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PLANT SECONDARY COMPOUNDS AND THE FREQUENCY OF FOOD TYPES AFFECT FOOD CHOICE BY MAMMALIAN HERBIVORES

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Abstract. We have investigated food choice in individual fallow deer (*Dama dama*) encountering different relative frequencies of food types in the form of bowls containing pellets with either high or low concentrations of hydrolyzable tannin. We performed two similar experiments, one with large and one with small differences in tannic acid concentration. With small differences in tannic acid concentration, the ratio of the consumption per low- and high-tannin bowl was independent of frequency of occurrence, but with large differences in tannic acid concentration, we found frequency-dependent food choice. The deer ate proportionally less from high-tannin bowls if these occurred at low relative frequency. Variation between frequency treatments in the average order of encounter of bowl types might have produced this effect, because we found that the deer left a high-tannin bowl more quickly if they had switched to it from a low-tannin bowl. We argue that the perceived contrast between the tastes of different food types can play a role for food choice by mammalian herbivores.

Key words: apostatic selection; contrast effect; Dama dama; *fallow deer; frequency dependence; mammalian herbivory; plant secondary compounds; tannic acid; tannin.*

INTRODUCTION

Plant defenses consist of both structural feeding deterrents and chemical defenses (Lindroth 1989). Among the most widespread defenses are the numerous secondary compounds that act against different types of herbivores (Palo and Robbins 1991). The typical foraging situation for large herbivores is that they encounter a range of different plants that are distributed more or less unevenly in the environment. Although a complete generalist might simply consume plants according to occurrence, it is thought that the diets of most herbivores contain higher concentrations of nutrients and lower concentrations of toxins than the average of the food available (Freeland and Janzen 1974). Such selectivity could be achieved by consuming more (or with higher probability) when encountering a plant that is more suitable as food. In addition, the relative preference for one food type over another could depend on their frequencies of occurrence. It is this kind of frequency dependence that is our main interest in this work.

Frequency dependence has been investigated for interactions such as predation or herbivory, because of its potential relevance for the coexistence of different prey types (Greenwood and Elton 1979, Allen 1988, Weale et al. 2000). Applied to herbivory, frequency-independent food choice could mean that the consumption from a plant individual of a given type is independent of the relative frequency of that type. More generally, if the ratio between plant types of the consumption per plant is independent of the frequencies of the types, this is also referred to as frequency-independent food choice. One possible deviation from such frequency independence is that the relative consumption per plant individual of a given type increases with its frequency. This pattern of food selection is referred to as positive frequency dependence, and it will act to favor rare types and disfavor common types. Conversely, if the relative consumption per plant individual of a given type is highest for low frequency of that type, there is negative frequency dependence, which will disfavor rare types and favor common types. The terms apostatic and antiapostatic selection are sometimes used to denote positively and negatively frequency-dependent food choice (Greenwood and Elton 1979, Allen 1988).

At present, rather little is known about frequency-dependent food choice in mammalian herbivores. It has been suggested that mammalian herbivores choose food on the basis of taste and odor rather than on visual stimuli (Tuomi and Augner 1993, Provenza et al. 2000). Their preferences for tastes and odors are at least partly based on a learned association between plant properties and the subsequent nutritional rewards or toxic effects (Villalba and Provenza 2000). These preferences are likely to be of importance when foraging in an environment where plants occur in different frequencies, but it is not clear which kind of reaction to frequency of occurrence one ought to expect. A study by Danell and Ericsson (1986) of moose foraging on different species of birch was perhaps the first investigation of frequency-dependent food choice by mammalian her-

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bivores. Danell and Ericsson (1986) found that the proportions of two birch species, *Betula pendula* and *B. pubescens*, in the moose (*Alces alces*) diet were only weakly affected by their frequencies of occurrence. This would correspond to negative frequency dependence, because the moose would need to eat more from plants of a rare species to maintain a constant, mixed diet. In an experiment with sheep grazing on clover and grass, Parsons et al. (1994) found that the sheep did adjust their diet with changes in relative abundance, but they also sustained a mixed diet in a manner corresponding to negative frequency dependence. On the other hand, in a selection experiment with moose feeding on birch (*B. pubescens*) and rowan (*Sorbus aucuparia*), Lundberg et al. (1990) found that the relative preference for birch over rowan did not vary with their frequencies of occurrence, which corresponds to frequency-independent selection (Greenwood and Elton 1979). These studies then suggest that either negative frequency dependence (perhaps because of benefits of a mixed diet [Greenwood 1984]) or frequency-independent food choice might be expected for mammalian herbivory on different species of plants.

When plant types differ mainly in the concentration of some secondary compound, one would not expect any benefit per se of a mixed diet. Nevertheless, mammalian herbivores regularly feed on plants containing toxins, and are thought to avoid harmful effects by regulating intake in accordance with toxicity (Bryant et al. 1991). This kind of regulation could lead to frequency-dependent food choice. A herbivore attempting to limit the intake of a toxic compound should consume less per plant of a relatively toxic type if it occurs at higher frequency and, in addition, ought to consume more per plant of a less toxic type if it occurs at lower frequency. One might thus expect negative frequency dependence when types differ in the concentration of a secondary compound.

There are, however, also general reasons to expect positive frequency dependence for relatively toxic plant types. When a more bitter tasting plant type is rare, a herbivore might often encounter it right after having tasted a less bitter type, resulting in a sharp contrast in the perceived reward of ingesting it, and thus, perhaps, in a smaller consumption. The appearance of an exaggerated response to a shift in reward is referred to as ''incentive relativity'' in animal psychology, and is an empirically well-established phenomenon (reviewed in Flaherty 1982, 1996). Applied to herbivory (and other foraging based on taste), contrast effects could mean that several aspects of the distribution of food types can influence relative intake. In addition to frequency of occurrence, patchiness and spatial proximity of food types can play a role. Spatial proximity might increase the perceived contrast between the tastes of different types, whereas the perceived contrast might be reduced if types grow apart in separate patches.

In this work, we have investigated the food choice of individual fallow deer (*Dama dama*) exposed to artificial food types in the form of pellets with different concentrations of hydrolyzable tannin. Food types were presented in a number of bowls placed fairly close together, with the intention of mimicking a situation in which a forager encounters a patch where the plant types contain different amounts of a secondary compound and can occur in different frequencies. We have performed two similar experiments, one with a larger difference in tannin concentration and a second with a smaller difference. From previous work on food choice (Alm et al. 2002), we know that fallow deer prefer lower concentrations of tannin, but that their food choice with respect to tannin content is relative, in the sense that they will ingest more of a given concentration when the concentration in alternative food is higher. We also know that the manner of presentation of food (two-choice or cafeteria test) can have an influence. Our aim in the present study is to perform a systematic and thorough investigation of the effect of frequency of occurrence on relative preference.

Methods

We used 10 female fallow deer that were hand-raised during the summer of 2000 and about one year old at the time for the study. The deer were kept in a 4-ha enclosure with forest and meadow, situated at Tovetorp Zoological Research Station in south-central Sweden. The deer had ad libitum access to pasture, water, a mineral stone, and a salt stone during the study. There was no fasting before the experiments. The study was performed with permission from the Swedish National Board for Laboratory Animals and took place from June to October 2001.

An experiment consisted of a number of trials, to which different food presentation treatments were randomly allocated. These treatments represented different frequencies of food types. The trials were performed with one animal at a time in a small experimental enclosure (100 m^2) which had been built within the larger enclosure where the deer were kept. The entrance to the experimental enclosure had a small vestibule into which an animal could be led. To start a trial, the animal was released from the vestibule into the experimental enclosure, where an experimental arrangement was placed, while the observer remained in the vestibule. The walls of the experimental enclosure were solid and 1.4 m high, so that the deer were prevented from seeing through or over the wall. The general procedure corresponded to that used by Alm et al. (2002). Each deer performed about one trial per day at varying times during the day.

Two similar experiments were performed, the first with a large difference in tannin concentration and the second with a smaller difference. The food consisted of pellets with hydrolyzable tannin added (tannic acid from Sigma-Aldrich, Stockholm, Sweden; CAS #:

1401-55-4, EC NO: 215-753-2). The low-concentration food had 0.3% tannin (per wet mass) in both experiments. For the large-difference experiment, the high concentration was 1.5% (i.e., five times the low concentration), and for the small-difference experiment, it was 0.6% (i.e., twice the low concentration). The tannin was dissolved in water and sprayed over the pellets. New batches of pellets were prepared approximately every five days, with both concentrations needed for the ongoing trials being prepared on the same day. They were thus between one and five days old when used. The pellets (Viltfor from Odal, Gnesta, Sweden) were of a type intended for wild cervids and were made of corn, milling by-products, sugar beet by-products, minerals, vitamins, fat, and vegetable oils, giving 10.5 MJ digestible energy and 120 g crude protein per kilogram of pellets.

Before the start of the experiments, the deer were trained to walk in and forage from bowls with pellets in the experimental enclosure. They were also given pellets with tannin before the experiments started, so that they were accustomed to this type of food. Each deer performed in a total of 24 trials in each experiment. The experimental arrangement consisted of eight numbered bowls (1–8) that were placed equally spaced along the perimeter of a circle. The diameter of the circle was 3 m and it was positioned in the middle of the experimental enclosure. At the start of a trial, each bowl contained 400 g of pellets, and the amount remaining in the bowl was weighed at the end of the trial. A trial was considered to be finished when the animal stopped eating for more than a minute. In both experiments, there were three treatments or presentation patterns, in which the low-tannin concentration occurred in one, four, or seven of the eight bowls. Each deer went through the three treatments in a random order, but performed eight trials in sequence for each treatment. For such a sequence (within the same treatment), the positions of the bowls were changed following a schedule, in order to average out potential effects if certain positions along the perimeter of the circle were more preferred (cf. Alm et al. 2002). The schedule was devised so that in the treatments with only one low- or one high-tannin bowl, this bowl was placed in all of the eight possible positions. In the treatment with four low-tannin bowls, the positions of the low-tannin bowls were 1,3,5,7; 2,4,6,8; 1,2,5,6; 3,4,7,8; 1,2,3,5; 4,6,7,8; 1,2,3,4 or 5,6,7,8. The order in which the positions were used differed between individuals, but the same order of treatments and schedule were used in both experiments, so that each animal was treated in the same way in the two experiments.

During a trial, the animal's sequence of visits to the different bowls was recorded, together with the duration of each visit, using a hand-held computer. A visit was defined as starting when the animal approached a bowl and lowered its head into it, and ending when the animal moved away from the bowl. A visit might include eating, sniffing, and also standing in front of the bowl. Using these data, we computed the average time spent visiting a bowl of a given category (low vs. high tannin) and the average number of visits to a bowl of a given category.

After each experiment, a two-choice test with the previously used tannin concentrations (i.e., 0.3 vs. 1.5% and 0.3 vs. 0.6%) was performed. In these two-choice tests, each animal performed six trials, and the two bowls contained 300 g of pellets each. A trial was considered to be finished either when the animal stopped eating for one minute or when one bowl became empty.

Data analysis

Based on the consumption of pellet types and their tannin concentration, we computed the total tannin intake for each trial. The average tannin intake over the eight trials of a treatment was then used in an ANOVA. Next, to investigate frequency dependence, we used the amounts eaten per bowl for low- and high-tannin bowls, which we averaged over the eight trials of a treatment, thus obtaining two data points per individual and treatment. We first checked whether the deer differentiated between low- and high-tannin bowls in the small-difference experiment, and then performed an ANOVA of the log ratio of the two amounts (Elston et al. 1996), using the individual deer as block factor. The log ratio is defined as the logarithm of the ratio between the trial average amount eaten per low-tannin bowl and the trial average amount eaten per high-tannin bowl. The ratio can be regarded as a measure of the relative preference for low- over high-tannin food (cf. Greenwood and Elton 1979). We included the two experiments in the same analysis, although treatments were randomly assigned only within experiments. For post hoc testing, we employed the Student-Newman-Keuls (SNK) procedure.

In addition to the ratio, a comparison between frequency treatments of the absolute consumption per bowl is also of interest. Because the two amounts (low- and high-tannin food) together determine the ratio, we only performed an ANOVA on the amount per high-tannin bowl. We then made pairwise comparisons, both for low- and high-tannin bowls. To deal with the issue of multiple comparisons, we used the sequential Bonferroni correction (Quinn and Keough 2002).

We analyzed the visiting time per bowl and the number of visits per bowl following a similar procedure as for the amounts. These analyses are not likely to be entirely independent of each other, because the different variables might be correlated, but they serve the purpose of illustrating the aspects of deer behavior that lie behind the pattern of consumption.

In order to investigate if perceived taste contrast might play a role in determining the pattern of consumption, we analyzed the visiting times as a function of bowl content for the first two bowls encountered in

TABLE 1. ANOVAs of tannin intake and log ratio measures of relative preference for low-tannin over high-tannin food.

Response variable and factor	df	F	P
Tannin intake			
Presentation pattern	2	72.46	<0.0001
Concentration difference	1	0.33	0.57
Presentation pattern × concentration difference	2	8.08	0.001
Amount eaten per bowl (log ratio)			
Presentation pattern	2	1.43	0.27
Concentration difference	1	62.02	<0.0001
Presentation pattern × concentration difference	2	5.78	0.006
Visiting time per bowl (log ratio)			
Presentation pattern	2	1.99	0.15
Concentration difference	1	0.14	<0.0001
Presentation pattern × concentration difference	2	0.83	0.44
Visits per bowl (log ratio)			
Presentation pattern	2	4.95	0.01
Concentration difference	1	2.42	0.13
Presentation pattern × concentration difference	2	1.10	0.34

Notes: The log ratios for the different variables are defined as the logarithm of the ratio of the trial average value for a low-tannin bowl and the trial average value for a high-tannin bowl. The individual animal was also included in the models as a block factor with 9 df, leaving 45 df for the error term for all comparisons.

a trial. We distinguished three sequence positions: the first bowl, the second bowl after a low-tannin first bowl, and the second bowl after a high-tannin first bowl. We used only data from the presentation pattern with four low-tannin bowls (out of the eight), because for this treatment nearly all individuals had experienced low- and high-tannin bowls at the three different sequence positions. We then made pairwise comparisons of the time spent on high-tannin bowls in the large-difference experiment. Finally, we examined the searching strategy used by the deer by looking at the number of bowls visited at least once during a trial.

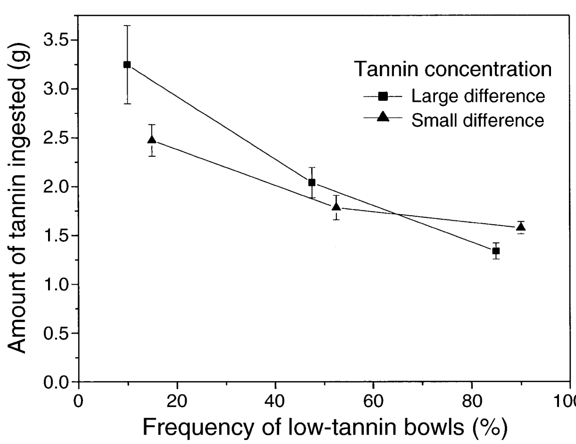

FIG. 1. Total intake of tannin per individual per trial ($\bar{x}$ ± SE for N = 10 individuals) in the different frequency treatments, which correspond to the three presentation patterns (low-tannin concentration in 1, 4, or 7 of the 8 bowls per trial). For clarity, points have been shifted left and right slightly. The low-tannin concentration was 0.3% in all trials. In the large-difference experiment (squares), high-tannin concentration was 1.5%; in the small-difference experiment (triangles), high-tannin concentration was 0.6%. See Table 1 for ANOVA of the influence of presentation pattern and concentration difference on tannin consumption.

RESULTS

The deer were quite willing to enter the experimental enclosure and forage from the bowls. They usually started to feed from a bowl close to the entrance and then moved on to the other bowls. A trial lasted, on average, 10 min (from 3 to 20 min). The ending of a trial was usually clear, with the deer walking away from the bowls, either to explore other parts of the enclosure or to just stand and wait.

Tannin intake

The consumption of tannin varied with the frequency of the bowl types, so that the highest per trial consumption of tannin occurred in the one low-tannin of eight bowls presentation pattern, both in the large- and the small-difference experiment (Table 1, Fig. 1). There was a statistically significant interaction between presentation pattern and concentration difference, providing evidence for a greater variation in tannin intake with frequency in the large-difference experiment (Table 1, Fig. 1).

Consumption per bowl

In the experiment with a small difference in concentration, the deer consumed more low- than high-tannin food per bowl (Fig. 2). We verified this with a t test of the treatment average log ratio consumption per bowl, with zero log ratio as the null hypothesis (t = 4.73, N = 10, P = 0.001). An ANOVA of the log ratio consumption per bowl (Table 1, Fig. 2A) further showed that there was an even stronger preference for

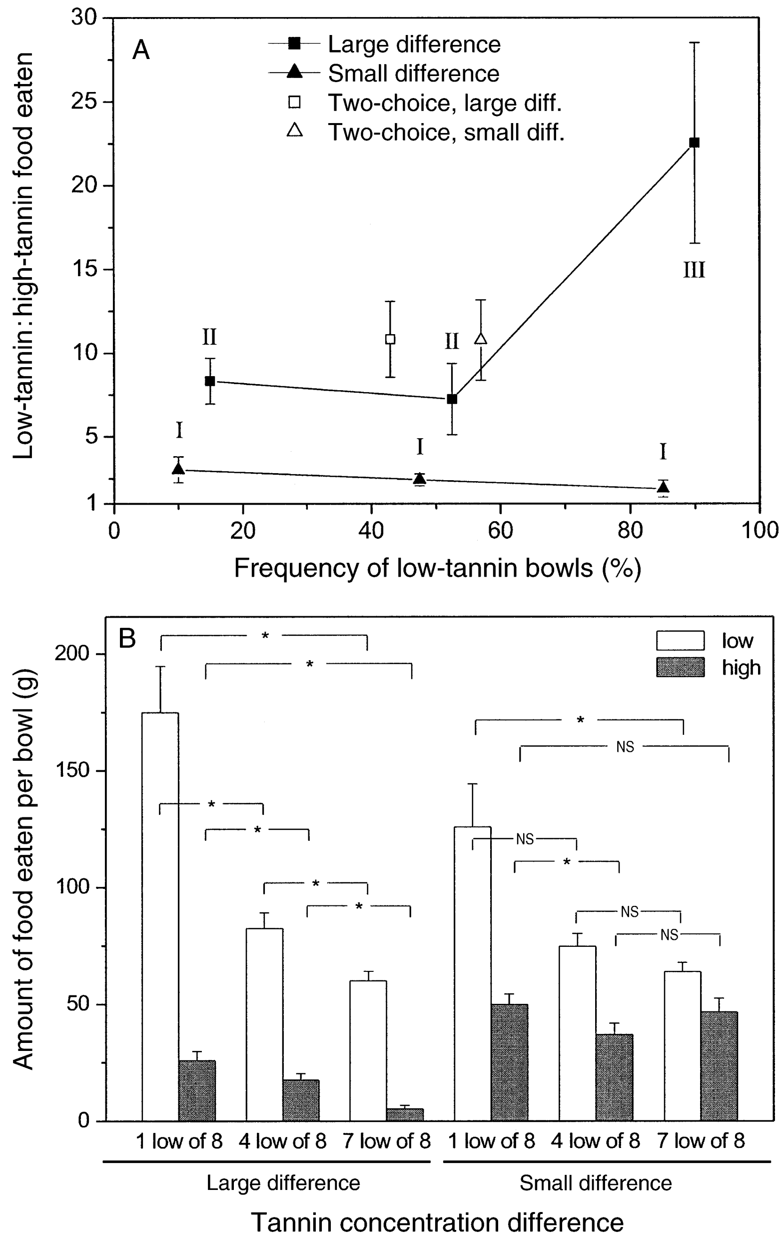

FIG. 2. (A) The relative preference for low- over high-tannin food ($\bar{x} \pm$ SE for $N = 10$ individuals), measured as the ratio of the trial mean amount eaten per low-tannin bowl and the trial mean amount eaten per high-tannin bowl (a ratio equal to 1.0 would mean a lack of preference). Open symbols correspond to the complementary two-choice tests, and solid symbols represent results from the main experiments; large- and small-difference experiments are as described in Fig. 1. See Table 1 for ANOVA of the influence of presentation pattern and concentration difference on relative preference. The symbols I–III indicate homogenous groups in the SNK post hoc tests. (B) The absolute amounts eaten per bowl, averaged over trials ($\bar{x} \pm$ SE for $N = 10$ individuals), for the different presentation patterns (1, 4, or 7 low-tannin bowls of 8 bowls per trial) in the two concentration differences. Asterisks indicate statistical significance ($P < 0.05$) in sequential Bonferroni-corrected comparisons between frequency treatments based on the six comparisons for each concentration difference. See Table 2 for ANOVA of the amount eaten per high-tannin bowl.

low-tannin food in the large-difference experiment. There was also a statistically significant interaction between presentation pattern and concentration difference (Table 1), and a post hoc test yielded three homogenous groups (I–III in Fig. 2A). For the small-difference experiment, there were no significant differences between the three frequency treatments (group I in Fig. 2A), indicating a lack of frequency-dependent food choice for small concentration difference. However, in the experiment with large difference, there was frequency-dependent food choice, so that a high-tannin bowl was relatively less utilized when occurring in low frequency

TABLE 2. ANOVAs of measures of absolute foraging intensity for high-tannin food.

Response variable and factor	df	F	P
Amount eaten per bowl†			
Presentation pattern	2	16.1	<0.0001
Concentration difference	1	79.7	<0.0001
Presentation pattern × concentration difference	2	13.3	0.001
Visiting time per bowl			
Presentation pattern	2	13.34	<0.0001
Concentration difference	1	28.74	<0.0001
Presentation pattern × concentration difference	2	1.07	0.35
Visits per bowl			
Presentation pattern	2	0.46	0.63
Concentration difference	1	54.63	<0.0001
Presentation pattern × concentration difference	2	1.06	0.36

Notes: The measures corresponding to the different variables are defined as the average value over eight trials for a high-tannin bowl. The individual animal was also included in the models as a block factor with 9 df, leaving 45 df for the error term for all comparisons.

† Square-root transformed to achieve homogeneous variances.

(III vs. II in Fig. 2A). Apart from the concentration difference and the frequency of occurrence, other aspects of the presentation also seemed to influence relative preference, at least in the experiment with small difference. The complementary two-choice tests both had 50% low-tannin food, but the deer showed a stronger preference for low-tannin food in the two-choice test with small difference than in the corresponding treatment of four low-tannin of eight bowls (paired t test: small difference, $t = 8.04$, $N = 10$, $P < 0.0001$; large difference, $t = 2.11$, $N = 10$, $P = 0.064$; see Fig 2A).

Concerning the consumption per bowl of high-tannin food, the ANOVA showed a statistically significant interaction between treatment and experiment (Table 2, Fig. 2B). Taking into account the paired comparisons for high-tannin food in Fig. 2B, we can conclude that there was a smaller consumption per bowl of high-tannin food in the large-difference experiment, and that this consumption was particularly small in the treatment with seven low-tannin bowls of eight (Fig. 2B). For low-tannin food, the paired comparisons (Fig. 2B) showed that the consumption per bowl in the experiment with large difference was greater when this bowl type occurred in lower frequencies (i.e., opposite to the pattern for high-tannin food). In the experiment with small difference, we could only establish a difference between the extreme treatments with one and with seven low-tannin bowls. Finally, considering overall consumption, the percentage of low-tannin food eaten by an individual during a trial varied from 27% up to almost 99% over the different frequency treatments and experiments (Table 3). Thus, the frequency of occurrence and the difference in tannin concentration had a rather strong influence on food intake.

Visiting time and visits per bowl

The consumption of different food types can be influenced by several components of foraging behavior. To shed some light on these kinds of relationships, we analyzed the pattern of visits to the different bowls. We first looked at the total visiting time per bowl. For the small-difference experiment, the time spent on low-tannin bowls was generally higher than the time spent on high-tannin bowls (Fig. 3A; t test of the treatment average of the log ratio visiting time: $t = 3.49$, $N = 10$, $P = 0.007$). The ANOVA of log ratio visiting time showed that this difference was even greater in the

TABLE 3. Percentage of low-tannin food consumed per individual per trial and the amount of food consumed per individual per trial in the different setups ($\bar{x} \pm$ SE for $N = 10$ individuals).

Concentration difference	Presentation pattern	Low-tannin consumption (%)	Total intake (g)
Large difference	1 low of 8	51.06 ± 4.72	356.38 ± 30.89
	4 low of 8	82.22 ± 2.91	399.74 ± 24.87
	7 low of 8	98.67 ± 0.40	425.31 ± 27.92
Two-choice large difference	—	89.01 ± 2.17	317.97 ± 15.56
Small difference	1 low of 8	27.26 ± 4.22	475.16 ± 25.08
	4 low of 8	66.46 ± 3.25	447.01 ± 27.01
	7 low of 8	91.23 ± 1.41	482.31 ± 21.65
Two-choice small difference	—	88.43 ± 2.48	331.13 ± 8.25

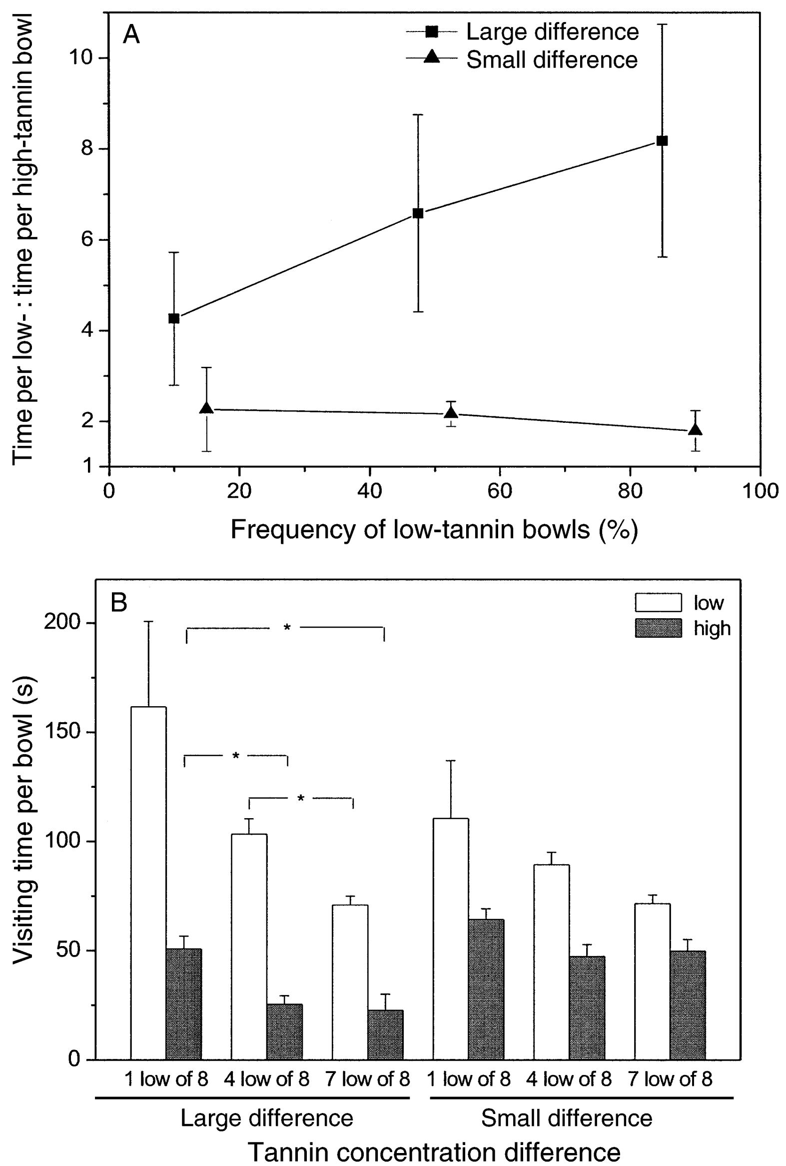

FIG. 3. Results for visiting time per bowl ($\bar{x} \pm$ SE for $N = 10$ individuals; see Fig. 2). (A) The ratio of visiting time per low-tannin bowl vs. high-tannin bowl. (B) The absolute visiting time per individual per bowl, by concentration and presentation pattern. Asterisks are as in Fig. 2B ($P < 0.05$), but nonsignificant comparisons are not shown. See Tables 1 and 2 for ANOVAs.

experiment with large difference (Table 1, Fig. 3A). Concerning the time spent on a high-tannin bowl, the deer spent more time in the experiment with small difference and also in the treatments in which the high-tannin bowls occurred at higher frequency (Table 2, Fig. 3B).

There was less variation in the number of visits per bowl, but overall there were more visits to low-tannin bowls than to high-tannin bowls (Fig. 4A; t test of overall average log ratio visits per bowl: $t = 2.47$, $N = 10$, $P = 0.035$). The ANOVA of log ratio visits per bowl (Table 1, Fig. 4A) showed no effect of experiment, but a significant effect of treatment, with higher log ratio in the treatment with one low-tannin bowl of eight. Concerning the absolute number of visits to high-tannin bowls, the only statistically significant effect was a higher number of visits in the experiment with large difference (Table 2, Fig. 4B).

Rate of consumption

The variation in visiting time per bowl (Fig. 3) showed largely the same pattern as the consumption per bowl (Fig. 2), except for the presence of statistically significant treatment by experiment interactions in the analysis of the consumption per bowl (Tables 1 and 2). To further investigate this effect, we also analyzed the

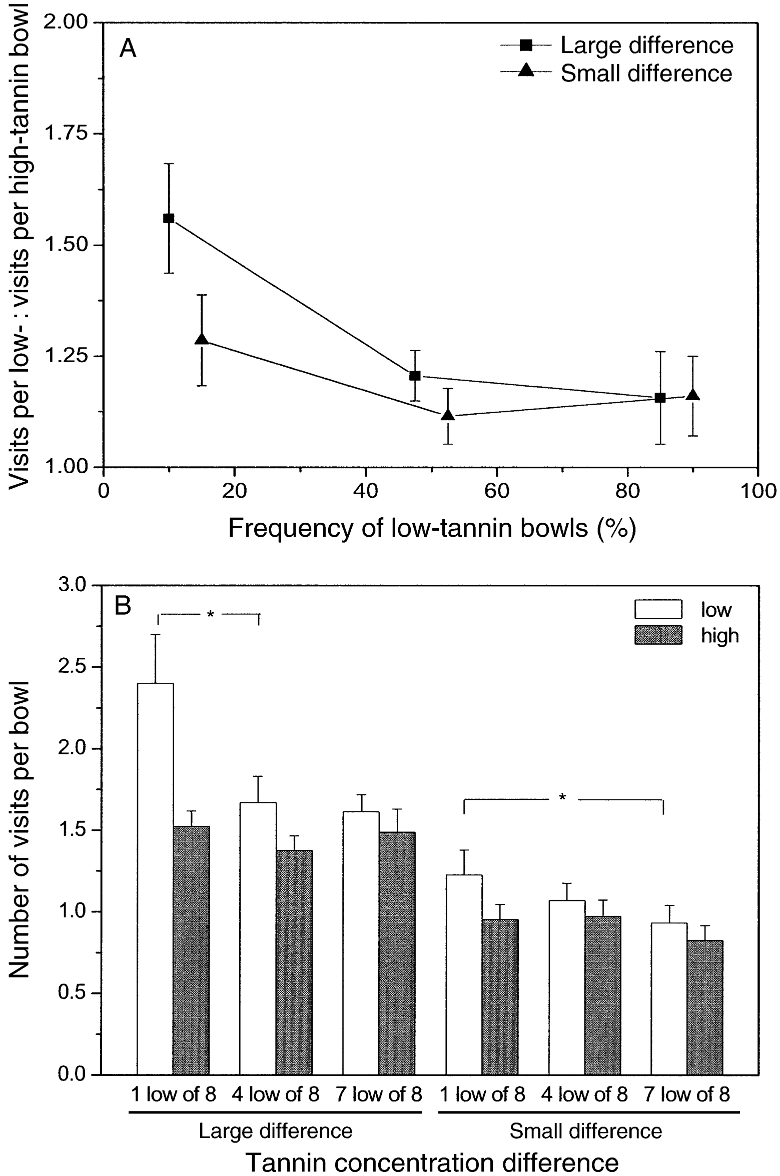

FIG. 4. Results for the number of visits per bowl ($\bar{x} \pm$ SE for $N = 10$ individuals; see Fig. 2). (A) Ratio of the number of visits per low-tannin bowl vs. high-tannin bowl. (B) Absolute number of visits per bowl, by concentration and presentation. See Tables 1 and 2 for ANOVAs.

rate of consumption, i.e., the amount eaten per time spent at low- and high-tannin bowls. In the experiment with small difference, the deer ate low- and high-tannin food at same rate (Wilcoxon one-sample test of the treatment average of the log ratio visiting time: $N = 10$, $T = 25$, $P = 0.8$). However, in the experiment with large difference, the low-tannin food was generally eaten at a higher rate than the high-tannin food (Wilcoxon one-sample test of the treatment average of the log ratio visiting time: $T = 0$, $N = 10$, $P = 0.005$). Concerning the effect of treatment in the experiment with large difference, we could see a nonsignificant trend that the treatment influenced the relative rate of consumption (Friedman test of the log ratio: $N = 10$, df = 2, $\chi^2 = 5.4$, $P = 0.067$). Thus, it is at least feasible that a low rate of consumption from the one high-tannin bowl in the treatment with seven low-tannin bowls was responsible for the relatively smaller amount consumed from this bowl.

Order of encounter in trials

To investigate if the consumption from low- and high-tannin bowls was influenced by the order in which the bowls were encountered, possibly as an expression of a contrast effect, we compared the time spent on the first and second bowls visited in relation to bowl con-

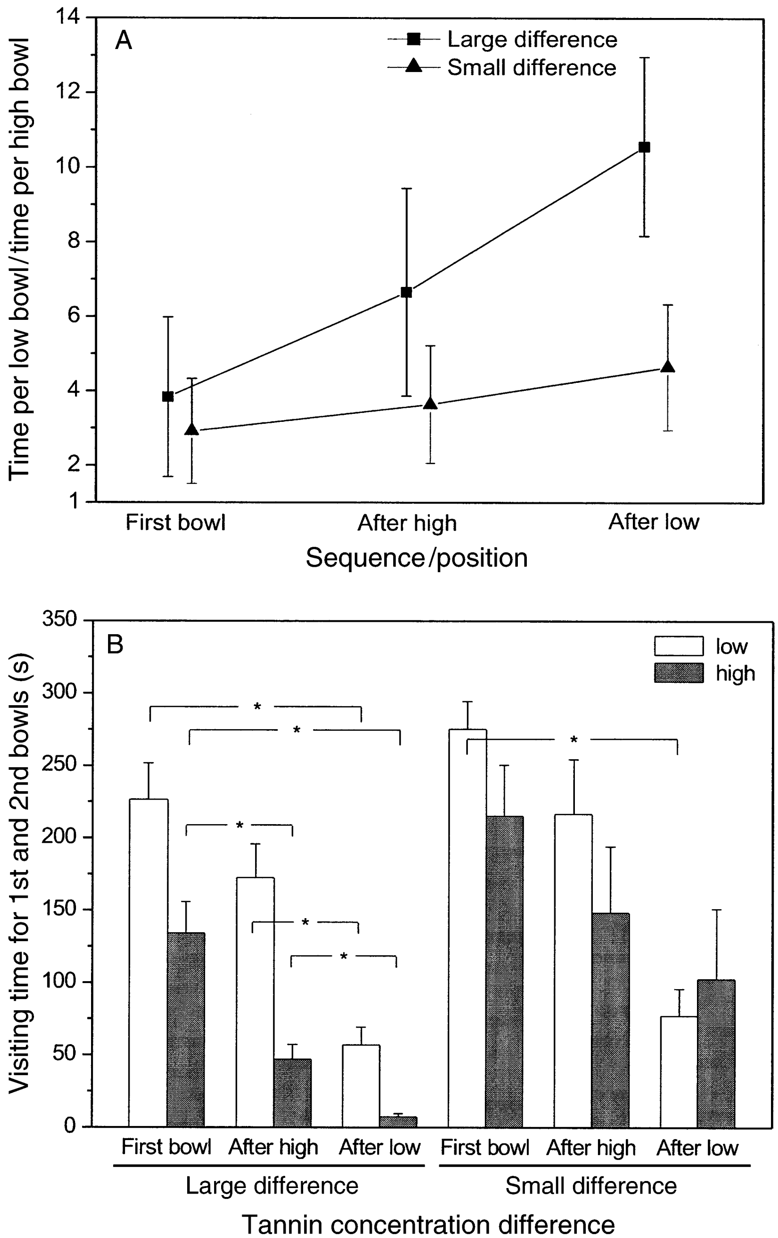

FIG. 5. Time spent on the first and second bowl visited ($\bar{x} \pm$ SE for $N = 10$ individuals). Visits to the second bowl are separated into two groups, depending on whether the first bowl visited was low or high in tannin. (A) The ratio of the visiting time (per low-tannin vs. high-tannin bowl); (B) The absolute visiting time. See text for statistical analysis.

tent. Visits to the second bowl were divided into two groups, depending on whether the first bowl visited was a low- or a high-tannin bowl. In the experiment with small difference, we could not establish statistically that the time spent on the first and second bowl visited depended of tannin concentration. However, in the experiment with large difference, the deer spent generally more time on a low-tannin bowl (Fig. 5A; Wilcoxon one-sample test of the position average log ratio visiting time: small difference, $N = 10$, $T = 10$, $P = 0.074$; large difference, $N = 10$, $T = 0$, $P = 0.005$). Because we found a difference between low- and high-tannin bowls for the large-difference experiment, we also tested whether the difference between low- and high-tannin bowls depended on sequence position. A nonparametric ANOVA of the log ratio visiting time in the large-difference experiment showed a statistically significant effect of sequence position (Friedman test: $N = 9$, df = 2, $\chi^2 = 6.89$, $P = 0.032$; Fig. 5A). This indicates that the preference for low- over high-tannin food depended on the previous experience. The absolute visiting time to a high-tannin bowl in the large-difference experiment also depended on sequence position (Fig. 5B; Friedman test of the time spent on high-tannin bowls: $N = 10$, df $= 2$, $\chi^2 = 16.22$, $P = 0.0003$).

Searching strategies

We checked for differences between experiments in searching behavior, because the deer made more visits per bowl in the experiment with large difference (Fig. 4B, Table 2). This could have been caused by repeated visits to certain bowls, but it might also be that the deer searched through more bowls in the large-difference experiment. The latter, in fact, was the case, because the number of bowls visited at least once in a trial was higher in the experiment with large difference ($\bar{x} \pm$ SE for $N = 10$ individuals; large difference, 6.71 $\pm$ 0.18; small difference, 5.33 $\pm$ 0.26; paired t test: $N = 10$, $t = 4.65$, $P = 0.001$). We also investigated whether the deer became more efficient in locating low-tannin bowls in successive trials. For one treatment, the four low-tannin of eight bowls in the large-difference experiment, there seemed to be such an effect, because 9 of the 10 individuals showed an increase over trials in the percentage of low-tannin food consumed. The average percentage increased from 70.12 $\pm$ 5.78% to 88.25 $\pm$ 3.64% over eight trials.

Discussion

Our study was designed to investigate the effect of frequency of occurrence on herbivore relative preference, with possible implications for plant defenses. In accordance with previous information (Alm et al. 2002), it was clear from both the two-choice tests and the frequency tests that the deer preferred lower tannin concentrations. Even though low-tannin food was always available, the deer consumed some high-tannin food in all of our setups. The percentage of high-tannin food ingested depended rather strongly on the percentage available, so the deer were far from keeping a constant, mixed diet of the presented food types. The idea of a regulation of intake in relation to the amount of toxin present in food, which should lead to negative frequency dependence, failed to find support in our data. Instead, we found frequency-independent food choice in the experiment with small concentration difference, and positive frequency dependence for high-tannin food in the experiment with large concentration difference. Note that the latter is opposite to what one would expect if the deer were trying to avoid an excessive intake of high-tannin food.

Frequency of occurrence can influence the sequence of encounter with different food items, and it might be this sequence, rather than the frequency per se, that has an effect on relative intake. For instance, in our two-choice tests, in which the deer were able to switch directly between low- and high-tannin bowls, the relative preference for low-tannin food tended to be stronger than in the four low-tannin of eight bowls treatment, although the availability of low-tannin food was 50% in both of these setups. Our finding that there was an influence of the order of encounter on the ratio of visiting times is consistent with an effect of perceived contrast. We suggest that the perceived contrast between a low-tannin bowl and a high-tannin bowl influenced food choice in our study. This could, for instance, explain why the deer left a high-tannin bowl more quickly when they had switched to it from a low-tannin bowl, and also could be the reason for the small consumption of high-tannin food in the presentation pattern with seven low-tannin of eight bowls.

Tannins are polyphenolic compounds of two types: condensed tannins, which are the most widely spread secondary compounds in vascular plants, and hydrolyzable tannins, which are only found in the dicotyledons (reviewed in Bernays et al. 1989). It is well-established that both types can be toxic, and the binding affinity to protein in vitro is similar for both types (Meyer and Karasov 1991), but the nutritional consequences are multifaceted and differ between condensed tannins and hydrolyzable tannins (McSweeney et al. 2001). It has been shown that the taste of both condensed and hydrolyzable tannins is correlated with the content in a plant (Mali and Borges 2003). Tannic acid is a type of hydrolyzable tannin, which was not found to reduce the uptake of proteins in cow and deer (Hagerman et al. 1992). It seems reasonable to assume that the preference for lower concentration of tannin displayed by the deer in our study was related to the astringent taste of the tannic acid. Because it is uncertain how well mammalian herbivores can distinguish between different types of tannins, the evolutionary explanation is perhaps the toxic or anti-nutritional effects of these similar substances. Concerning concentrations, both tannin and protein content vary between plants and between plant parts, and over the season (Palo and Robbins 1991). In 70 investigated plants eaten by roe deer (*Capreolus capreolus*) in France, the protein content varied between 4.0 and 33.5% of dry mass, and tannin content varied between 0.1 and 6.0% of dry mass (Tixier et al. 1997). However, plant parts can contain up to 40% tannin (Kraus et al. 2003). Thus, the concentrations of tannins and proteins used in our experiments are comparable to those found in plants.

Because the concentration of nutrients and metabolizable energy in plants generally is low, mammalian herbivores typically ingest large amounts. They then may need to utilize several species of plants simply to obtain sufficient quantities. Animals might also eat different plants to satisfy nutrient requirements (Westoby 1978), or because they detoxify different classes of secondary compounds through different pathways and eat different plants to avoid overloading individual detoxification pathways (Freeland and Janzen 1974). It also has been suggested that food choice is a trade-off between nutrient gain and toxicity (Freeland 1991, Provenza 1996). In our study, we have investigated a situation in which a mammalian herbivore deals with one specific secondary compound occurring in different concentrations, while the content of nutrients is the same in all foods. The preference for low-tannin food in our experiments ought to be interpreted as an attempt by the deer to limit the intake of tannin, but the deer were also more willing to eat high-tannin food when this kind of food was more common. In a broader context, it is likely that this latter aspect of their foraging behavior has an adaptive value, for instance to adjust the intake to changes in the quality spectrum of available food. Similarly, the contrast effect could be adaptive in adjusting foraging behavior to the current distribution of food types, e.g., when there is variation in palatability within a plant population. Perhaps the most basic implication of our results is that an unpalatable plant type also could benefit from its unpalatability when occurring at low frequency, and perhaps even in particular when occurring at low frequency. Our findings thus suggest that frequency-dependent food choice by mammalian herbivores could facilitate the evolution of plant defenses.

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