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Diet preference of the European ground squirrel [*Spermophilus citellus (Linnaeus, 1766)*] in Hungarian natural and seminatural grassland habitats investigation by floristic composition and microhistological faeces analysis

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1. INTRODUCTION OF THE TOPIC, OBJECTIVES

The focused European ground squirrel (*Spermophilus citellus*) is an indigenous species of the Carpathian Basin and endangered in most of Europe having fragmented, declining populations. Presently beside the remained natural habitats, they live in semi-natural grasslands with anthropogenic effects like on grassy airports. Hungary has a major number of individuals in the region thus local conservation has a high relevance inside the European Union, too. Traslocations were primarily made for the protection of the birds of prey but frequently unsuccesfully. It has revealed that more complex knowledge of the environmental requirements is necessary to the long-term conservation of the ground squirrels.

Literature about feeding habits of the ground squirrels are generally give scarce information about this species so we do not know whether and how the vegetation composition could restrict the sustain of the populations. Consequently it is essential to answer to the question: what kind of grassland management helps to keep or create eligible grasslands with rich food basis for the animals.

Summary, this research is aimed to get new scientific information about the animals' diet preferences in addition to support nature conservation efforts by botanical evaluation of the potential (distribution or translocation) habitats and make suggestions for „ground squirrel friendly" management of the grasslands.

Abovementioned, the objectives of the investigation are listed below:

- Botanical measurement of the vegetation in three tipical - machine-mowed, sheep-grazed and cattle-grazed grassland management types (by number of the plant species, diversity, relative vegetation cover) as potential food scale. Find the effects of the different treatments on the ground squirrels' diet.

- Identification of foodplants in Hungarian natural and semi-natural habitats (altogether in 17 localities) and determine the consuming ratios and preference orders.

- Application of the microhistological faeces analysis as a new method in ground squirrels to measure the diet. Within that testing the individual sampling and mix sampling methods in the three grassland management types. Besides, laboratorial preparation of epidermal tissue references (from the

plants collected on the field) is necessary for the microscopic identification of foodplants.

- Show seasonal changes in vegetation as food supply and in faecal pellets as food consumption. In connection, to measure seasonal changes in diet preference order of the ground squirrels. Comparation of data between the different grassland management groups both in larger and smaller plant taxons.

- Examination of the year effect in a mowed and a grazed sample site by comparation of two springtime data (2012, 2013) after a dry and wet period. It is targeted to detect changes in vegetation as potential food source and diet choice of the ground squirrels. In addition to this, measuring the scale and evaluate the results in the mirror of the different management types and seasonal changes.

- Measuring the scale of individual diet variance in three differently managed habitats between and within plant cathegories (taxons). Finding out whether individual diet preferences differ from the average preferences calculated for each local population.

- Assessment and comparation of relative densities of ground squirrels in sample sites according to the protocol of the national Conservational Information System, European Ground Squirrel Monitoring Action by counting the burrow entrances in paralel transects method. Measuring density in the same period and comparation of the sample sites and management types, also tracking changes in time by the analization of long-term database of some habitat sites.

- Presenting suggestions of the grassland management in the European ground squirrel habitats and for the succes of the translocations to choose appropriate target sites as well as sustaining grasslands in good condition for the animals.

2. MATERIALS AND METHODS

Phases of work during the investigation were the following ones:

1. Designation of the sample sites. From the database of the National European Ground Squirrel Monitoring (locality names and coordinates) and discuss with local experts also after personal review on field. European ground squirrel as steppe animal is frequently connected to the dry and incompact soiled grassy habitats, tipically to loess or sandy grasslands. First of all, the investigated sites

were Pannonic sandy grassland in natural or semi-natural (partly degraded) conditions. However the distribution map of this vegetation type is significantly overlaps with the area of the European ground squirrels in Hungary. Within that, most of the habitats as well as the sample sites are located along the Danube river valley and on the Homokhátság which means a sandy ridge between the Danube and the Tisza river.

2. Field work and collecting data. Relative population density assessment by counting the burrow entrances according to the protocol of National Conservation Information System and European Ground Squirrel Monitoring Action. Seasonally once fecal pellets of 10 inividuals were collected in all the sample sites, in August 5-5-5 areas of each management types and 3-3-3 in April similar way. Assessment of the vegetation as potential food supply was happened by semi-systematic quadrat method (5) and few plant specimens were collected into a herbarium as referece material.

3. Systematization and fixation of the collected samples. Identification, pressing and drying of the plants for putting into a herbarium were followed by drying and freezing of the fecal samples until the laboratorial process.

4. Work in the laboratory. Preparation of the fecal samples by microhistological faeces analysis, then observation and counting the plant epidermal tissues under optical microscope were carried out. Epidermal tissues were identified after the albums of Mátrai and Katona (2004) which contained the earlier result of the diet analysations of larger herbivors like red deer, roe deer and European hare, even so self-made referencies of the European ground squirrel was necessary. Latter were made of plant speciments collected on the field (herbarium), and the candidate took digital photo documentation, too.

5. Testing of methodologies. Sample size in quadrate method (minimum sufficient number of the 1 square meter-sized quadrates) was tested on field in a grazed and a mowed habitat both in April and August. During the laboratory work fecal analysis were firstly tested to adopt this to the ground quirrels and secondly to compare analyzation methods between the averages of 10 individual and mix of 10 feacal samples.

6. Elaboration and technical evaluation. Summary of the list and cover of the plant species were measured in quadrates by systematic recording (MS Excel), evaluation of the microscipic epidermal tissues and some chitinous (Arthropoda)

remains as additional food was carried out, too. Applied statistical methods were: Chi-square test, one-sampled t-test, one-way Anova, two-ways Anova, posthoc tests (Dunn's-test, LSD-test), Kruskal-Wallis non-parametri test, α-diversity, Jaccard similarity index, Jacobs selectivity index, relative density of the number of individuals (regarding of the Ground Squirrel Monitoring Database).

Research in numbers:

- 3 years of collecting data
- 17 different sample sites in ground squirrels' habitats
- 45 field works – sometimes for 2 days long,
- 216 quadrates recorded,
- 1100 < collected plant specimens into herbarium
- 300 individual fecal pellets,
- 6000 < counted and identified leaf epidermal tissues and referencies (45 species) processing

3. SUMMARY OF THE NEW SCIENTIFIC RESULTS (THESES)

1. First application of the microhistological faeces analysis for investigation of diet in the European ground squirrel, that was earlier succesfully used in case of larger herbivores like red deer, roe deer and European hare in literature. The author of the dissertation collected plants from all sites and put them into herbarium that was essential for the identification of the leaf epidermal tissues. After preparation of referencies, she took digital photos by a microscopic eye-piece and Webcam Companion software and edited a new album of ground squirrels' diet.

2. The author identified altogether 74 plant species during the vegetation survey in the sample sites. After coenosystematic classification it was found the main foodplants of the ground squirrels have tipically wider (eurasian, eurasian-mediterranean) distribution area, in addition to some rather local species. Consequently the distribution area of the foodplant species do not really restrict geografically the area of the ground squirrels. In point of Raunkiaer life forms two thirds of the species are hemikriptophytes, perennials. In thermoclima cathegories most of them belong to continental and submediterranean decidious forest belt, thus without active site management (mowing and grazing) most of the grasslands in Hungary would turn into forests. According to the water balance

the plants belong to varied cathegories, mainly to dry production sites, in case of nature conservation alignment they are species of natural, semi-natural and partly degraded grasslands and the number of protected species is low. As a result of designation of the sample sites, plant species are connected to calcareous or neutral soils, and among vegetation associations they are diverse, but they are rather tipical with fescue, fescue and brome, tall oatgrass or other species of disturbed grasslands.

3. Whereas testing the number of quadrates necessary to the botanical food supply estimation in April and in August in a grazed and a mowed habitat, the candidate has determined that having regard to a relative homogenous vegetation and the statistical minimum at a given time and site, 5 quadrates are enough to measure. Enhancement of the number of quadrates (up to 16) has not affect significantly the results of the potential food supply assessment.

4. Author of the disssertation identified altogether 37 foodplant species by the microhistological faeces analysis. Names in order they are listed below:

Astragalus glycyphyllos	*Elymus repens*	*Pimpinella saxifraga*	*Trifolium arvense*
Astragalus onobrychis	*Festuca pseudovina*	*Plantago lanceolata*	*Trifolium campestre*
Achillea collina	*Festuca rupicola*	*Plantago media*	*Trifolium dubium*
Achillea millefolium	*Leontodon hispidus*	*Poa pratensis*	*Trifolium pratense*
Achillea ochroleuca	*Lotus corniculatus*	*Potentilla arenaria*	*Trifolium repens*
Bromus hordaceus	*Medicago falcata*	*Potentilla argentea*	*Trifolium strictum*
Centaurea sadleriana	*Medicago lupulina*	*Rumex acetosella*	*Taraxacum officinale*
Centaurea scabiosa	*Medicago sativa*	*Salvia nemorosa*	
Coronilla varia	*Medicago minima*	*Thymus glabrescens*	
Dactylis glomerata	*Ononos spinosa*	*Thymus pannonicus*	

Studying the ratios of consumed plants on different taxonomic levels, the candidate has revealed that ground squirrels definitely prefer dicots to monocots that is clear also in the number of species: 6 monocots and 31 dicots were detected as the table shows above. Most of the monocots in diet was represented by fescue (*Festuca spp.)* while the primarily preferred dicots were leguminous plants (Leguminosae: *Medicago spp., Trifolium spp., Lotus corniculatus, Coronilla varia, Astragalus spp.*). In addition, yarrow (*Achillea*), plantain (*Plantago),* and in lower number cinquefoil (*Potentilla*), thyme *(Thymus)*, and in few percentages couch grass (*Elymus repens*), cock's foot (*Dactylis glomerata*), burnet saxifrage (*Pimpinella saxifraga*) appeared regularly in diet composition. Occasionally (under 1 percent ratio) recorded foodplants were *Centaurea spp.*, Poa spp., *Rumex spp.*, *Salvia spp., Bromus spp*., *Ononis spinosa*, T*araxacum officinale*, *Leontodon spp*..

5. The author of the dissertation has showed seasonality and management dependency of the potential food supply (floristical composition) in three type of grasslands: machine-mowed, sheep-grazed and cattle-grazed sample sites by the following methods. Based on the species composition and alpha-diversity the range of foodplants in sheep-grazed site was narrow, while it was much wider in the cattle-grazed site. Machine-mowed area was between them, but closer to cattle-grazed one being relatively rich in species. Total vegetation covers were generally above 95 percents representing unopened, stabilized grassland types. Monocots cover were between 75-95 percents in April, but averagely above 90 percents in August measured in all management types. However dicots covers have differed significantly in April: in sheep-grazed pastures (27%), in mowed grass (39%) in cattle-grazed pastures (43%). Oppositely, in August cover of dicots was the highest in sheep-grazed sites (49%), besides equal ratios (36%) in mowed and cattle-grazed grasslands.
Comparison of the floristical compositions of the sample sites by Jaccard similarity index have resulted that sheep- and cattle-grazed grasslands were the most different, which were followed by the sheep-grazed and mowed grasses, while the cattle-grazed and mowed habitats were the closest in floristical composition similarity. It can be declared, the way of grassland management although in variable degree but considerably affect the floristical composition of the habitats, namely the foodplants of the European ground squirrels.

6. Seasonality and management dependency have revealed in diet composition measured in three habitat types. In April food supply and consumption were poorly variable in all sites, detecting three tipical components: fescue (*Festuca spp.),* yarrow (*Achillea spp.),* and leguminous species (*Leguminosae*). Contrary in August diet composition was richer in species, supply and demand ratios have changed remarkably but different way depending the management type.
In August statistical analysis of the average values in mowed, sheep- and cattle-grazed areas has showed strong significant differences in diet between the managements. Similarly, in April significant differencies were found in the three management types.
In cattle-grazed pastures dicots were consumed in twice as much proportion than monocots both in April and August, beside well balanced diet of the ground squirrels during the year. In sheep-grazed pastures the average proportion of dicots is similar to the average proportion of monocots in diet, although consumption ratio of dicots has grown notably to August. In mowed sites the result was between the two grazed types. However at the end of the active season

of the ground squirrels more consumable dicots remained in mowed grasslands in the lack of the grazing animals (competitor herbivores).

From April to August the average of consumption ratios measured in lower taxons have changed as follows: 1. comsumption stayed proportional to supply: *Potentilla spp., Dactylis glomerata*; 2. consumptional ratio declined in comparison with supply: *Thymus spp., Achillea spp., Festuca spp.*; 3. consumption ratio growed more than supply: *Plantago spp.*.
In mowed habitats medium values of consumption were found between sheep- and cattle-grazed pastures which were rather closer to the cattle-grazed ratios.

7. Diet analysis has also revealed high individual variance in all management types. In larger taxons – monocots, dicots, seeds – were present in each individual fecal sample, beside a high degree of variance in proportions even within one habitat. The author has found remarkable individual variance in the level of smaller taxons, especially in the proportion of *Festuca ssp.* and Leguminosae as intensively consumed species. The author has established that preferred foodplants of European ground squirrels are tipical not only at population level, but also at individual level, although the animals feed on them in different proportions in a studied site at the same time. Consequently, individual variance in diet is rather quantitative than qualitative in all taxonomic cathegories, some qualitative difference can be found in small-scale (in the level of lower taxons).

8. In methodical tests the candidate have shown that beside individual faeces analysis the mix (of 10 faecal pellets) method is applicable like in case of larger herbivores. Analyzing faecal samples collected from three management types, none significant difference was found in the proportion of the consumed monocots, dicots and seeds, between the average values of individual samples and mixed samples. Likewise at smaller taxonomic levels there were no significant differences between the values of individual averages and mix samples. It can be determined, that in case of a general diet composition study of a local ground squirrel population, the mixed fecal analysis method is enough to use at this species too, thus saving time and costs in the laboratory work.

9. Seasonal differences have revealed by the author in food demand and supply ratios during the research of the European ground squirrel habitats in April and August. Comparisons of the sample sites and management groups have only

revealed significant difference in the proportion of consumed Leguminosae between the sheep-grazed and cattle-grazed pastures.
Among the results of August period there were significant ($p<0.05$) difference between the floristical and diet composition in case of dicots and these smaller taxons: *Festuca spp.,* Leguminosae*, Achillea spp., Plantago spp.*. There were not significant differences in case of monocots an the following taxons: *Dactylis glomerata, Thymus spp., Potentilla spp., Pimpinella saxifraga.*

10. The candidate has showed the alterations of the seasonal food preferece orders in points of taxons and habitat management types. In April the values of Jacobs' selectivity index in declining order were the next ones in mowed grasslands: 1. *Leguminosae* 2. *Achillea* 3. *Thymus* 4. *Festuca* 5. *Plantago.* 1,2,3 preferred, 4,5 avoided (consumed ratios were less than supply (vegetation cover) ratio. In sheep-grazed grasslands: 1. *Achillea* 2. *Plantago* 3. Leguminosae 4. *Potentilla* 5. *Thymus* 6. *Festuca.*1,2,3,4 preferred 5,6, avoided. In cattle-grazed grasslands: 1. *Achillea* 2. *Plantago* 3. Leguminosae 4. *Potentilla* 5. *Thymus* 6. *Festuca.*1,2,3,4 preferred 5,6, avoided.
In August the values of Jacobs' selectivity index in declining order were the followes in mowed grasslands: 1. Leguminosae 2. *Plantago* 3. *Thymus* 4. *Achillea* 5. *Festuca*, 6. *Potentilla.* 1,2,3 preferred, 4,5,6 avoided. In sheep-grazed grasslands: (1. Dactylis glomerata), 2. *Plantago,* 3. Leguminosae*, 4. Achillea*. 5. *Festuca 6. Thymus* 7. *Potentilla.*1,2,3, preferred, 4,5,6,7 avoided. In cattle-grazed grasslands: 1. Leguminosae 2. *Plantago* 3. *Potentilla* 4. *Thymus* 5. *Festuca,* 6. *Achillea.* 1,2,3, preferred, 4,5,6, avoided foodplants.

10. Significant year-effect was detected by the author both in vegetation and diet in a mowed and a sheep-grazed habitat of the ground squirrels compared two spring seasons.
In April of the dry year (2012) diet composition related more to the potential food supply (floristical composition) than in April of the the wet year (2013). Ground squirrels consumed the timely most frequent aromatic plant, *Achillea spp.* in the highest proportion and it was followed by the Leguminosae despite their scarce presence in the current vegetation state. A possible reason is why the animals also fed on the high fibre contained but dominant in vegetation cover *Festuca spp.* and frequent, but hairy leaved *Potentilla spp.* in remarkable degree, was the small scale of the potential foodplants. At this time of the year food supply were characterized by low number of species with a few foodplants, restricting the expression of the preference.

Due to the wet period in the next April (2013), the author has found differences comparing the previous dry year both in quantity and quality of the vegetations which were smaller in the mowed, larger in the sheep-grazed sample site. Squirrels could select from more dicot species, so the consuming ratio of the monocots (*Festuca spp*.) has reduced. More plants were recorded in diet like *Pimpinella saxifraga* and in „others" cathegory beside the increased animal components (*Arthropoda*) while the ratio of *Potentilla spp.* has decreased.
By the characteristic food preference there are deviations in degree of presence of the preferred plants in the diet also in food-scale of vegetation. Consequently a year-effect is important, sometimes it can be more considerable than the effects of the different management types at the same habitats.

11. The author of the dissertation has estimated relative density of the ground squirrels in April 2012 in three sample sites of each management types (3-3-3) according to the protocol of the National European Ground Squirrel Monitoring and Conservation Information System. The 5-10 cm height of the vegetation has not affect in visibility the result of the burrow entrance-counting. The average vegetation height was low in sheep-grazed, medium in mowed and higher in cattle-grazed pastures. Among the management types no significant differences in density was found, only tendency of the averages showed a slightly lower density in sheep-grazed and it was similar in cattle-grazed and little higher density in mowed sites; the variety among the sample sites was rather tipical in the same period of the year. Among the transects the deviation in the number of the counted burrow entrances was maximum double or triple in homogenous environment while in inhomogenous environment (because of micro-topography, anthropogenic effect) eight to ten times differences were also recorded.
In two sample sites the author has made long-term (12 years) dissections. At the examples of the mowed grassy airport of Budakeszi and the sheep-grazes grassy airport of Dunakeszi, the estimated values of density showed remarkable fluctuations of the local population size, meanwhile in each area after one year of a declining tendency a sudden two or three times growth of the population size was detected. Consequently viable populations living in regularly managed stable habitats are able to renew themselves soon after an unfavoured period.

4. CONCLUSIONS AND SUGGESTIONS

Before translocations into different grassland types first it would be necessary to know the food demand and preference of the ground squirrels, secondly to have

adequate information about the floristical composition of the source habitat to be able to choose similar targes sites, thus the animals have a better chance to adapt quickly to the new environment. Due to the practical skills, the first period after release is critical for longer survive of the population, and food supply is one of the most important factors. General aim of the conservation is to sustain grasslands with favourable floristical composition and to help creating such habitats by the appropriate site management as it is detailed below.

- Removal of the undesirable vegetation like shrubs, stinky weeds (e.g. *Eringium campestre, Ononis spinosa*) and poisonous plants (e.g. *Artemisia spp., Atropa spp.*) and (invasive) tall herb frings (e.g. *Erigeron*, *Asclepias*, *Solidago* species). Applicable methods are the traditional manual thinning as sheperds did earlier, also the autumnal cleaning or stem-crushing by machine-mowing.

- Avoidance of overgrazing. Low height of the grass can be sustained by sheep- or cattle-grazing, however it should be paused from October till April. This helps grassland vegetations to regenerate and grow eligible food supply (especially dicots, leguminous plants) by the time of emergence of the ground squirrels in spring. Because of the frequently mild winters in the last decades, the animals (mainly sheep flocks) are kept onwards in the pastures, whereas the wintertime grazing (between 31 October and 23 April) is a subject of authorization in Natura 2000 sites. This regulation is really necessary in the less productive, dryer areas like most of the habitats of ground squirrels.

- In deeper habitats with fluctuating ground water level to create „emergency hills" or drainage of the area for the safety of the ground squirrel populations would be essential. Field experiences showed that the first ensure the survival of at least some individuals in natural topographic conditions, the latter solution is working at many airfields athough it was developed not for the protection of the ground squirrels but for the safety of flying.

- Agri-environmental subsidies have been accessible from the European Agricultural Found for Rural Development (EAFRD) for the landusers of the designated Natura 2000 sites, for sustaining of extensive grasslands also for ecological (organic) farming, for developing natural grassland habitats, etc. Mowing regulations like for the benefit of the great bustard (*Otis tarda*) allow higher vegetation , than it would be optimal for the ground squirrels, however the present subsidies can be useful taking into consideration the

abovementioned „ground squirrel friendly" suggestions until introduction of a special subsidy for the protection of the European ground squirrel.

- Planning translocations, before selecting the new target site all the known environmental needs should be take account of as coenological mapping is essential. Appropriate state of the grasslands is reachable and sustainable by purposeful, long-term site-management. If all of them are ensured, only then should be start the translocation of the animals.

- For the release of the ground squirrels a wider and open vegetationed target area would be preferential, because the animals can easier find suitable feeding patches within a larger habitat; overlaps between the individual home-ranges would be lower also more space would be available for the offsprings and the growing population in the future.

- Both maintenance or new introduction of a grassland, to develop a meadow characterized by fescue (*Festuca spp.)* with numerous dicot species like yarrow (*Achillea spp.),* plantain (*Plantago spp.),* cinquefoil (*Potentilla spp*.), thyme (*Thymus spp*.) and mainly leguminous plants as clover (*Trifolium spp.)* and alfalfa (*Medicago spp*.) is suggested. In regards of coenology and fitogenetics to use local mix of seeds or mowed hay for spread out is the best.

- Mowing should be done flexible adapting to the local habitat and the target composition needs. Likely to grassy airports to keep the grass low in a medium-productive meadow during the whole vegetation period about five-time mowing are necessary according to the literature, however for some foodplants less mowing is favourable.

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