Hunting behaviour in arvicoline rodents

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Abstract. In laboratory experiments, purposeful predatory inter-relations with moving insects have been revealed and described in three species of herbivorous voles: the East European vole, the narrow-headed vole, and the Tuva silver vole. The appearance of the full hunting stereotype in these species does not require preliminary experience, which indicates its innate nature. The hunting behaviour of the Tuva silver vole differed from that of the East European vole and narrow-headed vole, which probably reflects the phylogenetic relationships between these species.

1. Introduction
It is known that invertebrates are included in the diet of almost all rodent species [1, 2]. However, for a long time it has remained unclear whether these animals are able to expand their diet due to active hunting, or whether they collect immobile invertebrates. Earlier, the hunting behaviour of rodents in relation to mobile prey was studied mainly on the example of carnivorous and omnivorous species [3-5], and only recently it was found in granivorous and herbivorous species [6-8].

Voles (Arvicolinae) are the most numerous rodent subfamily in the Northern hemisphere [9], they play a significant role in most terrestrial ecosystems, from tundra to forest and alpine. All voles are predominantly herbivorous [10], however, animal food was found in the stomachs of many species [11-13]. Active hunting for mobile insects was recently discovered in the bank vole (Clethrionomys glareolus) [6, 14], which is a food generalist with a very varied diet [15]. The hunting behaviour of voles with a predominantly herbivorous diet has not been studied.

The goal of our work is an experimental study and comparative analysis of responses to mobile prey in three species of herbivorous voles, in comparison with the hunting behaviour of rodents previously studied by us with various types of diet.

2. Materials and methods

2.1. Animals
The experiments were conducted in the laboratory on three species of voles. We used 22 East European voles Microtus levis (14 females and 8 males), 46 narrow-headed voles Lasiopodomys gregalis (23 females and 23 males), and 68 Tuva silver voles Alticola tuvinicus (33 females and 35 males). All three species are mainly herbivorous and live in family groups or colonies.
East European voles were descendants of animals bred in the Centre for collective usage “Gene pool of laboratory animals” of the Institute of Cytology and Genetics (ICG). All narrow-headed voles were caught shortly before the experiments in the Karasuk Field Station of the Institute of Systematics and Ecology of Animals (south of Novosibirsk region). Tuva silver voles are the descendants of the first and second generations of animals captured in the mountains of Altai in 2015. All the animals that were born in the laboratory have not previously encountered live insects.

All animals were housed in plastic cages containing cotton nesting material under a 16:8 light/dark cycle at 23–26°C. All animals had *ad libitum* access to water and food: mixed seeds, hay or fresh grass, fruits or vegetables, cottage cheese. As a movable prey elder imagoes and nymphs of marmoreal (*Nauphoeta cinerea*) with the average body length of 27.93 ± 0.40 mm were offered, as well as Turkmenian roaches (*Shelfordella lateralis*) with the average body length of 26.45 ± 0.50 mm, since they are innocuous, easily overcome, palatable for rodents and do not induce defensive reactions in small mammals (figure 1). Insects were kept and bred in our laboratory in separate containers.

![Figure 1. Narrow-headed vole L. gregalis (A) and Tuva silver vole A. tuvinicus (B) eating their prey – a lobster cockroach N. cinerea.](image)

All experiments with rodents were performed in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. The experimental protocol was approved by the Bioethics Committee of the Institute of Systematics and Ecology of Animals Siberian Branch Russian Academy of Sciences (protocol No. 1 from 14.04.2014).

2.2. Experimental procedures

To study the spontaneous manifestation of hunting behaviour, we applied the scheme that has become standard [8, 16]. We placed each rodent in a separate plastic arena (30 × 30 × 35 cm) covered with a transparent lid in order to prevent animals from getting out. In each trial, an insect was placed into the arena manually, 5 min after the rodent. Video recordings were made using a Sony Handycam DCR-SR68 camera (frame rate, 25 frames per second) and a Sony HDR-AS200V (60 frames per second). After each test, the arena was cleaned using 70% alcohol.

For technical reasons, we applied two test modes. The first mode was applied to all narrow-headed and East European voles and 16 Tuva silver voles. Each of these animals was tested once and received three insects in turn. In cases of unsuccessful hunting, we waited for 10 min since the last contact between the animal and the prey, and then finished the observation. The second mode was applied to 52 Tuva silver voles. Each animal was tested once a day with one insect only, during ten days, with a two-day break after the 5th day. Differences in test modes did not affect the results discussed in this article. In total, we obtained 1,138 video records with the total duration of 96.5 hours.
Similarly to [7, 16], we devised an alphabet of 19 letters for the analysis of hunting behaviours by assigning a letter to each of the elements of behaviour, in the order of their appearance, with no regard to their duration [16]. For the East European voles we introduced two new elements not previously found in other studied rodent species: nibbling the insect’s wings (K) and spontaneous shaking (O). Using the program ‘The Observer XT 12.5’ (Noldus Information Technology), we translated the observed behaviour of rodents into a sequence of letters, for example, SWE (“S” for walking, “W” for bite, “E” for seizing an insect with paws). We transferred the resulting sequences for each species through space into separate text files. We call these files “summary” files. We used them to further analyse the behaviour.

Statistical analysis was performed using Fisher’s exact test (R v.3.6.0) and Kruskal–Wallis H test (PAST 3.2). Similar to [17], to obtain ethograms (schemes of hunting stereotypes), we calculated matrices of probability estimates based on the relative frequencies of transition from a behavioural element to another one. Then we graphically expressed the obtained data as corresponding schemes where different transition probabilities (“p”) were represented by connecting arrows of different thickness.

2.3. Notions
We denote elementary movements and postures as minimal units of behaviour (“behavioural elements”, for brevity), and we call a “behavioural stereotype” a relatively stable chain of behavioural elements [16]. When considering the interaction of an animal with the prey, we call “successful” all cases of hunting that ended with catching the prey (even after several false attacks – for brevity, “successful stereotypes”.

3. Results
3.1. Composition of behavioural elements in successful stereotypes of voles
In total, we have obtained 18 successful hunting stereotypes in the East European vole, 34 in the narrow-headed vole, and 206 in the Tuva silver vole.
We consider behavioural elements associated with an attack, namely, bite (W) and seizing the prey with paws (E), as “key elements” since they may be critical for catching the prey. The group of “auxiliary” elements includes the ones related to pursuing the prey by walking (S) or running (Q), as well as sniffing (D), carrying the prey in the teeth (G), prey handling (R), nibbling the insect’s legs (H) and wings (K). The group of “noise” elements includes those that do not influence the performance of the stereotype at all: (C) freezing, (V) turning the body by 90°, (B) U-turn, (F) head turning, (Y) rearing against the wall, (I) free-standing rearing, (U) backward movement, (O) spontaneous shaking, (X) self-grooming, and (J) jump. The proportions of elements of the selected groups in successful stereotypes of the studied species are shown in figure 2.

![Figure 2](image-url)

Figure 2. The proportions of key, auxiliary, and noise elements of behaviour in hunting stereotypes of voles. The numbers indicate the amount of elements.
In hunting stereotypes of Tuva silver voles, the proportion of key elements is significantly lower, and the proportion of auxiliary elements is significantly higher than in East European and narrow-headed voles (Fisher’s exact test with Bonferroni’s amendment, p < 0.003 for all cases). The proportions of key and auxiliary elements in East European and narrow-headed voles, as well as the proportions of noise elements in all three species of voles, did not differ (Fisher’s exact test with Bonferroni’s amendment, p > 0.05).

3.2. Comparison of the composition of behavioural elements in hunting stereotypes of voles

In order to understand how different species of voles attack prey and how they manipulate it, we compared the number of behavioural elements associated with attack and prey processing that belong to one stereotype (table 1). The number of elements “bite” (W), “seizing with paws” (E), and “nibbling insect’s legs” (H) in the stereotypes of the Tuva vole is less than in the stereotypes of the narrow-headed and East European voles (H-test with Bonferroni amendment, p < 0.003 for all cases), and the stereotypes of the East European and narrow-headed voles did not differ in the amount of these elements (H-test, NS). In the stereotypes of the East European voles there were more “handling” (R) than in the stereotypes of the narrow-headed and Tuva silver voles, and in the stereotypes of the Tuva silver voles there were more of them than in the stereotypes of the narrow-headed voles (H-test with the Bonferroni amendment, p < 0.003 for all cases).

To understand how a hunter injures its prey, we examined the repeatability of the bite (W) element. To do this, we divided all the elements of the “bite” into single, double (two successive “bites”), and multiple (three or more “bites” in a row). Tuva silver voles inflicted fewer single, double and multiple bites than narrow-headed and East European voles (H-test with the Bonferroni amendment, p < 0.003 for all cases), while the latter did not differ among themselves in these parameters (H-test, NS).

### Table 1. Number of behavioural elements per hunting stereotype in the studied species of voles.

| Elements of behaviour | East European vole | Narrow-headed vole | Tuva silver vole |
|-----------------------|--------------------|--------------------|------------------|
| Bite (W)              | 11 (2–17)          | 6 (2–20.5)         | 2 (1–4)          |
| Single bite (W₁)      | 2 (2–6.5)          | 2 (1–7)            | 1 (1–2)          |
| Double bite (W₂)      | 1 (0–1)            | 0.5 (0–3)          | 0 (0–1)          |
| Multiple bites (Wₙ)   | 1 (0–2.75)         | 0 (0–1.8)          | 0 (0–0)          |
| Seizing with paws (E) | 4.5 (2–8.3)        | 2 (1–8.5)          | 2 (1–2)          |
| Handling (R)          | 2 (1–3)            | 0 (0–0)            | 0 (0–1)          |
| Nibbling insect’s legs (H)| 1 (0–3)           | 1 (0–2.8)          | 0 (0–0)          |

The median, first, and third quartiles are presented: Me (Q1–Q3).

3.3. Schemes of hunting stereotypes in voles

Based on the obtained matrices of probability estimates, we constructed the schemes of successful hunting stereotypes for all species studied (figure 1). In most cases, the successful stereotypes begin from approaching a potential prey by walking (S) or running (Q). Less commonly, if the insect itself approached the rodent, the stereotype could begin without a phase of pursuit. Then sniffing (D) could follow. After this, the prey attack phase began. The attack always started with a bite (W), followed by a seizing an insect with paws (E). After catching a cockroach, animals could carry it in their teeth (G) and seize it with their paws again, or they could proceed to manipulate with the prey: handle it (H), nibble its legs (H) or wings (K). Usually, at the end of the hunting stereotype, the insect would be eaten, but in some cases, the animals threw the immobilized prey and did not eat it. East European voles did so in 29.4% of cases (5 out of 17), and narrow-headed voles in 29.4% of cases (10 out of 34).
Figure 3. The schemes of hunting stereotypes in vole species studied. A thin dotted line indicates some unstable bonds between elements (\( p < 0.2 \)). A simple black line indicates stable bonds (\( 0.2 \leq p < 0.5 \)). The bold line indicates highly stable bonds (\( p \geq 0.5 \)). The “auxiliary” elements are indicated in parentheses.

4. Discussion

The hunting stereotype scheme was found to be universal in all three species of voles (figure 3). The attack on prey always began with the bite: in this respect, voles differ from the previously studied predatory and omnivorous hamsters [8, 18], which mainly seize the prey with their paws. The latter is an indicator of the predatory specialization of the species [19], and in this respect the stereotypes of voles are more primitive and close to the stereotypes of muroid rodents – field striped mouse (\( Apodemus agrarius \)) and brown rat (\( Rattus norvegicus \)) [7].

We compared hunting tactics, that is, a set of techniques used to damage prey and move on to eating it. Hunting tactics turned out to be similar in the East European and narrow-headed voles, but differed in the Tuva silver voles (table 1). The Tuva silver vole inflicted mostly single bites on prey during the attack, and also less often nibbling the legs of its prey. In other words, during the hunt, Tuva silver voles tried to quickly capture the insect in its paws and start eating it alive, without additional processing. In the stereotypes of the East European and narrow-headed voles, there is a significant proportion of double and multiple bites, as well as nibbling legs: these voles tried to damage and immobilize the insect before eating it.

Comparing the behaviour of voles with the hunting tactics of the previously studied rodent species, we can conclude that the tactics of the narrow-headed and East European voles are more similar to the behaviour of the granivorous field striped mouse, while the Tuva silver vole in this respect is more reminiscent of the brown rat, a generalist predator [7, 8]. These behavioural features can shed light on the phylogenetic relationship of the studied species. There is evidence that phylogenetically East European and narrow-headed vole are closer to each other than any of them to the Tuva silver vole [20, 21].
It should be noted that, although a general scheme of a hunting stereotype is similar in all species
(figure 3), some of them manifest species-typical traits of behavioural specialization. In the current
study, we found the East European vole is the only species that nibbles the wings (not legs) of a
cockroach and displays spontaneous shaking during hunting. The latter behaviour may indicate a
stressful reaction in rodents [22].

Our research shows that herbivorous voles are able to hunt effectively mobile insects. Hunting
sterotypes in three vole species were found to be similar both to each other and to the stereotypes of
previously studied species of granivorous and omnivorous rodents. This not only confirms the innate
nature of rodent hunting behaviour but also suggests that rodents have inherited their hunting
behaviour from common insectivorous ancestors.

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