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Natural animal food preference of Chinese mole shrew (*Anourosorex squamipes*) from an urban area: a laboratory study

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Abstract: Most wild animals are urban avoiders, but some others become adapters or exploiters successfully living in urban areas. Often, the latter is assumed to be attracted into cities by readily accessible and digestive anthropogenic food resources. Here, we quantified food preferences of sixteen (eight females and eight males) Chinese mole shrews (*Anourosorex squamipes*) captured from an urban area for “cafeteria tests” in laboratory. Shrews were presented with twelve foods allocated into three sets (natural animal, natural plant, and anthropogenic food). Once the most two highly consumed food items from each set were determined, six items were pooled together to form a mixed food. We found that these urban shrews tended to prefer raw pork, peanut, and cooked pork over others when offered three single food sets, respectively, whereas natural animal food was more preferred over the rest when the set of mixed food was offered. The results show that urban shrews acquired nutrition by consuming the significant preferred diets. Nevertheless, access to natural animal resources seems still mandatory for urban shrews, while animals could become more tolerant to disturbance because of these easily exploited and abundant fallback anthropogenic resources in urban environments.

Key words: Urban adapter, foraging behavior, food choice, anthropogenic food, cafeteria test, *Anourosorex squamipes*

Urban areas provide novel food resources that are not available in wildlands and opportunistic foragers can benefit from it (Withey and Marzluff, 2009). However, some urban dwellers only show extensive foraging for anthropogenic food when natural food production is poor, while switch back to natural food when available (Lewis et al., 2015). Apparently, anthropogenic food could be served as fallback resources, even if they are easily accessible (Murray et al., 2015) and provide a high amount of energy (Kaplan et al., 2011).

Shrews (Eulipotyphla: Soricidae), are opportunistic omnivores with a flexible diet being herbivorous, predacious and granivorous simultaneously (Churchfield, 1994). Almost all soricine shrews have extremely high metabolic rates and food requirements because of their small body size and short starvation time (Merritt and Vessey, 2000). Thus, the general opinion that shrews eat all preys which could overpower is correct to some degree (Rychlik and Jancewicz, 2002). Similarly, many shrew species have become adapted to urban environments and been observed feeding on anthropogenic food (Brack,

2006; Jakub et al., 2017), while their preference patterns are relatively unknown. To test whether natural food could be still preferred by urban shrews, we conducted cafeteria tests (free selection) under controlled experimental conditions (nearly identical food availability and environmental condition) with individuals of Chinese mole shrew (*Anourosorex squamipes*) captured from an urban area.

This study was conducted at the China West Normal University (Nanchong, China), an area of relatively high urbanization and human use. We captured sixteen adult *A. squamipes* (eight females and eight males) in March 2019 on the campus, using well-tested Sherman live traps (Funing, Hebei, China) that were equipped with mealworm larvae as baits. Trap checking intervals were short (maximal three hours) to minimize stress on animals.

Shrews were housed singly in plastic boxes (30 × 40 × 20 cm) in the experimental field. The bedding was changed each week, which was equipped with sand, sawdust, peat, and grass. Water and nontest food (living mealworm larvae) were available ad libitum. Light and temperature

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regimes approximated natural conditions. All animals underwent a 2-week-habituation in the same maintenance conditions. During this period shrews could become familiar with test food (see below), which was given in small amounts occasionally. Finally, after the experiment period, shrews were returned to the collecting sites.

“Cafeteria tests” (tests of free selection) (Rychlik and Jancewicz, 2002) were conducted individually in the experiment box (100 × 50 × 20 cm), which along with a nest box (50 × 40 × 20 cm) under the same humidity and temperature as housing conditions. To avoid any residual odor from the previously housed animal, papers were lined on the floor and walls and changed each time when a new individual was introduced into the experimental equipment. Tests were carried out between 9:00 and 18:00 h, during the period of decreased locomotor and foraging activity of shrews (Buchalczyk, 1972). Of course, to ensure that animals could feel some hunger but were not starved at the beginning of tests, we took nontest food out of housing boxes until 2 h before tests. Each individual corresponding number was placed at a visible location near the experiment box to prevent mistakes in recording.

Following the previous report on stomach contents and the food availability for the same population and sampling site (Nie et al., 2006), twelve foods with distinct nutritional compositions (Appendix I) were allocated into three sets of four food items. Set A (natural animal food): earthworm (*Eisenia fetida*), locust (*Locusta migratoria*), raw pork (a surrogate for mammalian corpses; *Sus scrofa*) and edible frog (*Lithobates catesbeianus*); set B (natural plant food): wheat (*Triticum aestivum*), peanut (*Arachis hypogaea*), mung bean (*Phaseolus aureus*) and foxtail millet (*Setaria italica*); set C (anthropogenic food): chicken ham (Shuanghui, Yunnan, China), white bread (Taoli, Shanghai, China), cooked pork (*Sus scrofa*) and dried bean curd (Jiabao, Chongqing, China). Once the most two highly consumed food items on average from each set were determined, six items were further pooled together to form a fourth set D (mixed food). Here, sets A and B are considered as a “healthy food, natural source, and without artificial additive” (van Vliet and Mbazza, 2011).

Test food was given 6 g of per item and prepared 10 min before the test. The weights of food were calculated with 1% error at most. To avoid positional biases, we randomized the relative position of food for each test. Next, we introduced shrews into the experiment box separately and left them in the box for 5 min. During this period, animals usually explored box and then hid in the nest box. A test began by randomly placing food dishes (0.9 cm height, 9 cm diameter) into the experiment box and allowing subjects to freely forage for 10 min. Finally, the mass of the food left by shrews on the dishes and/or abandoned out of dishes was weighed. In the first

experiment, shrews received single tests consisting of sets A, B, or C respectively, alternating three sets across 9 days, with 3 days (twice a day for each individual) for each set. After that, shrews then received a 3-days mixed session involving set D.

We calculated a dependent index (the “preference value”) to express the relative consumption of each food in that trial (Larrinaga, 2010). Due to the short duration and low rates of evaporative loss of each trial, we neglected autogenic changes and used a simplified formula:

$$X_{ijk} = \frac{t_{ijk1}}{\sum_{k=1}^n (t_{ijk1})/n}$$

where X_{ijk} means the relative preference value of subject i for food k in trial replicate j , t_{ijk1} means the weight of uneaten k -food at the end of trial, and n means the number of foods included in each test. Thus, the value of $X > 1$ indicates a relative preference for food k , while the opposite value of $X < 1$ indicates a relative avoidance. When focal food is neither preference nor avoidance, X takes a unity value “1”. Next, to analyze the effect of different factors on the response variables, we performed a repeated measures analysis of variance with three within-subject factors: food, trial, and sex. Meanwhile, we examined the differences between males and females in degree of preference by means of a general linear model with multiple dependent variables. More details can be found in Larrinaga (2010). All statistical analyses were conducted using SPSS 22.0 (IBM Corp., Armonk, NY, USA) and followed the recommendations by Zar (1999).

All experiments were conducted in accordance with the Chinese Wild Animal Conservation Law and the guidelines of the Institutional Animal Ethics Committee of the China West Normal University (Permission number CWNU20190101).

Set A. Given a choice among four natural animal foods, one subject was removed due to its failure to consume any of items, whereas the remaining *A. squamipes* (8 males and 7 females) showed a mean selective choice of food (sex effect, Appendix II), with no significant effect of trial or its interactions. The raw pork tended to be preferred (“raw pork vs rest” contrast, $F = 39.563$, $P < 0.05$), while locust was the least preferred food (“locust vs rest” contrast, $F = 103.125$, $P < 0.05$; Figure a). Despite small variations in food preferences, natural animal food choice pattern, as estimated from the mean of 15 individuals, did not change along the 6 trials (nonsignificant trial by food interaction, Appendix II).

Set B. Both sexes, considered together, showed the same degree of mean natural plant food choice (sex effect, Appendix II). The mean choice pattern indicated the significant preference for peanut (“peanut vs rest” contrast, $F = 81.441$, $P < 0.05$; Figure b). Neither the overall specific

choices nor the preference difference between sexes changed among trials (food by trial and food by sexes by trial interactions, Appendix II). In contrast, the wheat, mung bean, and foxtail millet tended to be relatively avoided.

Set C. There was no significant difference in the relative preferences of anthropogenic food choices between sexes. Simple effects (Appendix II) of food by sexes suggested that both sexes had highly preference for cooked pork (“cooked pork vs rest” contrast, $F = 50.389, P < 0.05$; Figure c). However, differences in degree of preference between sexes were significant ($F = 4.773, P < 0.05$; Appendix III). Males preferred cooked pork (2.481 ± 0.907) more than females did (2.016 ± 1.164). On the contrary, *A. squamipes* consumed less chicken ham, white bread, and dried bean curd.

Set D. The mixed food contained the two most highly consumed foods from three single sets including the natural animal food (earthworm and raw pork), natural

plant food (wheat and peanut), and anthropogenic food (chicken ham and cooked pork). Despite some fluctuations among 6 trials of relative consumption, mean food choices were constant over time, and with no significant preference effect of sexes (Appendix II). As seen in Figure d, the natural animal food tended to be the most preferred (“natural animal food vs rest” contrast, $F = 248.264, P < 0.05$), followed by anthropogenic food (“anthropogenic foods vs rest” contrast, $F = 95.931, P < 0.05$), whereas natural plant food was the least preferred diet (“natural plant food vs rest” contrast, $F = 334.676, P < 0.05$).

Overall, our results based on “cafeteria tests” show that urban *A. squamipes* exhibit diverse and flexible feeding patterns. We offered on average 6 g of each food per 10 min; however, shrews ate maximum 79% of available food (raw pork). Thus, the food choices were not affected by food scarcity. As generalist omnivores, shrews are able to discriminate among different functional food and adjust to their food choices flexibly. The access to

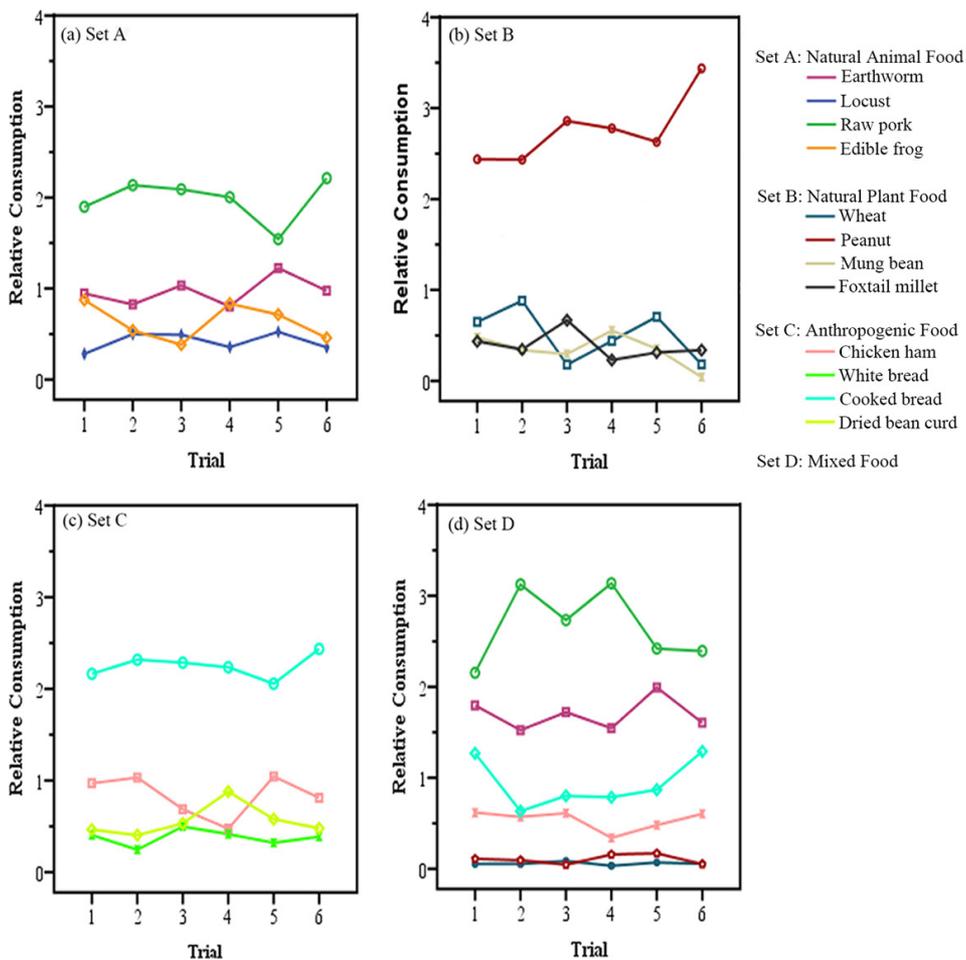


Figure. Food preferences of Chinese mole shrew (*Anourosorex squamipes*) from an urban area. **a, b, c, d.** Mean relative consumption along six trials for each food set are shown in a, b, c, d, respectively. (see materials and methods for details on the calculation of relative consumption).

natural animal food might predominate for urban shrews, while anthropogenic food could be exploited as fallback resources.

The overwhelming preferences suggest that food choices of urban shrews might be strongly related to nutritional quality when offered single food (Klenovšek et al., 2013). In the “cafeteria tests”, shrews were able to forage selectively the most rewarding food, particularly the food that rich in fat (Appendix I; Figure). In comparison to carbohydrate (4.04 kcal/g) and protein (5.64 kcal/g), fat provides twice the amount of the energy (9.44 kcal/g) (Karasov and del Rio, 2007). The clear preferences hence probably provide a higher energy intake rate. It also corroborates the expanding specialist foraging strategy (Brown and Morgan, 1995), that is, food is selected based on profitability. By contrast, the posteriorly consumed foods (earthworm, wheat, and ham sausage) were comparatively higher in carbohydrate such as starch (Appendix I), which was considered as the most rapidly metabolized ingredient, providing energy quickly. Moreover, the forager starts by selectively focusing on profitable food until it is depleted to a certain threshold below which it is included in less foods (Molokwu et al., 2011). This suggests a possible relationship among fuel storage, food utilization, and food choice.

The increasing urbanization influences the abundance and quality of available food for shrews. Accordingly, changes to the feeding patterns of shrews are thought to reflect an increased abundance of food resources and often associated with urban environments (Lowry et al., 2013). The main driver is anthropogenic food (e.g. garbage), which is considered as a high-caloric and valuable source for urban animals (Bateman and Fleming, 2012). Moreover, a higher food quality in urban areas, as described by other studies (Otoni et al., 2009; Maibeche et

al., 2015), is independent from the artificial infrastructure, while it depends on the diets with internal processing (i.e. digestion rates). Digestive constraints from consuming such anthropogenic food may be decreased (Whelan and Brown, 2005); hence, forager could complementally switch to the relative high-quality and digestible anthropogenic food. We, therefore, infer that mobile shrews could be the great urban adapters and urbanites as their dietary plasticity enables animals to satisfy viable needs using various urban resources.

The unlimited anthropogenic food resources may have beneficial effects on the increment of survival and reproduction. Nevertheless, it is also important to underscore negative impacts produced by this food subsidy. The health level of wild animals may decline due to their selectivity to use anthropogenic food, which could increase the risk of pathogen transmission, endangering subsequently human health (Abrahão et al., 2009). Furthermore, the 2014 Ebola outbreak in West Africa and 1998 emergence of Nipah virus in Malaysia also highlight the importance of understanding how anthropogenic resources bring wildlife reservoirs of zoonotic pathogens, which might facilitate the cross-species transmission of pathogens among wildlife, humans, and domestic animals (Pulliam et al., 2012; Alexander et al., 2015). Therefore, further studies are warranted to elucidate the effects of anthropogenic food resources on human health and/or individual health of urban tolerant wild animals.

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Appendix I. Nutritional composition (wet weight) of foods in the cafeteria tests.

Food category	Food item	Crude fat (g/100g)	Crude protein (g/100g)	Carbohydrate (g/100g)	Source
Natural animal food	Earthworm	9.04	58.78	0.20	Stafford, 1984
	Locust	19.62	50.42	4.78	Mohamed, 2015
	Raw pork	23.20	16.70	0.00	Pearson and Gillett, 1996
	Edible frog	1.11	14.50	3.60	Ayres et al., 2015
Natural plant food	Wheat	2.00	12.80	62.10	Ranhotra et al., 1996
	Peanut	49.23	25.80	21.70	Sauter et al., 2006
	Mung bean	0.80	21.60	55.60	Food label (Renmin, Jilin)
	Foxtail millet	7.70	15.90	70.10	Ravindran, 1991
Anthropogenic food	Chicken ham	13.00	11.00	10.00	Food label (Shuanghui, Yunnan)
	White bread	1.70	9.10	43.30	Food label (Taoli, Shanghai)
	Cooked pork	26.90	24.20	0.00	Pearson and Gillett, 1996
	Dried bean curd	3.20	12.30	9.40	Food label (Jiabao, Chongqing)

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Appendix II. Results of repeated measures analysis of variance (ANOVA) for food choices of *A. squamipes*. Two factors are within-subject factors. The response variable is defined as relative consumption over the total consumption per trial, and hence these effects are uninformative, showing sum of squares and mean squares smaller than 0.0001. P-values are corrected by the Huynh–Feldt epsilon (significant level = 0.05).

Food category	Effect	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Natural animal food	Food	128.63	3	42.88	19.53	< 0.05
	Sex	< 0.0001	1	< 0.0001	2.12	0.17
	Trial	< 0.0001	5	< 0.0001	0.91	0.37
	Food × Sex	7.07	3	2.36	1.07	0.37
	Food × Trial	9.93	15	0.66	1.04	0.42
	Sex × Trial	< 0.0001	5	< 0.0001	0.84	0.39
	Food × Sex × Trial	16.37	15	1.09	1.71	0.08
	Natural plant food	Food	398.89	3	132.96	56.45
Sex		< 0.0001	1	< 0.0001	0.07	0.79
Trial		< 0.0001	5	< 0.0001	0.39	0.85
Food × Sex		5.93	3	1.98	0.84	0.42
Food × Trial		22.19	15	1.48	2.19	0.05
Sex × Trial		< 0.0001	5	< 0.0001	1.44	0.22
Food × Sex × Trial		19.26	15	1.28	1.90	0.08
Anthropogenic food		Food	208.40	3	69.47	46.21
	Sex	0.01	1	0.01	1.11	0.31
	Trial	0.04	5	0.01	1.02	0.34
	Food × Sex	8.02	3	2.67	1.78	0.20
	Food × Trial	8.26	15	0.55	1.01	0.44
	Sex × Trial	0.04	5	0.01	0.98	0.35
	Food × Sex × Trial	15.88	15	1.06	1.94	0.05
	Mixed food	Food	494.43	5	98.89	48.23
Sex		< 0.0001	1	< 0.0001	0.44	0.52
Trial		< 0.0001	5	< 0.0001	0.34	0.86
Food × Sex		2.03	5	0.41	0.20	0.79
Food × Trial		23.08	25	0.92	1.67	0.08
Sex × Trial		< 0.0001	5	< 0.0001	0.63	0.65
Food × Sex × Trial		43.11	25	1.72	3.13	< 0.05

Appendix III. Results of GLM multiple dependent variables testing difference on the degree of preferences of foods between males and females (significant level = 0.05).

Food category	Food item	<i>df</i>	MS	F	P
Natural animal food	Earthworm	1, 88	1.82	3.14	0.08
	Locust	1, 88	0.86	3.45	0.07
	Raw pork	1, 88	2.81	2.38	0.13
	Edible frog	1, 88	1.57	2.32	0.13
Natural plant food	Wheat	1, 94	0.21	0.31	0.58
	Peanut	1, 94	4.38	2.84	0.10
	Mung bean	1, 94	0.72	1.80	0.18
	Foxtail millet	1, 94	0.62	1.61	0.21
Anthropogenic food	Chicken ham	1, 94	0.36	4.10	0.05
	White bread	1, 94	0.11	0.72	0.40
	Cooked pork	1, 94	5.20	4.77	0.03
	Dried bean curd	1, 94	0.36	1.05	0.31
Mixed food	Earthworm	1, 94	1.18	1.33	0.25
	Raw pork	1, 94	0.45	0.20	0.66
	Wheat	1, 94	0.01	0.59	0.45
	Peanut	1, 94	0.04	1.61	0.21
	Chicken ham	1, 94	0.01	0.05	0.82
	Cooked pork	1, 94	0.34	0.41	0.53