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Original Investigation

Seasonal changes in the concentrations of plant secondary metabolites and their effects on food selection by *Microtus oeconomus*

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ABSTRACT

Using cafeteria trials conducted during June–September 2008 in Qinghai Province, China, we investigated the selection of 20 plant species by root voles (*Microtus oeconomus*). It was found that both favored and edible plant groups of root voles comprised 6 species, while the remaining 8 species were anorectic plants. Three plant secondary metabolites (PSMs): flavonoids, condensed tannins, and total phenols, exhibited seasonal changes in concentration; being lowest at June and gradually increasing from July to August/September. Total phenols was the only factor included in the best model of generalized linear models, indicating that total phenols was the most important factor deterring food selection by root voles. In contrast, tannins had a weak effect on food selection by root voles. This study indicated that PSMs play an important role in food selection by root voles; however, the effects of PSMs depend on the type of PSMs. Furthermore, this finding partly verifies the hypothesis that PSMs contribute to the defense strategy of plants, significantly influencing plant selection by root voles.

Introduction

Food selection by herbivores is influenced by many factors, such as the abundance and distribution of plant resources, plant nutrient concentration and availability, odor and flavor of plants, plant physical and morphological traits, plant phenophase, habitat occupied by plants, predation risk, climate, and human disturbances (Black, 1990; Scott and Provenza, 1999; Ettle et al., 2004). Plant secondary metabolites (PSMs) are chemical compounds (e.g., alkaloids, terpenes, phenolics, non-protein amino acids) that have no direct role in the growth, development, or physiology of plants, but are toxic or detrimental to the physiological functioning in herbivores. Freeland and Janzen (1974) hypothesized that PSMs may influence the nutritional physiology and foraging behavior in herbivores. This hypothesis received widespread attention, resulting in PSMs becoming the subject of extensive research with focus on interactions among plants and mammalian herbivores (Villalba et al., 2002; Iason, 2005; Moore et al., 2005; Sorensen et al., 2005; Torregrossa and Dearing, 2009; Stolter et al., 2010, 2013; Borchard et al., 2011). During the long co-evolution of herbivores and plants, there might have been some correlations between PSM concentrations and food selection by herbivores (Lindroth, 1991; Iason and Villalba, 2006). Phenolic compounds are secondary metabolites abundant in all plant materials, and one of the well-known and important functions of phenols is their action in plant defensive mechanisms (Benett and Wallsgrove, 1994; Dixon and Paiva, 1995; Treutter, 2001; Ruechmann et al., 2002; Stolter et al., 2010, 2013). Flavonoids and tannins are two important phenolic compounds. Flavonoids are evolved as chemical defenses effective against herbivores (Feeny, 1976), while tannins bind to proteins, thereby reducing the availability of nitrogen to herbivores (Robbins et al., 1987).

The root vole (*Microtus oeconomus*) is widely distributed in northern Eurasia. In China, this species is primarily distributed within the Qinghai, Sichuan, Shanxi, Ningxia, and Sinkiang provinces. This small rodent dwells in alpine meadows; it is dominant in the *Dasiphora fruticosa* shrublands of the Haibei Alpine Meadow Ecosystem in Qinghai (Liu et al., 1982), and favors a wide variety of herbaceous monocotyledons and dicotyledons (Batzli and Jung, 1980; Batzli, 1985; Liu et al., 1991). Therefore, this species is a good candidate for studying the influence of PSMs on food selection by herbivores in the Alpine Meadow Ecosystem. However, the relationship and interactions between root voles and coexisting alpine meadow plants have not been studied adequately and remain poorly understood. We hypothesized that PSMs influence the plant selection of root voles, with root voles selecting plants that have low PSM concentration, while avoiding plants with high PSM concentration. We investigated changes in the seasonal concentrations of total phenols, condensed tannins, and flavonoids in

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plants growing in the habitat used by root voles in the Qinghai-Tibetan Plateau, and assessed the effects of these compounds on food selection by root voles.

Material and methods

Our study was conducted during June–September, 2008, at the Haibei Alpine Meadow Ecosystem Research Station of the Chinese Academy of Science in Qinghai Province, China. Twenty plant species growing near the research station, which might be used as potential forage matter by the voles, were chosen according to previous studies (Nie et al., 1995; Cui et al., 2005). These 20 species included both favored and less favored plants by the voles, with the aim to determine the relationship between plants consumed by root voles and plant PSMs. The specimens of 20 plants were preserved at Yangzhou University, China. The names of the 20 plants were confirmed by the ecologist at the Haibei Alpine Meadow Ecosystem Research Station.

Animal feeding trials

From June to September, 2008, we captured more than 10 adult root voles (releasing any pregnant or lactating females) monthly near the Haibei Station. We housed the voles in separate cages (45 cm × 30 cm × 20 cm) for 3 days before the experiment, and chose 6 healthy root voles (male = 3, female = 3) (18–25 g) for the experiment. The temperature was maintained at 20 ± 2 °C. Filtered tap water was available ad libitum, while sufficient quantities of carrot were fed daily to the root voles from 13:30 to 20:30. However, from 20:30 to 08:30 the next day food was not provided to the voles. Each root vole was transferred to a new clean cage, and the previously used cages used were washed clean for the experiment on the following day. For the feeding trials, we picked 40 g each of plant species growing in natural conditions near the field station each month. After collection, each plant was divided into 8 equal portions, of which 6 portions were fed to each of the 6 captive root voles. One portion was divided into 5 equal 1 g portions that were used to determine the concentration of flavonoids, condensed tannins, and total phenols in plants. The remaining one portion was used as the control sample to calculate the rate of water loss over 3 h. Before the actual experiment, we conducted a preliminary test to determine which of the 20 plant species are favored and avoided by the root voles. Then, we divided the 20 plant species into 4 random groups, each of which contained 5 plant species, ensuring that each group contained both favored and avoided plants by the root vole. Each month, we conducted the experiment from 08:30 to 11:30 daily for 4 consecutive days, with the 5 different plant species from one of the groups being simultaneously fed to each root vole over the 3 h period for the plant choice experiment. Thus, each root vole was exposed to all 20 plant species over 4 consecutive days. The root voles were released at the site from where they had been captured after the end of the experiment each month.

Uneaten plant material was reweighed at the end of the 3 h experiment. The water loss rate of the plants (E_i) was calculated by $E_i = (G_i - H_i)/G_i \times 100\%$, where E_i is the water loss rate of species i, G_i is the fresh mass of species i, and H_i is the mass of species i after 3 h. The amount of plants ingested by the root voles (i.e., plant intake amount) (Y_i) was determined by $Y_i = (A_i - B_i)/(1 - E_i)$, where Y_i is the intake amount of species i over 3 h, A_i is the supplied fresh mass of species i, B_i is the uneaten mass of species i after 3 h, and E_i is the water loss rate of species i. The food intake rate (F_i) was determined by $F_i = Y_i / \sum_{i=1}^{20} Y_i$. The selectivity index (SI) was determined by $SI = F_i / \left(A_i / \sum_{i=1}^{20} A_i \right)$. We characterized the selectivity index of palatability into 3 categories as favored ($1 < SI$), edible ($0.5 < SI \leq 1$), and anorectic ($0 < SI \leq 0.5$).

Plant secondary metabolites assay

For each sample, 100 mg of tissue was ground to determine the concentration of flavonoids by using a modified spectrophotometry method (Caldwell, 1977). The absorption value was read at λ = 380 nm by using a spectrophotometer (Jingke, 752N, Shanghai, China).

For each sample, 200 mg of tissue was extracted for 30 min in 600 ml acetone:distilled water at a 70:30 ratio. Samples were sonicated during extraction. After extraction, the samples were centrifuged for 10 min (3000 rpm at 4 °C) to remove particulates. The supernatant was collected and stored at 0 °C for the condensed tannins and the total phenols quantity assay. Condensed tannin concentration was determined using the modified BuOH–HCl technique (Porter et al., 1985), and the absorption value was read at λ = 550 nm. Condensed tannins, as the leucocyanidin equivalent, were calculated by the formula: A 550 nm × 78.26 × dilution factor, where the dilution factor was: 0.5 ml/(volume of extract taken). Total phenol concentration was determined using a modified Folin-Denis method (Makkar et al., 1993), and the absorption values at λ = 725 nm were recorded using a spectrophotometer.

Statistical analysis

All variables were tested for normality and homogeneity of variance. The data of 3 PSMs were log-transformed, and the amount of intake was square root transformed prior to analyses. We used paired-samples t-test to compare the differences in the concentration of 3 PSMs between 2 adjacent months. We used a general linear model repeated measures analysis, followed by post hoc LSD (Least Significant Difference), to examine the differences in the concentrations of the 3 PSM based on palatability by using SPSS16.0 (Chicago, SPSS Inc., 2007).

GLMs (generalized linear models) were used to explore the relationship between food intake and month and the 3 PSMs. We used Akaike's Information Criteria (AIC) with second order correction for small sample size (AICc) as the selection criteria of the most appropriate model (Burnham and Anderson, 2002) in R 2.10.1 with the MuMIn library. ΔAIC values exceeding 2 were considered indicative of substantial differences in support for the compared models, whereas models with a ΔAIC value within 2 of the models with the lowest AIC are equally likely to be the best model (Burnham and Anderson, 2002). According to the GLM results, we conducted linear regression with the factors in the best model using SPSS16.0. For all comparisons, differences were considered to be statistically significant ($P < 0.05$).

Results

Plant selection by root voles

The intake amount of each plant species by root voles varied among months (Table 1). According to the selectivity index (Table 1), *Plantago asiatica*, *Oxytropis* sp., *Pedicularis kansuensis*, *Saussurea superba*, *Taraxacum* sp., and *Potentilla nivea* were the 6 plant species favored by root voles. *Gentiana straminea*, *Medicago ruthenica*, *Saussurea nigrescens*, *Carex crebra*, *Kobresia pygmae*, and *Anemone cathayensis* were in the edible group, while the remaining 40% of the plant species presented to root voles (*Leontopodium nanum*, *Thalictrum petaloideum*, *Potentilla anserina*, *Lancea tibetica*, *Morina chinensis*, *Aster flaccidus*, *Ajania tenuifolia*, and *Belamcanda chinensis*) were classified as anorectic.