-hoc* test H-L: $P = 0.141$, H-H20: $P = 0.962$, L-L20: $P = 0.161$, H20-L20: $P = 0.792$). Concerning the beech seeds ($F_{(3,62)} = 3.711, P = 0.016$; *post-hoc* test H-L: $P = 1.000$, H-H20: $P = 0.040$, L-L20: $P = 0.932$, H20-L20: $P = 0.059$).

intercepts represent the underlying/fundamental Ivlev's electivity indices for individual seeds.

As shown (for all seeds) in Table 3, acorns were avoided slightly ($E = -0.087$); nevertheless, generally, their selection increased among days (0.007 day^{-1}). However, bank voles avoided them much more than yellow-necked mice (coeff. = -0.505). Lime seeds were preferred ($E = 0.281$), but their selection decreased among days (-0.004 day^{-1}). These seeds were more preferred by bank voles (coeff. = 0.148). Spruce seeds were preferred ($E = 0.350$), but their selection decreased among days (-0.005 day^{-1}). Also, in the exploration duration personality trait (s), the selection decreased slightly (-0.0009 s^{-1}). Similarly, pine seeds were selected ($E = 0.318$), and among days, their preference decreased (-0.004 day^{-1}). In the exploration duration personality trait (s), the selection decreased slightly (-0.0008 s^{-1}). Beech seeds were preferred slightly only ($E = 0.071$); however, the seed selection during the days increased (0.006 day^{-1}). In the exploration duration personality trait (s), the seed selection also increased slightly (0.0007 s^{-1}). Hornbeam seeds were generally strongly avoided ($E = -0.897$), but among days, their selection

increased (0.005 day^{-1}). Also, selection increased slightly positively in the exploration personality trait (s) (0.001 s^{-1}). Cherry seeds were avoided ($E = -0.430$), and among days, their preference further decreased (-0.005 day^{-1}). In the exploration personality trait (s), their selection decreased (-0.002 s^{-1}). These results obtained using GLMM (Table 3) were further supplemented by ANOVA calculations and graphical visualisation.

Initially, the selectivity of acorns by the yellow-necked mouse (M) was around zero; significantly higher than in the bank vole (V) (*post-hoc* test V-M: $P < 0.001$). After 20 days, the selectivity in the bank voles increased significantly, but stayed significantly lower than in the yellow-necked mouse (*post-hoc* test V20-M20: $P = 0.006$, Fig. 2). Selectivity of lime seeds was high overall; at the beginning, selectivity in the yellow-necked mouse was lower than in the bank vole and after 20 days this difference was significant (*post-hoc* test V20-M20: $P = 0.050$, Fig. 2).

The selection of spruce seeds was high overall, and differences between groups with high and low exploratory intensity were not significant after

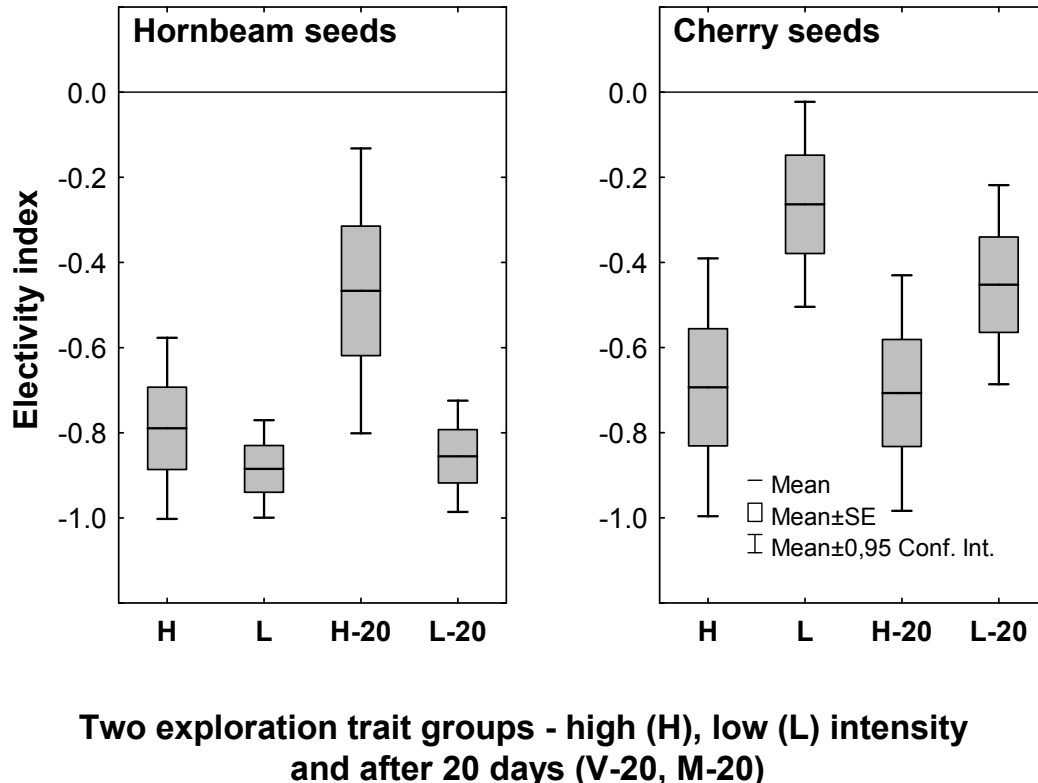


Fig. 4. Comparison of Ivlev's electivity index of hornbeam seeds according to the higher (H) and lower (L) exploratory personality traits on the beginning and after 20 days under laboratory conditions ($F_{(3, 62)} = 5.053$, $P = 0.003$; *post-hoc* test H-L: $P = 0.793$, H-H20: $P = 0.068$, L-L20: $P = 0.899$, H20-L20: $P = 0.013$). Concerning the cherry seeds ($F_{(3, 62)} = 2.893$, $P = 0.042$; *post-hoc* test H-L: $P = 0.092$, H-H20: $P = 0.999$, L-L20: $P = 0.613$, H20-L20: $P = 0.499$).

Table 4. Fixed effect estimates of the best GLMM for individual Ivlev's index of electivity extracted from R calculation of models shown in Table S2 and the nutritional composition of the tested seeds.

Seed Species	Electivity Index	Percentage				
		Proteins (n)	Lipids	Glucose	Starch	Cellulose
Oak	-0.0868	0.89	2.12	16.6	52.0	2.70
Lime	0.2814	4.35	26.9	5.89	5.47	9.15
Spruce	0.3495	3.52	40.3	2.61	1.15	14.7
Pine	0.3180	5.97	32.0	3.99	0.58	11.5
Beech	0.0708	4.02	48.2	5.21	4.63	2.86
Cherry	-0.4297	4.63	42.2	12.4	4.56	3.87
Hornbeam	-0.8968	0.79	1.93	0.30	0.60	35.0

20 days (Fig. 3). Similar results were obtained in the pine seeds (Fig. S1). Initially, the selection of beech seeds was low. However, selection of these seeds increased significantly after 20 days in individuals with higher exploration intensity (*post-hoc* test L-L20: $P = 0.040$, see Fig. 3).

Hornbeam seeds were avoided; however, after 20 days, the avoidance in individuals with higher exploration intensity was weaker, and the difference

to lower exploration individuals was significant (*post-hoc* test H20-L20: $P = 0.013$, Fig. 4). Selection of cherry seeds was overall low, and differences between groups with high and low exploratory intensity were not significant nor after 20 days (Fig. 4).

Nutritional composition of the used seeds

The determined levels of the main nutritional substances and Ivlev's electivity indexes in the used seeds are presented in Table 4. Preference in both

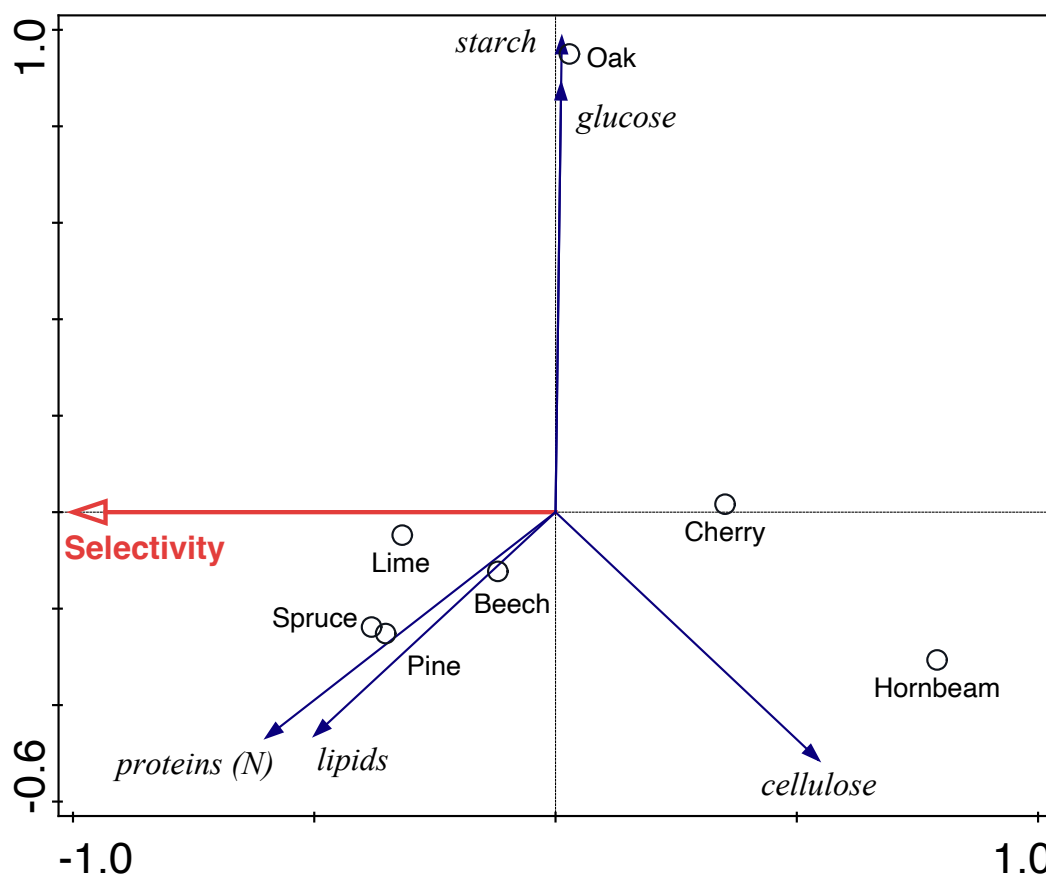


Fig. 5. Redundant analysis RDA of the data from Table 4. Explained cumulative variation: axis 1 – 18.1 %, axis 2 – 67.3 %. The graph shows that seed selection correlates positively with proteins and lipids.



rodent species for the most nutritious seeds was similar. Seeds of beech, spruce, pine and lime were chosen most often (Table 4, Fig. 5). Generally, they had the highest levels of proteins and lipids. On the other hand, seeds of hornbeam and oak were consumed the least: seeds of hornbeam contain less-digestible cellulose and were not eaten much by either rodent species (Fig. 4); acorns, which contain a lot of digestible glucose and starch were avoided by the bank vole, but consumed by the yellow-necked mouse moderately (Fig. 2). The highly nutritious cherry seeds were not eaten much, probably because of the hard seed coat (Fig. 4).

Discussion

Nutritional composition of the used seeds

The nutritional quality of seeds is of fundamental importance for small terrestrial rodents, which have high-intensity metabolism (Wereszczyńska et al. 2007). Beech seeds can provide house mice with both the energy and protein required for growth and reproduction (Ruscoe et al. 2005). Under conditions of higher population density, mice and voles consumed food richer in nitrogen (Jánová et al. 2005). The rapid growth of rodent densities in the forests of Central Europe is associated with mast seasons of deciduous trees, mainly oak and beech (Wereszczyńska & Nowakowski 2004). Bilberry seed production seems to be a causal driver of bank vole fluctuations; however, it remains to be seen to what extent the chemical composition of bilberry plants influences vole performance (Selås et al. 2021). Reil et al. (2015) examined the dependence of bank vole abundance on beech fructification and found a tight correlation. Red-backed voles (*Myodes gapperi*) and deer mice (*Peromyscus maniculatus*) decreased in body mass in a long-term test. Plant secondary metabolites likely played a major role in the deterioration of the body condition of voles fed subalpine fir and white spruce seeds. These results indicate that conifer seeds are a sufficient food resource for mice but cannot be used by primarily herbivorous voles as a major/sole food source (Lobo & Millar 2011).

In this study, seed selectivity was related to proteins and lipids content (Fig. 5). The three most abundant compounds, i.e. carbohydrates: 16.7 kJ g^{-1} ; proteins: 18.8 kJ g^{-1} ; lipids: 39.7 kJ g^{-1} are very different in their caloric values (Eckert et al. 2000) and can be digested in several ways by rodents (Pflumm 1989). Grodziński & Sawicka-Kapusta (1970) revealed that beech seeds have the most energy (29 kJ g^{-1}), followed by lime, pine and spruce seeds (about 25 kJ g^{-1}). Levels in acorns

are around 17 kJ g^{-1} . Seeds of hornbeam, which were eaten only in small quantities (because of the large amount of hard digestible cellulose), proved to have a lot of energy. These energy data correspond to the calculations of energy content from the actual share of nutritional compounds content (Table 4) and the published caloric values (Eckert et al. 2000).

The data mentioned above indicate that small mammals trying to optimise their diets so that on the one hand valuable nutrients (such as fats and proteins) are preferred and on the other hand indigestible food (such as fibres, cellulose or poisonous substances) are avoided (Harju & Hakkarainen 1997, Heroldová et al. 2008). Seeds with high lipids and protein (nitrogen) content from trees such as beech, spruce, pine, and lime were positively selected by bank voles and yellow-necked mice (Tables 3, 4), but the highly nutritious seeds of cherry were not. One reason could be the hard coat around the edible core, which was not sufficiently attractive to crack compared with other food, which was easier to obtain in higher quantities. Another reason for the low palatability could be the presence of plant secondary metabolites (PSM) such as tannins and polyphenols, which are known from acorns and chestnuts. Their effects on rodents and other mammals were investigated by Lindroth & Batzli (1984), Bryant et al. (1991), and Xiao et al. (2008). PSM can influence the metabolism in various ways. Gollig (2008) found some polyphenols (chlorogenic acid, gallic acid, ellagic acid) in acorns and detected metabolites of polyphenols in the urine of pigs just a few hours after consumption. Shimada & Saitoh (2003) found in experiments lasting 15 days with *Apodemus speciosus* (fed with acorns of *Quercus serrata*, *Quercus mongolicus* and a tannin-free control diet) that tannins (contained in acorns) can strongly influence body weight, nitrogen digestibility and even kill the animals (six of 16 animals). Independent of the high concentrations of PSM in seeds, preference in rodents was influenced by the quality of the food, namely the content of nutrients (Pucek et al. 1993, Verhagen et al. 2000, Čermák & Ježek 2005).

Changes in the electivity index during the test

Learning strongly shapes rodent seed choices and may be advantageous over inherited behaviours in unpredictable situations, such as acorn infestation rates that vary substantially among years and trees (Muñoz & Bonal 2008). During the test, variable selection of tree seeds was observed, probably due to the different taste experience of the rodents. Despite the yellow-necked mouse being a more pronounced granivorous rodent, species affiliation was not the



dominant factor. Both tested species are known as more or less omnivores with ecological and trophic preferences and adaptations (Niethammer 1978, Viro & Niethammer 1982) and can adapt quickly to new situations in nature and in experiments as well. Although the day of testing always played a significant role in the models, seed selection showed a non-uniform pattern over time. In spruce, pine and lime seeds, the clear positive selection at the beginning decreased during the test. On the other hand, the seeds of hornbeam and oak, which were avoided, showed an increase during the test. Separate categories showed a preference for beech seeds with a subsequent further increase in interest; on the contrary, cherry pits were avoided, with interest then falling even further. These results correspond with observations under natural conditions showing that beech seeds, spruce, and pine are important food and energy sources (Drozd 1966, Grodziński & Sawicka-Kapusta 1970). On the other hand, despite being full of high-quality biomass inside the seed, cherry seeds are not easily accessible to small rodents (see chapter above). It is vital to mention that a lack of food did not influence selectivity under laboratory conditions, as is often the case in nature. We can conclude that the presented increase or decrease in selectivity mirrors fine cognitive processes using taste to evaluate the food on offer (e.g., Lobo & Millar 2011).

The role of personality trait

In exploring personality traits (latency of reaching the most distant point), the electivity index decreased in spruce, pine and cherry. The index increased in the other two seed species, beech and hornbeam. The pattern is not uniform it is possible to state that animals that search more carefully learn to consume beech and hornbeam seeds. Traits associated with diet choice may be important targets for selection, and secondary metabolites can act in carefully searching animals. Behavioural selection could play a role in defining the trophic niche of individuals (Hämäläinen et al. 2021). Animal personality can generate context dependence in plant-animal interactions and suggests that behavioural diversity may have important consequences for the functioning of mutualisms (Brehm & Mortellitia 2022). Consistent differences between animal individuals can explain most of the variance in seed management variables, such as dispersal distance and seed size selection. Feldman et al. (2019) highlight the importance of considering the individual component of behaviour in scatter-hoarding rodents. Both the tested rodents are able to create this type of store.

Intrinsic traits of seed dispersers provide an alternative way of making sense of the enormous variation in seed fates (Zwolak 2018). The overview by Zwolak & Sih (2020) shows the impact of various personality traits on consumption, seed dispersal and natural forest renewal. One is gross behavioural activity, which we also worked with in the form of latency in reaching the furthest point. In the model we present, this personality trait (latency of reaching the furthest point) is often a more critical factor than species; however, it depends on the type of seed. Zwolak & Sih (2020) suppose that higher activity (short latency) requires a higher energy income.

Our study is limited to the selection of seeds, i.e., the beginning of the entire process of consumption, propagation and forest natural renewal. In the mentioned process, for example, chemosensory information on predators and conspecifics influences the foraging decisions of seed-gathering rodents over short spatial and temporal scales. In sites where rodents perceive the risk of predation to be high, inefficient foraging behaviour may result in less successful seed dispersal. Conversely, the detection of conspecific scents may increase dispersal efficiency and seedling recruitment (Sunyer et al. 2013). Under different predation pressures and seed availability, different personality traits may play a role at the population level (Burgos et al. 2022).

The species difference

It appears that differences between species in preferred food may not be profound enough to contribute to contrasting ecological niches. For example, heteromyids of different genera and sizes have similar preferences for wheat particles of various sizes and for commercially available seed species that differ considerably in nutritional and morphological characteristics (Price 1983). Drozd (1966) compared the stomach content of free-living yellow-necked mice with those of bank voles and found only slight differences in the consumption of tree seeds, but there were significant differences between the seasons. Drozd (1966) could not find any significant distinctions between the yellow-necked mouse and bank vole in the consumption of beech, hornbeam, pine, lime, and oak seeds. Our laboratory experiments showed similar observations, except for lime and oak. Bank voles selected fewer oak seeds and more lime seeds out of the two seed species. Selection for the other seed species, cherry and spruce, was the same. To make more reliable predictions regarding the food preferences of these two rodents, data from long-term feeding experiments, both in the wild and



laboratory, as well as data from stomach content analysis, should be considered.

Differences between species become apparent when their food strategies are clearly different. Lu & Zhang (2008) mentioned that the observed rodents (David's rock squirrel, white-bellied rat, large field mouse) showed larder hoarding of certain seeds (Liaodong oak, wild apricot, wild walnut, cultivated walnut) but not other seeds. The large field mouse was observed scatter-hoarding wild apricots, and David's rock squirrel scatter-hoarding Liaodong oak acorns and wild walnuts. Liaodong oak acorns, which have a soft seed hull, were more often eaten *in situ*, whereas wild walnuts, which have a hard seed hull and more tannin, were less hoarded by all rodent species.

In our tested rodents, we found different selectivity only in two seed species. A higher selectivity of the acorn in the yellow-necked mouse, where the index hovered around zero and in the bank vole near -1 . For the voles, the low nutritional quality and secondary metabolites of acorns were probably decisive factors. A less pronounced but still significant difference was found for the lime seeds. Here, selectivity was more pronounced in the bank voles.

Based on these primary results, some more general conclusions can be drawn. As might be expected, spruce forest rodents preferred spruce seeds and pine seeds, which look quite similar and had a very similar composition of major nutrients. The suitable but probably unfamiliar beech seeds were initially preferred somewhat less. This result changed during the test, especially in more exploratory individuals. Although there were a few oaks in the forest of capture, their attractiveness for mice was small, and voles even avoided them. Of course, the low nutrient content is a possible explanation. On the other hand, high preference is probably related to the high nutrient content; however, exclusive preference for seeds of a particular species (+1) was not observed. This finding indicates that preference did not lead to the consumption of a mono diet. For the natural

regeneration of the forest, it is crucial that even the most preferred seeds are not consumed exclusively, and some of them are left. When differentiating individuals into higher and lower exploratory intensity individuals, differences in their preferences and changes during the test were revealed. Although certain seeds had a high nutrient content (cherry, lime, beech), the animals had to learn to eat them, and there was a difference in how the higher and lower exploratory intensity individuals mastered it.

Acknowledgements

Our work was partly supported by the Ministry of Education, Youth and Sport of the Czech Republic (Grant 2370/2011/A). We also thank R. Pešková for animal husbandry and our English language editor, I. Steenbergen. The voles were bred and tested in accordance with the principles of animal welfare and guidelines of the Departmental Commission for Animal Protection of the Ministry of Education, Youth and Sports, permit number 7945/2010-30. After the experiments, the voles remained in the laboratory and were used for further breeding and behavioural testing.

Author Contributions

P. Mühlböck: conceptualisation, methodology, investigation, writing – original draft preparation. B. Šerá: methodology, management of seed analyses, interpretation of obtained seed data and contribution to the discussion. F. Sedláček: supervising, formal analysis, validation, writing – review & editing. All authors agreed with the content and gave explicit consent to submit this paper. All authors agreed with the participation in the study of the influence of behavioural traits on eating seeds. The authors have no relevant financial or non-financial interests to disclose.

Data Availability Statement

The data supporting this study's findings are available in the FigShare Digital Repository: https://figshare.com/articles/dataset/Data_seed_preference_Vertebrate_Biology_xlsx/26095399.



Literature

- Bates D., Mächler M., Bolker B.M. & Walker S.C. 2015: Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67: 1–48.
- Bäumler W. 1989: Von Mäusen angenagte Wurzeln bei Waldbäumen. *Anz. Schädlkd. Pflanzenschutz Umweltschutz* 62: 72–74.
- Bergmüller R. & Taborsky M. 2010: Animal personality due to social niche specialisation. *Trends Ecol. Evol.* 25: 504–511.
- Biro P.A. & Stamps J.A. 2008: Are animal personality traits linked to life-history productivity? *Trends Ecol. Evol.* 23: 361–368.
- Brehm A.B. & Mortellitia A. 2022: Small mammal personalities generate context dependence in the seed dispersal mutualism. *Proc. Natl. Acad. Sci. U. S. A.* 119: e2113870119.
- Bryant J.P., Provenza F.D., Pastor J. et al. 1991: Interactions between woody plants and browsing mammals mediated by secondary metabolites. *Annu. Rev. Ecol. Syst.* 22: 431–446.
- Budaev S.V. 2010: Using principal components and factor analysis in animal behaviour research: caveats and guidelines. *Ethology* 116: 472–480.
- Burgos T., Fedriani J.M., Escribano-Ávila G. et al. 2022: Predation risk can modify the foraging behaviour of frugivorous carnivores: implications of rewilding apex predators for plant–animal mutualisms. *J. Anim. Ecol.* 91: 1024–1035.
- Carere C., Grignani G., Bonanni R. et al. 2015: Consistent individual differences in the behavioural responsiveness of adult male cuttlefish (*Sepia officinalis*). *Appl. Anim. Behav. Sci.* 167: 89–95.
- Čermák P. & Ježek J. 2005: Effect of tree-seed crop on small mammal populations and communities in oak and beech forests in Drahany Upland (Czech Republic). *J. For. Sci.* 51: 6–14.
- Dell Inc. 2016: Dell Statistica (data analysis software system), version 13.2. https://download.su.se/Dell_Statistica_13_2_ReleaseNotes.pdf
- Dingemanse N.J. 2017: The role of personality research in contemporary behavioral ecology: a comment on Beekman and Jordan. *Behav. Ecol.* 28: 624–625.
- Drozdz A. 1966: Food habits and food supply of rodents in the beech forest. *Acta Theriol.* 11: 363–384.
- Eckert R., Randall D., Burggren W. & French K. 2000: Tierphysiologie, 3rd ed. Georg Thieme Stuttgart, New York, USA.
- Feldman M., Ferrandiz-Rovira M., Espelta J.M. & Muñoz A. 2019: Evidence of high individual variability in seed management by scatter-hoarding rodents: does ‘personality’ matter? *Anim. Behav.* 150: 167–174.
- Gębczyńska Z. 1976: Food habits of the bank vole and phenological phases of plants in an Oak-Hornbeam forest. *Acta Theriol.* 21: 223–236.
- Gollig K. 2008: Phenolische Inhaltsstoffe von Eicheln (Früchte von *Quercus* ssp.) sowie im Harn von Schweinen bei Nutzung von Eicheln als Futtermittel. *PhD thesis, Tierärztliche Hochschule Hannover, Germany.*
- Gosling S.D. 2001: From mice to men: what can we learn about personality from animal research? *Psychol. Bull.* 127: 45–86.
- Grodziński W. & Sawicka-Kapusta K. 1970: Energy values of tree-seed eaten by small mammals. *Oikos* 21: 52–58.
- Hansson L. 1971: Small rodent food, feeding and population dynamics. *Oikos* 22: 183–198.
- Harju A. & Hakkarainen O. 1997: Effect of protein and birch-bark powder on selection of food by root vole (*Microtus oeconomus*). *J. Mammal.* 78: 563–568.
- Hämäläinen A., Kiljunen M., Koskela E. et al. 2021: Artificial selection for predatory behaviour results in dietary niche differentiation in an omnivorous mammal. *Proc. R. Soc. B Biol. Sci.* 289: 20212510.
- Heroldová M., Tkadlec E., Bryja J. & Zejda J. 2008: Wheat or barley? Feeding preferences affect distribution of three rodent species in agricultural landscape. *Appl. Anim. Behav. Sci.* 110: 354–362.
- Ivlev V.S. 1961: Experimental ecology of the feeding of fishes. *Yale University Press, New Haven, USA.*
- Jánová E., Heroldová M. & Čepelka L. 2005: Rodent food quality and its relation to crops and other environmental and population parameters in an agricultural landscape. *Sci. Total Environ.* 562: 164–169.
- Jędrzejewska B., Pucek Z. & Jędrzejewski W. 2004: Seeds crops and forest rodents. In: Jędrzejewska B. & Wójcik J. (eds.), *Essays on mammals of Białowieża Forest. Mammal Research Institute, Polish Academy of Sciences, Białowieża, Poland: 129–138.*
- Kollmann J., Coomes P.A. & White S.M. 1998: Consistencies in post-dispersal seed predation of temperate fleshy-fruited species among seasons, years and sites. *Funct. Ecol.* 12: 683–690.
- Krebs C.J. 1989: Ecological methodology, 2nd ed. *Harper & Row, New York, USA.*



- Lantová P., Šíchová K., Sedláček F. & Lanta V. 2011: Determining behavioural syndromes in voles – the effects of social environment. *Ethology* 117: 124–132.
- Li H.J. & Zhang Z.B. 2003: Effects of rodents on acorn dispersal and survival on the Liadong oak (*Quercus liaotungensis* Koidz.). *For. Ecol. Manag.* 176: 387–396.
- Lindroth R.L. & Batzli G.O. 1984: Plant phenolics as chemical defenses: effects of natural phenolics on survival and growth of prairie voles (*Microtus ochrogaster*). *J. Chem. Ecol.* 10: 229–244.
- Lobo N. & Millar J. 2011: The efficacy of conifer seeds as major food resources to deer mice (*Peromyscus maniculatus*) and southern red-backed voles (*Myodes gapperi*). *Mamm. Biol.* 76: 274–284.
- Lu J. & Zhang Z. 2008: Differentiation in seed hoarding among three sympatric rodent species in a warm temperate forest. *Integr. Zool.* 2008: 134–142.
- Mather J.A. & Logue D.M. 2013: The bold and the spineless: invertebrate personalities. In: Carere C. & Maestripieri D. (eds.), *Animal personalities: behaviour, physiology, and evolution*. The University of Chicago Press, Chicago, USA: 13–35.
- Muñoz A. & Bonal R. 2008: Seed choice by rodents: learning or inheritance? *Behav. Ecol. Sociobiol.* 62: 913–922.
- Nicolaus M., Brommer J.E., Ubels R. et al. 2013: Exploring patterns of variation in clutch size-density reaction norms in a wild passerine bird. *J. Evol. Biol.* 26: 2031–2043.
- Niethammer J. 1978: *Apodemus flavicollis* (Melchior, 1834) – Gelbhalsmaus. In: Niethammer J. & Krapp K. (eds.), *Handbuch der Säugetiere Europas I/1 Rodentia (Sciuridae, Castoridae, Gliridae, Muridae)*. Akademische Verlagsgesellschaft, Wiesbaden, Germany: 327–336.
- Pflumm W. 1989: *Biologie der Säugetiere. Pareys Studentexte 66*, Verlag Paul Parey, Germany.
- Pickett K.N., Hik D.S., Newsome A.E. & Pech R.P. 2005: The influence of predation risk on foraging behaviour of brushtail possums in Australian woodlands. *Wildl. Res.* 32: 121–130.
- Price M.V. 1983: Laboratory studies of seed-size and seed species selection by heteromyid rodents. *Oecologia* 60: 259–263.
- Pucek Z., Jędrzejewski W., Jędrzejewska B. & Pucek M. 1993: Rodent population dynamics in a primeval deciduous forest (Białowieża National Park) in relation to weather, seed crop and predation. *Acta Theriol.* 38: 199–232.
- R Core Team 2018: R: a language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*.
- Réale D., Dingemanse N.J., Kazem A.J.N. & Wright J. 2010: Evolutionary and ecological approaches to the study of personality. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 365: 3937–3946.
- Réale D., Reader S.M., Sol D. et al. 2007: Integrating animal temperament within ecology and evolution. *Biol. Rev.* 82: 291–318.
- Reil D., Imholt C., Eccard J.A. & Jacob J. 2015: Beech fructification and bank vole population dynamics – combined analyses of promoters of human puumala virus infections in Germany. *PLOS ONE* 10: e0134124.
- Ruscoe W., Elkinton J., Choquenot D. & Allen R. 2005: Predation of beech seeds by mice: effect of numerical and functional responses. *J. Anim. Ecol.* 74: 1005–1019.
- Schaeffer L.R. 2004: Application of random regression models in animal breeding. *Livest. Prod. Sci.* 86: 35–45.
- Selås V., Framstad E., Rolstad J. et al. 2021: Bilberry seed production explains spatiotemporal synchronicity in bank vole population fluctuations in Norway. *Ecol. Res.* 36: 409–419.
- Shimada T. & Saitoh T. 2003: Negative effects of acorns on the wood mouse *Apodemus speciosus*. *Popul. Ecol.* 45: 7–17.
- Stamps J.A. & Krishnan V.V. 2014: Combining information from ancestors and personal experiences to predict individual differences in developmental trajectories. *Am. Nat.* 184: 647–657.
- Stamps J.A. & Krishnan V.V. 2017: Age-dependent changes in behavioural plasticity: insights from Bayesian models of development. *Anim. Behav.* 126: 53–67.
- Sunyer P., Muñoz A., Bonal R. & Espelta J.M. 2013: The ecology of seed dispersal by small rodents: a role for predator and conspecific scents. *Funct. Ecol.* 27: 1313–1321.
- Ter Braak C.J.F. & Šmilauer P. 2018: *Canoco reference manual and user's guide: software for ordination, version 5.1x*. Microcomputer Power, Ithaca, USA.
- Tomkins J.L. & Brown G.S. 2004: Population density drives the local evolution of a threshold dimorphism. *Nature* 431: 1099–1103.
- Urbánková G., Šíchová K., Riegert J. et al. 2020: Lifetime low behavioural plasticity of personality traits in the common vole (*Microtus arvalis*) under laboratory conditions. *Ethology* 126: 812–823.
- Verhagen R., Leirs R. & Verheyen W. 2000: Demography of *Clethrionomys glareolus* in Belgium. In: Bujalska G. & Hansson L. (eds.), *Bank vole biology: recent advances in the*

- population biology of a model species. *Pol. J. Ecol. (Suppl.)* 48: 113–123.
- Viro P. & Niethammer J. 1982: *Clethrionomys glareolus* (Schreber, 1780) – Rötelmaus. In: Niethammer J. & Krapp K. (eds.), *Handbuch der Säugetiere Europas, Band 2/I, Rodentia II (Cricetidae, Arvicolidae, Zapodidae, Spalacidae, Hystricidae, Capromyidae)*. *Akademische Verlagsgesellschaft, Wiesbaden, Germany*: 109–146.
- Watts C.H.S. 1968: The food eaten by wood mice (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*) in Wytham Woods, Berkshire. *J. Anim. Ecol.* 37: 25–41.
- Wereszczyńska A.M. & Nowakowski W.K. 2004: What food allows bank voles to stay fit in spring? *Electron. J. Pol. Agric. Univ.* 7: 01.
- Wereszczyńska A.M., Nowakowski W.K., Nowakowski J.K. & Jędrzejewska B. 2007: Is food quality responsible for the cold-season decline in bank vole density? Laboratory experiment with herb and acorn diets. *Folia Zool.* 56: 23–32.
- Xiao Z., Chang G. & Zhang Z. 2008: Testing the high-tannin hypothesis with scatter-hoarding rodents: experimental and field evidence. *Anim. Behav.* 75: 1235–1241.
- Zwolak R. 2018: How intraspecific variation in seed-dispersing animals matters for plants. *Biol. Rev.* 93: 897–913.
- Zwolak R. & Sih A. 2020: Animal personalities and seed dispersal: a conceptual review. *Funct. Ecol.* 34: 1294–1310.

Supplementary online material

Table S1. Overview of trapped and tested animals.

Table S2. Selection of the best GLMM for Ivlev's electivity index response variables with different fixed factors (day, species, sex, personality trait, locomotor activity, body weight). All models used (day|id) specification as their random effects. Presented likelihood-ratio tests compare the particular model with a reduced model of the ~ +day + (day|id).

Fig. S1. Comparison of Ivlev's electivity index of pine seeds according to the higher and lower exploratory personality traits on the beginning and after 20 days under laboratory conditions ($F_{(3, 62)} = 3.052$, $P = 0.035$; *post-hoc* test L-H: $P = 0.077$, H-H20: $P = 0.999$, L-L20: $P = 0.287$, H20-L20: $P = 0.756$).

(<https://www.ivb.cz/wp-content/uploads/JVB-vol.-73-2024-MuhlbockP.-SeraB.-SedlacekF.-Tables-S1-S2-Fig.-S1.pdf>)