

Molecular evidence for historic long-distance translocations of brown bears in the Balkan region

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Abstract We tested the hypothesis that brown bears were translocated from the Romanian Carpathians to Bulgaria via air transportation during the communist regime in the 1970s and 1980s. Microsatellite analysis was performed on 199 bear samples from Bulgaria and Romania. Assignment and admixture tests revealed the existence of seven genotypes (=2.8 %) in Bulgaria that were assigned with high probabilities to the Romanian population, supporting the translocation and successful establishment of Carpathian bears in Bulgaria. While we cannot rule out the possibility that active long-distance dispersal contributed to the observed pattern, the spatial distribution and sex ratio of the detected Romanian genotypes strongly favor the translocation hypothesis.

Keywords *Ursus arctos* · Large carnivores · Balkan · Assignment test · Bear conservation

Introduction

Intentional or unintentional human-mediated translocations of wildlife may lead to unexpected outcomes in

phylogeographic and population genetic surveys. In large mammals, translocations happen mostly with a specific intention, for instance in order to restock populations of game species or in the course of reintroduction programs for endangered species (Fischer and Lindenmayer 2000). While it may be difficult to functionally link historic events with present patterns of genetic population structure visualized by molecular methods, the latter have the potential to reveal otherwise not verifiable hypotheses concerning past anthropogenically driven range expansions and translocations.

Here we aimed to test the unproven myth that past translocations of brown bears from Romania occurred from the Romanian Carpathians to mountain ranges in Bulgaria. The leader of the Romanian Communist Party, Nicolae Ceaușescu (1918–1989) was a passionate bear hunter. During the second half of the 20th century, bears were protected, fed, and sometimes even raised in enclosures in the Romanian Carpathians, in order to provide a constant supply of bears for Ceaușescu's hunting purposes. In contrast, the bear population appeared to be far more restricted in size in Bulgaria, where the Bulgarian president, Todor Zhivkov (1911–1998), shared the passion of bear hunting with his Romanian equivalent. In order to restock the Bulgarian bear population, it has been reported that brown bears from the Romanian Carpathian Mountains were transported during the 1970s and 1980s by military planes to Bulgaria and released in both major Bulgarian bear habitats, the Stara Planina mountains and the Rhodopes (Ovidiu Ionescu, Forest Research and Management Institute, Romania; personal communication). Interestingly, there is no publicly available document or paper which clearly proves the bear translocations. Personal communications from involved foresters and game wardens suggest that bears were transported to large fenced enclosures, of which some do still exist in the two main translocation areas, Mazalat in the

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Table 1 Genetic diversity across brown bear samples from Bulgaria (N ind. = 125) and Romania (N ind. = 74)

Locus	Total			Bulgaria					Romania			
	Allele range	N_A	H_O	H_E	N_A	H_O	H_E	pA	N_A	H_O	H_E	pA
Msut-2	74–86	7	0.71	0.77 ^a	6	0.72	0.73	–	7	0.70	0.80	1
UarMu-26	181–199	7	0.20	0.37 ^a	7	0.26	0.44 ^a	4	3	0.12	0.23 ^a	–
G1A	104–118	8	0.65	0.71 ^a	6	0.63	0.63	–	8	0.69	0.78	2
Mu23	142–158	8	0.68	0.74	8	0.64	0.70	2	6	0.74	0.76	–
G10C	90–116	11	0.84	0.85	9	0.84	0.84	–	11	0.84	0.83 ^a	2
Mu51	106–126	8	0.71	0.80	7	0.69	0.77	1	7	0.76	0.77	1
G10P	140–164	11	0.73	0.82	9	0.67	0.76 ^a	–	11	0.85	0.83	2
G1D	167–181	8	0.80	0.84 ^a	8	0.80	0.82 ^a	1	7	0.79	0.78	–
Mu10	113–133	9	0.72	0.73	9	0.67	0.66	2	7	0.81	0.81	–
G10U	157–183	14	0.60	0.87 ^a	13	0.58	0.87 ^a	5	9	0.62	0.79 ^a	1
G10L	136–166	10	0.74	0.80 ^a	9	0.68	0.74 ^a	1	9	0.83	0.85	1
G10 J	73–103	11	0.71	0.82 ^a	10	0.70	0.77 ^a	2	9	0.73	0.78 ^a	1
G10H	214–252	15	0.72	0.87 ^a	13	0.70	0.82 ^a	–	15	0.75	0.78	2
Average		9.8	0.68	0.77	8.8	0.66	0.73	1.4	8.4	0.71	0.75	1.0

Provided are the allele length range in bp, number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosity, and the number of private alleles (pA)

^a Significant departures from HWE are indicated

Central Balkan region (GPS: 42.729303°/25.170391°), and Kormisosh State Hunting Enterprise (Rhodope mountains; GPS: 41.772493°/24.922523°) (anonymous, personal communication).

Given the lack of sound documentation a genetic assessment provides the only opportunity to test the hypothesis of significant and successful bear translocation from the Romanian Carpathians to Bulgaria. For this purpose, we used bear samples collected for population genetic studies in Bulgaria and Romania and used assignment tests to check for potentially translocated individuals or their progeny.

Materials and methods

Genetic material from bears in Bulgaria was collected with the purpose to provide estimates of population size and subdivision. Samples from Romania were added to test the hypothesis of bear translocation. In total we collected 133 hair samples (Bulgaria $n = 126$; Romania $n = 7$), 126 scat samples (Bulgaria $n = 108$, Romania $n = 18$) and 71 tissue samples (Bulgaria $n = 16$, Romania $n = 55$). The samples were collected between 2004 and 2012 by means of hair trapping with lured barbed wire traps, hair collection from marking trees, hair collection from bears captured for telemetry, opportunistic scat collection, and tissue sampling from legally harvested individuals.

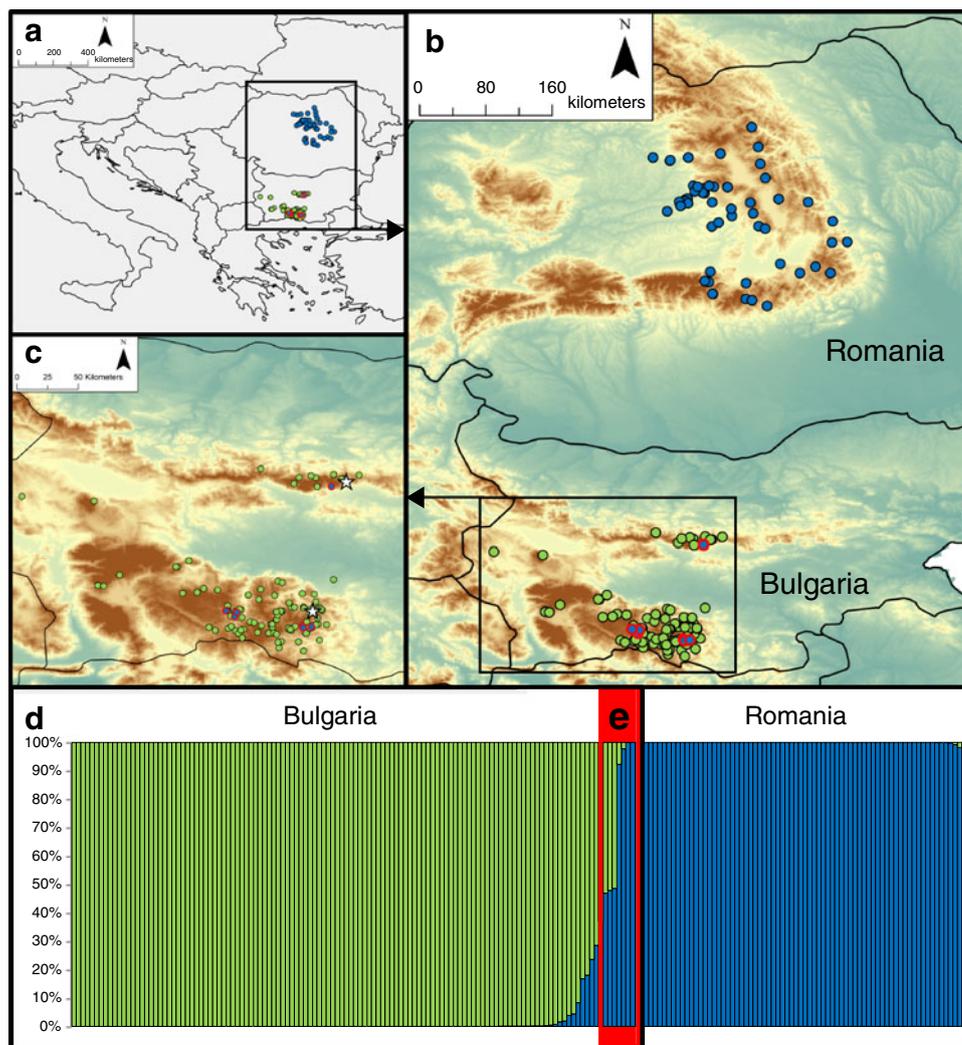
DNA was extracted using either the Stool extraction kit (scats), the Investigator kit (hairs) or the Blood & Tissue kit (muscle tissue) (Qiagen, Hilden, Germany) as described in

Frosch et al. (2011). Multilocus genotype profiles were obtained from 13 polymorphic microsatellite markers (Msut2, Kitahara et al. 2000; G10C, G10P, Paetkau et al. 1995; G1A, G10L, G1D, Paetkau and Strobeck 1994; UarMU26, Taberlet et al. 1997; G10H, G10 J, G10U, Paetkau et al. 1998; Mu10, Mu23, Mu51, Bellemain and Taberlet 2004) (Table 1) using three multiplex PCR reactions and four PCR replicates for hair and scat samples. Sample handling, PCR amplification and fragment length analyses were performed as described in Frosch et al. (2011).

Only samples with at least ten successfully amplified microsatellites were used. Consensus genotypes were constructed manually and recaptured individuals were identified using the software Coancestry 1.0.1.1 (Wang 2011). Genotyping error rates were estimated using GIMLET 1.3.3 (Valière 2002) including the consensus genotypes in the input file. The software GenAlEx 6.1 (Peakall and Smouse 2006) was used to estimate allele frequencies by locus and population, observed (H_O) and expected unbiased (H_E) heterozygosity, departures from the Hardy–Weinberg equilibrium (HWE), mean number of alleles per locus (N_A), and number of private alleles.

Sex identification was performed as described in Bidon et al. (2013) in a single multiplex with three replicates per sample. To assign individuals to source populations, we initially applied the Bayesian model-based clustering method which is implemented in the software Structure version 2.3.3 (Pritchard et al. 2000). We used the no admixture model with independent allele frequencies and ran the software for 1,000,000 steps with an initial burn-in of 200,000.

Fig. 1 Geographic distribution of bear samples and genotypes in the study region. **a** Map of Southeastern Europe. **b** Detail map of Romania and Bulgaria. Dots display Structure results; green Bulgarian population; blue Romanian population; red outlines show migrants detected with GenClass2. **c** Detailed map of Bulgaria including seven migrants. The location of enclosures used for bear translocation, breeding and release is marked with a white star symbol (north = Mazalat, south = Kormisosh). **d** Structure results, $K = 2$, samples from Bulgaria (left side) and Romania (right side). **e** Samples identified as migrants using GenClass2. (Color figure online)



We tested the range of K s from 1 to 11. To quantify the statistics of the posterior probability of population structure for a given K , we performed ten independent runs for each K . The most likely number of clusters was then inferred using Evanno et al.'s (2005) method in Structure Harvester (Earl and vonHoldt 2012).

In addition, an assignment test implemented in the software GeneClass2 Version 2.0 (Piry et al. 2004) was used to identify potential migrants in the Bulgarian bear population. We used the likelihood computation as described in Paetkau et al. (2004) to detect first generation migrants ($L = L_{\text{home}}/L_{\text{max}}$). We used the frequency-based method with a default frequency for missing alleles of 0.01 as described in Paetkau et al. (1995) for polar bears. Monte-Carlo resampling algorithm was used following Paetkau et al. (2004) with a Type I error (α) of 0.01 and 100,000 simulated individuals.

Results and discussion

Microsatellite PCR amplification success rate was 0.91, while genotyping errors occurred at rates of 0.10 (allelic dropout) and 0.01 (false alleles). Individual assignment of samples resulted in 125 individual genotypes from Bulgaria and 74 additional individuals from Romania. Structure analysis resulted in a clear division between the Romanian and Bulgarian samples, indicating reproductive isolation between bears from the Carpathians and Bulgarian mountain regions (Fig. 1a–d). Both the assignment test implemented in GeneClass2 and the admixture analysis in Structure software gave similar results and confirmed the existence of at least seven individuals in Bulgaria (Stara Planina: $n = 1$; Rhodopes: $n = 6$) that were assigned to the Carpathian populations (threshold p value = 0.03 in GenClass2) (Fig. 1b–e). Sex discrimination was successful for

six migrant samples, resulting in three male and three female individuals.

Interestingly, two different locations of migrant occurrence were found in the district of Smoljan in the Rhodopes. In the eastern part three “pure” migrants (Structure = 100 %; GenClass2 threshold value = 0.01) were located next to the village of Slaveyno. In the other location in the more western part of Smoljan near the village Devin two individuals were identified as migrants with Structure assignment probabilities of 92 and 98 % and p values of 0.021 and 0.013 in the assignment test (Fig. 1e). The additional sample from the western part of Smoljan and the sample from Stara Planina showed Structure assignment probabilities of 49 and 48 % with p values of 0.028 and 0.029, respectively. Four additional samples from the Rhodope Mountains showed Structure assignment probabilities to the Romanian population between 18 and 47 %, while two more samples from Stara Planina displayed signs of Romanian introgression with values of 17 and 37 % (Fig. 1d). For these samples we did not get signs of translocation in GeneClass2.

The finding of Carpathian genotypes in Bulgarian mountain ranges raises the question of their origin. There is an ongoing discussion on the existence of a potential wildlife corridor between the Romanian Carpathians and the Stara Planina in Bulgaria that leads over the easternmost parts of Serbia and spans over several hundreds of kilometers (Kaczensky et al. 2012). While there are strong barriers along this potential corridor, such as the Danube River, bear presence has been repeatedly confirmed along parts of the corridor (e.g. Paunovic and Cirovic 2006). For other parts, however, no evidence for recent bear presence has been reported (Kaczensky et al. 2012, Paunovic and Cirovic 2006), questioning the suitability of this corridor for effective long-distance bear dispersal. While we cannot rule out the possibility of natural migration due to the high active dispersal capacity of bears, there is strong evidence that these bears or their ancestors were translocated by humans. First, three of the six successfully sexed animals were females, which are known to be less prone to long-distance dispersal (Stoen et al. 2006). Second, all migrant genotypes were not found close to the potential migration corridor in the western part of the Southern Carpathians and the Stara Planina, but six of them occurred in the Rhodopes, which would require to pass across a second corridor between Stara Planina and the Rhodopes (Kaphegyí et al. 2013). These facts, as well as the finding of four of the seven Carpathian genotypes within a few kilometers of the two known bear enclosures that were used to keep the bears after translocation (Fig. 1) provides clear evidence in favor of the translocation hypothesis.

It has to be noted that the release of bears did not happen directly after translocation. Based on our own investigation, the Kormisosh enclosure in the Rhodopes does still

exist, potentially even holding bears of Carpathian origin, or their progeny. The fact that bears might have been bred in the translocation enclosures and were released consecutively explains the finding of pure Carpathian genotypes and generally low admixture grades. However, we were not able to collect samples from the existing enclosure.

While our sampling does not allow to finally test if natural dispersal via a wildlife corridor between the Carpathians and the Balkan region contributed to our findings, we urge the importance of such a study, including sophisticated habitat modeling in combination with genetic sampling along the potential corridor in Romania, Serbia and Bulgaria. In our case, however, human-mediated translocation provides a more likely explanation for the existence of Carpathian bear genotypes in Bulgaria, providing an example of aerial long-distance transfer of bears and a case study on the importance of considering past human impacts when interpreting genetic patterns in wild populations.

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