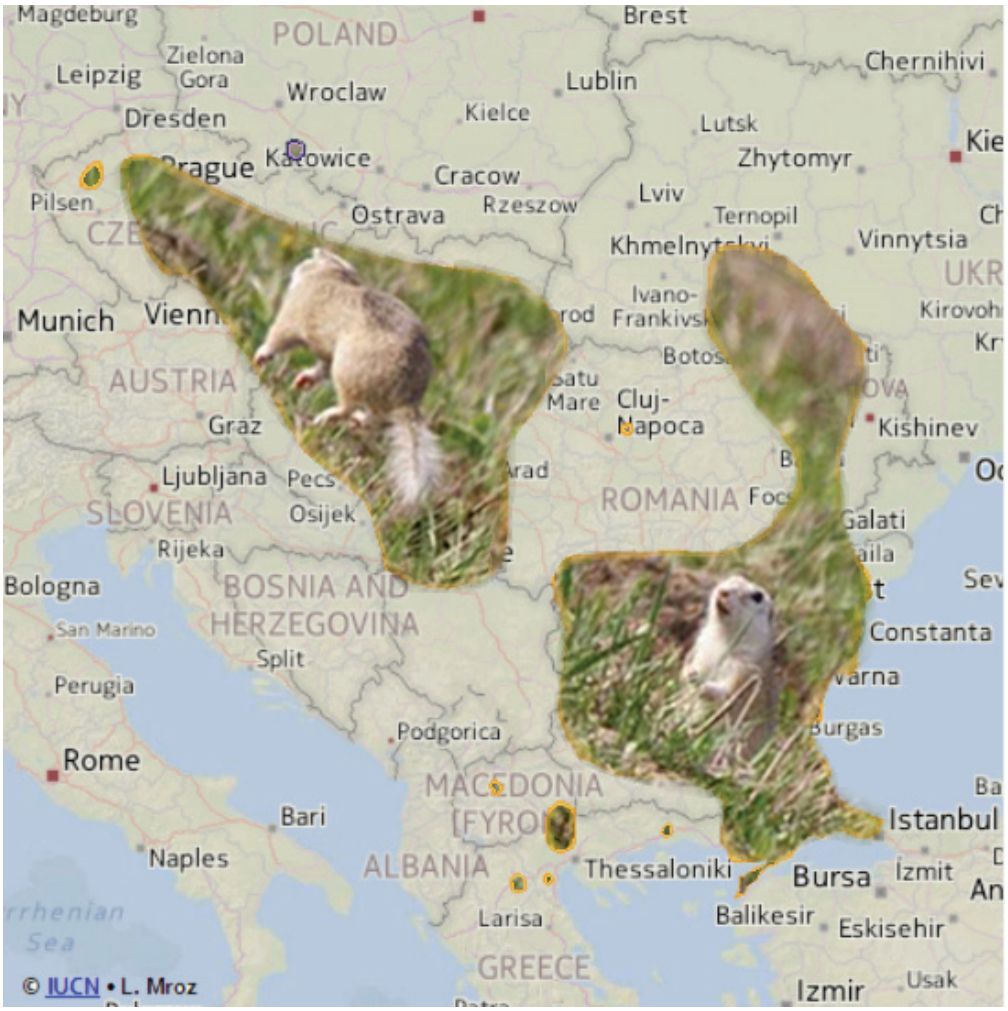
5th European Ground Squirrel Meeting

Perspectives on an endangered species

ABSTRACTS



EGSM 5

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Preface

The European ground squirrel (*Spermophilus citellus*) typically inhabits short-grass steppes and dry grasslands in central and south eastern Europe. Similar to other obligate hibernators, its active season follows a rather strict annual schedule. Nevertheless, European ground squirrels show a number of intriguing adaptations to optimize the timing and course of various seasonal processes like reproduction and the allocation of body fat reserves prior to hibernation.

Unfortunately, habitat destruction, fragmentation, and alteration due to intensification of agricultural practices have led to dramatic declines throughout most of its geographic range and the population trend is still decreasing. The species is currently listed as vulnerable on the IUCN Red List of Threatened Species, listed in Annex II and IV of the EU Habitat Directive and is critically endangered in Austria and several other countries.

The European Ground Squirrel Meeting (EGSM) has been established to facilitate an exchange of knowledge among international researchers and thus to better understand the requirements of the species. Furthermore the EGSM is an excellent forum to coordinate and evaluate research and conservation activities.

This volume summarizes contributions presented at the 5th European Ground Squirrel Meeting in Rust, Austria, in October 2014. Recent results on the geographic range, monitoring and re-introduction projects as well as on ecology, behaviour and physiology of the European ground squirrel help broaden our knowledge about this fascinating species and promote international networks to plan, implement, coordinate and evaluate management plans.

Eva Millesi
on behalf of the Organizing Committee

Session 1

Behavioural Ecology

Seasonal shift in the diet of the European ground squirrel (*Spermophilus citellus*) in Hungarian dry grasslands

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Seasonal diet choice of the European ground squirrel was investigated in sheep-grazed, cattle-grazed and mowed dry grasslands by comparing vegetation composition and microhistological faecal analysis. Data were collected in April and August focusing on the post-hibernation and pre-hibernation period in 2012 and 2013. We examined food availability in terms of local vegetation composition by quadrate method and Jaccard index, while food preferences were analysed by Jacobs' selectivity index.
In April, plant species were present in low numbers and cover on all grassland types (but higher in cattle-grazed, lower in sheep-grazed sites), and ground squirrels fed on dicots and monocots in similar proportions. The characteristic species groups were *Festuca, Achillea* and Leguminosae, but only the latter had significantly different proportions in sheep- and cattle-grazed pastures. In August, the food supply was richer, and diet composition varied on a larger scale. The proportions of the consumed species differed not only seasonally but also by grassland management type. Differences between botanical and faecal composition were significant in four species groups (dicots: Leguminosae*, Achillea* and *Plantago*; monocots: *Festuca*; $p<0.05$; 2-way ANOVA, Kruskal-Wallis tests), but not in other dicots (*Thymus, Potentilla, Pimpinella saxifraga*) and monocots *(Dactylis glomerata)*. Both in vegetation and diet composition, the highest differences occurred between sheep- and cattle-grazed habitat types; the features of mowed grasslands were closer to the cattle-grazed areas. From April to August the proportion of monocots declined and the proportion of most dicots increased in the diet, but not in all dicot species groups: the proportions of *Achillea* and *Potentilla* species decreased, while Leguminosaeup to one third.
Our results indicate that cattle-grazing ensures the best conditions regarding food supply throughout the active season while sheep-(over)grazing results in poor choice for ground squirrels in April.

Relations between genetic, geographic and acoustic distances in five populations of speckled ground squirrels *Spermophilus suslicus*

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Integrative study of genomic and acoustic variation helps to understand the relationship between the phenotypic traits that are important for survival of individuals and their underlying genomes. We compared alarm call structures and genetic polymorphism of a mitochondrial control region of five geographically isolated populations of the speckled ground squirrel *Spermophilus suslicus*. Alarm calls were recorded from individually marked adult animals sitting singly in live-traps. We analyzed 733 alarm calls from 75 individuals (15 ground squirrels per population, 5 to 10 calls per individual). We calculated acoustical distances between all populations based on Mahalanobis distances of discriminant functions using 10 acoustic variables of calls, averaged for each individual; their values varied from 0.99 to 7.32. Genetic distances between populations were calculated based on data of genetic polymorphism of the full-sized control region of mtDNA (999-1001 b.p.), obtained by sequencing of animals included into the acoustic analysis. Genetic distances ranged from 0% to 0.9% within populations and from 0.5% to 4.7% between populations. Ground squirrels from the Eastern part of the species distribution area displayed considerably lower genetic diversity compared to the Western populations of this species. Geographical distance between populations varied from 12 to 1274 km. Comparison of acoustic, genetic and geographic distances showed a significant positive correlation between genetic and geographical distances among populations ($r=0.85$, $p<0.005$). The acoustical distances were neither correlated with genetic ($r=0.28$, $p=0.44$) nor with the geographical distances ($r=0.52$, $p=0.12$). This study should be expanded, with adding more populations and by applying more variable genomic markers.

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Ontogeny of the alarm call in the European ground squirrel (*Spermophilus citellus*): findings from a semi-natural enclosure

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The European ground squirrel emits an alarm call to warn conspecifics against potential danger. Although it has been observed that inexperienced juveniles emit alarm calls that sound similar to those of adults, studies focusing on juvenile calls are missing. The aim of our study was to analyze the acoustic structure of alarm calls produced by juveniles at the age before their dispersal. Since we conducted our research in an enclosure in Prague zoo where the animals were permanently marked, we were also able to evaluate changes in the acoustic structure of their alarm calls within a time span of one year.
Alarm calls changed significantly with age, and interestingly, in the same way in all studied individuals. All juveniles emitted exclusively calls comprising one element with constant frequency, and added a frequency-modulated element as adults. Duration and frequency modulation of the element with constant frequency decreased in all individuals, whereas fundamental frequency parameters and peak location increased with age. All individuals produced calls with lower fundamental frequency as juveniles. This is in contrary to an expectation that juveniles with shorter vocal folds should produce calls with higher fundamental frequencies, but consistent with similar findings in other Palaearctic ground squirrels. However, the "vocal mimicry" hypothesis, assuming that ground squirrel juveniles produce alarm calls structurally indistinguishable from those of adults to avoid infanticide and age-dependent predator risk, is not fully verified by our study, because alarm calls produced by juveniles differed considerably from those of adults, and thus could be distinguished easily. We suggest that a follow-up comparative study based on more individuals from the wild as well as from captive or semi-captive populations is needed to better understand the ontogeny of alarm calls in European ground squirrels, and to evaluate possible influences of captivity on this process.

The early bird catches the worm: the time of emergence affects female reproduction in yellow ground squirrels

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In female hibernators, the time of vernal emergence is one of the most important factors among other life-history traits (e.g., body condition or age) that set constraints on reproduction and may affect reproductive decisions. Late emergence provides females and their offspring with less time to grow and accumulate fat reserves before hibernation, and can reduce the chances to reproduce successfully. We studied variation in reproductive effort, annual reproductive success, and survival among females in the yellow ground squirrel (*Spermophilus fulvus*) in relation to age, body mass, previous reproduction, and time of emergence. We observed individually marked squirrels (N=160) in a free-living colony (Saratovskaya oblast', Russia) in 2004-2008. The probability of weaning a litter did not depend on female age, body condition, time of emergence or previous reproduction. Litter size, litter mass and offspring survival did not vary with female age or body condition upon emergence. Reproduction did not influence female survival, physical condition upon emergence next spring or subsequent reproductive efforts. The only factor that affected the extent of female reproductive effort and offspring survival was the date of emergence: the later a female emerged, the lower the total and mean offspring mass and fewer offspring survived. Thus, the extent of female reproductive effort in this long-hibernating species was modulated by only the timing of vernal emergence. An independence of female reproduction from other individual characteristics can be explained by the high costs of missed reproductive opportunity because of short longevity combined with low costs of reproduction when resources are abundant enough to meet both somatic and reproductive needs. The variation among females in the probability to reproduce remains unexplained and suggests that other factors are involved, possibly related to the social or ecological environment.

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Session 2

Hibernation

Differences in the patterns of heterothermy in two free-living populations of Anatolian ground squirrels

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Many small mammals overcome energetic constraints by employing heterothermy, a temporary and controlled reduction of metabolism, body temperature, and other physiological functions, as an energy-saving strategy. Expression of heterothermy can be highly flexible among species and even among individuals of a single species, although two common patterns of heterothermy have been most commonly recognized: daily torpor (lasting < 24 hours) and hibernation (consisting of multiday torpor bouts, interrupted by brief inter-bout euthermic intervals). Surprisingly, little information is available on how populations of the same species living in different environments vary in regard to the use of torpor and thermal physiology. In this study, we aimed to understand differences in the patterns of heterothermy in two free-living populations of Anatolian ground squirrels, Spermophilus xanthoprymnus, whose range encompasses highly different climatic and ecological conditions. Accordingly, this study was conducted at two study sites, approximately 900 km away from each other, one in central Anatolia, and the other in north-eastern Anatolia, at different times (2005-2006 and 2006-2007 heterothermal seasons in central Anatolia and 2013-2014 heterothermal season in north-eastern Anatolia). Body temperature was continuously recorded from late summer to spring by intraperitoneally implanted temperature-sensitive data loggers and soil temperature by a temperature-sensitive data logger at a depth of 1 m in the field. Based primarily on climatic and ecological differences at each study site, we found significant differences in the patterns of heterothermy between the two populations. For example, as expected, Anatolian ground squirrels in colder, more seasonal north-eastern Anatolia remained in heterothermy longer than those in warmer, less seasonal central Anatolia.

Energy challenges and oxygen (un)availability – key players in pre-hibernation's molecular changes essential for euthermy abandonment in *Spermophilus citellus*

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Explaining the regulatory mechanisms responsible for the ability of hibernators to cycle between physiological states that differ dramatically in terms of metabolic rate, oxygenation and body temperature, without almost any adverse effects, would be highly beneficial for proceedings in human pathology. Although entrance into torpor occurs in a very short time, recent studies proclaim early metabolic/molecular reprogramming in the pre-hibernation period as essential for hibernation onset.
In this study, we investigated involvement of key regulators of energy homeostasis: hypoxia-inducible factor-1 and AMP-activated protein kinase (AMPK), in regulation of thermogenic processes in both brown adipose tissue and skeletal muscle, but also their role in white adipose tissue and adrenal gland functioning. Special emphasis was placed on the regulation and pattern of changes of mitochondrial oxidative and antioxidant capacity in these tissues/organs. All these molecular perturbation were examined in two physiological extremes – cold-exposure and in hibernation. To do this, ground squirrels were exposed to 4 ± 1 ºC, resulting in two groups: 1) animals that fell in torpor and 2) animals that stayed euthermic and active.
In summary, our results suggest that molecular reprogramming essential for euthermy abandonment during euthermy-torpor transition starts long before hibernation actually begins. Data showed that metabolically-uncompensated induction of thermogenic processes leads to energy deletion, which, in turn, activates AMPK and prevents further maintenance of euthermy. In addition, one of the main reasons for metabolic disengagement seems to be compromised oxygenation of the tissues, which limits its oxidative capacity, i.e. energy-producing potential. Interestingly, we detected up-regulation of antioxidant defense in these tissues, which suggests that its elevated level in thermogenic tissues is necessary for maintenance of redox-homeostasis in hibernation.

Poster Session

Molecular characteristics of oxidative and antioxidant capacity of *Spermophilus citellus* kidney in hibernation - initiation of a resistant phenotype well before hibernation induction

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Mammalian hibernators offer the unique opportunity to examine what is essentially nature's version of organ preservation. Given that the kidney is the most frequently transplanted organ, we aimed to examine its molecular remodelling in a hibernator in two physiological states that differ dramatically regarding energy demand, metabolic rate, and perfusion: euthermy and torpor.
To do this, European ground squirrels were exposed to 4 ± 1 ºC and then divided into two groups: 1) hibernating and (2) active. We examined structural remodelling of kidney tissue, as well as protein expression of oxidative phosphorylation (OXPHOS) components, antioxidant enzymes and regulators of molecular/metabolic reprogramming: hypoxia-inducible factor-1 (HIF-1), nuclear respiratory factor 1 (Nrf1), peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) and nuclear factor (erythroid 2-related)-like 2 (NFE2L2).
Results showed that OXPHOS capacity of the kidney in hibernation was considerably suppressed. This suppression was paralleled by a decreased protein level of transcription regulators responsible for mitochondriogenesis and the expression of OXPHOS components: PGC-1α and Nrf1. Nevertheless, protein expression of CuZn superoxide dismutase and catalase was elevated in hibernation parallel with their transcription regulator NFE2L2. All above-mentioned changes were yet visible in euthermic animals readily after exposure to cold. In addition, initiation of such molecular remodelling seems to coincide with accumulation of O_2-sensitive α subunit of HIF-1 and increase of glomerulus size.
In summary, these results suggest that a resistant phenotype of kidney in hibernation is characterized by suppression of mitochondrial oxidative metabolism parallel with maintenance or even increase of antioxidant capacity. This molecular signature seems to be initiated by cold (and consequent drop in perfusion and oxygenation), well before hibernation induction.

Population size, ecological isolation, and genetic diversity in European ground squirrels

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Genetic diversity is of paramount importance for the evolutionary potential in populations or for individual fitness. For conservation issues, it is crucial to know how genetically diverse an endangered species is, and what factors may affect genetic variation among populations. We studied whether genetic diversity of local populations (4 in Hungary and 5 in Austria) of European ground squirrels was influenced by census population size and ecological isolation of the populations. We genotyped 144 individuals at 11 polymorphic microsatellite loci to assess population-specific allelic richness (AR) by a rarefaction approach to account for different sample sizes. We based estimates of population sizes on standardized counts of numbers of burrow entrances and used the index of Rodríguez & Delibes to obtain population-specific values of ecological isolation (using grid-based presence/absence data of European ground squirrels around each studied population). General linear models and model averaging indicated higher AR in the Austrian populations than in the Hungarian populations („relative variable importance" – RVI=0.98), but no marked effect of ecological isolation (RVI=0.40) or census population size (RVI=0.03). The unexpected region effect does not correspond to the „core/margin hypothesis of genetic diversity of a species"; it may partly result from higher population densities in Hungary, which in turn might reduce effective genetic population sizes, because of relatively high proportions of non-reproducing yearling males. Overall, effects on the larger geographic scale, possibly independent from the currently studied factors, seem to be involved in the spatial variation of genetic diversity of European ground squirrels from their western sub-range.

The structure of the *Spermophilus erythrogenys* superspecies according to sequencing data for the control region of mtDNA

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The polymorphous species *Spermophilus erythrogenys* sensu lato, widespread in the former USSR, is now under intensive study in Russia. Molecular genetic studies of this superspecies are the most effective means for revealing their phylogenetic structure. This is because morphological characteristics formerly used for systematic arrangement often have cline variability and possible adaptive importance throughout the range. Differentiation of Red-cheeked ground squirrels according to sequencing data of the control region of mtDNA has already been conducted (Ermakov et al., 2013). Our study specifies the structure of groups inhabiting Altai Krai and Novosibirsk Oblast in Russia.
In this study, material collected in Altai Krai and Novosibirsk Oblast in May-June 2013 was used. A C-region fragment of mtDNA was amplified and sequenced for 26 individuals from 9 localities in the studied area. Data were processed using MEGA6 software suite and Test Maximum Likelihood Tree was plotted.
On the basis of the obtained sequences, a phylogenetic tree was plotted in which all samples were divided into two groups corresponding to the populations of the right and left banks of the Ob River. There were no samples for which the mitotype typical for the opposite bank was found. This confirms that the Ob River is a reliable isolation factor. This difference between the two mitotypes of the two groups of Red-cheeked ground squirrels from opposite banks of the Ob River raises the following: to which of the two groups does the lectotype of this species belong? Johann Friedrich von Brandt (1841) did not specify the exact location where the lectotype was caught. But later he cited the city of Barnaul (Brandt, 1843) as the type locality. However, we found both mitotype groups in the vicinity of Barnaul on opposite banks of Ob River.

Age of maturation in yellow ground squirrel males (*Spermophilus fulvus*)

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Life-history theory predicts that in hibernators age of maturation is related positively to body size and negatively to the duration of active season aboveground. Yellow souslik *Spermophilus fulvus* is a large-sized ground squirrel with long hibernation, which suggests late maturation. Our aim was to determine the age of maturation in males through analysis of age-dependent variation in body size, mass, androgen status, timing of spring emergence, ranging patterns and social behavior during the mating season.
We observed permanently marked squirrels (N=88) in a free-living colony (Saratovskaya oblast', Russia) during mating seasons in 2004-2007. We recorded body mass, structural size, the level of testosterone in feces as well as social contacts and location of males during daily observations.
Yearling males were smaller, lighter, had lower levels of testosterone, emerged later and had smaller home ranges than older males. Social activity and the number of females encountered did not differ between age classes. After the second hibernation, none of the studied parameters varied with age. Cluster analysis revealed two behavioral tactics: "active" males (adults only) emerged earlier, ranged more widely, initiated more contacts, encountered more females and were heavier than "passive" males (both yearling and adult) who resided close to their hibernacula. Thus, males of *S. fulvus* reached adult size and matured after two hibernations, which is relatively fast for such a big species with a short active period. Indirect evidence for copulations and high variation among yearlings in all parameters suggest that some of them might successfully compete with adults. Active tactic of wandering and searching for females is energetically costly, and probably only adult males in good physical condition can afford it, whereas passive tactic of residing is energy saving and good for adults in poor condition and for yearlings that are continuing to grow.

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Ten years of European-ground-squirrel reintroduction in Poland

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In Poland, European ground squirrels disappeared in the 1970s, and their reintroduction began in 2004, when the first group of animals was imported from Hungary to establish a breeding program in the Poznań Zoo. In the years 2004, 2005, 2007, 2011 and 2014, ground squirrels were brought from Hungary and Slovakia to the Zoos of Poznań (135 individuals from Hungary and 65 from Slovakia) and Opole (54 individuals from Slovakia and 5 from Poznań Zoo), to six large (100 m^2) enclosures, three in each of the zoos. Since 2005, reintroductions have been attempted in five locations within the historical range of European ground squirrels in southern and south-western Poland: Kamień Śląski (Opolskie Voivodeship) where we released 246 individuals, Głębowice – 170 individuals, Jakubowo Lubińskie – 108 individuals, Rościsławice – 45 individuals, and Jemielno – 64 individuals (all four in Dolnośląskie Voivodeship). At translocation in the field, a soft method of reintroduction was used: the animals are released into an acclimatization enclosure (40 m^2 each), where they are +/- safe from predators and can leave the enclosure themselves by burrowing their way outside. The breeding program in the Poznań Zoo has been successful, but in the Opole Zoo it finished with failure. Until 2014 reintroductions in two locations have been successful – in Kamień Śląski and Głębowice – and in two finished with failure – in Jakubowo Lubińskie and Rościsławice. One – in Jemielno, was started in 2014, and it is too early to evaluate its results.

The program is supported by EU in frame of the Operational Programme 'Infrastructure and Environment' and The National Fund of Environmental Protection and Water Management.

Habitat maps of the European ground squirrel (*Spermophilus citellus*) in the south-eastern Pannonian plain (Vojvodina, Serbia)

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In the lowland of Vojvodina, the population of the European ground squirrel (*Spermophilus citellus*) is fragmented due to land use changes (e.g. habitat conversion and infrastructure), and restricted to very small patches of suitable habitat, mostly grazed pastures. These small habitat fragments are often outside of protected areas. Moreover, not all species' habitats are mapped and defined, so there are no complete data on the species' potential and actual distribution, and habitat connectivity. Gathering data on species habitat suitability and mapping occupied and unoccupied habitat patches within the available area will enable us to study the current status and future perspectives of European ground squirrels in Serbia. The general population trend is decreasing, but data on the exact number of individuals within certain areas is scarce, as is the case with number of suitable habitat patches and occupied patches within available habitat. The main objective of this work was to produce habitat suitability maps and actual distribution maps in Vojvodina and to investigate the current status of populations within the area of Central Banat (Vojvodina). To access this goal we first calculated a potential distribution map with MaxEnt software. Then we investigated 82 previously known localities (published or calculated) and mapped 54 habitat patches occupied by European ground squirrels in 2014. Furthermore, we delineated polygons of used patches within available habitats for the species in ArcMap 10 and overlaid it with the existing network of protected areas and future Regional Eco Network (REN). The results revealed patterns of European-ground-squirrel distribution overlapping more frequently with potential ecological network areas than with current regional conservation practice. Further on, outcomes of this work will be used to evaluate spatial characteristics of species available and occupied habitat. Further fieldwork will be carried out in 2015.

Vulnerability of European ground squirrel colonies in the Pannonian region of Romania

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Distribution of the European ground squirrel in western Romania is patchy, nevertheless these isolated colonies are among the largest within the central-European population of the species, and as such, have a remarkable implication for conservation. Our goal was to collect basic geographic information about the size of ground squirrel habitats, the isolation among them and the frequency of the two major threats leading to habitat destruction and fragmentation in the region, i.e. transformation to arable land and construction activities. Distribution of ground squirrels was mapped between 2006 and 2014, with 151 colonies located. The surface of each colony was assessed as the surface of the largest continuous grassland area apparently suitable for the species. The area of ground squirrel habitats was drawn using the most recent Google Earth satellite imagery, as well as own GPS data. The shortest distances of discrete colonies to the nearest neighbour colony, as well as to settlements were measured in order to assess their degree of isolation, and the likelihood of facing threats owing to the close proximity of humans, respectively. By comparing recent satellite images with previous ones, we were able to assess the frequency of grassland habitat losses owing to transformation into arable land or to construction. The mean area of colonies was 133 ha, with 39% being smaller than 50 ha. The mean shortest distance between colonies was 3018 m. 40% of the colonies have witnessed losses owing to transformation into arable land in the past decade, while construction activities affected 17%. Colonies near settlements were significantly more affected by both construction and agriculture compared to those remote from settlements. However, overlap with protected areas did not decrease the effect of these threats. With habitat destruction progressing at an alarming rate, our results suggest that immediate action is required to save these shrinking colonies from extinction.

Distribution and habitat selectivity of the European ground squirrel (*Spermophilus citellus*) in Eastern Romania

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The European Ground Squirrel (*Spermophilus citellus*) is known as a common species for pastures in Romania, even if its distribution and ecology is weakly documented. During 2013, we studied the distribution and habitat selectivity of European ground squirrel colonies in the Moldova region (Romania). Population density was assessed by counting the vertical spring burrow entrances on transects (3 x 150 m) within a distance of 2.5m on every side and by direct counting of animals by visual observation. In total, 872 squares (250 x 250 m) were examined, with a cumulated area of 5450 ha. The number of colonies identified is higher at lower altitudes (0-150 m). Population density increases with slope: at a gradient of 0-4°, the average density recorded is 10.3 individuals/ha, whereas on a slope greater than 12.1 to 17°, the values reach 19.4 individuals/ha. Also, the density varies according to slope orientation ($Fr = 75.12$, $p < 0.0001$), from 10.7 individuals/ha on south-western slopes to 15.2 individuals/ha on eastern slopes. In the Moldova region, European ground squirrels prefer natural habitats, especially pastures, with a vegetation height below 15 cm. Occasionally, the species was also found in hayfields, where the vegetation height frequently exceeds 20 cm. Population density of European ground squirrels decreased with an increase of tree and shrub coverage. For our study area, the highest density (15.2 individuals/ha) was in areas with less than 5% cover. The distribution of the species is fragmented and an effect of environmental factors was observed on the occurrence of European ground squirrels. The majority of colonies was found in grassland at low altitudes with a high slope gradient. Grazing, mowing and limiting succession of trees and shrubs are measures apt to maintain the species' habitat.

Conservation program for the European ground squirrel (*Spermophilus citellus*) in Burgenland

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From 2008 to 2014 we examined the current occurrence and distribution of the European ground squirrel in the Austrian province Burgenland. Differences in habitat characteristics and quality were mapped and alterations recorded.
Except the common pastures of the area around Apetlon/Lange Lacke (National Park Neusiedler See – Seewinkel), all squirrel colonies in the study area turned out to be endangered due to various reasons.
During the investigation period, most of the ground squirrel colonies either showed a dramatic decrease to estimated sizes of less than 20 individuals or disappeared completely.
In Burgenland, ground squirrels are confined to dry grassland, pastures, mowed meadows and green anthropogenic areas like camping sites or airfields. With one exception, vineyards are only colonised temporarily, which might be due to changes in viniculture. In contrast to other small mammals such as the Common hamster or the Mound-building mouse, ground squirrels seem to have not adapted to agroecosystems in this part of eastern Austria. Not even Lucerne meadows could be verified as current ground squirrel habitat, possibly because population densities are not high enough to facilitate dispersal and colonisation.
Obvious problems are habitat loss due to conversion of grassland into construction ground, building projects and parking areas, habitat deterioration due to missing landscape management and misusing meadows as storage areas, and direct threats such as cats, dogs and poisoning.
In Burgenland, the European ground squirrel has retreated into rare and precious habitats. Landscape management, landscape protection and public relation activities are most important issues for future conservation work.

Session 3

Geographic Range: Past and Present

Understanding past climate-driven range shifts and demographic events: the story of Anatolian ground squirrels

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Global climate changes through the Quaternary glacial-interglacial cycles have had significant impacts on the geographic distribution and genetic structure of numerous taxa. The effects of these climatic oscillations have already been well documented especially for temperate European species by molecular phylogeography. Ecological niche modelling has also been become widely used to understand further the response of species to global climate changes through the Late Quaternary glacial-interglacial cycles. The present study aimed to understand how Anatolian ground squirrels, *Spermophilus xanthoprymnus*, have responded to global climate changes through the Late Quaternary glacial-interglacial cycles. Accordingly, ecological niche modelling and molecular phylogeography were used together. Using species occurrence data and the maximum entropy machine learning algorithm in MAXENT, an ecological niche model was developed to predict the geographic distribution of Anatolian ground squirrels under reconstructed past (the Last Interglacial, 130 000-116 000 years ago and the Last Glacial Maximum, 21 000 years ago) and present (1950-2000) bioclimatic conditions. Using previously published and new cyt *b* mtDNA sequences and Bayesian Markov chain Monte Carlo and Approximate Bayesian computation methods in BEAST and DIYABC, respectively, demographic events were assessed over the history of Anatolian ground squirrels. The results supported that continental species, adapted to drier climate with greater seasonal variation, such as Anatolian ground squirrels, have been in refugia during the interglacial periods. It is these species that are of most immediate concern because they currently contract their range into interglacial refugia and face increased threat with further rises in global temperatures. In conclusion, glacial range expansion and interglacial range contraction is not a pattern only for high-latitude (northern) and -altitude (alpine) species.

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The European Ground Squirrel (*Spermophilus citellus*) in Burgenland, Austria: 1950ies to 2013

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Early data on the distribution of the species in Burgenland are rare. Nevertheless, they corroborate the conclusion that any adequate habitat in the Burgenland once was inhabited by squirrels. High population densities were described especially from pasture areas north and east of Neusiedler See. Altogether, historical data suggest a good connectivity among clusters of squirrel colonies.
From 1968 onwards, more detailed data are available: numbers of squirrel holes per hectare varied from 15 on a ploughed meadow to 500 on a grassy farm track, a figure not to be found now anymore in Burgenland. In the 1970ies, several large squirrel colonies were destroyed through afforestation, intensified agriculture and the change of dry meadows in building land.
All this led to the splitting of colonies into groups of just a few individuals inhabiting suboptimal relics of former ideal habitats.
Nowadays, viable colonies are, apart from those in and adjacent to the National Park Neusiedler See - Seewinkel, completely isolated, and their long-term persistence is uncertain.
One possible measure to show the development of ground squirrel populations is the number of quadrants (1 longitudinal minute by 1 latitudinal minute) with evidence of ground squirrel occurrence:
From 1970 to 1999, we counted 63, in 2007 only 39, and in 2012, meagre 32 inhabited quadrants had remained. From 2007 to 2012, 10 groups of just a few animals and 5 larger colonies disappeared for various reasons, and the decreasing trend might continue.
Main reasons for this decline are insufficient habitat management, changes in viniculture, recultivation of fallow land and last but not least, the insufficient awareness of many people considering the importance to support the survival of the species. One of the main goals of our future work is to improve this situation throughout the species' range in general, and in Burgenland in particular.

On the state of the European ground squirrel (*Spermophilus citellus*) in the Republic of Moldova

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The European ground squirrel has the eastern limit of its distribution on the territory of the Republic of Moldova, but there are only few data about the species in this area. The first information on the occurrence of *S. citellus* on the territory of Moldova was recorded about 100 years ago, and only in the northern part. In the middle of the past century, when complex faunistic studies began, the species was not found in the northern part of the republic, but it was recorded in some localities from the central zone.

At present, 25 colonies of European ground squirrels are registered between 47°11'-48°01'N and 28°19'-28°52'E, with the corresponding localities Napadova village (northern limit, 48°00.42'N and 28°38.92'E, 114m a.s.l.), Paharniceni-Piatra village (eastern limit, 47°22.24'N and 28°52.14'E, 30m a.s.l.), and Sadova village (south-western limit 47°11.40'N and 28°19.20'E187 m a.s.l.). Most of the colonies are located in pastures covered with sparse brush vegetation, on rocky sites in Nistru river valley and along its tributaries Raut, Cula and Cogylnic. The species' abundance is continuously decreasing in the last decades, in spite of its protection.

During a long-term study period of about 25 years there were recorded different phases of population size among years. Individuals form stationary aggregations at any stage of such fluctuations, on areas ranging from 350-400 m^2 to 1500-2000 m^2, depending on population density and size (from 4 to 18-20 individuals). Also, at any stage of a fluctuation can be determined the centre (core) of the colony with a higher population density, and the periphery with a more scattered distribution of the individuals. The most dense populations from Moldova (Sănătăuca village) have cores with a density of 25-30 individuals/ha, in other colonies the overall density varies from 2-4 to 10-12 individuals/ha. Colonies with higher densities usually occupy larger surfaces (Sănătăuca – about 70-90 ha) with less pronounced fluctuations.

Long-term, countrywide biodiversity monitoring of European ground squirrels in Hungary

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The Hungarian Biodiversity Monitoring System (HBMS) was funded in 1998. One of the first programmes of this system was the national monitoring of the European Ground Squirrel (HBMS EGS). This countrywide survey has been carried out since 2000 with the assistance of volunteers, every year at the week of Earth Day (22 April), in 63 permanent sample plots.

Based on earlier experience, the Department of Ethology of the Eötvös Loránd University, Budapest, developed a simple method to estimate population size. Having established the average number of burrow entrances per individual, population size can since be assessed by simply counting used ground-squirrel holes. This method is suitable for a rapid, standardised, and synchronous estimation of the relative number of individuals even in the case of low-density ground squirrel populations at independent locations, without employing special expertise.

The success of the project is also shown by the wide range of participants: school groups led by teachers, nature conservation activists and experts from all national park directorates also contributed reports.

The results clearly illustrate the long-term dynamics of the Hungarian populations and spotlights that European ground squirrels have disappeared from some localities since the year 2000. An additional conclusion from the surveys is that grassy airports can strongly contribute to the long-term survival of the species in Hungary.

The results of the HBMS EGS, which operates on permanent sample plots, are completed with data reported exclusively by volunteers and compiled by the programme WildWatcher. In this way, presence data from 16 easily identifiable plant and animal species including the European ground squirrel have been collected since 2009. Several new occurrences of European ground squirrels have been localized by the help of people reporting observations in the home page of the programme (www.vadonleso.hu).

Eleven years of European ground squirrel monitoring in the Czech Republic

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The European ground squirrel (*Spermophilus citellus*) is one of the most endangered mammal species in the Czech Republic. As such, its populations have been regularly monitored since 2004. The monitoring consists of visual counting of active individuals in each colony, mapping of inhabited area and estimation of total number of individuals. To reduce subjective errors, monitoring is performed by a relatively stable team over time and across populations annually in July. In 2004, monitoring started on 30 populations, and 33 were monitored in 2014. Contrary to this seemingly small variation, 9 localities vanished and 12 others were discovered as new since 2004.
During the years, the total estimated number of ground squirrels has increased to currently ca. 4,000 individuals, but this trend is slightly biased due to discoveries of new populations. Sizes of observed populations were highly variable from year to year; however, there was no obvious synchronicity in population dynamics among them. The largest populations count hundreds of individuals, but the majority contain only several tens. Monitoring revealed some particular results: declines in abundance can be caused by unusual weather events, inappropriate management of vegetation cover or high predation pressure. In addition, some cases of dispersal, and establishment of both natural and artificial new populations have been documented.
To conclude, the status of the European ground squirrel in the Czech Republic is similar or slightly better than in 2004, since management of ground-squirrel habitats has much improved since 2008, when the Action Plan for the European ground squirrel was adopted. Nevertheless, long-term existence of isolated populations, irrespective of their size, seems to be unsustainable without some artificial "metapopulation" manipulations. On the contrary, increasing abundance of ground squirrels in some south Moravian vineyards inspire optimism.

Session 4

Conservation: Implications and Side-effects

Conservation of the European ground squirrel (Mammalia: Rodentia) in Slovakia: Results of the current reintroduction program

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Conservation of the European ground squirrel (*Spermophilus citellus*) was supported by the LIFE - Nature project "Conservation of *Falco cherrug* in NE Bulgaria, Hungary, Romania and Slovakia" in the period 2010 – 2014. The repatriation program was carried out at two sites in Slovakia: Piesočná (Moravský Svätý Ján) and Pod Okrúhlou skalou (Tisovec). The source sites situated in the middle and east of Slovakia were 6 pastures in or near Gemerské Dechtáre, Jánovce, Jesenské, Muráň (Biele vody), Turňa nad Bodvou (Skalistý potok) and Zádiel (Zemné hradisko), 2 airports (Košice, Spišská Nová Ves), and a hayfield at Spišská Nová Ves (Piesočná). Additional individuals originated from source localities in west Slovakia: 2 pastures at Chtelnica and Kuchyňa, 3 airports (Bratislava, Trnava, Nové Zámky), and the Zoo of Bojnice. Altogether 174 individuals were released at Pod okrúhlou skalou and 284 individuals at Piesočná target site. Monitoring of both repatriated colonies focused on the area of distribution and natality. At both target sites successful hibernation and reproduction has been confirmed. The repatriation date was strongly influenced by weather conditions, especially during the rainy season. Therefore, the target sites required more care than initially planned. Several times per season, it was necessary to cut the grass in order to ensure appropriate conditions for survival of individuals and it was a concern to protect them from predators. Housecats hunting European ground squirrels can be considered as the most serious threat at Piesočná site. Squirrels at Pod Okrúhlou skalou were overly attacked by foxes. Intensive care (appropriate management of sites, additional feeding) ensured stability of colonies, their survival and increase. We think that there is a potential chance to improve individual body condition by additional feeding (proved by supplementing seeds of barleycorn and sunflower) to reduce the percentage of ground squirrel's mortality during hibernation.

Semi-natural breeding of European ground squirrels within its Action Plan in Prague Zoo – development over years and gained experience

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Breeding of European ground squirrels in Prague Zoo was established in 2006 as part of the Action Plan (AP) for this species in the Czech Republic (CR). Individuals are bred with the main purpose of later reintroductions to selected suitable sites, as planned in the AP. This contribution tries to summarize the development of the breeding program since its beginning (using data from regular control trappings) and gained experience, which should promote the success of further breeding programs in the CR currently in preparation.

The program started in 2006 with 4 individuals (3 ♂♂, 1 ♀) in an enclosure 14.5 x 9.5 m and 2 m high, with sides laid vertically into the ground. After some adjustments to the enclosure and to the feeding regime, 25 animals (12 ♂♂, 13 ♀♀) were added in 2007, but no distinct increase of present individuals was observed during the following years. Therefore, 44 more ground squirrels (16 ♂♂, 28 ♀♀) were placed in the enclosure in 2011. This addition – though not immediately – probably stimulated reproduction, as shown by large proportions of trapped juveniles in 2012 (46% of 35 captures) and 2013 (60% of 58 captures). In 2014, however, 20 of the 39 captures were yearlings that did not yet reproduce, and only 8 juveniles were trapped. The total number of animals in the enclosure was estimated to be 50-60 each year since 2011, with quite stable and balanced sex ratios. Control trapping data also provided information on the condition of bred animals (body mass, ecto- and endoparasites, physical flaws), which was then compared to data from natural localities.

Due to the preference for semi-natural breeding by the AP-team, the enclosure was finally opened for the ground squirrels in 2013 in order to colonize its surroundings, which constitute suitable habitat, managed by regular pasture. This step showed successful and animals now inhabit both the enclosure and the nearby area.

Reintroducing European ground squirrels: Stress coping in a soft release enclosure

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The European ground squirrel is severely threatened, mainly due to habitat loss and degradation. To counteract the species decline, reintroduction programs are often being considered. The soft release method allows the animals to habituate to the new habitat while being protected from predators and provided with food in a field enclosure, before they can voluntarily explore the surrounding area and settle within an existing colony. However, it cannot be excluded that translocation and captivity could produce a high stress load for the ground squirrels and by that, negatively affect their physical condition. We therefore implemented a pilot study in Vienna, monitoring the translocation of free-ranging female European ground squirrels into a soft release enclosure. Adrenal activity reflected in faecal cortisol metabolite (FCM) concentrations and body mass changes were investigated to gain information on stress levels and physical condition. The females were captured and immediately transferred to an enclosure built within the natural habitat of the species. FCM-concentrations and body mass were measured in the week before (phase 1) and after (phase 2) the transfer as well as in the subsequent 4 weeks (phase 3). Results showed that FCM levels significantly peaked shortly after the translocation and decreased to baseline levels thereafter. Body mass was not affected throughout the study. The results indicate that although transport and release into the enclosure led to increased adrenal activity, the females coped with the new situation within one week. Interestingly, phase-3-FCM-levels of translocated females in the enclosure were significantly lower than those of females in the source population during the same time of year. We conclude that the period in the enclosure allowed the animals to recover from the stressful translocation in a save environment. Based on these results, the soft release method appears to be suitable for reintroduction programs. Future research should focus on monitoring ground squirrels after they have left the enclosure.

Minimum viable population size of the European ground squirrel (*Spermophilus citellus*)

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Once reduced in size and geographical range, species face a considerably increased risk of extinction. Population size is one of the major determinants of extinction risk, and one of the main questions in conservation biology is, how large populations need to be to ensure persistence. We used population viability analysis to estimate minimum viable population size (MVP) for the European ground squirrel. Sensitivity of the estimated MVP to demographic and environmental variability over time was assessed by a set of simulations with different parameters. As inbreeding can play an important role in survival of small populations, we assessed the influence of this parameter in an additional set of scenarios. Basic scenario indicated that a population size of 300 individuals is sufficient to maintain population viability over 50 years. However, if we take into account different levels of demographic and environmental stochasticity, MVP should be considerably larger. We found that the simulated population is most sensitive to variations in litter size and juvenile survival rates. Our study also indicates the importance of the inclusion of genetic considerations when inferring MVP, given that a much larger population size is needed to maintain sufficient genetic diversity in the population than the one required to keep the population merely extant. To our knowledge, this study represents the first population viability analysis of the European ground squirrel.

The case of the 'Brünnerstrassler Ziesel': Incidence of a lightish phenotype in an Austrian population of *Spermophilus citellus*

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During hair growth, specialized cells in the hair bulb and the skin produce pigments that are deposited in the hair cortex and medulla and result in a variety of colours, ranging from yellows and paler reds to brown or black. When pigmentation is reduced due to defects in cell differentiation and/or migration from the neural crest during development, the outcome is leucism: a phenotype due to either patches or the entire body surface lacking cells capable of producing pigment. Whereas leucism - mainly albinism - is fairly good documented in a variety of tree and ground squirrels, this is the first reported case of a lightish phenotype in the European ground squirrel: members of this species were - apart from the occasional pale blotch on juvenile noses - known to consistently have an agouti coat, with paler areas on muzzle, cheeks, throat, chest and inner side of fore legs.
From April 2012 to June 2014, we performed a capture-mark-recapture study in frame of a conservation management plan for a sub-population of S. citellus inhabiting 7ha land zoned for urbanisation in Vienna. A by-product of data acquisition on demography, life history and ranging behaviour was the detection of a pale morph, manifested by the capture of an adult whitish male initially considered as senescent. A further capture some weeks later - a female with white tail and belly, and interspersed white blotches on head, dorsum and tail - suggested evidence for a pale phenotype in this sub-population. This condition was corroborated by altogether 4 ♂♂ and 3 ♀♀ (12.7% of all captures) that exhibited partial leucism, ranging from a single pale spot to a predominantly pale aspect including a non-pigmented sclera. Additional 1-11 individuals were observed and/or photographed by visitors.
We assume that this feature has prevailed because of the isolated character of the population studied. Further monitoring, completed by genetic analyzes and comparisons with museum specimens are required to reveal its underlying mechanisms.

Round Table

Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora

Dealing with Article 16 of the Habitats Directive by the example of *Spermophilus citellus*

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Introduction to the specifications of Article 16, acknowledging the Guidance document on the strict protection of animal species of Community interest. The contribution examines its implementation in the federal laws of Vienna, Lower Austria and Burgenland, highlighting current examples and approaches to solutions from these three Austrian provinces.

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