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Genetic differentiation of capercaillie populations in the Western Carpathians based on non-invasive samples

Key words: *Tetrao urogallus*, gene flow, habitat fragmentation

1. Introduction

Long-term conservation efforts and plans require an examination of information on population genetic structure, gene flow and connectivity (Moritz 1994). Small and isolated populations of the *Tetraonidae* family might be exposed to a higher risk of extinction due to a negative genetic impact (Höglund et al. 2006). In Central and Western Europe, the Western capercaillie is considered to be the biggest grouse species of the old high-altitude coniferous forests (Storch 2007) with restricted dispersal abilities (median of natal dispersal: 5–10 km; Storch & Segelbacher 2000). Human impact and topography are considered the two most important factors causing a patchy metapopulation structure due to fragmentation (Segelbacher & Storch 2002, Segelbacher et al. 2003a, 2008).

Habitat fragmentation and population decline are considered to be major threats for the capercaillie in Central and Western Europe (Storch 2000, Saniga 2003). The Western-Carpathian population is exposed to rapid population decline. Small isolated populations survive only in high-altitude patchy habitats at the upper tree limit. The estimated population size in 1972 was 3700 males. In 1992 it was estimated at 1100–2000 males (Saniga 1992). Recent estimates of capercaillie abundance in the Slovak range of the Western Carpathians assume 500–600 males (Saniga 2011). The decline of old, well-structured forests is considered the main factor for such a dramatic decline in the capercaillie population of the Western Carpathians (Saniga 2012). Information on habitat connectivity, capercaillie dispersal, genetic diversity and structure is unavailable. In addition, basic information relating to the total area of suitable habitats and the population abundance of the Western capercaillie is unknown in the whole Western Carpathian range. In neighbouring Poland the population of 550–750 individuals is subdivided into 4 isolated regions and the Polish part of the Western Carpathians is occupied by 120–150 males (Tomiałojc & Stawarczyk 2003). Populations in the Moravian-Silesian Beskyds (Moravsko-Sliezske Beskydy) and the High Tatras are neighbouring the Slovak population. Rutkowski et al. (2005) found significant heterozygosity deficiency (F_{IS} = 0.36) in the Polish Carpathian population, which is considered the edge of the Western Carpathian population. The study of capercaillie in the Alps revealed a vital metapopulation system and high levels of genetic variation (Segelbacher & Storch 2002). The situation in the Western Carpathians with lower altitude and a more heterogeneous landscape might be different.

In this study, using nuclear microsatellites, we assessed the differences in genetic variation levels, effective population sizes and gene flow among six sampled populations in the Western Carpathians. Our main objective was the identification of the most threatened populations where urgent conservation action is necessary to avoid extinction of capercaillie in the area.

2. Material and Methods

2.1. Sampling

In total, 431 moulted feathers and faeces were collected from 2011 to 2014 across the Western Carpathian range of capercaillie in Slovakia (Fig. 1, Tab. 1). For each sample, the geographical coordinates in S-JTSK were recorded. Reliable sexing was ensured by molecular genetic techniques.

Based on the dispersal ability and the presence of geographical barriers sample sites were grouped into 6 subpopulations (Volovské Mountains, Muráň Plateau, Low Tatras – east, Low Tatras – west, Great Fatra, High Tatras). Geographical distances between the different groups varied from 16 to 110 km (Table 1). Samples were collected during lek seasons. We used a silica-gel-based method for sample storage. In total, 163 individual genotypes were used for statistical processing.

2.2. DNA extraction

Samples were stored dried at room temperature. DNA was extracted using an extraction kit developed for DNA extraction from human stool (QIAGEN). Feather samples were extracted with a NucleoSpin Tissue DNA extraction kit (Machery Nagel). Extraction was performed with a small modification. A 1–1.5 cm feather barb or blood clot was cut into small pieces and placed into tubes. All the following steps were performed according to the manufacturer's instructions. DNA extraction and pre-PCR steps were performed in a sterile box equipped with UV radiation light, which was run before each procedure.

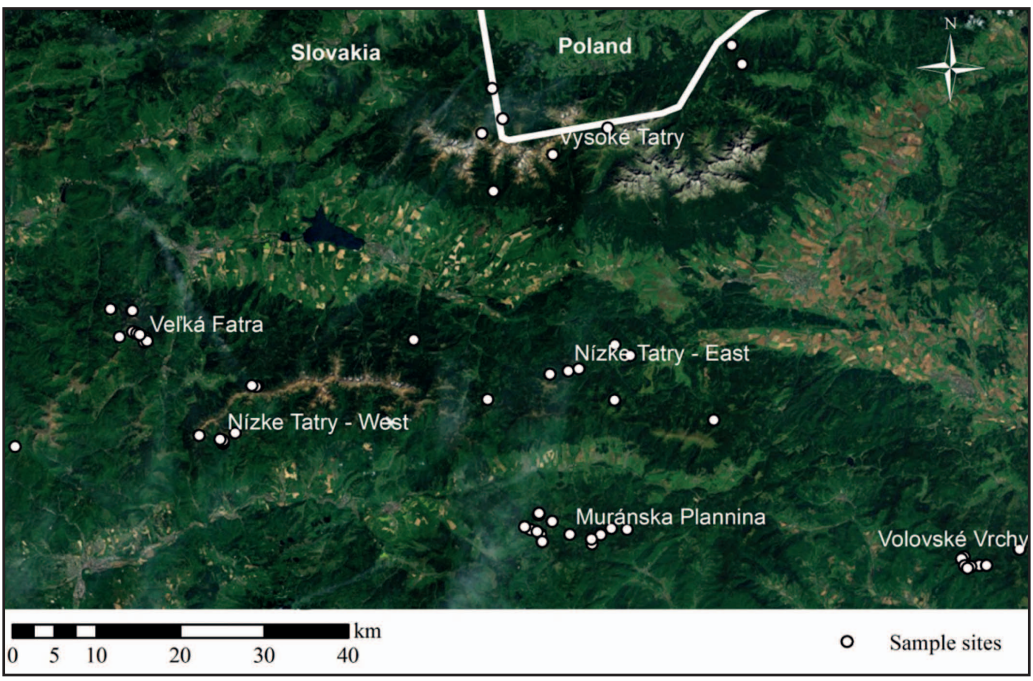

Fig. 1 The map of sampling sites in Volovské Mountains (Volovske vrchy), Muráň Plateau (Muranska planina), Low Tatras – east (Nizke Tatry – East), Low Tatras – west (Nizke Tatry – West), Great Fatra (Velka Fatra) and High Tatras (Vysoke Tatry).

Table 1 Genetic variation indices per each of 6 population sample size (N), allelic richness (AR), private allelic richness (priv. AR), mean number of alleles per locus (NA/loci), expected heterozygosity (H_E), observed heterozygosity (H_O), fixation index (F_{IS}) (Weir & Cockerham 1984) with confidence interval (CI) 95 %.

Sampling area	N samples	AR	Priv. AR	NA/loci	H_E	H_O	F_{IS}	CI (95 %)
Volovské Mountains	20	3.77	0.33	3.89	0.53	0.42	0.23	(0.08 – 0.33)
SD (±)					0.21	0.21		
Muráň Plateau	44	4.27	0.19	5.22	0.58	0.65	–0.10	(–0.19 – –0.04)
SD (±)					0.13	0.14		
Low Tatras – east	32	4.6	0.42	5.56	0.57	0.51	0.12	(–0.01 – 0.22)
SD (±)					0.13	0.13		
Low Tatras – west	25	3.56	0.09	3.89	0.53	0.45	0.16	(–0.02 – 0.29)
SD (±)					0.17	0.22		
Great Fatra	27	4.05	0.11	4.56	0.56	0.57	–0.01	(–0.13 – 0.10)
SD (±)					0.13	0.14		
High Tatras	15	4.11	0.14	4.11	0.55	0.55	0.04	(–0.15 – 0.15)
SD (±)					0.18	0.21		

2.3. PCR amplification

All samples were PCR-amplified at least 3 times to ensure reliable genotyping. The DNA amplification was performed at 11 microsatellites in the following concentrations: 0.1 µM LEI98 (Gibbs et al. 1997), 0.08 µM ADL184, 0.08 µM ADL230 (Cheng & Crittenden 1994), 0.08 µM BG15, 0.40 µM BG16, 0.10 µM BG18 (Piertney & Höglund 2001), 0.13 TUT1, 0.05 µM TUT2, 0.08 µM TUT3 and 0.08 µM TUT4 (Segelbacher et al. 2000). For male vs. female recognition we amplified a fragment of the chromo-helicase-DNA-binding (CHD) gene using the primer pair P2 and P8 0.04 µM (Griffiths et al. 1998 in Jacob et al. 2009). DNA was amplified in a 10 µL reaction mixture containing 1.3 µl extracted DNA, 5 µl 2× Qiagen Multiplex Kit, 1 µl 5× Q-solution, nanopure H_2O.

DNA was amplified according to the following protocol: 15 minutes initial denaturation at 94°C, 35 cycles of 30 sec. denaturation at 94°C, 45 sec. annealing of primers at 60°C, 45 sec. of DNA extension at 72°C with final 20 min. elongation at 60°C. PCR fragments were analysed on the ABI 3130 (Appled Biosystems, USA). To detect any unexpected contamination of samples with exogenous or amplified DNA a negative control was ensured.

2.4. Reliability of typing of non-invasive samples

Genotyping was performed using the GeneMapper 3.7 (Applied Biosystems). To identify consensus genotype and control possible genotyping errors due to stutter peaks or false alleles the software Gimlet (Valière 2002) and Microchecker (Van Oosterhout et al. 2004) were employed. Marker BG16 was not amplified in most of the samples, so it was excluded from further analyses. Samples with false alleles were excluded from the analyses. In total, 163 individual genotypes were included in statistical processing. All loci were tested for departure from Hardy-Weinberg equilibrium in Genepop on the Web and linkage disequilibrium between all pairs of loci at a 0.05 level of significance were assessed in Genetix (Belkhir et al. 2004).

2.5. Data analysis

Genetic diversity

Mean number of alleles, allelic richness (*AR*) and private allelic richness (*priv. AR*) were calculated by the rarefaction method implemented in HP-rare 1.0 (Kalinowski 2005). Mean number of alleles per locus (*NA*/loci), expected heterozygosity (H_E), observed heterozygosity (H_O) with their standard deviations (SD) in 99 % threshold of polymorphism *P(0.99*), fixation index (F_{IS}) (Weir & Cockerham 1984) with confidence interval (*CI*) 95 % confirmed by 10,000 bootstraps to test for deviation from Hardy-Weinberg equilibrium (HWE) were calculated in Genetix 4.05 (Belkhir et al. 2004).

First-generation migrants

To evaluate possible migration between studied populations we identified first-generation migrants using software GeneClass2 (Piry et al. 2004). The software detects first-generation migrants based on the computation of likelihood of an individual that originates from a certain population (L) (Paetkau et al. 2004). The probability that an individual is a resident at the 1 % significance level was computed using Monte-Carlo resampling with a simulation algorithm of Paetkau et al. (2004) with 10,000 simulated individuals. The assignment of sampled individuals to a source population was tested using the frequencies-based simulation method Paetkau et al. (2004) with default frequency for missing allele set to 0.01 following the same setting as in the Bayesian method.

Population differentiation

The pairwise F_{ST} (Nei 1978) was tested for significance by 10,000 permutations in Genetix 4.05 (Belkhir et al. 2004). The linear pair-wise geographic distances between centres of the six sampling areas were measured in Google Earth (http://earth.google.com). Pairwise distances created two semi matrices used as an input file for Genepop 4.0 (Raymound & Rousset 1995), where F_{ST} (Nei 1978) was converted to $F_{ST}/(1–F_{ST})$ (Rousset 1997) Isolation by distance comparing correlation of genetic and geographic distances was tested using the Mantel test (Mantel 1967) with 10,000 randomizations implemented in its software IBDWS (Jensen et al. 2005). The principal coordinate analysis (PCoA) performed in R-package PopgenReport was used to visualize possible population substructuring in two-dimensional space.

Creating genetic landscape

To investigate genetic boundaries we first determined the presence of isolation by distance (IBD) pattern. Patterns of genetic divergence were mapped within the ArcGis software package (ESRI, Redlands, CA) Genetic Landscapes GIS Toolbox (Perry et al. 2010). Isolation by distance pattern was not significant, therefore we used Rousset's (1997) genetic distances. Genetic distances were visualized as a genetic landscape in the Single Species Divergence Tool using Monmonier's algorithm. The cell size for the output genetic surface was set to 120×120 m. Such a cell size is about 10 % of the capercaillie's annual home range size (Braunisch et al. 2010).

Bayesian clustering

To assess the population genetic structure, a Bayesian clustering method implemented in the nonspatial clustering program Structure 2.3.3 (Pritchard et al. 2000) and a spatial clustering program TESS (Durand et al. 2009) were performed. Both programmes used 100,000 Markov Chain Monte Carlo (MCMC) algorithms discarding with the first 10 % of iterations discarded as a burn-in. Ten runs for each K 1–20 were performed using the admixture model with assuming correlated allele frequencies (Falush et al. 2003). The STRUCTURE results were processed in software Structure-Harvester (Earl et al. 2012) using Evanno's method (Evanno et al. 2005) resulting in the identification of 3 clusters (K = 3). The Q coefficients were calculated by averaging these 10 runs in Clumpp 1.1.2 (Jakobsson & Rosenberg 2007). Outputs were visualised with Distruct 1.1 (Rosenberg 2004). TESS was run 10 times for each 2–20 K values using a conditional autoregressive admixture model

(CAR) considering discrete sampling across the sampling range. The best K = 2 was identified according to the lowest deviance information criterion (DIC) (Chen et al. 2007). The results were averaged in Clumpp 1.1.2 (Jakobsson & Rosenberg 2007) and visualised in figures produced by Distruct 1.1 (Rosenberg 2004).

3. Results

3.1. Quality of noninvasive samples

From 431 samples of faeces and feathers collected in 2011–2014 we analysed 163 individual genotypes in the six populations across the Western Carpathians. In all, we analysed 65 females and 98 males resulting in a female to male sex ratio of 1 : 1.5.

3.2. Genetic variability

In the analysed Western Carpathian populations allelic richness varied from 3.56 in the Low Tatras – west to 4.6 in the Low Tatras – east. The Low Tatras – east recorded the highest allelic richness and high expected heterozygosity. It might be considered as the core capercaillie population in the Western Carpathians. The lowest private allelic richness was observed in the Low Tatras – west (*Priv. AR* = 0.09) followed by the Great Fatra contrasting with the highest value in the Volovské Mountains (*Priv. AR* = 0.33) (Table 2). The remainder of the easternmost population in the Volovské Mountains exhibits a significantly high deficiency of heterozygotes. Populations from the Great Fatra and the High Tatras seem to be close to Hardy-Weinberg equilibrium.

3.3. Genetic differentiation

The level of genetic differentiation measured by pairwise genetic distances (Nei 1978) indicates significant moderate genetic differentiation of capercaillie populations in the Volovské Mountains from the Muráň Plateau, the Low Tatras – east and west, the Great Fatra and the High Tatras. Genetic differentiation between the remaining capercaillie populations was low. However, the population in the High Tatras shows low genetic differentiation from the Muráň Plateau, both populations in the eastern and the western part of the Low Tatras, and the Great Fatra. The maximum linear geographic distance between two populations was 110 kms.

*Table 2 Pairwise genetic distance (Nei 1978) (above the diagonal) vs. linear geographic distance matrix [km] (below the diagonal). All pair-wise genetic distance values are significant P < 0.05 after 10 000 permutations. Significant very high differentiation of the Volovské Mountains popu*lation is highlighted in bold.

Pairwise distances	Volovské Mountains (20)	Muráň Plateau (44)	Low Tatras – east (32)	Low Tatras – west (25)	Great Fatra (27)	High Tatras (15)
Volovské Mountains (20)	0	**0.154**	**0.222**	**0.19**	**0.143**	**0.21**
Muráň Plateau (44)	50	0	0.022	0.034	0.014	0.071
Low Tatras – east (32)	46	17	0	0.03	0.05	0.072
Low Tatras – west (25)	100	37	35	0	0.046	0.087
Great Fatra (27)	110	58	57	16	0	0.061
High Tatras (15)	53	40	25	56	58	0

The Mantel test did not detect a significant correlation Z = 99.5924, r = 0.4732 (P = 0.1247) between geographic distance and estimate of $F_{ST}/(1–F_{ST})$. We also performed the Mantel test with the logarithm of geographic distances with a non-significant result ($P = 0.1027$).

Rousset's (1997) genetic distances were processed in S in Genetic Landscape GIS Toolbox (Perry et al. 2010) within the software ArcGIS 10.2 (ESRI, Redlands, CA) and resulted in a population surface with genetic divergence values transformed to colour shading. The constructed map clearly differentiated populations of the Low Tatras – east and west, the Great Fatra, the High Tatras and the Muráň Plateau from a single population in the Volovské Mountains. Light blue shading of the High Tatras population indicates a low differentiation from those of the Great Fatra, the Low Tatras and the Muráň Plateau (Figure 2).

The two-dimensional coordinate analysis of 163 genotypes indicated that the population in the Volovské Mountains is genetically different from the other Western Carpathian populations. The populations in the Muráň Plateau, the Low Tatras, the Great Fatra and the High Tatras seem to be admixed due to ongoing gene flow (Figure 3).

The level of differentiation of the Volovské Mountains from the rest of the Western Carpathian populations was also corroborated by the Bayesian approach implemented in Tess and Structure. Evanno's method indicated 3 clusters. The first cluster comprised the Volovské Mountains genotypes, the second and third clusters were generally equally represented in individuals of the remaining populations. Therefore we also visualized the structure with $K = 2$ clusters. Tess results provided a clear differentiation of the Volovské Mountains popula-

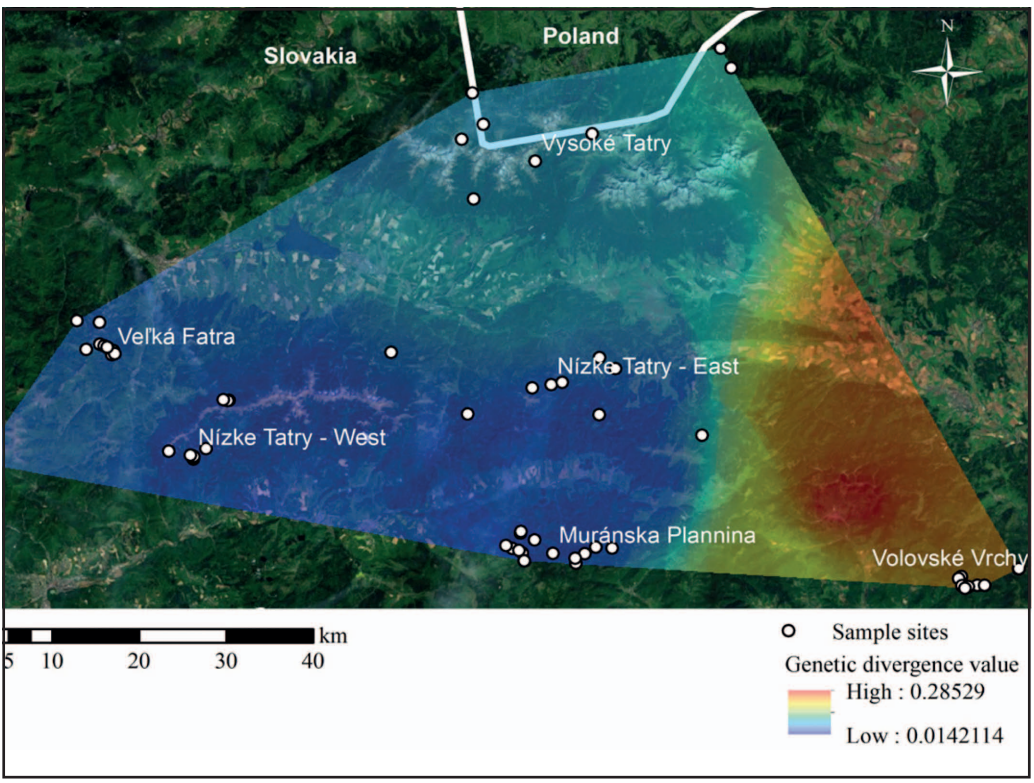

Fig. 2 The map of genetic divergence of capercaillie populations in the Western Carpathians based on reduced $F_{ST}/(1-F_{ST})$ (Rousset 1997) visualised in space (Keller & Largiader 2003, Manel et al. 2003) implemented in Genetic Landscape GIS Toolbox (Perry et al. 2010). The value of genetic differentiation increases with reddish colour.

tion from the other Western Carpathian populations. Both approaches confirmed 2 distinct clusters within the Western Carpathians with the Volovské Mountains forming the single genetic cluster (Figure 4).

Identification of first generation migrants

The results calculated in GeneClass2 (Piry et al. 2004) indicate significant dispersal between sample sites. The Bayesian method (Rannala & Mountain 1997) indicated only six first generation dispersals with the probability below the $P < 0.006$ after 10,000 simulations. The High Tatras population was identified in three cases as a source population. From seven dispersing individuals only one was male. This fact suggests sex specific dispersal with females having more dispersive behaviour.

Conservation consequences

Conservation management should focus on units that are significantly genetically differentiated (Moritz 1994). Therefore, the increased

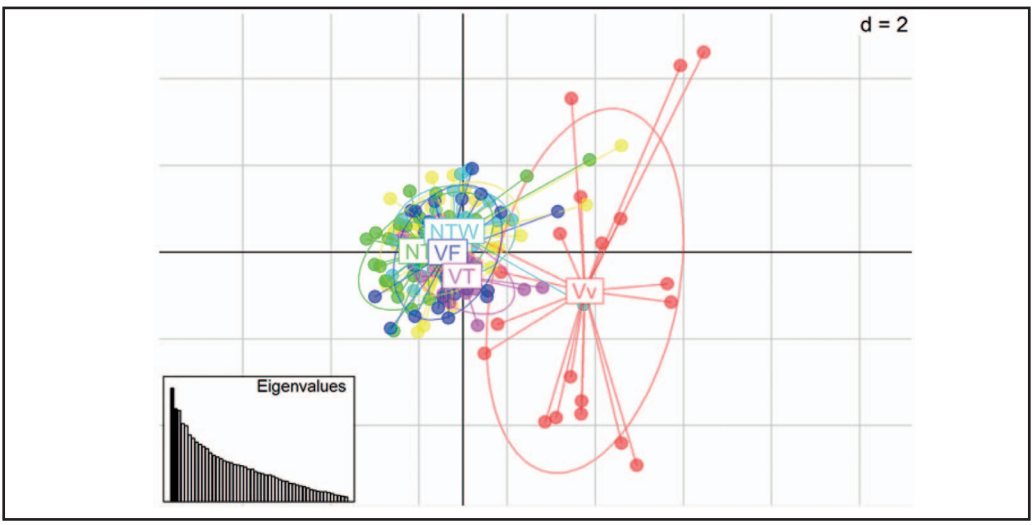

Fig. 3 Principal coordinate analysis differentiated population in the Volovské Mountains (Vv) from the Muráň Plateau (MP), Low Tatras – East (NTE), Low Tatras – West (NTW), Great Fatra (VF) and High Tatras (VT).

Table 3 Identification of the first generation immigrants F_0 in populations with ongoing gene flow was performed by GeneClass2 with implemented Monte Carlo resampling method (Piry et al. 2004).

Individual ID	Sample site	Sex	Origin	Distance [km]	P-value L_home
SKVv1069	Volovské Mts.	F	Great Fatra	110	P = 0.0032
SKMP0308	Muráň Plateau	F	Volovské Mts.	50	P = 0.0042
SKMP0338	Muráň Plateau	F	High Tatras	40	P = 0.0057
SKNTE0368	Low Tatras – east	M	High Tatras	25	P = 0.0053
SKNTE0779	Low Tatras – east	F	Muráň Plateau	17	P = 0.0009
SKNTW0413	Low Tatras – west	F	Muráň Plateau	37	P = 0.0063
SKNTW0894	Low Tatras – west	F	High Tatras	56	P = 0.0006

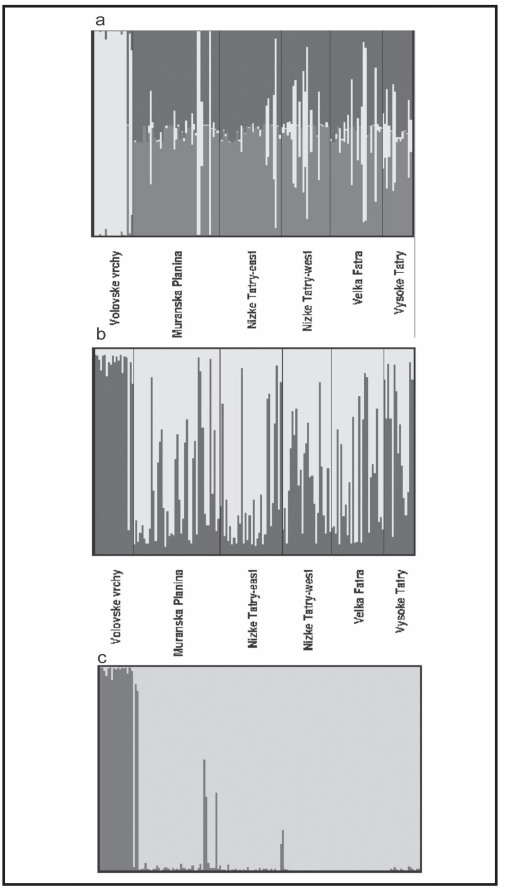

Fig. 4 Distruct plots for the Western Carpathian populations at (a) K3 (b) K2 after averaging Structure *runs with* Clumpp *(c) after averaging* Tess *runs with* Clumpp

risk of extinction and the high genetic differentiation of the population in the Volovské Mountains highlight the need to adopt and apply a specific conservation management plan for Western Carpathian habitats, including capercaillie-friendly silviculture techniques to create a network of suitable habitat patches enabling migration and gene flow of differentiated capercaillie population in the Western Carpathians. In future studies it will be necessary to accomplish genetic analyses of edge populations in the Orava region, the Kremnické Mountains, the Malá Fatra, Poľana, and the Levočské Mountains. It will also be necessary to perform analyses of environmental variables using a spatially explicit approach (Braunisch et al. 2010) to identify potential migration corridors and hybrid zones among core and edge habitats. A combination of genetic analyses results together with data on habitat suitability of forest stands will be crucial in the development of an effective conservation management plan for capercaillie in the Western Carpathians.

4. Discussion

The Low Tatras population having preserved the highest allelic richness can be assumed as a core population of the Western Carpathians, and supports the view of Mikoláš et al. (2014). The geographic position of the Low Tatras Mountains ensures them an important role as a potential crossroad or a stepping stone for birds dispersing from the surrounding populations in the Muráň Plateau, the Great Fatra and the High Tatras. Such an assumption was confirmed by the identification of first-generation migrants discovered in the Low Tatras originating from the High Tatras and the Muráň Plateau. Also, low genetic distances between the Low Tatras on one side and the Muráň Plateau, the High Tatras or the Great Fatra on the other side indicate the existence of gene flow. In our study we revealed long distance dispersal and identified first-generation migrants in the Volovské Mountains originating from the Great Fatra (a linear distance of 110 km). Identified migration over extremely long distances might be due to rapid destruction of old spruce (*Picea abies*) high altitude habitats from inappropriate forestry techniques or, from the occurrence of natural disasters such as windstorms followed by a bark beetle invasion in the last decade. Among the 163 individuals analysed seven were identified as first-generation migrants. Identification of three first-generation migrants originating from the High Tatras might indicate increased emigration from damaged habitats due to the windstorm in 2004 and the still persistant reduction of spruce forest patches due to a bark beetle invasion following the windstorm. The capercaillie population in the High Tatras is isolated from other populations by a strongly human-affected basin and a linear distance varying from 25 to 58 kilometers. We have identified possible gene flow across this expected barrier.

Promotion or restoration of the source-sink population dynamic to the maintenance of genetic connectivity among the sink regions is recommended (Jacob 2006). Median natal dispersal of capercaillie was estimated to 5–10 km and the median dispersal of juvenile birds was estimated at 5–6 km (Storch & Segelbacher 2000). Dispersal over extremely long distances was recorded in released birds in Massif Central in France. The longest dispersal distance observed in two males was 100 and 140 kms. Documented unexpected long distance migration of analysed individuals may be caused by ongoing habitat deterioration and high predation occurring in the last decade in the Western Carpathians. Within the Western Carpathians we identified two clusters with individuals from the Volovské Mountains belonging to a distinct cluster. Population structuring may be the result of historical processes or ongoing limited gene flow due to isolation by distance or geographical barriers such as unsuitable habitats or human settlements.

Genetic analyses show a sex ratio favouring males. Our results suggest a male to female ratio of 1.51 : 1. Genetic sexing of the fragmented populations in the Black Forest (Segelbacher et al. 2008), the Swiss Alps (Jacob 2010) and the Cantabrian Mountains (Vázquez et al. 2013) favoured males. Different estimates from field observations and molecular sexing might be caused by the migration of females between several closely spaced leks resulting in the observation of more females than males. The deficit of females in capercaillie populations could be the result of increased predation risk during the nesting period due to habitat lost and clearcuts. Increased predation pressure on nesting birds was observed at the edge of the clearcut and the forest stand (Andrén 1994).

In our analyses of the first-generation migrants we identified six female individuals and only one male. This might indicate that the females in Western Carpathian habitats disperse more than the males. Microsatellite analyses of black grouse (*Tetrao tetrix*) with mating behaviours similar to capercaillie, consider female-biased dispersal to play an important role in reducing the risk of inbreeding (Lebirge et al. 2010).

Dramatic habitat loss and population decline result in low allelic richness, a high deficit of heterozygotes and a significantly high deviation from Hardy-Weinberg equilibrium These are the consequences of reduced gene flow, a loss of alleles and high genetic differentiation within the currently easternmost population of the Western Carpathians. Ferianc (1954) identified the easternmost isolated patchy population in Branisko. In the last decade no records of capercaillie evidence were found in Branisko, therefore we can consider this population already extinct.

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Summary

The capercaillie population has experienced a dramatic decline in the Western Carpathians and has become extinct in large areas of its former natural range. For this reason there is a strong need for a complex genetic structure study of the surviving populations in an attempt to support appropriate conservation management actions. Exact data on capercaillie density, sex ratio, dispersal possibilities and the genetic structure of the population in the studied region are missing. From 431 samples of faeces and feathers collected in 2011–2014 we analysed 163 individual genotypes in six populations across the Western Carpathians. We identified more males than females in a ratio of 1.5 male to 1 female. Analyses of effective population size indicated population decline in two analysed generations. We reviewed the highest allelic richness in core populations of the Low Tatras. The population in the Volovské Mountains was identified as the most distinct population with low genetic diver-

sity and a high deviation from Hardy-Weinberg equilibrium. Low population size and habitat deterioration cause an interruption of gene flow and a decline in genetic diversity which results in lower individual fitness and increased risk of extinction. Therefore, there is an urgent need for active conservation management to focus on promotion of suitable habitats to ensure gene flow among fragmented populations in the Western Carpathian Mountains.

Zusammenfassung

Genetische Differenzierung von Auerhuhn-Populationen in den westlichen Karpaten basierend auf nicht-invasiven Proben

Die Auerhahnpopulation hat einen dramatischen Rückgang in den Westkarpaten erfahren und ist in weiten Teilen seiner früheren natürlichen Verbreitungsgebieten ausgestorben. Aus diesem Grund gibt es einen starken Bedarf für eine komplexe genetische Untersuchung der überlebenden Populationen um geeignete Erhaltungsmaßnahmen zu unterstützen. Genaue Daten über die Dichte, Geschlechterverhältnis, Ausbreitungsmöglichkeiten und die genetische Struktur des Auerhahns in den Westkarpaten fehlen. Von 431 Kot- und Federproben, gesammelt in 2011–2014, wurden 163 Einzelgenotypen in sechs Populationen festgestellt. Wir identifizierten mehr Männchen als Weibchen (1,5 : 1). Die Analysen der effektiven Populationsgröße deuten einen Populationsrückgang in beiden analysierten Generationen an. Der höchste allelische Reichtum ist in den Kernpopulationen der Niederen Tatra zu finden.

Im Gegensatz wurde in Volovské Vrchy die geringste genetischen Vielfalt und eine hohe Abweichung vom Hardy-Weinberg-Equilibrium identifiziert. Kleine Populationsgröße und Lebensraumverlust verursachen eine Unterbrechung des Genflusses und einen Rückgang der genetischen Vielfalt, die zu einer niedrigeren „Fitness“ und verstärktem Aussterbenrisiko führt. Es besteht daher ein dringender Bedarf für aktives Naturschutz-Management und auf die Förderung von geeigneten Lebensräumen, um den Genfluss zwischen den fragmentierten Populationen in den westlichen Karpaten zu gewährleisten.

References

Andrén, H. (1994): Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat. – Minireview OIKOS **71**: 355–366.

Belkhir, K.; Borsa, P.; Chikhi, L.; Raufaste, N.; Bonhomme, F. (1996–2004): GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France).

Bohonak, A.J. (2002): IBD (Isolation by distance): a program for analysis of isolation by distance.

Braunisch, V.; Segelbacher, G.; Hirzel, A.H. (2010): Modelling functional landscape connectivity from genetic population structure: a new spatially explicit approach. – Molecular Ecology **19** (17): 3664–3678.

Do, C.; Waples, R.S.; Peel, D.; Macbeth, G.M.; Tillett, B.J.; Ovenden, J.R. (2014): NeEstimator V2: reimplementation of software for the estimation of contemporary effective population size (Ne) from genetic data. – Molecular Ecology Resources **14**: 209–214.

Durrant, C.J.; Beebee, T.J.C.; Greenaway, F.; Hill, D.A. (2009): Evidence of recent population bottlenecks and inbreeding in British populations of Bechstein's bat (*Myotis bechsteinii*). – Conservation Genetics **10** (2): 489–496.

Earl, D.A.; von Holdt, B.M. (2012): STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. – Conservation Genetics Resources **4** (2): 359–361.

Evanno, G.; Regnaut, S.; Goudet, J. (2005): Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. – Molecular Ecology **14**: 2611–2620.

Excoffier, L.; Laval, G.; Schneider, S. (2005): Arlequin ver. 3.0: An integrated software package for population genetics data analysis. – Evolutionary Bioinformatics Online **1**: 47–50.

Falush, D.; Stephens, M.; Pritchard, J.K. (2003): Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. – Genetics **164**: 1567–1587.

Ferianc, O. (1954): Rozšírenie lesných kúr na Slovensku. – Biológia **9** (2): 182–209.

Höglund J.; Larsson J.K.; Jansman H.A.H.; Segelbacher G. (2006): Genetic variability in European black grouse (*Tetrao tetrix*). – Conservation Genetics **8**: 239–243.

Jacob, G.; Bollman, K.; Schmid, B.; Gugerli, F. (2006): Landscape barriers induce genetic structuring in the capercaillie (*Tetrao urogallus* L.) in the Swiss Alps – Implications for the management of the species. p. 37–60. – In: Jacob, B. (2006) Dissertation thesis: Conservation genetics of the capercaillie (*Tetrao urogallus*) in the Swiss Alps. 123 pp.

Jacob, G.; Debrunner, R.; Gugerli, F.; Schmid, B.; Bollmann, K. (2010): Field surveys of capercaillie (*Tetrao urogallus*) in the Swiss Alps underestimated local abundance of the species as revealed by genetic

analyses of non-invasive samples. – Conservation Genetics **11** (1): 33–44.

Jakobsson, M.; Rosenberg, N.A. (2007): CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. – Bioinformatics **24** (14): 1801–1806.

Jensen, J.L.; Bohonak, A.J.; Kelley, S.T. (2005): Isolation by distance, web service. *BMC* Genetics **6**: 13. v.3.23 http://ibdws.sdsu.edu/.

Kalinowski, S.T. (2005): HP-Rare: a computer program for performing rarefaction on measures of allelic diversity. – Molecular Ecology Notes **5**: 187–189.

Lawler, R.R. (2008): Testing for a historical population bottleneck in wild Verreaux's sifaka (*Propithecus verreauxi verreauxi*) using microsatellite data. – American Journal of Primatology **70**: 990–994.

Lebigre, C.; Alatalo, R.V. & Siitari, H. (2010): Female-biased dispersal alone can reduce the occurrence of inbreeding in black grouse (*Tetrao tetrix*). – Molecular Ecology **19** (9): 1929–1939.

Mantel, N.A. (1967): The detection of disease clustering and a generalized regression approach. – Cancer Research **27**: 209–220.

Mikoláš, M.; Kalafusová, I.; Tejkal, M.; Černajová, I.; Michalová, Z.; Hlásny, T.; Barka, I.; Zrníková, K.; Bače, R.; Svoboda, M. (2013): Habitat conditions of the core population of the Western Carpathians: Is there still place for the species in Slovakia? – Sylvia **(2)**: 79–98.

Moritz, C. (1994): Defining 'evolutionary significant units' for conservation. – Trends of Ecology and Evolution **9**: 373–375.

Nei, M. (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. – Genetics **76**: 379–390.

Nève, G.; Verlaque, R. (2010): Genetic differentiation between and among refugia. – In: Habel J.C.; Assmann, T. (editors) (2010) Relict species: phylogeography and conservation biology. – Springer-Verlag, Berlin, Germany.

Paetkau, D.; Slade, R.; Burden, M.; Estoup, A. (2004): Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. – Molecular Ecology **13**: 55–65.

Perry, W.; Lugo, R.; Hathaway, S.A.; Vandergast, A.G. (2010): Genetic Landscapes GIS Toolbox: Tools to create genetic divergence and diversity landscapes in ArcGIS. U.S. Geological Survey.

Piry, S.; Alapetite, A., Cornuet, J.-M.; Paetkau, D.; Baudouin, L. & Estoup, A. (2004): GENECLASS2: a software for genetic assignment and first-generation migrant detection. – The Journal of Heredity **95** (6): 536–539.

Pritchard, J.K.; Stephens, M.; Donnelly, P. (2000): Inference of population structure using multilocus genotype data. – Genetics **155**: 945–959.

Rannala, B.; Mountain, J.L. (1997): Detecting immigration by using multilocus genotypes. – Proc. Natl. Acad. Sci. USA **94**: 9197–9201.

Raymond, M.; Rousset, F. (1995): GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. – Journal of Heredity **86**: 248–249.

Rosenberg, A.N. (2004): DISTRUCT: a program for the graphical display of population structure. – Molecular Ecology Notes **4**: 137–138.

Rousset, F. (1997): Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. – Genetics **145** (4): 1219–1228.

Rutkowski, R.; Niewęgłowski, H.; Dziedzić, R.; Kmiec, M. & Goździewski, J. (2005): Genetic variability of Polish population of the capercaillie *Tetrao urogallus*. – Acta Ornithologica **40** (1): 27–34.

Saniga M. (1992): Desať rokov pozorovaní hlucháňa obyčajného (*Tetrao urogallus*) na lokalitách vo Veľkej Fatre a Nízkych Tatrách. – Tichodroma **4**: 63–73.

Saniga, M. (2003): Ecology of the capercaillie (*Tetrao urogallus*) and forest management in relation to its protection in the West Carpathians. – Journal of Forest Science **49** (5): 229–239.

Saniga, M. (2011): Why the capercaillie population (*Tetrao urogallus*) in the mountain forests in the central Slovakia decline? – Folia Oecologia **38**: 110–117.

Saniga, M. (2012): Population dynamics of Capercaillie *Tetrao urogallus* on leks in Central Slovakia in the period 1981–2012. – Grouse News **44**: 5–9.

Segelbacher, G.; Cushman, S.A.; Epperson, B.K.; Fortin, M.J.; Francois, O.; Hardy, O.; Holderegger, R.; Taberlet, P.; Waits, L.P.; Manel, S. (2010): Applications of landscape genetics in conservation biology: concepts and challenges. – Conservation Genetics **11**: 375–385.

Segelbacher, G.; Höglund, J.; Storch, I. (2003): From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. – Molecular Ecology **12**: 1773–1780.

Segelbacher, G.; Manel, S.; Tomiuk, J. (2008): Temporal and spatial analyses disclose consequences of habitat fragmentation on the genetic diversity in capercaillie (*Tetrao urogallus*). – Molecular Ecology **17**: 2356–2367.

Segelbacher, G.; Storch, I. (2002): Capercaillie in the Alps: genetic evidence of metapopulation structure and population decline. – Molecular Ecology **11**: 1669–1677.

Segelbacher, G.; Storch, I.; Tomiuk, J. (2003a): Genetic evidence of capercaillie *Tetrao urogallus* dispersal sources and sinks in the Alps. – Wildlife Biology **9**: 267–273.

Slatkin, M. (1994): Gene flow and population structure. Ecological Genetics (Real LA, ed). Princeton, NJ: Princeton University Press; 3–17.

Sokal, R.R.; Rohlf, F.J. (1981): Biometry: the principles and practice of statistics in biological research, 2nd ed. Freeman: San Francisco, 859 pp.

Storch I.; Segelbacher, G. (2000): Genetic correlates of spatial population structure in central European capercaillie *Tetrao urogallus* and black grouse *T. tetrix*: a project in progress. – Wildlife Biology **6**: 305–310.

Storch, I. (2000): Status survey and conservation action plan 2000–2004: Grouse. IUCN, Gland, Switzerland,

and Cambridge, UK, and The World Pheasant Association, Reading, UK.; 112 pp.

Storch, I. (2007a): Conservation status of grouse worldwide: an update. – Wildlife Biology **13**: 5–12.

Storch, I. (2007b): Grouse: status survey and conservation action plan 2006–2010. IUCN Grouse Specialist Group. 124 pp.

Storch, I.; Segelbacher, G. (2000): Genetic correlates of spatial population structure in central European capercaillie *Tetrao urogallus* and black grouse *T. tetrix*: a project in progress. – Wildlife Biology **6**: 305–310.

Tomiałojć, L.; Stawarczyk, T. (2003): [The avifauna of Poland. Distribution, numbers and trends]. PTPP "pro Natura", Wrocław.

Valiere, N. (2002): GIMLET: a computer program for analysing genetic individual identification data. – Molecular Ecology Notes **2**: 377–379.

Van Oosterhout, C.; Hutchinson, W.F.; Wills, D.P.M.; Shipley, P. (2004): Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. – Molecular Ecology Notes **4**: 535–538.

Vázquez, J.F.; Pérez, T.; Albornoz, J.; Domínguez, A. (2013): Census and effective population size of the endangered Cantabrian capercaillie (*Tetrao urogallus*) estimated from non-invasive samples. – Grouse News 46: 12–26.

Waples, R.S.; Do, C. (2008): LDNE: a program for estimating effective population size from data on linkage disequilibrium. – Molecular Ecology Resources **8**: 753–756.

Weir, B.S.; Cockerham, C.C. (1984): Estimating F-statistics for the analysis of population structure. – Evolution **38**: 1358–1370.

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