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Using noninvasive DNA sampling to estimate abundance and some genetic properties of the Brown bear (*Ursus arctos*) in the Western Carpathians

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Abstract

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In Slovakia, there is a constant need for scientifically based information to manage its bear population after it has been allowed to increase in size and range. In this study we assessed population size, sex structure and genetic variability of a local brown bear population in Strážovské vrchy Mts (North-western Slovakia). This goal has been achieved by using noninvasive method of genetic sampling in 2011–2012. Brown bear DNA for analysis was obtained from 94 out of 232 samples (41%), among which 24 unique genotypes were identified. Average observed heterozygosity was 0.56 in 2011 and 0.63 in 2012. Minimum population size was determined from the number of unique genotypes and population size estimates were calculated via Lincoln-Peterson CMR method (n = 38) and Rarefaction models according Kohn method (n = 36), Eggert method (n = 25) and Chessel's equation (n = 19). Additionally, relative spatial activity and movement pattern of some individuals have been inferred from the distribution of typed samples.

Keywords

noninvasive genetic tracking, population size, rub trees, sex ratio, spatial activity pattern

Introduction

The brown bear (*Ursus arctos* Linnaeus, 1758) is the most widespread ursid in the world with a distribution in Europe, Asia and North America traversing from northern arctic tundra to dry desert habitats (Swenson et al., 2000). In Europe, almost all European bears belong to large populations occurring in Eastern and Northern European countries, whereas less than 1% of all European bears are found in western and south-western Europe (Zedrosser et al., 2001).

In Slovakia, the brown bear (*Ursus arctos*) population is increasing and expanding after that successful conservation measures were employed during the 20th century (Hell and Slamečka, 1999). Currently, the Western Carpathian bear population extends across all mountain ranges of central, northern, northwest and northeast Slovakia but shows only a fragmented, discontinuous habitat pattern due to topographic characteristics of the country's landscape resulting in mountain ranges with prime bear habitat separated by areas of denser human settlement and activity in broad river valleys (Rigg and Adamec, 2007). As a consequence, for Slovak society, there is a constant need for scientifically based information to successfully manage its bear population. Two important issues in conservation and management are of pressing concern: 1. to understand how bears use their habitat, at different spatial-temporal scales, and 2.

to estimate size, structure and trend of the population from regional to national scales.

However, such data are often difficult to obtain, especially for rare, elusive or species with large home ranges that overlap to certain degree (Bellemain et al., 2005). To overcome many of these obstacles several methods have been developed over a time to address the needs of conservation biologist and researchers. Particularly, the development of noninvasive DNA-based methods have brought several advantages over conventional methods as genetic samples (i.e., hairs or faeces) are easily collected without the need to see, disturb or trap the animal (Taberlet et al., 1999). Because each individual is characterized by a unique multilocus genotype, it is possible to determine the number of animals sampled and, through the use of statistical models, also to estimate population size (Kohn and Wayne, 1997; Mowat and Strobeck, 2000; Bellemain et al., 2005). Additionally, important population data on behavioural ecology like home range, habitat use and spatial activity pattern can be partially inferred from distribution of typed samples (Kohn et al., 1999).

Brown bears had been apparently absent from 1930s until the mid1960s (Hell and Slamečka, 1999; Rigg and Adamec, 2007) but re-colonised Strážovské vrchy Mts during the period 1967–1984, when the recovering Western Carpathian bear population expanded its range 40 km north-westwards (Janík et al., 1986). According to Turček (1949) and Feriancova (1955) bears still did not occur in this area until 1960 and the first reference to 4 migrating bears comes from range-wide bear census in 1966 and 1968 (Škultéty, 1970; Randík, 1971). Currently, Brown bears in Strážovské vrchy Mts form a stable sub-population in the westernmost portion of their Carpathian range.

Before the beginning of this study, bear numbers were estimated up to 30 individuals based on snow and mad tracking, direct observations and camera trapping (Pepich and Pepich, 2012; Pepich and Pepich, 2013). We, however assume that some of them might have home ranges extending beyond the study area (data from telemetry or DNA studies are not yet available from nearby mountain ranges). The main aim of our study was to examine the degree of genetic variability and several population parameters such as minimum population size, population size estimates and social structure. Relative spatial activity and movement pattern of some individuals have been consequently inferred from distribution of typed samples as described by Kohn et al. (1999).We also tried to evaluate whether it is feasible to use hairs from rub trees to produce population estimates.

Material and methods

Study area

Fieldwork was conducted in the Protected landscape area (PLA) Strážovské vrchy (included in the NATURA 2000), which extends over an area of 300 km^2 in northwest Slovakia, forming part of the Inner Western Carpathian Mountains. To obtain more credible data, some additional areas of 200 km^2 that lie immediately to the PLA in the east, west and south were also included in the fieldwork because all these parts form relatively compact bear habitat and there is an assumption that this area is shared by one sub-population unit. Total size of the study area (Fig. 1) was 500 km^2. Elevations range from c. 200 m in walleyes to 1,213 m in the central part of Strážovské vrchy Mts and 1,352 m in the south of Lučianska Malá Fatra Mts. Most of the area is covered with intensively managed deciduous and mixed forest (78%), dominated by European beech (*Fagus syl-*

Fig. 1. Location of the study area in Slovakia and its total size (500 km^2).

vatica L.) followed by Norway spruce (*Pices abies* L.) and Sycamore maple (*Acer pseudoplatanus* L.), 19% accounts for mountain meadows and farmland and 3% for built-up and water areas (Pepich and Pepich, 2012). The study area covered fully 8 and partially 20 hunting grounds.

Sample collection

The noninvasive sampling was conducted in two consecutive years 2011 and 2012. Each year, there were two study periods (spring-early summer and autumn). The study area was divided into 22 parts (transects), where regular searches for bear hair and faeces were conducted in 2–3 weeks' time periods. Samples for genotyping were detected only in the southern parts (200 km^2) of the study area (Fig. 2).

A total 232 samples were collected of which 191 were hair samples and 41 were faeces samples. Hair samples were collected regularly from 48 out of 90 known bear rub trees (n = 181), several day beds (n = 4) and four dens (n = 6), whereas all 41 faeces samples were collected opportunistically throughout the whole year on forest roads and animal trails. Individual hairs or clumps of hair stuck in bark were scanned visually and those that showed probability of the dry cells were placed in an envelope with cautious approach to prevent contamination by human DNA. Consequently, all remaining hairs were removed mechanically (using a brash) from the bark to prevent recollection of the same hair in next sampling collection. No hair plucking devises (barbed wire) were used for hair collection at rub trees, only hair naturally stuck in bark by bear rubbing against a tree was used. Searches for bear faeces were conducted throughout the study area, even in parts, where bears are usually rare or non-existent (Pepich and Pepich, 2012). When a bear scat was discovered, sample of 1–2 cm^3 was picked up with a stick of wood and put in a labelled plastic bag. A different stick and plastic bag were used for each sample. Only samples from relatively fresh faeces were taken (subjective opinion). For each sample, a sampling date, a geographical location (WGS84) and the collector's name were recorded.

Sample preservation and DNA extraction

To limit the degradation of DNA before extraction, we preserved hair dry or with silica gel. Each hair sample (5–50 hairs) was placed in a different labelled envelope, whereas all faeces samples were stored dry in a freezer (–20 ºC) as described by Walsh et al. (1991), Taberlet et al. (1997).

All extractions were carried out in a room dedicated to processing noninvasive samples like hair and faeces.

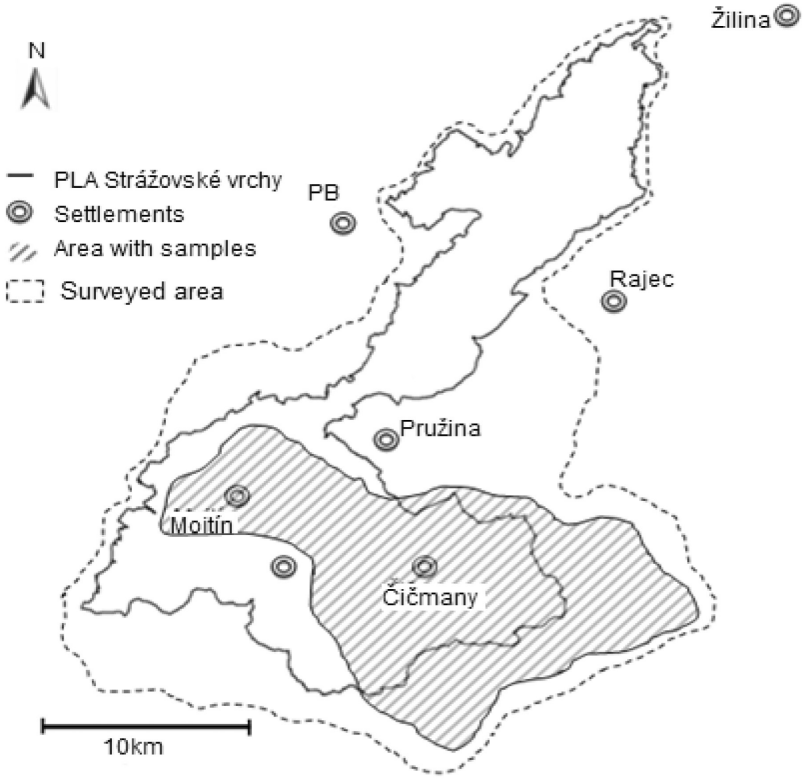

Fig. 2. Distribution of samples within the study area in 2011–2012.

For all collected hair samples, only one extraction was carried out per sample and only one hair was used per extraction. The suitable hairs were chosen by binocular magnifier loupe according to the presence of hair bulbs (dry cells). The root part (5 mm) of the hair was cut and added to 50 µl of extraction buffer (10 mM Tris-HCL pH 8.0, 50 mM KCL, 0.5% Tween20) with addition of 1 µl of the Proteinase K (20 mg ml^{-1}). For every collected faeces sample, DNA extractions were performed using the QIAmp DNA Stool mini Kit (Qiagen 51504) according the manufacturer's instructions. This kit has been developed especially for this type of material. To detect cross-contamination, tubes without bear faeces or without hair root were treated identically as samples through both the extraction procedure and the subsequent amplification (Walsh et al., 1991).

Genetic typing and sex determination

For individual genotyping, ten polymorphic microsatellite loci Mu10, Mu50, Mu23, G10L, Mu15, G10C, Mu59, G10P, Mu09, G1D, SRY (Table 1) were amplified using PCR. These markers were chosen for their discriminatory power (loci with the lowest PI) based on previous brown bear noninvsive data set (Skrbinšek at al., 2010).

Bear sex was determined by amplification of SRY fragment on the Y chromosome together with other microsatellites (Skrbinšek at al., 2010). Primers were fluorescently labelled. All markers were amplified in one reaction and products were amplified using Qiagen multiplex Mix (Qiagen), the final mixture volume for PCR reaction was 6 µl, DNA volume was 2 µl. Preliminary analyses were carried out and only samples that could be typed in some loci were kept for subsequent analysis. Consequently, PCR reactions and electrophoresis were carried out three times. PCR products (fragment lengths) were analysed using automatic sequencer ABI 3130 (Applied Biosystems). The final mixture volume consisted of 9 µl formamide; 0.2 µl size standard and 0.8 µl PCR product. Genotypes were determined by using GeneMapper 4.0.

Table 1. Characteristics of used markers

Primer	Microsatellite sequence	Flourescence	Allele size range (bp)	Reference
Mu10F	ATTCAGATTTCATCAGTTTGACA	FAM	121–127	Bellemain et al., 2004
Mu10R	TCAGCATAGTTACACAAATCTCC			
Mu50F	GTCTCTGTCATTTCCCCATC	FAM	94–100	Bellemain et al., 2004
Mu50R	AACCTGGAACAAAAATTAACAC			
Mu23F	TAGACCACCAAGGCATCAG	NED	143–157	Bellemain et al., 2004
Mu23R	TAGACCACCAAGGCATCAG			
G10LF	ACTGATTTTATTCACATTTCCC	PET	141–161	Bellemain et al., 2004
G10LR	GATACAGAAACCTACCCATGCG			
Mu15F	CTGAATTATGCAATTAAACAGC	PET	117–129	Taberlet et al., 1997
Mu15R	AAATAAGGGAGGCTTGGGT			
G10CF	AAAGCAGAAGGCCTTGATTTCCTG	VIC	122–138	Paetkau et al., 1995
G10CR	GGGACATAAACACCGAGACAGC			
Mu59F	GCTCCTTTGGGACATTGTAA	NED	98–118	Bellemain et al., 2004
Mu59R	TGACTGTCACCAGCAGGAG			
G10PF	TACATAGGAGGAAGAAAGATGG	VIC	141–173	Taberlet et al., 1997
G10PR	AAAAGGCCTAAGCTACATCG			
Mu09F	AGCCACTTTGTAAGGAGTAGT	VIC	190–196	Taberlet et al., 1997
Mu09R	ATATAGCAGCATATTTTTGGCT			
G1DF	CTACTCTTCCTACTCTTTAAGAG	FAM	171–178	Paetkau et al., 1995
G1DR	ATCTGTGGGTTTATAGGTTACA			
SRYF	GAACGCATTCTTGGTGTGGTC	PET	75	Taberlet et al., 1997
SRYR	TGATCTCTGAGTTTTGCATTTG			

Reliability of the DNA results

To distinguish individual samples among themselves with confidence we used sufficient number of polymorphic markers with low probability of identity – PI (Paetkau and Strobeck, 1994; Waits et al., 2001). Using five markers with lowest PI, the probability of finding identical non-kin genotypes would be 1 : 3.5 million what highly exceeds total world brown bear population and it is therefore very little probable that in the study area occur two bears with the identical genotype (Table 2). Reliability of genotypes was assessed with the program RELIOTYPE with default settings.

Table 2. Probability of identical genotypes

	PI (biased)	PI (unbiased)	PI (sibs)
Mu59	$9.131\ 10^{-2}$	$5.316\ 10^{-2}$	$3.891\ 10^{-1}$
Mu23	$8.826\ 10^{-3}$	$2.956\ 10^{-3}$	$1.539\ 10^{-1}$
G1D	$1.242\ 10^{-3}$	$2.859\ 10^{-4}$	$6.688\ 10^{-2}$
G10P	$2.054\ 10^{-4}$	$3.143\ 10^{-5}$	$3.086\ 10^{-2}$
Mu15	$3.471\ 10^{-5}$	$3.594\ 10^{-6}$	$1.431\ 10^{-2}$
G10C	$7.286\ 10^{-6}$	$5.801\ 10^{-7}$	$7.039\ 10^{-3}$
Mu09	$1.916\ 10^{-6}$	$1.154\ 10^{-7}$	$3.692\ 10^{-3}$
Mu10	$5.052\ 10^{-7}$	$2.631\ 10^{-8}$	$2.002\ 10^{-3}$
Mu50	$1.475\ 10^{-7}$	$6.487\ 10^{-9}$	$1.105\ 10^{-3}$
G10L	$8.064\ 10^{-8}$	$2.994\ 10^{-9}$	$8.296\ 10^{-4}$

Genetic variability

Observed (H_o) and expected (H_e) heterozygosity were calculated using Cervus 3.0 software (Field Genetics). Observed descriptive statistics for each locus (mean number of alleles per locus, heterozygosities and polymorphic information content) were calculated from genotypes.

Population size estimates

Minimum population size was determined by number of unique genotypes successfully typed from collected samples in 2011 and 2012 (Taberlet et al., 1997; Bellemain et al., 2005). Total population size estimates were subsequently calculated from faecal and hair genotypes by using Lincoln-Peterson CMR estimator and Rarefaction statistical models.

CMR estimator

The CMR (Capture-Mark-Recapture) estimator was based on grouping identical multilocus genotypes and compiling a "capture" and "recapture" history for each individual by dividing the data set into 20 weekly sampling period for 2011 and 16 weekly period for 2012 or by dividing the data set into spring – early summer sampling period and autumn sampling period in 2011 and 2012 respectively. Only the weeks with typed samples were considered for estimating population size. If an individual's hair or faeces were captured two or more times within the same capture period, only one capture was considered. Consequently, the following Lincoln-Peterson CMR estimator was applied to estimate the population size (Seber, 1982):

$$N = \frac{(C+1)(M+1)-1}{R+1},$$

where N = estimate of total population size, M = number of unique genotypes typed in 2011 or in autumn sampling period, C = number of unique genotypes typed in 2012 or in spring early summer sampling period, R = number of unique genotypes that were typed in both years 2011 and 2012 or were retyped in both sampling periods within the same year.

Rarefaction indices

Following the method described in Kohn et al. (1999), we compared the multilocus genotype of each sample with all those drawn previously and calculated the population size as the asymptote of the relationship between the cumulative number of unique genotypes and the number of samples typed. This curve is defined by the equation: $y = (ax) / (b + x)$, where a is the asymptote, x is the number faeces sampled, y is the number unique genotype, and b is the rate of decline in the value of slope. Chessel proposed to use equation ($y = a - a(1 - 1/a)^x$) which in the case of heterogeneity of capture probability seems to underestimate the population size. Eggert et al. (2003) derived another estimator with a similar equation: $y = a(1 - e^{bx})$.

Results

We managed to collect 232 samples (191 hair and 41 faeces samples) in two consecutive years 2011 and 2012 (Table 3). In 2011, 20 faecal samples and 150 hair samples were collected and 68 (41%) were successfully amplified for ten loci (including the sex locus). From these 68 samples, 16 unique genotypes were obtained (50% males and 50% females). Each multilocus genotype was found from 1 to 29 times. In 2012, 21 faecal samples and 41 hair samples were collected and 26 (42%) provided enough DNA for a complete genetic typing at all ten polymorphic loci (including the sex locus). From these 26 samples, 13 unique genotypes were obtained (46% males and 54% females). Each multilocus genotype was found from 1 to 6 times. In 2012 we sampled 5 individuals (32%) identified also in 2011. In total, sufficient brown bear DNA for analysis was obtained from 94 (41%) out of 232 samples.

Table 3. Sampling for DNA analysis in 2011 and 2012

	Hair		Faeces		Total		Unique genotypes
	Samples	Typed	Samples	Typed	Samples	Typed	
2011	150	54	20	14	170	68 (41%)	16
2012	41	15	21	11	62	26 (42%)	13
Total	191	69 (37%)	41	25 (63%)	232	94 (41%)	24

Using programme Gimlet (Valiére, 2002), a total of 31 different genotypes were identified, but only 24 were identified with confidence among these 94 samples. Other seven genotypes were considered not to be reliable due to the missing alleles. If only one allele was missing and other alleles matched with other genotype such samples were believed to belong to the same individual. Three samples (3.1%) that showed no available data on more than two alleles were not considered for further analysis and in some samples (5%) sex of individuals could not be determined.

Minimum population size was determined by number of unique genotypes successfully typed from collected samples in 2011 (16 individuals) and 2012 (13 individuals) and 24 individuals in 2011–2012. Total population size estimates were calculated via Lincoln–Petersen CMR estimator. The CMR estimate was calculated using the number of unique faeces and hair genotypes typed in 2012 (C = 13), number of unique genotypes typed in 2011 (M = 16) and number of unique genotypes that were typed in 2011 and then retyped in 2012 (R = 5). Using the Lincoln–Petersen CMR model the total population size has been estimated to 38.6 individuals. The Lincoln-Petersen CMR model was also used to estimate population size in 2011 and 2012 respectively, where number of unique genotypes typed in autumn 2011 (C = 14) and autumn 2012 (C = 8), number of unique genotypes typed in spring-early summer 2011 (M = 7) and spring–early summer 2012 (M = 7), number of unique genotypes that were typed in spring-early summer 2011 and then retyped in autumn 2011 (R = 5) and number of unique genotypes that were typed in spring-early summer 2012 and then retyped in autumn 2012 (R = 2). Total population size was calculated by statistical models Lincoln-Petersen CMR and was estimated at 19 individuals in 2011 and 23 individuals in 2012 and 38 individuals in 2011–2012. The population size was also estimated by programme GIMLET to be 36 according to Kohn's method (Kohn et al., 1999), 25 according Eggert's method (Eggert et al., 2003), 19 according to Chessel's equation for joint analysis 2011 and 2012. Kohn method in 2011 accounted for 23 individuals and 53 in 2012. According to Eggert method there were 15 bears in 2011 and 26 in 2012, whereas according to Chessel's equation there were 11 bears in 2011 and 17 in 2012 (Fig. 3).

It seems that the best results were provided by Kohn method (n = 36) and Eggert (n = 25) method, which are relatively consistent with population size (n = 32) obtained in range-wide census conducted in 2012 (Pepich and Pepich, 2012). Chessel's equation provided underestimation of total population size as it was lower than obtained minimum population size (n = 24). Our findings confirm that Chessel's method has tendency to underestimate population size, especially in cases of heterogeneity of capture probability like is also our situation. Chessels method gave even lower estimates than minimum number of unique genotypes found.

Fig. 3. Population size estimates based on statistical models and minimum population size.

The number of alleles per locus ranged from three to six, with a mean observed heterozygosity of 0.55 in 2011. The number of alleles per locus ranged from four to seven, with a mean observed heterozygosity of 0.63 in 2012 (Table 4).

Five individuals (21%) out of 24 were typed in both years 2011 and 2012. Genotypes of ten individuals (42%) were sampled more times (2–29), whereas fourteen (58%) unique genotypes were sampled only once. All typed samples were found in an area of c. 200 km^2, in the central and south-eastern part of study area, which represents 40% out of the total study area (Fig. 2). Although, the presence of bears is occasionally reported even in other areas (south-western and northern) during our study we did not detect any hair or faeces samples in these parts of the study area. The sampling location of each genotype was plotted on a map to see their distribution (Fig. 4).

Table 4. Observed and expected heterozigosity and effective number of alleles in 2011–2012

	H_{exp}		H_{obs}		n_a		n_e	
Locus	2011	2012	2011	2012	2011	2012	2011	2012
G10C	0.62	0.6524	0.62	0.7419	5	5	2.6406	2.8772
G10L	0.27	0.1920	0.23	0.1724	3	4	1.3740	1.2377
G10P	0.66	0.6383	0.77	0.7333	4	4	2.9391	2.7650
G1D	0.70	0.6778	0.46	0.5862	4	4	3.3465	3.1033
Mu09	0.58	0.6296	0.62	0.6207	4	4	2.3960	2.6998
Mu10	0.55	0.5541	0.77	0.7097	3	4	2.2092	2.2427
Mu15	0.66	0.7598	0.54	0.7241	4	5	2.9157	4.1634
Mu23	0.76	0.7320	0.54	0.6129	6	7	4.1220	3.7320
Mu50	0.54	0.6165	0.31	0.5862	3	4	2.1818	2.6078
Mu59	0.77	0.7384	0.69	0.7931	5	7	4.2985	3.8227
Mean	0.61	0.6191	0.55	0.6281	5	4.8	2.6406	2.9252

H_{exp}, expected heterozygozity; H_{obs}, observed heterozygosity; n_a, observed number of alleles 59 n_e, effective number of alleles.

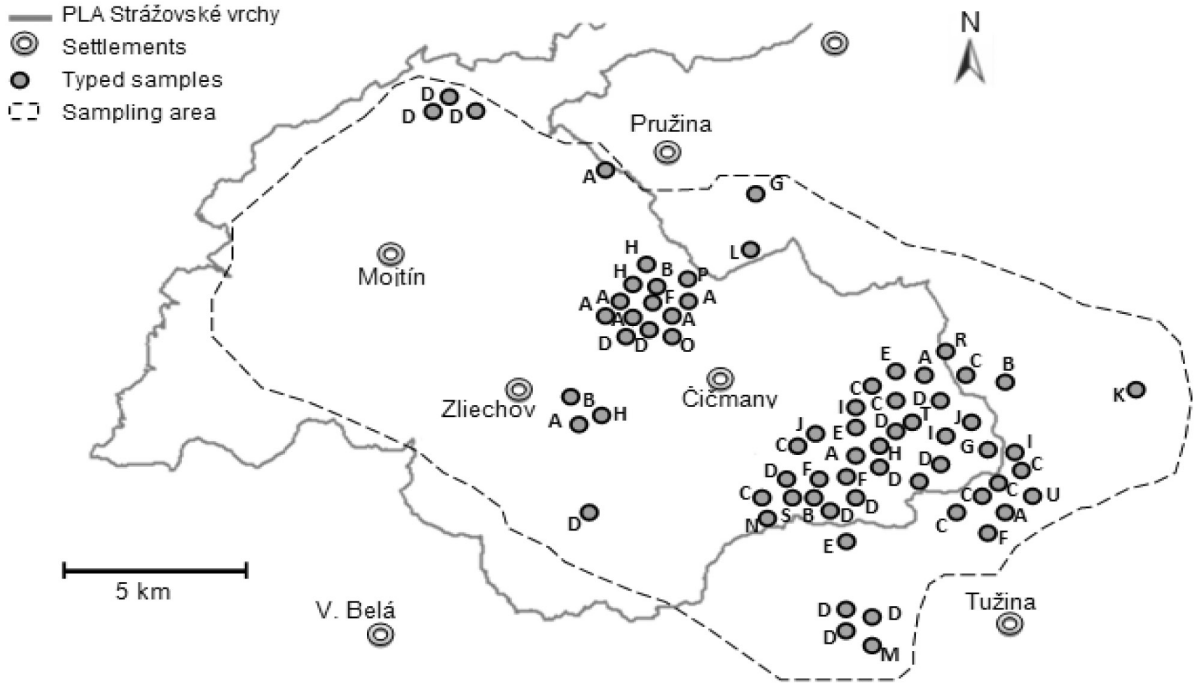

Fig. 4. Location of typed samples in the study area. The sampling area (250 km^2) indicates where hair and faeces samples were collected. Dark-grey circles represent unique genotypes. Sex structure of genotypes is as follows: A♂, B♀, C♂, D♂, E♀, F♂, G♀, H♂, I♀, J♂, K♂, L♂, M♀, N♂, O♀, P♂, R♂, S♀, T♀, U♀, V♀, X♀, Y♂, Z♀.

The spatial activity pattern was consequently estimated from distribution of typed samples. This was particularly possible for 10 unique genotypes whose samples had been typed more times and their subsequent polygons (MCP) could have been portrayed from the distribution of typed samples. The best results were obtained for one male (D) with 29 typed samples covering an area of 175 km^2 (Fig. 5) within seven different hunting grounds and ten cadastres in 30 days. All individuals with more typed samples showed wide spatial activity from spring to autumn but their late autumn / early winter and early spring samples were predominantly detected in one area, which indicates the main den site in Strážovské vrchy Mts.

Discussion and conclusion

This study demonstrated that it is feasible to obtain several population parameters like minimum population size, population size estimate and sex structure from noninvasively collected samples without capturing or even seeing the animals.

To facilitate this genetic study, we utilized data from a 10 year long fieldwork, where positions of all known bear rub trees, day beds and den sites were recorded (GPS). Data on bear distribution and spatial activity pattern obtained by annual ground tracking survey (2010–2012) and range-wide census (2010, 2012) were also taken into consideration. Very useful data were provided by camera trapping method on the feasibility of rub trees for genetic study because these trees proved to be visited not only by dominant males but also by other categories of bears such as females with cubs and young independent bears. Average number of bears visiting a particular rub tree (n = 10) accounted for 4.4 bears in 2011 and for 4.8 bears in 2012 (data supported by camera trapping).

Minimum population size was obtained by unique genotypes typed in each year. Population size estimate was calculated by statistical models: Lincoln-Petersen

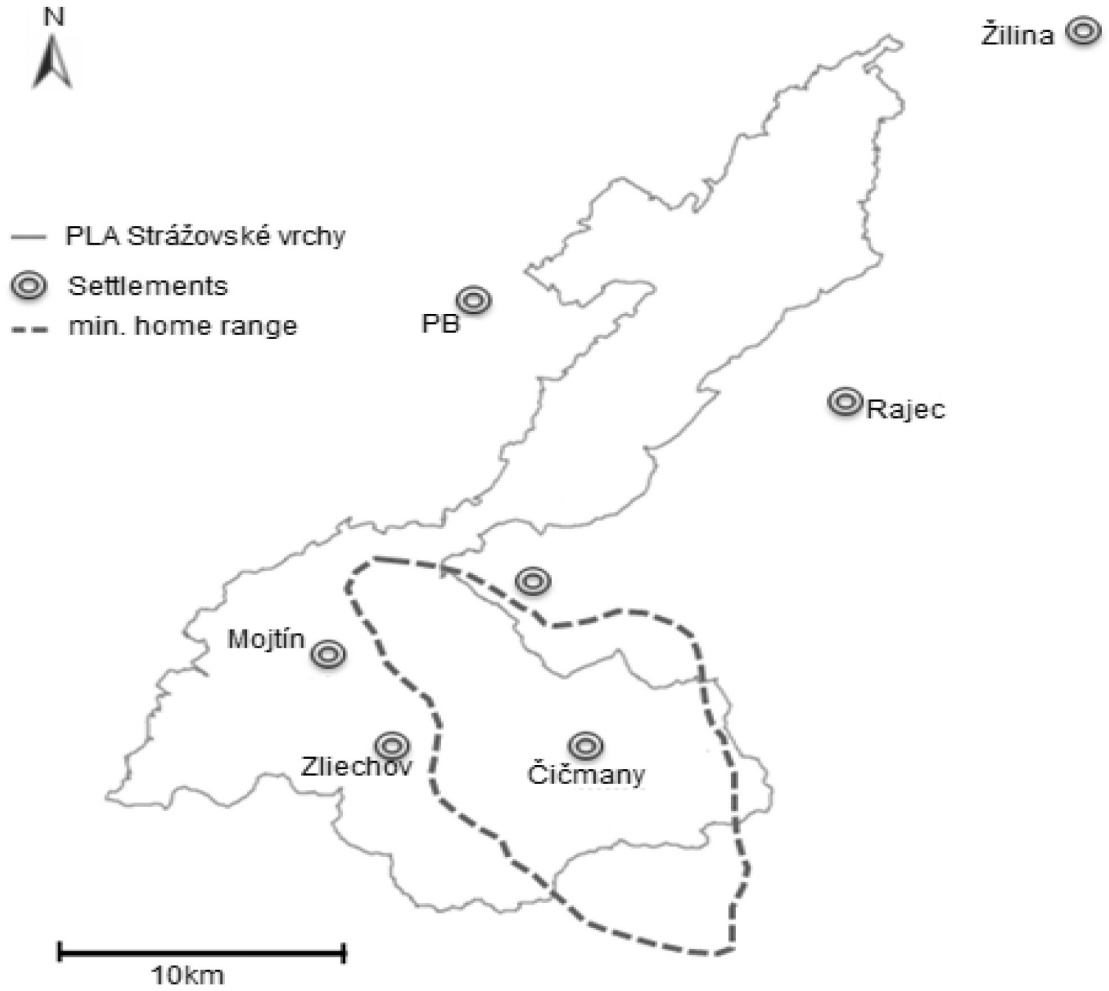

Fig. 5. Spatial activity pattern of a male bear (D) with MCP of 175 km^2.

CMR (n = 38) and rarefaction models according Kohn method (n = 36), Eggert's method (n = 25) and Chessel's equation (n = 19) for joint analysis 2011 and 2012. These population estimates were subsequently compared with reliable census results (excellent time and weather conditions) from October 2012 (32 individuals). Results of all statistical models ranged between 19–38 individuals. We can thus conclude that in all conducted research methods and statistical models in the study period (2011–2012), population size of brown bear in PLA Strážovské vrchy Mts (c. 300 km^2) and surroundings areas (c. 200 km^2) does not exceed 40 individuals.

Not only fundamental population parameters like size and structure but also some important genetic properties have been detected from typed samples. The relatively high level of heterozygosity and low degree of inbreeding detected in this study or in the study conducted by Graban et al. (2013) imply that the sub-population of bears in Strážovské vrchy is not geographically isolated and gene exchange with other segments of the Western Carpathian population is maintained to certain degree. Although the results of the present study shows relatively high variability, microsatellite analysis of brown bears in Malá Fatra National Park (Janiga et al., 2006) found higher numbers of alleles per locus. Moreover, the difference between observed and expected heterozygosity was greater in Malá Fatra than in Strážovské vrchy and low values of FIS for each locus demonstrated a higher occurrence of heterozygotes (Graban at al., 2013). Higher variability has also been found in central and northern Slovakia (Straka et al., 2012). Lower genetic variability of bear population in the study area might be a result of its smaller size and lower migratory level when compared with populations in other mountain ranges in Slovakia.

Our sampling efforts have been biased by uneven distribution of collected samples because there are considerable variations in bear distribution, spatial activity pattern, and density of bears within the study area (Pepich and Pepich, 2013). The study area is also characterised by uneven distribution of rub trees, from which hair samples were systematically collected for genotyping. Large amount of such trees can be found only in the central and south-eastern parts of the study area, where high densities of bears can be found. No bear rub trees had been found before or throughout the study period in south-western and northern parts. Considerable variations in bear densities but mainly a fact that some parts of the study area are inhabited by bears only seasonally (transient individuals) biased also our faecal sampling. Bear faeces for genotyping were not detected in northern and south-western parts of the study area as a consequence that bears inhabit these parts only seasonally when they migrate in search for food or mating opportunities.

Low number of successfully typed hair samples (37%) might be results of low quality and quantity of DNA but is mainly due to the financial limits for DNA analysis. However, proportion of typed faeces samples (63%) in this study is relatively consistent with other studies conducted in Slovakia or elsewhere in the world. In other studies, the successful portion of typed samples was for faeces 70% (Bellemain et al., 2005) in Scandinavia and in the DNA study conducted the Pyrenees only 57 samples (36 hair and 21 faeces) out of 352 samples provided enough DNA for a complete genetic typing at all polymorphic loci (Taberlet et al., 1997). In Slovakia, the successful portion of typed samples was for faeces 48% in Veporské vrchy and 65% in Poloniny (Straka et al., 2009). In the study conducted by Graban et al. (2013), 39% of samples provided enough DNA for a genetic typing.

To gain better picture of the studied population we matched four unique genotypes (17%) with corresponding track sizes (faeces or hair samples were found when tracking bears in snow) and nine unique genotypes (38%) were matched with remote sensing camera photos (10 rub trees were fitted with trail cameras). Three genotypes were supplemented with both track sizes and remote sensing camera photos, one genotype was matched only with track size and six genotypes were matched only with remote sensing camera photos. No track data of dominant male (width of front paw at least 15 cm) were matched with genotypes and only one dominant male captured by remote sensing camera photos was matched with typed samples.

Our results represent a comprehensive study of a subpopulation which has hitherto received little attention from researchers. We demonstrated that noninvasive genetic methods have become an efficient tool and are especially appropriate for use with elusive species in small populations. We believe that obtained data on population size, population structure, spatial activity pattern, movement pattern, and genetic variability will be used for further work building on our study to contribute to comprehensive knowledge of this charismatic species and that knowledge will be finally employed for proper conservation and management of bears within and between protected areas.

We also hope that our results might confute spreading disinformation about bears in this part of Slovakia (e.g.: large number of bears in every cadastre, total population size exceeding 100 individuals, large number of dominant males) but mainly will be used as a means of providing credible and reliable data to inform both experts and the public in Slovakia.

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