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Landscape genetics highlight the importance of sustainable management in European mountain spruce forests: a case study on Western capercaillie

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Abstract The mountain spruce forests of the Western Carpathians have experienced a dramatic deterioration in the last decades increasing the landscape fragmentation. This considerably affected the Western capercaillie population recently surviving within small habitat patches surrounded by unfavourable habitats. Our study shows that the long-term isolation resulted in genetic differentiation with decreasing trend in allelic richness towards the most adjacent western subpopulations. We evaluated dispersal possibilities within the landscape and identified barriers and the most critical corridors between genetically distinct subpopulations. Landscape genetic analysis confirmed that the isolation by environmental features explains the observed genetic patterns better than straight geographical distance. We highlight the urgent need for an active conservation management in the critical habitats where dispersion might be constrained or "bottlenecked" in order to ensure gene flow within the fragmented capercaillie metapopulation of the Western Carpathian mountain forests.

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Introduction

Forest management integrating the protection of biodiversity is one of the major objectives of sustainable forestry in the 21st century (Hofstetter et al. 2015). Management platforms of the past which prioritised exclusively the economic benefits resulted in considerable loss of natural habitats with serious consequences, such as fragmentation of the original habitats, reduction of habitat patches and increased distances among patches (Andrén 1994; Rolstad 1991). Habitat fragmentation is generally considered as negative process, subdividing originally continuous habitat into smaller patches mostly due to the impact of human activities (Wright 1974). It became the most critical factor for the viability of many vertebrate species within the last decades (Pascual-Hortal and Saura 2008). Mitigation of these negative impacts of the past is one of the biggest challenges of the recent and future forestry because it directly influences movement abilities and viability of free-ranging organisms. This is only possible by incorporating information on the spatial genetic structure of the threatened species as well as by addressing the importance of the remaining habitat patches in terms of large-scale gene flow (Moritz 1994).

Western capercaillie (*Tetrao urogallus*) occupying high-altitude old coniferous forests of Central and Western Europe (Storch 2007) is a typical umbrella species. It is, however, facing substantial habitat deterioration (Mikoláš et al. 2013, 2015, 2016) and consequently a rapid population decline within the last decades (Saniga 1992, 2011). The estimated capercaillie population size in the Slovak range of

the Western Carpathians in 1972 was 3700 males, while in 1992 it had decreased to approximately 1100–2000 males (Saniga 1992). Recent estimates are only around 500–600 males (Saniga 2011). Habitat patches placed at the upper tree limits are surrounded by unforested landscape or fragments of forest habitats with unsuitable spatial structure for the capercaillie. It has been shown previously that patch size reduction may result in birds' population decline and limited dispersion into new areas, as well as fewer nesting sites resulting from the lack of critical resources (Rolstad 1991). Deterioration of the old mountainous spruce forests with mosaic structure is likely the main factor causing this dramatic decline (Saniga 2012). Continuous application of clearcutting techniques, salvage logging and intensive tourism has considerable negative impacts on reproduction process and substantially diminished and fragmented suitable capercaillie habitat in Carpathians (Mikoláš et al. 2015, 2016). Moreover, the restricted dispersal abilities of the species (median of juvenile dispersal: <2–3 km; Myberget 1978; Swenson 1991) together with fragmentation of the existing habitats are considered the major threats to the capercaillie in Central and Western Europe (Storch 2000; Saniga 2003), resulting in a patchy distribution of small isolated populations which are prevented from dispersion into new areas (Segelbacher and Storch 2002; Segelbacher et al. 2003, 2008).

It is known that a long-term separation of distant subpopulations may lead to a site-specific adaptation and genetic isolation, which might affect their persistence. To test whether capercaillie population in Slovakia underwent these changes, we evaluated the pattern in genetic variation among six subpopulations using nuclear microsatellite markers. Subsequently, we assessed whether the gene flow is affected purely by the geographical distance between subpopulations (isolation by distance, IBD) or by the complex interaction of environmental factors including human activity (isolation by environment, IBE). Finally, we applied least-cost corridor models and circuit theory to identify areas important for sustaining habitat connectivity for capercaillie in the region and highlighted the most critical corridors for which we suggest high priority in forest management. Our study is one of the few that analyses the consequences of the long-term deterioration of mountainous spruce forests for the viability of its flagship species and provides particular spatial information on key habitats crucial for future survival of the capercaillie population in the Western Carpathians.

Methods

Sampling

In total, 28 moulted feathers and 403 faeces were collected from 2011 to 2013 across the capercaillie range in Central Slovakia. Samples were collected in cooperation with NGO "OZ Prales", foresters and conservationists. We surveyed existing spruce habitats according to the documented historical capercaillie distribution as a part of the preparatory phase for the national inventory of capercaillie habitats. We divided the capercaillie population into subpopulations in forested mountain ranges separated by basins with human settlements. The low numbers of capercaillie and the limited number of suitable habitats with patchy distribution complicated the survey and sample collection. Surveys were repeated twice a year within each potential site of species occurrence. For each sample, geographical coordinates in WGS84 format were recorded. Due to sexual dimorphism in capercaillie, feathers and some faeces could be identified as originating from either female or male individuals. Reliable sexing was ensured by molecular markers (Griffiths et al. 1998). Based on geographical distance and the presence of expected geographical barriers (i.e. basins), the sampled sites were grouped into six subpopulations (Volovec Mountains, Muráň Plateau, Low Tatras—east, Low Tatras—west, Great Fatra, High Tatras; Fig. 1). The Euclidean distance between the subpopulations varied from 10 to 98 km which is far beyond the median natal dispersal of capercaillie in such conditions (<2–3 km; Myberget 1978; Swenson 1991). Samples were collected during the lek seasons and stored in plastic tubes filled with silica gel or 96% ethanol and stored at room temperature.

DNA extraction and PCR amplification

DNA was extracted using an extraction kit developed for human stool DNA extraction (QIAGEN). Droppings with a length of 2–3 cm were placed in 15 ml tubes and covered with ASL solution and gently washed over night at room temperature. The following day, 1.6 µl of solution was transferred into new tubes with an InhibitEX tablet. The remaining procedures were done according to the protocol provided by the manufacturer. Feather DNA samples were extracted using a NucleoSpin Tissue DNA extraction kit (Macherey-Nagel). Extraction was performed with a minor modification. We cut 1 to 1.5-cm feather barb (Johansson et al. 2012) or blood clot (Horváth et al. 2005) into small pieces and placed them into tubes. All the following steps were performed according to the manufacturer's instructions. To avoid any contamination with amplified DNA, extraction, preparation of the master mix and PCRs were all performed in separate pre- and post-PCR rooms using pipette tips equipped with an aerosol-resistant filter. Details of the PCR protocol can be found in the Supplementary Material.
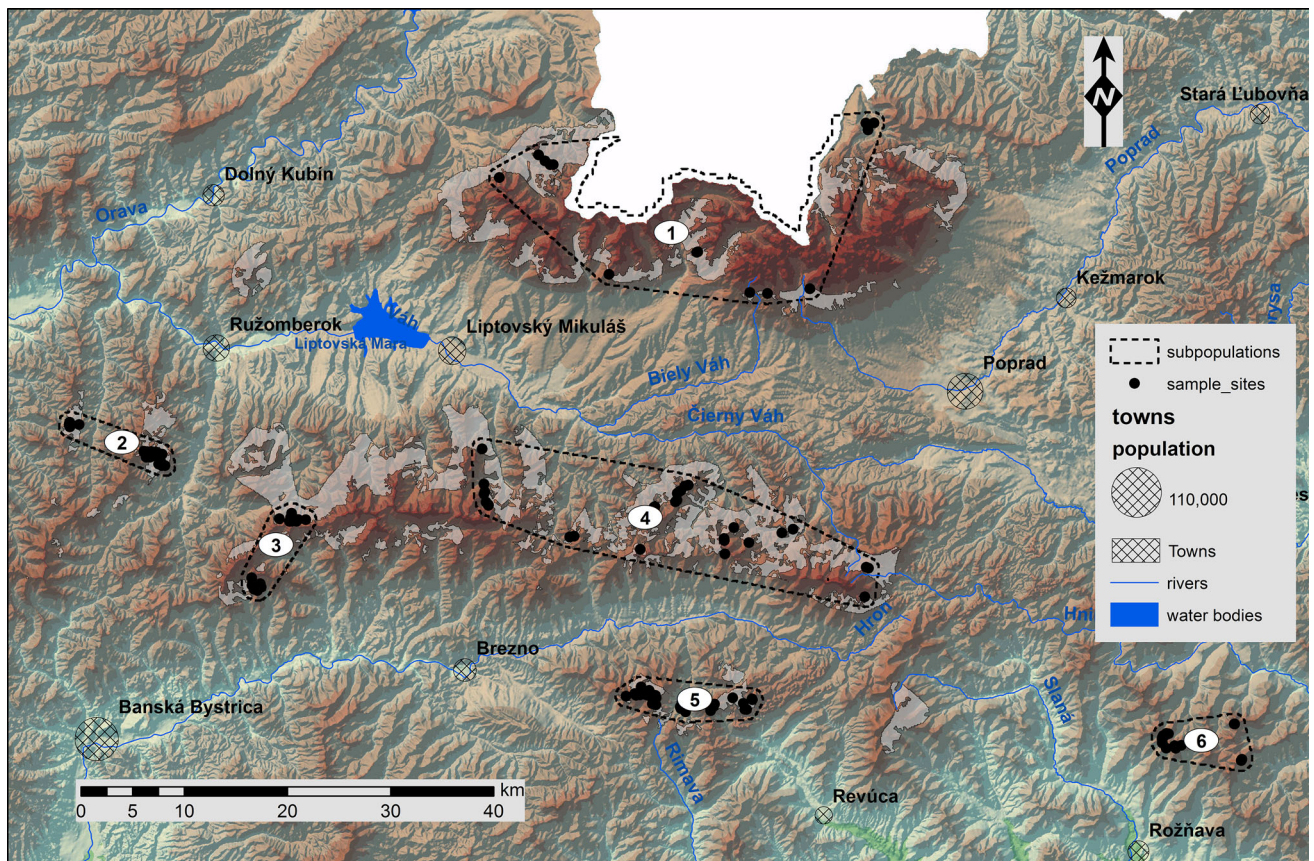
Fig. 1 Location of the sample sites and distribution of the capercaillie (*T. urogallus*) subpopulations in Slovakia: High Tatras (*1*), Great Fatra (*2*), Low Tatras—east (*3*), Low Tatras—west (*4*), Muráň Plateau (*5*), Volovec Mountains (*6*)

Reliability of genotyping

Genotyping was performed in GENEMAPPER 3.7 (Applied Biosystems). To identify the consensus genotype and to control for possible genotyping errors due to stutter peaks or false alleles, the software GIMLET (Valière 2002) and MICROCHECKER (Van Oosterhout et al. 2004) were employed. Duplicate genotypes with at least 95% matching alleles were excluded from the analyses. All loci were tested for departure from the Hardy–Weinberg equilibrium in GENE-POP on the Web (Raymond and Rousset 1995; Rousset 2008), and the linkage disequilibrium between all pairs of loci was assessed in GENETIX (Belkhir et al. 2004).

Genetic variation and differentiation analyses

Allelic richness and private allelic richness (*priv. AR*) were calculated in HP-RARE software (Kalinowski 2005). Expected heterozygosity (H_E), observed heterozygosity (H_O) with their standard deviations (SD) and fixation index (F_{IS}) with confidence interval (CI) 95% were confirmed by 1000 bootstrap pseudoreplicates. The pairwise G'_{ST} (Hedrick 2005), G''_{ST} (Meirmans and Hedrick 2011), D_{Jost} (Jost

2008) were tested by 10,000 bootstraps in package DiveRsity (Keenan et al. 2013) for R (R Development Core Team 2013).

Bayesian clustering

To assess the population genetic structure, a Bayesian clustering method implemented in the non-spatial clustering software STRUCTURE 2.3.3 (Pritchard et al. 2000) and the spatial clustering using the software TESS (Chen et al. 2007, Durrant et al. 2009) were performed. Both programmes use a Markov Chain Monte Carlo (MCMC) algorithm to simulate genotypes under the Hardy–Weinberg equilibrium and linkage disequilibrium assumption. In STRUCTURE twenty runs, 100,000 burn-ins and 1,000,000 MCMC iterations were performed for each K 1–10 using an admixture model with the assumption of correlated allele frequencies (Falush et al. 2003). The STRUCTURE results were processed with the software STRUCTURE HARVESTER (Earl and vonHoldt 2012) using Evanno's method (Evanno et al. 2005). The Q-coefficients were calculated by averaging these 20 runs in CLUMPP 1.1.2, and the "greedy" algorithm plus the "all possible input order" options were used (Jakobsson and

Rosenberg 2007). Outputs were visualised with DISTRUCT 1.1 (Rosenberg 2004). TESS was run 20 times for each 2–20 K values using the conditional autoregressive admixture model (CAR) considering discrete sampling across the sampling range with interaction parameter 0.7, 200,000 burn-ins and 1,000,000 sweeps. The results were averaged with CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) and visualised with DISTRUCT 1.1 (Rosenberg 2004). We created a template map using a minimum convex hull of sampling sites with ArcMap Version 10.1 (Esri Corp, Redlands, CA) as an input into TESS in order to generate posterior predictive maps of admixture proportions for each run. To predict the admixture proportions across space, we visualised the spatial interpolation of the Q-matrix from posterior predictive maps of admixture proportions with an algorithm implemented within the software TESS AD-MIXER (Mitchell et al. 2013).

Resistance surface, isolation by distance (IBD) and isolation by environment (IBE) analyses

Land cover data used to develop the resistance surface layer for connectivity analyses were derived from the Corine Land Cover (CLC) layer (EEA 2012) and the layer of cool mountainous, very humid climate (Lapin et al. 2011) which were collapsed into ten land cover categories (Table SM-1, Supplementary Material). Based on the knowledge that the capercaillie is an umbrella species of high-altitude spruce forests in Central Europe, we overlaid the layer of coniferous forest (EEA 2012) with the cool mountainous very humid climate layer (Lapin et al. 2011). Then we extracted all coniferous forests matching the cool mountainous very humid climate. The resulting land category was called the "mountain coniferous forest" and considered as the least resistant surface feature (Table SM-1, Supplementary Material). Based on the literature review (Storch 1994, 2002; Saniga 2004; Jacquin et al. 2005; Pascual-Hortal and Saura 2008; Thiel et al. 2008) and expert knowledge on capercaillie habitat selection, we assigned a resistance value 1–10 to each land cover category (Table SM-1, Supplementary Material) where 10 was the most resistant surface. Boundaries of the subpopulations were delineated by the minimum convex polygon (MCP) including all sample sites with a buffer of 2.5 km (median of capercaillie dispersal movements). The delineated MCPs were then used in LINKAGE MAPPER (McRae and Kavanagh 2011) to detect dispersal paths requiring the least cost of energy for dispersal movement (least-cost paths, LCP). Pairwise Least-Cost paths analysis using cost-weighted distances allowed the quantification of the energy expenditure by capercaillie individual moving

from one area to another. In the PINCH POINT MAPPER (McRae 2012b), we detected the "pinch points" representing sections of narrowed or "bottlenecked" corridors where capercaillie movement is restricted due to unfavourable habitat conditions caused by physical features and where alternative pathways are not available (McRae et al. 2012). The pinch point areas represent hot spots of the conservation priorities because further deterioration of these habitats can disproportionally disrupt overall habitat connectivity (Dutta et al. 2015). Three alternative cost-weighted cut-off distance values (2.5, 5, 10 km) were tested to optimise reasonably narrow corridor widths without the loss of alternate, redundant branches in several linkage zones. Cost-weighted corridor "width" was set to 2.5 km. We calculated the values of landscape resistance between adjacent pairs of pinch points using CIRCUITSCAPE (McRae et al. 2008) implemented in the PINCH POINT MAPPER (McRae 2012b). All areas restricting movement between two locations within the landscape should be considered as barriers when implementing any restoration measures to promote connectivity (McRae et al. 2012). In case of the capercaillie, such features may include agricultural land and industrial zones. These features were mapped in the Barrier Mapper (McRae 2012a) with detection radius ranging from 1 to 5 km and the radius step value of 1 km. Landscape data based on satellite imagery often suffer from classification errors, and thus, we examined the permeability of the detected barriers and the least-cost paths to reduce the effect of classification error in connectivity analysis products (McRae et al. 2012). We physically verified all the pinch points and barriers in the field. Further, to find out whether genetic distances are reflecting the isolation by distance (IBD) or the complex isolation by environment (IBE) patterns, we fitted a linear mixed effect models in R package *lme4* (Bates et al. 2015) using three different types of genetic distances as response variable and three types of standardised isolation matrices as explanatory variables with the population ID as random effect. We used G'_{ST} (Hedrick 2005), G''_{ST} (Meirmans and Hedrick 2011) and D_{Jost} (Jost 2008) as response variables and the pairwise Euclidean distance matrix, the pairwise least-cost path distances and the pairwise resistance matrix (circuit theory) as fixed effects. Due to the small sample size and the high correlation between explanatory variables ($r > 0.7$), we tested them separately ending up with nine models (3 response × 3 explanatory variables). Then we applied an information-theoretic approach using the corrected Akaike's Information Criterion (AICc) for small sample sizes (Anderson and Burnham 2002) to choose the most parsimonious models of the three sets. All tests were considered significant at $P \leq 0.05$.

Results

Genetic variation

In total, we identified 163 individual genotypes. The microsatellite marker BG16 was excluded from the statistical analyses due to low amplification success. Nine loci evolved independently in randomly mating populations, and at any particular loci no deviation was detected from either Hardy–Weinberg equilibrium or from linkage disequilibrium (LD) across all studied populations. Altogether, we identified 73 female and 90 male individuals resulting in the sex ratio 1:1.23.

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 Volovec Mountains (*Priv. AR* = 0.33). The remains of the easternmost population in the Volovec Mts. significantly exhibited the highest deficiency of heterozygotes and deviation from HWE. A significant excess of heterozygotes was found only in the Muráň Plateau. Subpopulations from the Great Fatra and the High Tatras seemed to be close to Hardy–Weinberg equilibrium (Table 1).

The level of differentiation found in the Volovec Mts. compared to that observed in the rest of the Slovak populations was corroborated by the Bayesian approaches implemented in TESS and STRUCTURE. Evanno's method implemented in STRUCTURE HARVESTER indicated two clusters. The best $K = 2$ was determined when the deviance information criterion (DIC) reached a plateau (Chen et al. 2007). The first cluster comprised the Volovec Mts. genotypes, and the second cluster was represented by all individuals from the remaining populations. The TESS results also provided clear differentiation of the Volovec Mts. from the other subpopulations. Thus, both approaches confirmed two distinct clusters within the Western Carpathians. The map of the spatially interpolated

Q-matrices clearly differentiated the subpopulations of the Low Tatras—east and west, the Great Fatra, the High Tatras and the Muráň Plateau from a subpopulation in the Volovec Mountains (Fig. SM-1).

Connectivity and gene flow analyses

We derived the pairwise genetic distance, the Euclidean distance, the least-cost distance and the resistance distance between all six studied subpopulations. Majority of the sample sites were located in the coniferous forests with cool mountainous very humid climate. Only the elusive peripheral population of the Volovec Mountains and four individuals of the Great Fatra Mountains were located out of the mentioned conditions.

The genetic distance between examined capercaillie subpopulations was confirmed to be significantly affected by all three types of the isolation matrices at comparable magnitudes; however, the resistance matrix explained most of the variation in all three types of the genetic distances (Table 2). This supports the assumption that isolation of the subpopulations is determined by the complex of environmental features rather than just the straight distance between them.

The pairwise least-cost paths distance matrix truncated to 2.5 km path width varied from 35.65 to 286.41 km (Fig. 2) and corresponded to real landscape features. The critical corridors (also called the pinch points) as well as the barrier centres precisely corresponded to the real landscape features verifying the reliability of our results. The most critical corridor which is overlapped by a barrier is located in the Tatra Basin in the area between Važec and Východná villages (Fig. 3). All pairwise corridors between subpopulations except the Low Tatras east and west were separated by landscape barriers (Fig. 3). Bayesian clustering analyses (Fig. SM-1) confirmed the existing gene flow across the permeable barriers, where the capercaillie

Table 1 Genetic variation indices (mean and standard deviation SD) of six capercaillie subpopulations: 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 and Cockerham 1984) with confidence interval (CI) 95%

Sampling area	N	AR	Priv.AR	NA/loci	H_E	H_O	F_{IS}	CI (95%)
Volovec Mountains	20	3.77	0.33	3.89	0.53	0.42	0.23	(0.08–0.33)
SD (±)		1.07	0.58		0.21	0.21		
High Tatras	15	4.11	0.14	4.11	0.55	0.55	0.04	(−0.15–0.15)
SD (±)		0.99	0.32		0.18	0.21		
Muráň Plateau	44	4.27	0.19	5.22	0.58	0.65	−0.10	(−−0.19–0.04)
SD (±)		1.22	0.28		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 (±)		0.87	0.30		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 (±)		0.88	0.27		0.17	0.22		
Great Fatra	27	4.05	0.11	4.56	0.56	0.57	−0.01	(−0.13–0.10)
SD (±)		1.11	0.18		0.13	0.14		

Table 2 List of three sets of the AICc-based candidate models explaining the relation of genetic distances G'_{ST} (Hedrick 2005), G''_{ST} (Meirmans and Hedrick 2011) D_{Jost} (Jost 2008) and Euclidean distances and environmental measures between capercaillie subpopulations with regression coefficients (β) and standard errors (SE) of the isolation matrices and AICc difference and Akaike's weights (w) of the models

Response variable	Explanatory variable	β	SE	ΔAICc	w
G'_{ST}	Least-cost distance	0.070	0.0158	3.89	0.12
G'_{ST}	Euclidean distance	0.074	0.0150	5.46	0.05
G'_{ST}	Resistance	0.071	0.0178	**0.00**	**0.83**
G''_{ST}	Least-cost distance	0.068	0.0188	3.09	0.16
G''_{ST}	Euclidean distance	0.069	0.0187	3.68	0.12
G''_{ST}	Resistance	0.067	0.0205	**0.00**	**0.73**
D_{Jost}	Least-cost distance	0.032	0.0086	2.66	0.17
D_{Jost}	Euclidean distance	0.032	0.0088	2.45	0.19
D_{Jost}	Resistance	0.031	0.0096	**0.00**	**0.64**

The top models explaining the variation in genetic distance between populations are in bold
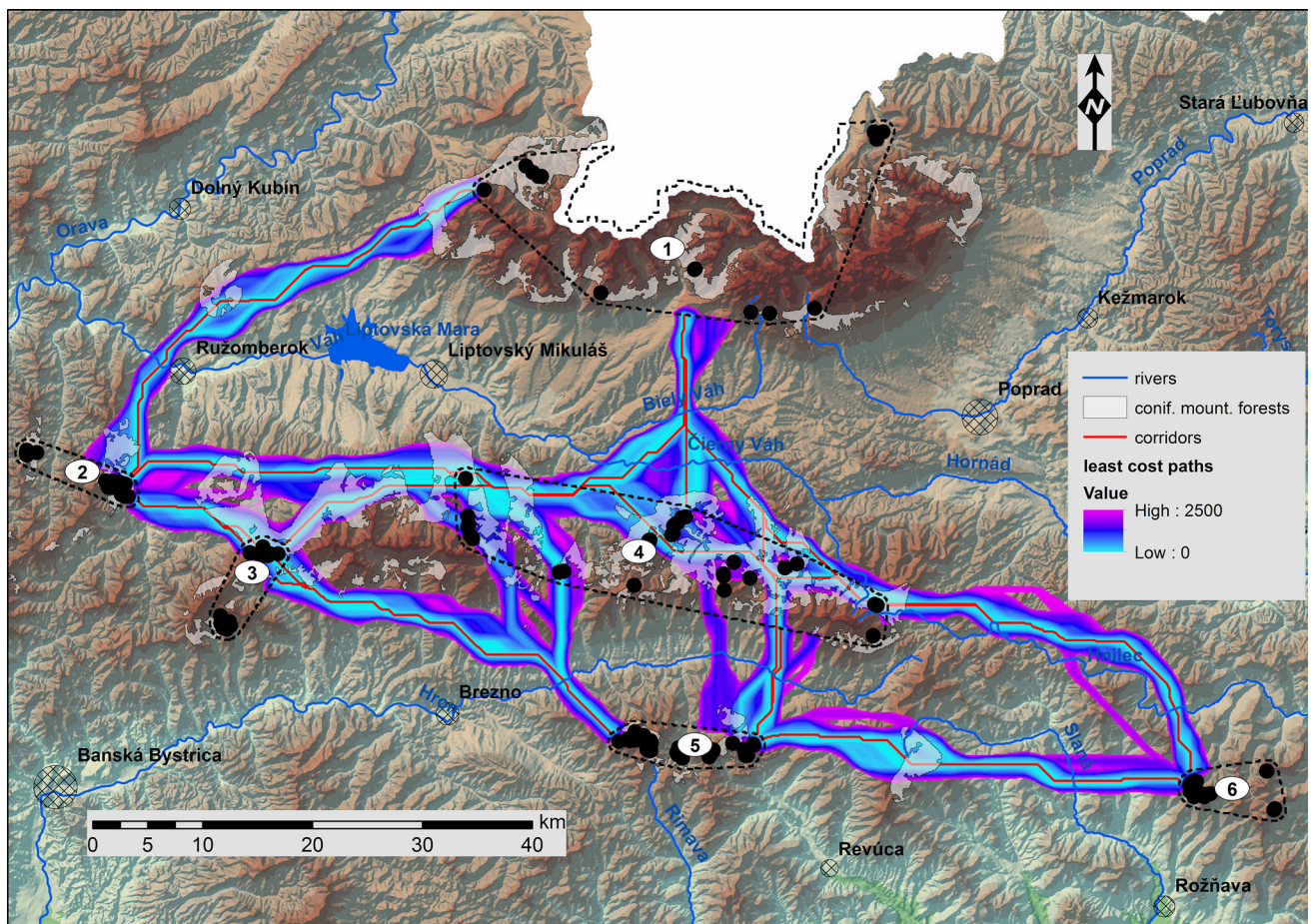
Fig. 2 Location of the least-cost paths (scaled from *blue* to *purple*) truncated to 2.5 km width (median of the capercaillie dispersal) among subpopulations: High Tatras (*1*), Great Fatra (*2*), Low Tatras—east (*3*), Low Tatras—west (*4*), Muráň Plateau (*5*), Volovec Mountains (*6*). (Color figure online)

dispersal is likely possible mostly westwards from the Volovec Mts. The critical corridor (the pinch point) overlapping with barrier centre between the High Tatras and the Low Tatras was considered a permeable barrier according to the genetic analyses. Our habitat connectivity analysis confirmed the pattern of isolation by the complex of
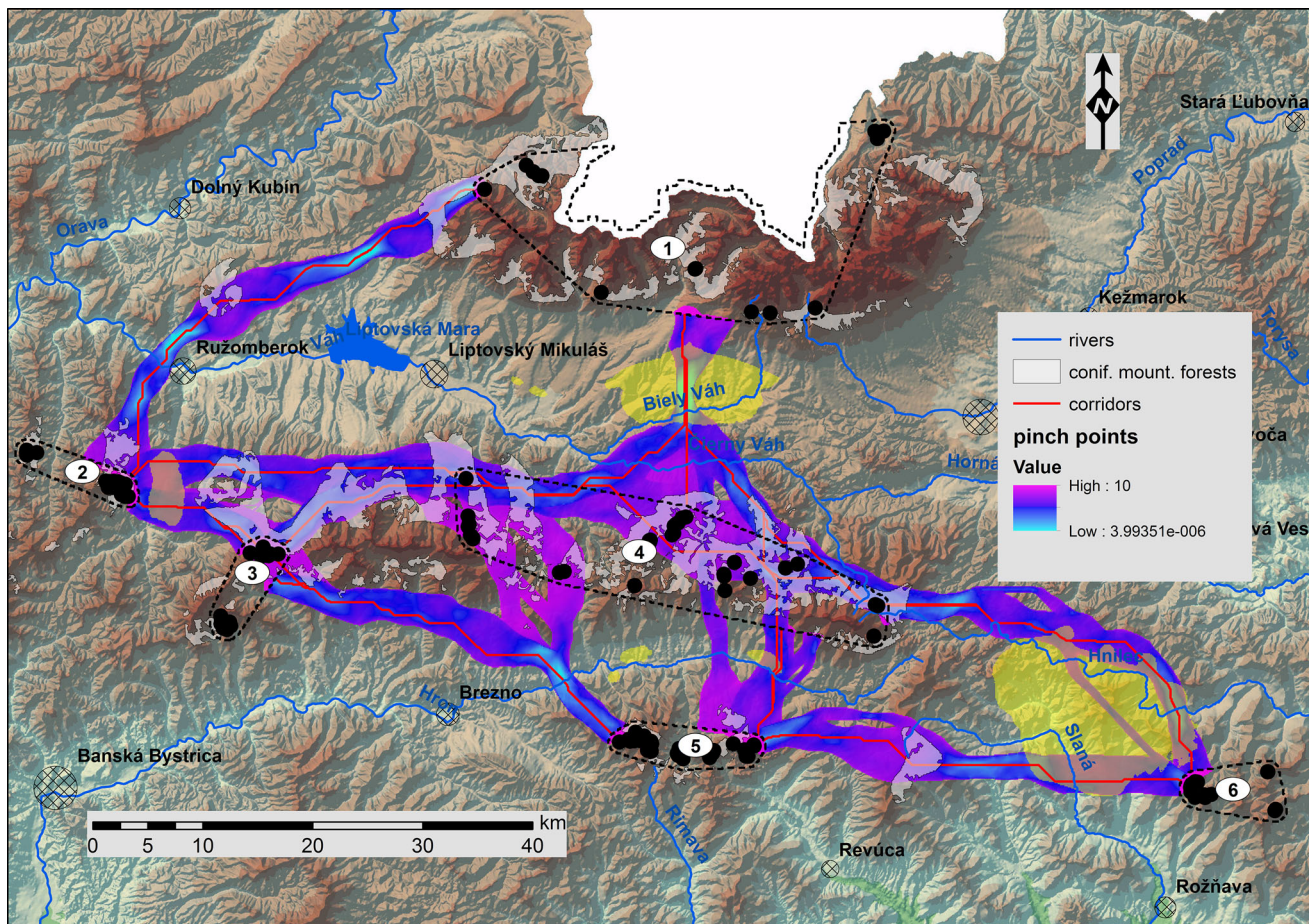
Fig. 3 Map of the barriers (*yellow polygons*) and the critical habitats (*pinch points*) illustrating the most important areas (*light blue colour*) for sustaining the gene flow. *Colouration* of the critical habitats reflects the permeability of the particular corridor from low to high. When low-permeability corridors overlap with barriers, the corridor might be impermeable and the management of this area should be focused on restoration of the migration. Subpopulation spots: High Tatras (*1*), Great Fatra (*2*), Low Tatras—east (*3*), Low Tatras—west (*4*), Muráň Plateau (*5*), Volovec Mountains (*6*). (Color figure online)

environmental factors (Table 2). In the easternmost population of the Volovec Mountains, corridors were interrupted by the landscape barrier (Figs. 2, 3) which is located westward of the Volovec Mountains, and according to the genetic data, this barrier decreases the permeability of the least-cost paths connecting the Volovec Mountains with their nearest neighbours.

Discussion

Within the Western Carpathians, we identified two genetically distinct groups of the capercaillie suggesting a long-term isolation. We also found evidence of the isolation by the complex of environmental factors limiting the gene flow between those two capercaillie groups. The landscape genetic analysis confirmed low permeability and restriction of the gene flow at several locations especially across the barrier between subpopulations of the High Tatras and the

Low Tatras which are separated by a wide basin with high human activity (Fig. 3). Recent population differentiation studies of the Western capercaillie along the Carpathian mountain ridge also clearly distinguished the Western Carpathian population from adjacent populations inhabiting the Ukrainian and the Romanian Carpathians (Klinga et al. 2015). This might be either a result of the different population origins or more likely it is caused by the long-term isolation between the Western and the Eastern Carpathian population. Indeed, the continuing trend of habitat fragmentation and consequently further isolation of subpopulations within the Western Carpathian plays the most important role for future survival of the species.

In order to maintain a genetic connectivity in the peripheral regions and to sustain the gene flow capabilities of the landscape, a promotion or a restoration of the source-sink population dynamics is recommended (Jacob et al. 2006). Several studies reported a median natal dispersal of the adult capercaillie of 2–3 and 5–6 km for juvenile birds

(Myberget [1978], Swenson [1991]). However, the studied subpopulations were 10–98 km apart, indicating restriction of the gene exchange between them as well as underlining the importance of the transient habitats. The long-distance dispersal movements (over 100 km) have also been described by several studies (Nappée and Douhéret [2004], Borchtchevski and Moss [2014]); however, it is likely that only a fraction of the population would be able to perform such movements since migration is known to be associated with high energy costs and predation risk. Therefore, besides protection of the habitats with permanent capercaillie presence, the identification and protection of the potential dispersal corridors is necessary to sustain large-scale gene flow and thus an effective conservation of the species. Especially important are those corridors where the capercaillie movement might be restricted due to unsuitable forestry management.

The capercaillie in Slovakia is listed as endangered species (Demko et al. [2013]). However, besides the official declaration of the Special Protection Areas included in the Natura 2000 ecological network, set up under the Habitats Directive 92/43/EEC within the Birds Directive 79/409/EEC later emended as Directive 2009/147/EEC, the specialised habitat needs have not been implemented in the forest management plans. Forest management affects the structure, density and canopy composition of the forest and consequently the ground vegetation cover due to the amount of light reaching the forest floor. For instance, the amount of light in capercaillie habitats influences the balance between bilberry (*Vaccinium myrtillus*) and cowberry (*Vaccinium vitis-idaea*). Forest ground vegetation with more than 20% bilberry cover is considered as a good-quality habitat for capercaillie (Broome et al. [2014]). In coniferous habitats where the bottlenecked critical corridors (pinch points) are located, altering light levels by management to favour bilberry through the thinning and felling could significantly increase suitability of the habitat. Forest management in such habitats should be focused on silvicultural techniques favouring the capercaillie habitat requirements in order to create a network of suitable habitat patches enabling steady gene flow among the fragmented capercaillie subpopulations. Management practices preserving the natural vertically and spatially differentiated structure of the capercaillie habitats with respect to its special requirements need to be prioritised over timber production (Mikoláš et al. [2015]). Otherwise, further habitat loss resulting in observed population decline may reduce the gene flow to a critical level deepening genetic differentiation and thus vulnerability of the currently easternmost capercaillie subpopulation in the Western Carpathians.

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