Research Article

The role of landscape history in determining allelic richness of European ground squirrels (*Spermophilus citellus*) in Central Europe

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Abstract

Genetic diversity is of paramount importance for individual fitness and evolutionary potential of populations. For conservation planning it is crucial to know how genetically diverse a species is and what factors may explain variation of genetic diversity among populations. Our aim was to evaluate the effects of landscape history, ecological isolation, and local population size on allelic richness of local populations in European ground squirrels (*Spermophilus citellus*).

We genotyped 144 individuals from nine local populations collected in two neighbouring regions with decades of different landscape history. We assessed allelic richness, ecological isolation and local population size by eleven polymorphic microsatellites, the isolation index of Rodríguez and Delibes, and standardised counts of burrows openings, respectively.

Statistical models indicated a strong effect of landscape history on allelic richness of local populations. Ecological isolation of local populations apparently played only a marginal role, and local population size was an unimportant factor.

Our modelling results highlight the dominant role of landscape history for the genetic diversity of *S. citellus*. The strong landscape history effect encountered presently includes a different region-specific socio-economic development due to distinct agricultural systems in the two regions, especially after World War II. Levels of ecological isolation of local populations have diverged in an extent too small to explain variation of local allelic richness. The lack of a significant effect of local population size suggests that census sizes of the populations studied are all not critically low. Moreover, census and estimated effective population sizes were not closely related. Establishing corridors or translocating *S. citellus* in the species' historical range should be encouraged to promote gene flow between local populations and counteract the loss of genetic diversity by drift, provided that no conflicting factors (ecological, epidemiological, etc.) exist.

Introduction

Allelic richness as a surrogate for genetic diversity is considered of paramount importance to maintain evolutionary potential or individual fitness (Frankham, 1996; Paetkau and Strobeck, 1998). The increase of genetic diversity is one of the key issues in reintroduction and re-enforcement programs in small, isolated populations (IUCN, 1998). Therefore it is important to know how genetically diverse a species is, and what factors may explain the variation or the loss of genetic variation within and among local populations. In addition to population census (Nc) and effective size (Ne) various ecological, behavioural and historical factors shape the genetic diversity of populations via genetic drift, gene flow and natural selection. Such factors may be ecological and genetic isolation, mating system and social structure, meta-population and spatial structure, and population history (Amos and Balmford, 2001; Booy et al., 2000; Amos and Harwood, 1998). Nevertheless, region specific landscape history and land use or biogeographical differences (Cousins et al., 2014; Taylor and Hoffman, 2014; Ibáñez et al., 2013; Cobben et al., 2012; Antolin et al., 2006; ETC/BD,

2006; Holderegger et al., 2006; Johansson et al., 2005) may also affect genetic diversity of populations.

According to neutral molecular theory (Kimura, 1984), genetic diversity is positively correlated with Nc of natural populations (Allentoft et al., 2009; Frankham, 1996): if ecological attributes of populations are more or less identical, then smaller populations carry less genetic diversity than larger ones (Henle et al., 2004; Amos and Harwood, 1998; Frankham, 1996; Young et al., 1996). Nevertheless, Amos and Harwood (1998) set out several alternative explanations (e.g., recent bottleneck, inbreeding, meta-population structure) when the positive correlation of Nc and genetic diversity is weakened.

Isolated natural populations with low probability of immigration are more prone to the disadvantageous consequences of genetic drift (Wang and Bradburd, 2014; Ricketts, 2001; Amos and Harwood, 1998; Young et al., 1996) or to the negative effect of inbreeding, because isolation could potentially disrupt gene flow among colonies and reduce Ne. Certain life-history traits, such as limited dispersal ability, special habitat requirements, and high degree of sociality, further increase the likelihood of becoming ecologically and also genetically isolated (Bradburd et al., 2013; Henle et al., 2004). However, the relationship between the magnitude of isolation and genetic diversity either within or among populations is complicated (Saunders et al., 1991).

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European ground squirrels (hereafter as EGS) (*Spermophilus citellus*, Linnaeus 1766) serve as a good example for investigating the effect of landscape history, local population size, and ecological isolation on genetic variation. They are obligatory hibernators and produce one litter annually. Young adults show male-biased dispersal, and their polygynous mating system (Millesi et al., 1998) involves pronounced intra-sexual competition at high densities (Millesi et al., 2004; Hoffmann et al., 2003a,b). Local EGS populations occur mainly in isolated habitat fragments in the anthropogenic landscape (e.g., Spitzenberger and Bauer, 2001; Mitchell-Jones et al., 1999; Ružič, 1978), which is characteristic for relic populations (Begon et al., 1990). Such residues likely constituted meta-populations (*sensu* Hanski and Gilpin, 1997), which formerly occupied continuous areas in the Pannonian biogeographical or ecoregion (Horváth et al., 2012), north and south of the River Danube in Central, Eastern, and South-eastern Europe (Hoffmann et al., 2003a).The nowadays fragmented local populations may consist of as few as three reproductive individuals in Austria (Hoffmann, 2005) or less than 50 individuals in more than 50% of all Czech colonies (Matějů et al., 2008), but can occasionally reach large population sizes in Central Europe at the margin of the species' distribution range (F. Suchentrunk unpublished). EGS occur on dry, short-grass steppes which are often maintained by human activities, such as grazing by livestock, or by mowing on grassy airports, golf courses or vineyards (Janak et al., 2013; Enzinger, 2006). They are possibly able to pass large rivers, such as Tisza, on bridges (Ćosić et al., 2013). The species is endangered throughout its range (e.g., Coroiu et al., 2008), with causes of population declines and extinction risks differing locally (Hoffmann et al., 2003b). Recent studies suggested more depleted genetic diversity and a higher level of fragmentation in Czech populations compared to Hungary and Slovakia, and continuous disintegration of populations, as well as higher genetic drift than migration (Ben Slimen et al., 2012; Říčanová et al., 2011). These studies provided the base for further investigations of differences in genetic diversity among local populations in two neighbouring regions with different landscape history within the Pannonian biogeographical region (Horváth et al., 2012; ETC/BD, 2006; Kilian et al., 1994) and of possible key factors that may explain its variation.

Our objective was to investigate whether diverging landscape history after World War II may have shaped genetic diversity of nine local ground squirrel populations. Different political systems resulted in profound socio-economic, cultural and life-style differences between communist (1949–1989) Hungary and increasingly capitalist Austria (Cousins et al., 2014; Reif et al., 2011; Gaddis, 2006). In our interpretation of landscape history we tightly follow the approach and understanding of Marcucci (2000). He argues that a particular and current landscape is formed by both natural and human influences and their interactions. This results in strong spatial and temporal dimensions with keystone processes, such as region-specific politically driven economic activity. He pointed out that flow of energy, material and organisms, including people, into and out of a landscape had profound effect on its evolutionary path. The local populations in this study dwell in the north-western portion of the species' distribution range (see e.g., IUCN range map; Coroiu et al., 2008). The two studied regions were separated from each other for at least 40 years by the so called "Iron Curtain" until 1989. The separation resulted in distinct differences in landscape history includinglong-term land management practices. EGS inhabit both regions, and local populations may have earlier been variably connected to form a large meta-population (Spitzenberger and Bauer, 2001; Hoffmann et al., 2003a).

Therefore "landscape history", "ecological isolation", and "census population size" were investigated for their possible effects on overall population-specific allelic richness (hereafter as *Rs*), which is considered to decline faster than heterozygosity (Sielezniew and Rutkowski, 2012). We used *Rs* as surrogate of genetic diversity, because it is more sensitive to ecological isolation and fragmentation than expected heterozygosity (*He*), and because rare alleles do not contribute much to *He* but to *Rs* (Cornuet and Luikart, 1996; Nei, 1978). We estimated population-specific *Rs* in the two neighbouring regions. The investigated populations differed in degree of ecological (habitat) isolation and census population sizes. The local populations of central Hungary have been studied genetically already (Ben Slimen et al., 2012).

We tested the following hypotheses: 1) Ecological (habitat) isolation of local populations has led to an erosion of genetic diversity (*Rs*); on the basis of historical data (Spitzenberger and Bauer, 2001), we presumed that all these local populations have earlier been part of a large meta-population. 2) We expected census size of local populations to be an important parameter in explaining *Rs*. In accordance with the expectations of neutral genetic theory, larger local populations should have higher *Rs* at neutral loci than smaller ones. 3) Locally smaller agricultural field size along with less rigorous landscape alterations in eastern Austria compared to central Hungary may have affected natural gene exchange between local populations in Austria less than in Hungary; thus, eastern Austrian populations may still harbor higher *Rs* than Hungarian ones.

Materials and Methods

Sampling and Study sites

A total of 144 EGS was trapped in 2006 by snaring or Tomahawk live traps. A detailed description of the capture technique appears in Hoffmann et al. (2003b) and Gedeon et al. (2011). We sampled four local populations from central Hungary and five from eastern Austria (Fig.1). Despite their different region-specific landscape histories, all local populations dwell in the Pannonian biogeographical region within the central European range of EGS. The grasslands in this region are the "cultural product of long-lasting traditional land use" (Ibáñez et al., 2013). Having created and shaped primary and secondary habitats of EGS, this historic process likely has been of keystone importance for this landscape and the species.

We also described certain soil characteristics related to burrowing activity, as those might have influenced local population density and size. We estimated mean bulk density (kg/m^3), clay and sand content (%) at the depths of 0, 5, 15, 30, 60 and 100 cm for a 1 km square grid around the centre of each local population (Hengl et al., 2014) in both regions. Since these measures had different measurement dimensions, we log$_{10}$-transformed the data first. Then we applied a principal component analysis (PCA) on the 18 variables. Finally the component scores were compared by a MANOVA to test for significant differences in these soil properties between the regions.

The mean (median and mode) of the number of samples per local population was 16, while values of the local population-specific expected heterozygosity (*He*) ranged between 0.4 and 0.6. This range repres-
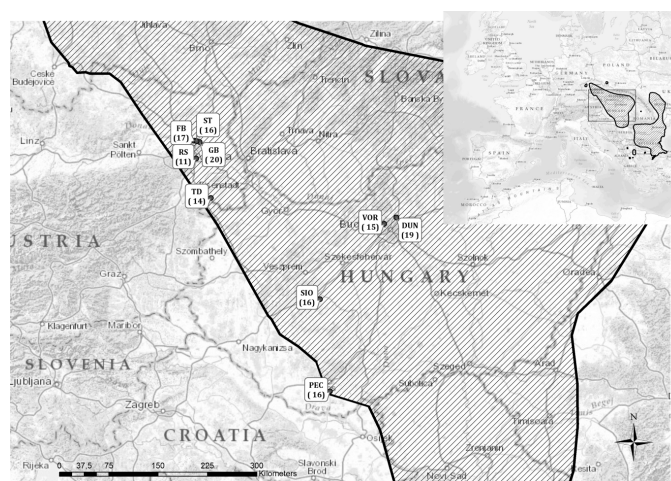
Figure 1 – Topographic illustration of *Spermophilus citellus* Central European (northwestern) distribution range (larger image). Number of sampled individuals is written in parenthesis after each acronym that represents the study sites. For Eastern Austria, TD is Trausdorf, RS is Radiostation, GB is Gerasdorf Badeteich, ST is Stammersdorf, and FB is Falkenbergwiese. For Central Hungary, PEC is Pécs, SIÓ is Siófok, VOR is Vöröskő, and DUN is Dunakeszi. The smaller image in the top right corner illustrates the total distribution area of the species.

ents *He* of loci with moderate polymorphism. As a result we assumed that He could yet be estimated moderately well with lower sample sizes (Hale et al., 2012).

Genetic samples and microsatellite genotyping for allelic richness estimates

Ear tissue and hair-bulb samples were collected from each individual for DNA extraction during live capturing and samples were stored frozen at −18 °C or in 96% ethanol for later DNA extraction. The traps were distributed evenly throughout the area to avoid non-random sampling of related individuals. Detailed information on the localities and numbers of analyzed individuals per locality is given in Fig. 1. Live trapping and sampling was approved by the Austrian Advisory Committee for Animal Experiments (BMBWK-66.006/0012-BrGT/2006) and by the local authorities for nature conservation. Field procedures were carried out according to the Ethical Committee for Animal Experiments at Eötvös University, and followed the rules detailed in ASM guidelines (Gannon and Sikes, 2007).

Total genomic DNA was extracted from tissue and hair-bulb samples using the GenElute™ Mammalian Genomic DNA Miniprep kit (Sigma) and individual genotypes at eleven microsatellite loci (Ssu1, Ssu5, Ssu13, Ssu15, Ssu16, IGS-1, IGS-110b, IGS-BP1, MS53, MS56, SX) were determined following Ben Slimen et al. (2012).

GENEPOP 3.4 (Raymond and Rousset, 1995) was used to test for genotypic linkage disequilibrium (LD) separately for each pair of loci in each local population (Markov chain method, default parameter settings). Allele frequencies, observed and expected heterozygosity for each locus and local population, as well as pairwise Cavalli-Sforza & Edwards (CSE) distances between local populations and associated permutation tests for statistical significance were calculated using GENETIX 4.05.2 (Belkhir, 2004). GENETIX was also used to test genotype frequencies for deviations from Hardy-Weinberg (HW) expectations separately for each local population by calculating significance of *Fi*s values through permutations (10000) of alleles. It was further used to run a Mantel test of correlation of pairwise *Fst* values (transformation: $\frac{Fst}{(1-Fst)}$) and linear geographic distances between local populations, i.e., to check for genetic isolation by geographic distance.

FSTAT 2.9.3.2. (Goudet, 2001, 1995) was used to calculate *Rs* from allele frequencies by rarefaction (based on 6 diploid individuals) to account for varying local population sample sizes. Local population-specific *Rs* values were obtained as arithmetic means of locus-specific *Rs* values. FSTAT was also used to test for a significantly higher level of relative genetic differentiation (pairwise *Fst* values between local populations) in the eastern Austrian than in the central Hungarian region, as suggested by the on average longer geographic distances between local populations and as suggested by the tendency for genetic isolation by geographic distance in the whole data set (see Results). Specifically, a one-sided permutation (1500) test was applied for pairwise *Fst* values (eastern Austrian region with five populations vs. central Hungarian region with four populations).

ARLEQUIN vers. 3.11 © L. Excoffier 1998–2007 (Excoffier et al., 2006) was used to run a hierarchical Analysis of Molecular Variance (AMOVA) with two levels (local populations within biogeographical regions) to estimate the genetic variance components due to partitioning into the two regions and into the local populations within the regions. To further explore and confirm the AMOVA results, STRUCTURE 2.3.4 (Pritchard and Wen, 2004; Falush et al., 2003; Pritchard et al., 2000) was used to calculate likelihood ($ln(P(D))$) values when assuming *K* genetic clusters (HW populations) for Bayesian genetic admixture models without population priors, with allele frequencies correlated among populations, 500000 MCMC repetitions after burn-in of 150000 repetitions, and 15 runs per *K* (*K*=1–10). Mean and standard deviation of estimated Ln probabilities per *K* (*L(K)*), and Evanno et al. (2005) ΔK ($\frac{mean(|L(K)|)}{SD(L(K))}$) were calculated by using the on-line STRUCTURE HARVESTER (Earl and von Holdt, 2012) to infer the most likely number of *K* genetic clusters inherent to the whole data set.

For assessing spatial population clustering and for determining the most likely number of clusters (*K*), two spatial Bayesian methods im-

plemented in BAPS (Corander et al., 2009) and GENELAND (Guillot et al., 2005) were used. In BAPS, spatial clustering of individuals was used with five independent runs, *K* ranging from 1 to 15, with each *K* value replicated ten times. After obtaining the most likely number of clusters (*K*), additional runs with fixed obtained *K* value were performed, using the same settings. GENELAND was conducted using both, uncorrelated and correlated allele frequency models, with 1000000 MCMC iterations in five independent runs, with *K* ranging from 1 to 15 and saving every thousandth iteration (10% burn-in value). Additional runs were performed with fixed *K* according to the determined most likely number of clusters. Based on the spatial clustering results, STRUCTURE models and F-statistics were applied to the three identified clusters.

Estimating census local population size (*Nc*)

We estimated census size of local populations by multiplying local population density data with the estimated size of the area (ha) that was inhabited by individuals of the respective local populations. Firstly, local population density was estimated by counting the number of active EGS burrow openings along a line transect of 1000 m (5 times 200 m) long and 2 m wide shortly after hibernation in 2006, when sampling occurred. Then this number of burrow openings (per 0.2 ha) was extrapolated to one ha, which gave the burrow density of a local population. Finally, burrow density was divided by six because in this period of the year the number of used burrow openings per individual is approximately six (Hut and Scharff, 1998; Katona, 1997). Counting of burrow openings is a reliable method of estimating and comparing differences in *Nc* or density of local populations (Koshev and Kocheva, 2008; Váczi, 2005; Váczi and Altbäcker, 2005a; Katona et al., 2002; Váczi and Altbäcker, 1999). Monitoring the *Nc* and density of local EGS populations has been successfully carried out since 2000 in Hungary according to the protocol of the Hungarian Biodiversity Monitoring System (Váczi and Altbäcker, 2005b). Although this scheme is not used in the national monitoring system of Austria, it can be applied to any EGS population for census size estimates and comparisons. Therefore the EGS monitoring protocol was extended to the currently studied local populations in Austria. We estimated each local population area by determining the local population perimeter with GPS receivers in the field and then by calculating surface area within the perimeter.

We used *Nc* instead of effective population size (*Ne*) to estimate local population sizes and for our statistical model because of two main reasons. First, data on the ratio of effective and census population sizes of hardly social ground squirrels indicate that *Nc* is about twice as large as *Ne* (McEachern et al., 2011; Antolin et al., 2001) and second, our *Ne* estimates showed wide confidence intervals (including infinity); consequently, its estimation would have been meaningless. The current local populations have been monitored and studied for several years, and we could infer reliably that their census size exceeded 150 individuals. This suggested that our sample sizes per local population must have been smaller than their *Ne* estimated from the ratio of *Ne*/*Nc*. England and co-authors (2006) and Weckworth et al. (2013) argue that *Ne* can be estimated confidentially only when sample sizes are larger than the *Ne* estimate. Therefore we decided in favour of data of census population size.

Calculating ecological isolation

Ecological isolation was quantified by the isolation index (hereafter as ISOL INDEX) according to Rodriguez and Delibes (Rodríguez and Delibes, 2003), with

$$\text{ISOL INDEX} = \sum_{i=1}^{k} \frac{w_i \times e^{D_i}}{S_t} \qquad (1)$$

where *k*=number of neighbouring local populations, *D*=distance to the nearest neighbouring local population, and S_t is the sum of all the neighbouring local populations' sizes. This formula assumes that (1) all neighbouring local populations contribute immigrants, (2) the contribution of each neighbouring local population decreases geometrically with distance (estimated by the exponential function e^{D_i}), and in-

Table 1 – General population characteristics of local populations. Size: census local population size; *Rs*: local population-specific allelic crichness; ISOL INDEX: ecological isolation index; *He*: expected heterozygosity corrrected for small sample sizes; *Ho*: observed heterozygosity; *A*: mean number of alleles per locus.

Colony ID	Country	Size	ISOL INDEX	Rs	He	Ho	A
TD*	Eastern Austria	496	0.00029	3.300	0.49540	0.32880	3.6364
RS*	Eastern Austria	624	0.02822	3.449	0.48460	0.36570	3.5455
GB	Eastern Austria	289	0.00160	3.175	0.51300	0.44290	3.6364
ST*	Eastern Austria	6705	0.00056	3.449	0.57610	0.44320	3.8182
FB	Eastern Austria	179	0.00064	3.260	0.50720	0.44620	3.6364
DUN*	Central Hungary	3770	0.04000	2.998	0.44520	0.38120	3.7273
VOR	Central Hungary	2833	0.06250	2.925	0.43990	0.39910	3.2727
SIO	Central Hungary	17125	1.00000	2.558	0.43710	0.34820	2.7273
PEC	Central Hungary	6000	0.11110	2.792	0.46830	0.41330	3.0000

* Significant deviation from HW ewuilibrium at *p*<0.05 after strict Bonferroni corrections.

creases linearly with neighbouring local population's size, (3) immigrant contribution is additive, and (4) the effect of distance is weighted (*w*) by neighbouring local population's size, where $w_i = \frac{\text{local population}_i}{S_t}$. This way of modelling ecological isolation is conceptually similar to that used by Hanski (Hanski, 1997). For the ISOL INDEX a neighbouring or sampled local population was determined as a continuum of occupied adjacent grid cells connected either by sides or corners. We defined local populations as inhabited areas by EGS with clear and recognizable margins. The inhabited area and interconnections of the nine local populations were defined by the freely available 1 km UTM reference grid for Europe (Van Liedekerke and Panagos, 2009) combined with Google Earth® satellite map, and our a priori field knowledge about the sampled local populations and their environs. Based on data indicating short (<1 km) dispersal distances of ground squirrels (Turrini et al., 2008; Neuhaus, 2006; Martin and Heske, 2005; Hoffmann et al., 2004; Sutherland et al., 2000), an unoccupied, adjacent 1 km grid cell was considered enough to separate a local population from a neighbouring one. As a result local populations farther than 1 km (1 km grid cell) and separated from a sampled local population did not contribute immigrants and did not modify the ISOL INDEX. The higher the ISOL INDEX, the more isolated a local population is.

Determining region-specific landscape histories

According to Kilian et al. (1994); Horváth et al. (2012) and others (Fekete et al., 2014; Lukács et al., 2013), the two investigated regions are part of the Pannonian biogeographical region (but see ETC/BD, 2006). The landscape of eastern Austria and Hungary has been managed differently during at least the past century (Cousins et al., 2014). Especially after World War II, traditional agricultural practices were abandoned and agriculture intensified in both countries (Austria: "consolidation of farming", Hungary: "program of modernization"), but diverging in timing, method and extent of implementation: In Hungary, traditional agricultural practices and structures were rapidly replaced by a comprehensive industrialisation of agriculture (Kovács, 2013). Such changes, however, have not altered all open landscapes of eastern Austria, and the traditional small-scale and sustainable farming has persisted locally. The region-specific agricultural and land management practices over more than 40 years have affected biotic and abiotic characteristics of the soils along the border significantly and differently (Rampazzo et al., 1999a,b), whereas climate and biographic characteristics remained similar.

The effect of such differences is detectable in both species richness and diversity (Cousins et al., 2014). Therefore we hypothesised that different landscape histories have significantly influenced genetic processes. Specifically, such different regional landscape changes over decades could have affected gene flow between and drift within local EGS populations through the quality and connectivity of habitats as well as life-history traits (such as social structure, density, birth rates, and mortality). In our understanding and interpretation, region and different landscape histories combine not hierarchically structured conditions and parameters not yet explored in detail that may have shaped ecological and genetic characteristics of local EGS populations.

Statistical analysis (B)

We applied a generalized linear model (GLM) approach (McCullagh and Nelder, 1989) to estimate the amount of variation in local population-specific *Rs* by two ratio (ecological isolation, *Nc*) and one nominal variable (landscape history or region). Our global model included "landscape history" (hereafter as LAND HIST), "ecological isolation" (ECOL ISOL) and "census local population size" (CENSUS SIZE) and their interactions as predictors (lm(formula = Rs~(ECOL ISOL + CENSUS SIZE + LAND HIST)^2)) and we based our model ranking on Akaike's Information Criterion for small sample sizes (AICc) (Burnham and Anderson, 2002; Akaike, 1973). Following Burnham and Anderson (2002), we also calculated the Akaike weights (w_i), the relative variable importance (*RVI*), the percentage of variation in *Rs* accounted for by each model (adjusted R^2) and ΔAICc or simply Δ*i* values. Smaller values of AICc and larger values of R^2 indicate a better-fitting model; therefore, the best-fitting model has the smallest AICc value. All model calculations were performed with the R program (R Core Team, 2005). Most importantly, however, *RVI* values greater than 70–80% would identify factors significantly affecting the dependent variable (Burnham and Anderson, 2002).
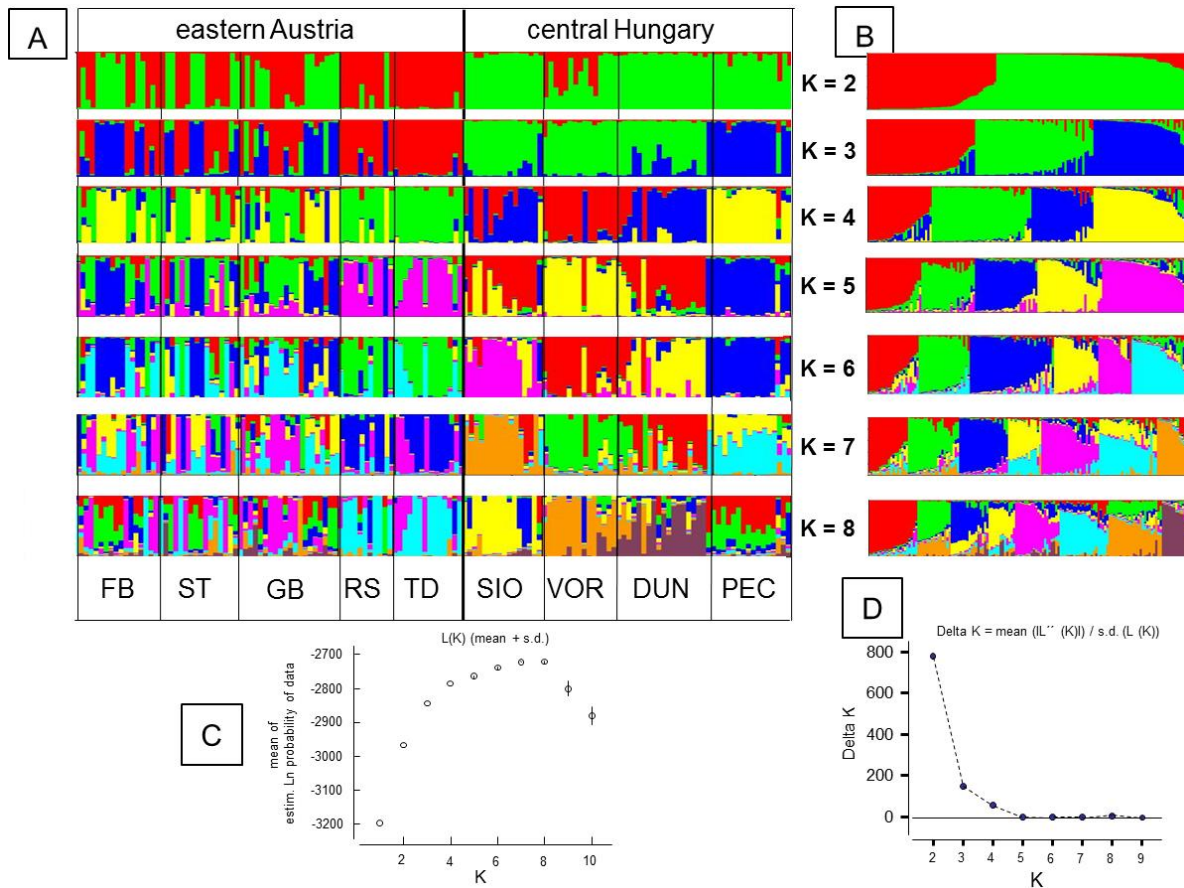
Results

Similarities in soil properties between the two regions

As we had expected we did not find a significant difference between the two regions in soil properties (F=2.35, *p*=0.19), that could otherwise have had a significant effect on burrowing activity and population density. The Principal Component Analysis resulted in three Components accounting for 95% of total variation of the original dataset where we had 18 variables in relation to bulk density, clay and sand content at six depth levels.

Genetic variation and structure of EGS populations

A total of 63 alleles was revealed at the eleven loci studied, resulting in an overall mean number of 5.7 alleles per locus. However, the mean number of alleles per locus (*A*) ranged between 3.0 and 3.82 for single local populations. Local population-specific *A* values are detailed in Tab. 1 along with *Ho*, *He*, and *Rs* values and indications of significant deviations from HW expectations.

No significant LD was found in any local population. Pairwise *Fst* values were significantly (*p*=0.049; one-tailed permutation test, FSTAT) higher in central Hungary (*Fst*=0.156; four local populations) than in eastern Austria (*Fst*=0.049; five local populations), and there was a tendency for genetic isolation by geographic distance over all nine local populations (Pearson r=0.393, *p*=0.059; Mantel test, GENETIX). The hierarchical AMOVA indicated that 4.49% (*p*=0.032±0.0018) of the relative genetic variation was partitioned between the two regions, whereas 9.45% (*p*<0.0001) was partitioned among the local populations within regions, 12.4% (*p*<0.0001) among individuals within local populations, and 73.66% (*p*<0.0001) were due to variation within individuals. Pairwise CSE distances ranged between 0.045 and 0.104

Figure 2 – Results of the Bayesian STRUCTURE analysis; A) individual admixture (Q in %) according to the $K=2$–8 assumed genetic clusters inherent to the whole data set; B) ordered Q values for runs of $K=2$–8. C) likelihood ($ln(P(D))$) distributions of K genetic clusters underlying the overall data set for $K=1$–10 (means and s.d. over all 15 runs per K, respectively); D) Evanno's ΔK distribution for $K=2$–9 (for $K=1$ and $K=10$ ΔK could not be calculated due to mathematical reasons).

with a mean of 0.0782 for local populations in Austria and between 0.088 and 0.222 with a mean of 0.129 for local populations in Hungary. Whereas all (n=26) pairwise CSE distances for Hungarian populations were significantly ($p<0.00192$; nominal $\alpha=0.05$) above zero after strict Bonferroni corrections, for local populations in Austria only two out of ten pairwise CSE distances were significantly ($p<0.005$; nominal $\alpha=0.05$) above zero.

The admixture models in STRUCTURE (Fig. 2A, B) suggested five or six most likely genetic clusters ($K=5$ or 6), despite the $L(K)$ distributions for $K=7$, and $K=8$ suggested higher probabilities (Fig. 2C). This conclusion was reached, because ordered individual Q distributions for $K=7$ and $K=8$ runs (Fig. 2B) did constantly indicate relatively low maximal Q values for one or two genetic clusters, respectively. Moreover, models of $K=7$ and $K=8$ did not provide essentially additional information on genetic partitioning and admixture for individuals or local populations (Fig. 2A). Assuming $K=2$ genetic clusters produced no clear distinction between local populations from Austria and Hungary, and $K=3$ fairly distinguished the Hungarian PEC population from all other studied Hungarian populations. At $K=4$ still further genetic differentiation among the Hungarian populations was observed: PEC and VOR were distinct, respectively, whereas DUN and SIO were genetically similar to each other, and no obvious additional genetic differentiation was observed for eastern Austria. Finally, at $K=5$ and $K=6$ some little genetic differentiation was evident within TD and SIO. Moreover, assuming $K=6$ clearly distinguished SIO from DUN (Fig. 2A). Evanno's ΔK suggested $K=2$ clusters for the overall data set (Fig. 2D), which was clearly underestimating the most likely number of STRUCTURE clusters $K=5$ or $K=6$ (see above), and our BAPS and GENELAND results as well as our results of absolute (pairwise CSE distances) and relative (Fst) genetic differentiation (see above).

Overall, the STRUCTURE results, in line with AMOVA, indicated some genetic differentiation between the two regions on the one hand

and somewhat more genetic partitioning into local populations in central Hungary than in eastern Austria on the other hand. Additionally, the STRUCTURE plots suggested slightly lower levels of individual genetic admixture in central Hungarian populations than in eastern Austria.

The spatial clustering in BAPS (Fig. 3) and GENELAND based on uncorrelated allele frequencies suggested $K=3$ clusters inherent to the overall data set. One spatial cluster ("Austrian local population cluster 1 plus Hungarian population PEC") encompassed FB, ST, GB, PEC, a second cluster ("Austrian local population cluster 2") combined RS and TD, and a third cluster ("Hungarian local population cluster without PEC") combined the three Hungarian populations SIO, VOR, and DUN. Even though the assignment probabilities for individuals for each cluster were relatively low (66%) in all five consecutive GENELAND runs, all three pairwise Fst values for those spatial clusters were significantly higher than zero (FB-ST-GB-PEC population vs. RS-TD population: $Fst=0.0702$, 95% c.i.=0.03442–0.1008; FB-ST-GB-PEC population vs. SIO-VOR-DUN population: $Fst=0.081$; 95% c.i.=0.0346–0.1328; RS-TD population vs. SIO-VOR-DUN population: $Fst=0.1847$, 95% c.i.=0.06523–0.3112). Our GENELAND results based on correlated allele frequencies returned $K=6$ spatial clusters, further splitting "Hungarian local population cluster without PEC" into three separate clusters (SIO, VOR and DUN), and also "Austrian local population cluster 1 plus Hungarian population PEC" into two separate clusters: one comprised of FB, ST, GB, and one comprised of PEC. Even though with a low (<40%) individual assignment probability, the GENELAND results based on correlated allele frequency were in favour of the interpretation of our initial STRUCTURE results of $K=5$ or 6 genetic clusters. According to Guillot et al. (2005) models based on correlated allele frequencies are biologically more meaningful and may show subtle differentiation, even though such models might be more prone to algorithm instability.
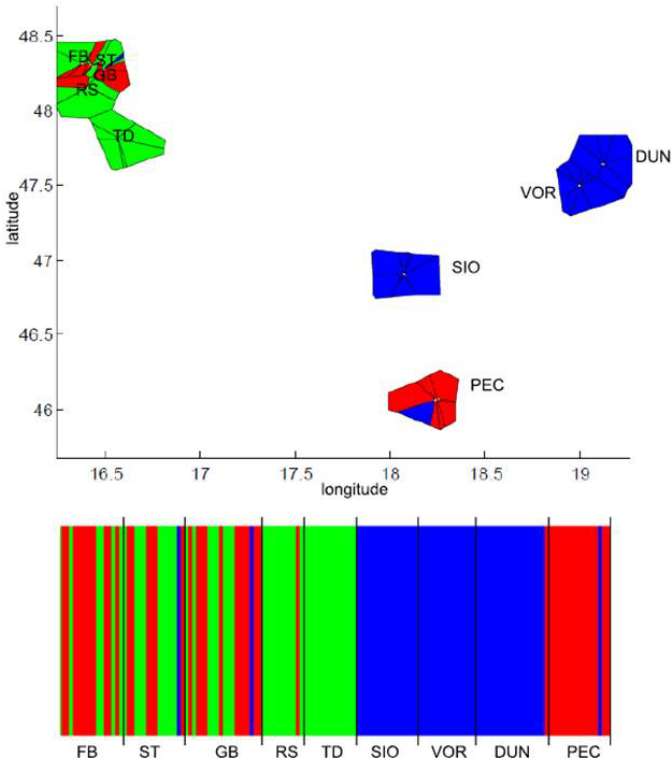
Figure 3 – Graphical representation of the spatial clustering of EGS individuals in the program BAPS returned an optimal partition value of *K*=3, with each cluster indicated in red, blue and green colour. The barplot is showing the memberships of each individual to one of the three clusters detected in BAPS across 9 sampling locations. (X-axis represents longitude, while y-axis represents latitude).

Modelling allelic richness

According to the AICc values the "landscape history-model" [S(LAND HIST)] appeared the best approximating model for our data (AICc=-4.95). This model explained 77% of variation in *Rs* (adjusted R^2=0.77). The second best model, with "landscape history" and "ecological isolation" [S(LAND HIST + ECOL ISOL)] as fixed factors, however, had only 0.8 AICc units more than the best model, and is thus equally plausible, as ΔAICc<2 (see generally Burnham and Anderson, 2002 for model selection). All further models were clearly less plausible. The Akaike weight for the best model is not large (*wi*=0.573), relative to the weight of the second best model (*wi*=0.384). This ratio for the next three models ranges from 32 to 96. Our results indicate that region specific landscape history and possibly also ecological isolation do shape local population-specific allelic richness. However, the RVI value of 0.42 for ECOL ISOL suggests basically no importance of it. In contrast to our expectations, there was no support for the effect of local census population size on *Rs*, which is usually related to the evolutionarily important effective population size. Also, possible interaction factors did not play any role in explaining variation of allelic richness.

RVI estimates of predictor variables across all models are detailed in Tab. 2.

Discussion

The variation of genetic diversity in the EGS populations studied as expressed by allelic richness is explained predominantly by one factor according to our model, namely by the region-specific landscape history resulting from contrasting regional land management histories and uses in eastern Austria and Hungary, particularly after World War II. Ecological isolation apparently did not affect allelic richness; variation of genetic diversity might rather have resulted from a complex interaction of several factors: Male-biased and short-distance dispersal ability, habitat fragmentation, and restricted gene flow (Ćosić et al., 2013) might have concealed effects of ecological isolation. Also, there was no support for the hypothesis of a positive effect of local census size on *Rs*. The lack of this latter relationship may be explained by two different mechanisms: (1) all currently studied local populations are still above a critically low level of population size, or (2) census and effective population sizes are not closely related.

Effect of different landscape histories

After World War II, Austria and Hungary increasingly diverged in terms of politics and socio-economy (Gaddis, 2006). Our results suggest that different landscape histories attributable to distinct landscape management schemes for decades (i.e. agro-environmental practices) may have resulted in higher degrees of gene-flow disruption and loss of genetic diversity due to impaired dispersal, leading to more dismembered meta-population structure and unbalance between local extinctions and recolonisations in central Hungary compared to eastern Austria. As Marcucci (2000) pointed out, landscape history encompasses the flow of different biotic and abiotic factors, such as energy, material and organisms including people from a temporal perspective. We argue that this historical aspect acts differently compared to other mechanisms influencing gene flow and dispersal, such as current environmental or ecological isolation (Bradburd et al., 2013). The importance of temporally diverging landscape histories becomes apparent in significant differences between physical, chemical, microbiological and zoological soil characteristics along the Austrian-Hungarian border (Rampazzo et al., 1999a,b). Several authors found differences in species diversity (Cousins et al., 2014), population trends of birds (Reif et al., 2011) or exotic bird species diversity (Chiron et al., 2010, 2009) between the studied regions. On the other hand, soil properties of local populations in relation to burrowing activity were similar in both regions. However, EGS burrows are critical for their survival (Lagaria and Youlatos, 2006) as they serve life supporting functions, such as the hibernaculum (Nemeth et al., 2009), and soil characteristics can affect habitat use and as a result local population density, size, and distribution within the local landscape (Lohr et al., 2013; Van Vuren and Ordeñana, 2012; Laundré and Reynolds, 1993).

As a result of state-directed agricultural modernisation and industrialisation in Hungary, private property was collectivized, and many sustainably cultivated lands were abandoned in the late 1950s and early 1960s (Kovács, 2013). This collectivization involved more than 1.2

Table 2 – Model selection information for the first five models. Parameter values (Θ) indicate if it is included in the specific model. Adjusted R^2 indicates the percentage of variation accounted for by each model. AICc indicates the Akaike's Information Criterion corrected for small sample size; Δi indicates the AICc difference relative to the smallest AICc value in the set of models; Akaike's weight (*wi*) indicates the probability of fitting of the model from the model candidates or in other words the relative likelihood of the model. Relative variable importance (RVI) indicates the cumulative *wi* over all models that include the explanatory variable *j*.

Model No.	Θ$_{ISOL INDEX}$	Θ$_{CENSUS SIZE}$	Θ$_{LANDS HIST}$	adjusted R^2	AICc	Δ$_i$	w$_i$
1			-0.4046	77	-4.95	0	0.573
2	-0.00268		-0.3251	87	-4.15	0.8	0.384
3	-0.00001	-0.4356		74	1.95	6.9	0.018
4	-0.00521			43	3.16	8.11	0.01
5	-0.00312	0.00003	-0.3834	89	4.28	9.23	0.006

Relative variable imnportance (RVI): landscape history: 0.96; ecological isolation: 0.42; census size: 0.03; ecological isolation × landscape history: 0.00; census colony size × landscape history: 0.00; ecological isolation × census colony size: 0.00.

million peasants and their lands. The agrarian sector employed 52% in 1948, but in 1995 only 8.5% of the total employment, and the number of privately-owned properties decreased from 1600000 in 1949 to 145000 in 1962 (Kovács, 2013). These changes in the agrarian sector, together with other socio-economic factors, lead to the progressive abandonment of rural areas after World War II.

EGS favour short, dry grasslands on various soil types, but with good water retention. These habitat characteristics are usually maintained by mowing or grazing on natural, such as Pannonian grasslands and steppes, or semi-natural grasslands, such as golf courses, grassy airports, playgrounds and recreational areas. The species avoids industrialised farmland, but is abundant in vineyards, orchards, and gardens in some areas (Janak et al., 2013), indicating that suitable habitat has been shaped by human activities. Indeed, the species had started to spread across its recent distribution range after Neolithic deforestation (Spitzenberger and Bauer, 2001). The land use history in Hungary during the past decades, when animal husbandry and pasture farming collapsed, disfavoured EGS habitats, and may have caused severe declines of both population size and successful dispersal in central Hungary. The lack of long-distance gene exchange via stepping stones may explain the lower genetic diversity of the Hungarian local populations compared to those studied in eastern Austria, given that local isolation levels and census population size have not significantly affected *Rs*. While the political and socio-economical separation between the two study regions has ended in 1989, ecological, economical, and sociological effects are still noticeable (Chiron et al., 2010, 2009).

In addition to studies of Chiron et al. (2010, 2009), Cousins et al. (2014), and Reif et al. (2011), our result is in accordance with a study on Perote ground squirrels (*Xerospermophilus perotensis*, Merriam 1983) that indicated a reduction of genetic diversity in an increasingly fragmented landscape (Ochoa et al., 2012). Similarly, region-associated differences of genetic diversity related to historical and natural demographic factors were found in local populations of European common frogs (*Rana temporaria*, Linnaeus 1758) by Johansson et al. (2005), and according to Koen et al. (2014) environmental conditions affected neutral genetic diversity and structure of Canadian lynxes (*Lynx canadensis*, Kerr 1792).

However, there is no data on the genetic diversity of EGS from the last century. The available evidence from the reports of the Hungarian National Biodiversity Monitoring since 2000 has supported that the species' natural habitats (Gedeon et al., 2010; IUCN, 2010) have been fragmented and lost in Hungary. Approximately 50% of the local populations monitored are now on grassy airports, and national monitoring data suggest that these refugia show more stability (O. Váczi and Cs. Gedeon, *pers. comm.*). Short grass and protection are maintained on grassy airfields continuously for hobby flying, whereas in natural habitats protection is less practicable, grass height is frequently suboptimal, and consequently, unexpected extinction occurs more frequently (Gedeon et al., 2012; Váczi and Altbäcker, 1999; Kis et al., 1998). Decrease of animal husbandry and grazing or mowing on ground squirrel habitats since the 1990s has worsened the conditions for EGS. Nevertheless, data for Lower Austria indicate that local populations with low density and census size have been stable (Enzinger, 2006). Less disturbance in a locally more stable or predictable landscape could have helped to maintain this status quo in some populations in eastern Austria. However, this population stability could basically remain unnoticed because eastern Austria lies at the edge of the distribution area and there is no long term, systematic monitoring of census sizes of local populations.

In summary, the importance of landscape history in our models indicates that different socio-economic conditions can shape distinctly the genetic diversity of local EGS populations and consequently their evolutionary potential, ecological and conservation status.

Effect of "Ecological isolation"

Our model results did not particularly suggest an effect of ecological isolation on allelic diversity of local populations. Generally, ecological isolation means only (very) limited or (strongly) biased dispersal

and thus reduced or interrupted gene flow (Bradburd et al., 2013; Edelaar and Bolnick, 2012). Ecological isolation of populations is one of the essential parameters in meta-population dynamics, and its effect on *Rs* appears to be complex. Strong ecological isolation and resultant fragmented populations may in the long run lead to extinction of local populations (Hanski, 1998). The index of ecological isolation we applied only accounts for recent isolation effects. However, the different land-use patterns in eastern Austria and Hungary, to which local populations of EGS have been exposed over many generations, may reflect differences in long-term population isolation (e.g., different levels of disappearance of local populations that have acted as stepping stones); hence, the significant effect of region-specific landscape history on allelic richness may be a correlate of long-term ecological isolation on the genetic diversity of local populations in the larger geographic context.

Presently, we found higher genetic differentiation (*Fst* and *CSE* values) between local populations in central Hungary than between the populations from eastern Austria, which conforms to the simultaneously revealed higher ecological isolation of the former. The higher level of genetic differentiation between the Hungarian populations may indicate stronger drift effects than in the Austrian populations, but could also reflect the currently observed tendency of genetic isolation by geographical distance, as the Hungarian populations are on average dispersed across a larger area than the Austrian populations. Genetic isolation by geographical distance seems to play a role at least on the larger geographic scale, as indicated by our hierarchical AMOVA that revealed significant partitioning of relative genetic variability into the two regions in addition to the general effect of differentiation among local populations. On the other hand, our STRUCTURE results suggest somewhat more genetic admixture in eastern Austria than in central Hungary, which would accord to the hypothesis of stronger drift and loss of allelic diversity in the Hungarian populations, despite their on average bigger census sizes than in Austria.

In principle, this inference is in accordance with an earlier study of EGS populations across a larger geographical region, including several of the currently studied local Hungarian populations that suggested an absence of a migration-drift equilibrium and dominance of drift over gene flow (Ben Slimen et al., 2012). However, our current finding of a tendency of genetic isolation by geographic distance across the whole study area does not allow us to conclude that genetic drift is higher among the local Hungarian populations than the Austrian ones.

Long-term changes in the agricultural landscape and supposedly more pronounced fragmentation in central Hungary have triggered the disintegration, blocking or even disappearance of habitat corridors that formerly may have facilitated gene flow between local populations. The unexpected and relatively quick extinctions of small local populations in Hungary likely have aggravated a reduction of natural gene flow. However, it is also worth noting that deviations from the Hardy-Weinberg equilibrium were found in three local populations from eastern Austria (TD, RS, ST) and one from central Hungary (DUN). It suggests that drift cannot be neglected in either region, which is in accordance with earlier results (Ćosić et al., 2013; Ben Slimen et al., 2012). Our findings suggest that long-term landscape alterations involving the loss of habitat connectivity on the larger geographic scale negatively affected genetic diversity of local populations; short-term and small-scale reduction of suitable habitats as conveyed by our index of ecological isolation may be too recent to demonstrate a negative effect on genetic diversity of local populations.

Implications for conservation

The natural meta-population structure and continuous grassland habitat of EGS have been recently fragmented by anthropogenic activities (Hoffmann et al., 2008), and local populations are exposed to different degrees of ecological or environmental constraints and its consequences, such as reductions of local census population sizes. Our genetic results suggest that regional landscape differences due to diverging landscape histories between eastern Austria and central Hungary do affect the genetic diversity and therefore the evolutionary potential

of EGS in local populations. Thus, conservation management and planning should take into account such regional differences when attempting to maximise genetic diversity of local populations of endangered species. Given the limited dispersal potential of EGS, potential barriers to gene flow between local populations, as a result of adverse landscape management must be identified and eliminated or re-established. Relocations of wild individuals within its historical range can provide a tool to increase or at least to maintain a certain level of genetic diversity locally, should there be no chance of establishing habitat corridors for gene flow and no other ecological or epidemiological objections. ✍

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