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TECHNIQUES AND STRATEGIES

Comparison the Brown bear (*Ursus arctos* L.) population genetic characteristics based on the microsatellite markers - 'DNA fingerprints' around the world and their application for biotracking

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Abstract. The evaluation of selected Brown bear population genetic attributes based on microsatellites is reviewed in this article. In order to design a study comparative population genetics a group of markers which comparable to other bear studies had to be selected. Different projects around the world offer a selection of the most frequently used microsatellite loci and their properties. Microsatellite variability can be explored in different terms. Differences in genetic characteristics between populations can be reflected via expected and observed heterozygosity, polymorphic information or coefficient of inbreeding. The question of how many microsatellites markers are necessary for discerning individuals had to be answered. Degree of the allelic variability can reflect the gene flow between populations and genetic drift or mutation rates. Acquired microsatellites data evaluation and in particular in finding answers to the question of individuals distribution on the site led them to propose procedures for comparation of microsatellite data was processed using our original Java script software methods. A global overview of microsatellite loci tested in different areas in the Europe, Asia or America is also presented in the summary table with the collection of all applied loci.

Key words: Brown bear, *Ursus arctos*, microsatellites, conservation genetics, biotracking

Introduction

Recent developments in molecular technology have allowed to perform genetic analyses of any organism with relative ease, bringing new possibilities for studying Brown bear (*Ursus arctos*) ecology. In the last two decades there has been a proliferation of genetics studies describing bear populations at different ecological scales from regional (DeBarba *et al.* 2010) to continental (Tammeleht *et al.* 2010). Researchers can now routinely utilize genetic information in DNA to address questions about the behaviour, ecology, life history, and evolution of Brown bear populations. Non-invasive genetic methods, especially appropriate for use with elusive species in small, endangered populations or over large areas, are now available to allow identification of individual animals, census populations and monitor migration and gene flow (Graban *et al.* 2013).

The Brown bear is one of the best-studied mammalian species. The microsatellite DNA or 'DNA fingerprints' - highly variable nuclear markers can be used in various applications to identify individuals and their immediate relatives (Servheen *et al.* 1999). In particular, mitochondrial cross-sectional studies on European scale have shown an interesting phylogeographic dichotomy in Brown bears. Taberlet and Bouvet (1994) identified two highly divergent lineages which on average differed by more than 7%. The western lineage is found in Spain, the Pyrenees, Norway, southern Sweden, Italy (Alps and Apennines), Romania and the Balkans whereas the eastern lineage occurs in Slovakia, Estonia, Romania, Russia, Finland, northern Sweden and also in the Russian Far East. Summary of loci used in studies aimed at mapping the movement of the bear gives us the possibility to compare the values of expected and observed heterozygosity between different locations (Graban *et al.* 2013). The studied locations show particular site characteristics that have a specific impact on the bear population.

How many loci are required for an optimal population-genetic analysis?

A significant number of the Brown bear population-genetics studies was carried out in the European region. The conventional (effective) determination of bear population size in the selected locations is based on the cooperation between wildlife conservationists, park managers and researchers. (Gervasi *et al.* 2008, Rigg 2005, Zedrosser *et al.* 2001). Population genomics involves sampling many loci across the genome in addition to the sampling of many individuals from each population. It also includes many loci from functional genes with known map locations. An important reason to sample many loci is to increase power to identify 'outlier' loci

that might be under selection (Manel *et al.* 2003, Vitalis *et al.* 2001). In the study of the 24 microsatellite markers previously reported by Paetkau and Strobeck (1994), Paetkau *et al.* (1995) and Taberlet *et al.* (1997), the range of microsatellite loci for animal identification proved to be more efficient. Seven loci were sufficient to identify individuals (Kindberg *et al.* 2011) until enough data have been accumulated, and 12 or 15 loci could be used to assign parentage. Thus we can assume that the number of markers used for parentage tests can be decreased when the study area is extended and the sample size is increased (Itoh *et al.* 2009). Selection of a more variable genetic marker set is advantageous for analysing genetic diversity in wildlife populations (Paetkau 2003, Waits *et al.* 2001) (Table 1, 2).

Microsatellite DNA data is often used for studying evolutionary relationships of closely related species or populations. Various genetic distance measures used for gene frequency data have been described by Nei (1987). The selection of microsatellite loci carries an inherent limitation due to the fact that the mutational pattern is often irregular and

Location/Locus		CXX20	G1A	G10B	G10C	G1D	G10H	G10J	G10L	G10M	G10O	G10P	G10X
Scandinavia NN (n=29) (Waits *et al.* 2000)	NA		7	6	4	6	8	7	7	5	2	5	3
Scandinavia NS (n=108) (Waits *et al.* 2000)	NA		7	7	5	6	10	6	6	7	3	6	5
Scandinavia M (n=88) (Waits *et al.* 2000)	NA		5	6	5	5	7	6	8	5	3	6	4
Scandinavia S (n=155) (Waits *et al.* 2000)	NA		5	5	5	4	6	6	7	4	3	6	4
Malá Fatra Slovakia (n=23) (Janiga *et al.* 2006)	NA					4							5
Northern Slovakia (n=71) (Straka *et al.* 2011)	NA			5	7	6		6	6	6		5	4
Central Slovakia (n=96) (Straka *et al.* 2011)	NA			4	6	6		5	6	6		6	5
Eastern Slovakia (n=16) (Straka *et al.* 2011)	NA			5	5	5		5	5	5		4	6
Western Slovakia (n=57) (Graban *et al.* 2013)	NA			2		4			2				
Romania (n=109) (Straka *et al.* 2011)	NA			8	9	7		8	8	7		8	10
Romania (n=16) (Zachos *et al.* 2008)	NA		5	7	7	5			8			9	
Central Austria (n=22) (Kruckenhauser *et al.* 2009)	NA			4		4			3			6	
Grevena Greece (n=49) (Karamanlidis *et al.* 2010)	NA				6	6		6				8	
Italy (n=17) (Zachos *et al.* 2008)	NA		2	2	2	2			2			2	
Abruzzo Apennines (n=30) (Lorenzini *et al.* 2004)	NA	2	2	2	3	2	2		2			2	2
Cantabriam Moutains Spain Eastern subpopulation (n=8) (Pérez *et al.* 2009)	NA		2	1	1	2		2	1		1	2	2
Cantabriam Moutains Spain Western Subpopulation (n=39)(Pérez *et al.* 2009)	NA		3	3	3	1		3	6		1	2	4
Hokkaido Japan (n=38) (Itoh *et al.* 2009)	NA		7	6	3	4	3	4	6	5		5	3
Deosai National Park Pakistan (n=28) (Bellemain *et al.* 2007)	NA		3	2	2	4	4	4	5		2		4
Prudhoe Bay Region Alaska (n=36) (Cronin *et al.* 1999)	NA	8	9	7	5	9	8	5	6	6	5	7	5

Table 1. Comparison of the numbers of microsatellite alleles (NA) in selected Brown bear populations around the world (n - number of investigated samples in the tested population).

Locality/Locus		Mu05	Mu09	Mu10	Mu15	Mu23	Mu26	Mu50	Mu51	Mu59	Mu61	Mu64
Scandinavia NN (n=29) (Waits *et al.* 2000)	NA	5		7	5	6		5	6	9	2	
Scandinavia NS (n=108) (Waits *et al.* 2000)	NA	6		6	5	6		7	7	10	3	
Scandinavia M (n=88) (Waits *et al.* 2000)	NA	6		8	4	6		7	8	9	3	
Scandinavia S (n=155) (Waits *et al.* 2000)	NA	7		6	4	7		6	6	8	3	
Malá Fatra Slovakia (n=23) (Janiga *et al.* 2006)	NA						4					9
Northern Slovakia (n=71) (Straka *et al.* 2011)	NA			8		7		6	6	7		
Central Slovakia (n=96) (Straka *et al.* 2011)	NA			7		7		6	7	7		
Eastern Slovakia (n=16) (Straka *et al.* 2011)	NA			5		6		4	6	7		
Western Slovakia (n=57) (Graban *et al.* 2013)	NA						2	3	3			3
Romania (n=109) (Straka *et al.* 2011)	NA			7		8		8	7	15		
Romania (n=16) (Zachos *et al.* 2008)	NA				9				7	13		
Central Austria (n=22) (Kruckenhauser *et al.* 2009)	NA					5	4	4		5		
Grevena Greece (n=49) (Karamanlidis *et al.* 2010)	NA							5		7		
Italy (n=17) (Zachos *et al.* 2008)	NA				3				3	4		
Abruzzo Apennines (n=30) (Lorenzini *et al.* 2004)	NA	3						2		2		
Cantabriam Moutains Spain Eastern subpopulation (n=8) (Pérez *et al.* 2009)	NA	3	2	2		3		1	2	1	1	1
Cantabriam Moutains Spain Western Subpopulation (n=39)(Pérez *et al.* 2009)	NA	4	4	4		3		5	4	4	2	3
Hokkaido Japan (n=38) (Itoh *et al.* 2009)	NA	4	4	2	2	6		5	4	7	3	5
Deosai National Park Pakistan (n=63) (Bellemain *et al.* 2007)	NA			5	3	5		4	3	7		
Prudhoe Bay Region Alaska (n=36)(Cronin *et al.* 1999)	NA							8		5		

Table 2. Comparison of the numbers of microsatellite alleles (NA) in selected Brown bear populations around the world (n - number of investigated samples in the tested population).

there seems to be an upper limit of the number of repeats (Forbess *et al.* 1995, Goldstein *et al.* 1995b). Furthermore a microsatellite locus can be highly polymorphic in some populations or species but monomorphic in others (Takezaki and Nei 1996).

Heterozygosity

Allele frequencies allow descriptive statistics for each locus (mean number of alleles per locus, heterozygosities and polymorphic information content (PIC). There is a well-known relationship between the size of the repeat unit of a microsatellite locus and the variability of the locus, with larger repeat units leading to lower heterozygosity. In agreement with this trend, smaller repeat unit size was also found to lead to a higher mean number of repeats, and a higher mean number of repeats led to higher heterozygosity (Pemberton *et al.* 2009). Heterozygosity reflects the proportion of heterozygotes within a population, whereas allelic richness represents the number of alleles at each locus. Variable microsatellite loci, such as those used by molecular ecologists, have a particularly high degree of polymorphism, with heterozygosity frequently exceeding 70% (Webster *et al.* 2002).

If heterozygosity at microsatellite loci reflects genome-wide heterozygosity, then the dispersion of females choosing males in larger territories would suggest that a selection for males more heterozygous group mates and more heterozygous potential fathers for their offspring is taking place. However, if the relationship between heterozygosity of the microsatellite markers is strong and the genome-wide heterozygosity is weak, the findings may have little to do with inbreeding and the link between male quality and heterozygosity would instead have probably arisen through heterosis at one or more specific loci (Hansson and Westerberg 2002, Seddon *et al.* 2004). Genetic characteristics between populations are reflected via the expected heterozygosity and the numbers of alleles per locus. Examination of the degree of isolation of the population reflects occurrence of private alleles, suggesting that they could by migrants or individuals from beyond the study area (Bellemain *et al.* 2007).

Although researchers have used different sets of microsatellite loci, thus limiting the comparability different studies, the results show a clear dichotomy, where the high expected heterozygosity and allelic diversity occurs in the largest populations and low heterozygosity occurs in the smallest populations (Swenson *et al.* 2011). The changes in number observed and expected heterozygotes and heterozygote excess have been used to estimate effective immigration in Brown bear populations (Swenson *et al.* 2011, Tallmon *et al.* 2004). The low detected heterozygosity level indicates the isolation of the tested population. Consequently the PIC is also low and the inbreeding coefficient (FIS) ranges between -1 and 0. Migration of the animals to other locations leads to great heterozygosity, connected with great PIC values and with the inbreeding coefficient ranging between 0 and +1 values (Table 3, 4).

Variations of microsatellites

Microsatellite variability can be explored in different terms. High heterozygosity and extended range of allele size indicates massive population expansion. It is well known that the length of microsatellites is variable in different populations and individuals. Variations of alleles can reflect gene flow between populations and genetic drift. Relative low degree of genetic variability may be caused by geographic separation from other bear populations or a too small effective size of the studied population.

Microsatellite DNA sequences mutate at higher rates than the rest of DNA. If two individuals started with exactly the same allelic length polymorphisms in their genetic history, then current divergence could be reflected by the individual mutational jumps. Genetic shortage is reflected mainly by limited allelic variation, rather than by the average heterozygosity. A maximum of three alleles was found in two out of the 12 polymorphic microsatellites, while, on average, from five to nine alleles were found at the same loci in other brown bear populations (Cronin *et al.* 1999, Paetkau *et al.* 1998b, Waits *et al.* 2000).

Loci G10B and G1D showed two alleles with size

Locality/Locus		CXX20	G1A	G10B	G10C	G1D	G10H	G10J	G10L	G10M	G10O	G10P	G10X
Scandinavia NN (n=29) (Waits *et al.* 2000)	HO		0.53	0.67	0.80	0.83	0.90	0.70	0.77	0.77	0.10	0.70	0.53
	HE		0.68	0.77	0.67	0.76	0.81	0.74	0.76	0.74	0.10	0.68	0.54
Scandinavia NS (n=108) (Waits *et al.* 2000)	HO		0.64	0.61	0.73	0.80	0.76	0.82	0.73	0.80	0.09	0.76	0.51
	HE		0.68	0.67	0.66	0.72	0.75	0.74	0.80	0.79	0.11	0.79	0.48
Scandinavia M (n=88) (Waits *et al.* 2000)	HO		0.75	0.71	0.64	0.63	0.44	0.69	0.59	0.56	0.34	0.77	0.63
	HE		0.71	0.72	0.65	0.63	0.50	0.66	0.70	0.63	0.32	0.76	0.62
Scandinavia S (n=155) (Waits *et al.* 2000)	HO		0.64	0.68	0.68	0.60	0.60	0.55	0.81	0.63	0.37	0.77	0.60
	HE		0.64	0.69	0.67	0.62	0.59	0.57	0.78	0.69	0.33	0.77	0.57
Scandinavia - south 1985-1987 (n=22) (Tallmon *et al.* 2004)	HO		0.73	0.77	0.59	0.55	0.68	0.55	0.64		0.91	0.55	0.73
	HE		0.65	0.72	0.63	0.57	0.50	0.57	0.87		0.81	0.41	0.58
Scandinavia - south 2000-2002 (n=127) (Tallmon *et al.* 2004)	HO		0.65	0.69	0.68	0.58	0.44	0.54	0.76		0.75	0.36	0.54
	HE		0.58	0.66	0.68	0.58	0.39	0.57	0.75		0.76	0.33	0.50
Norwegian South (n=62)(Eiken *et al.* 2009)	HO					0.58			0.83				
	HE					0.59			0.76				
Norwegian Northeastern (n=75)(Eiken *et al.*2009)	HO					0.85			0.63				
	HE					0.84			0.61				

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Norwegian Middle (n=43)(Eiken *et al.* 2009)	HO					0.74			0.73						
	HE					0.67			0.68						
Norwegian Northwestern (n = 26)(Eiken *et al.* 2009)	HO					0.73			0.78						
	HE					0.70			0.79						
Malá Fatra Slovakia (n=23)(Janiga *et al.* 2006)	HO					0.63									0.65
	HE					0.43									0.47
Northern Slovakia (n=71) (Straka *et al.* 2011)	HO			0.59	0.73	0.68		0.80	0.41	0.73		0.57	0.81		
	HE			0.63	0.73	0.79		0.79	0.48	0.74		0.62	0.69		
Central Slovakia (n=96) (Straka *et al.* 2011)	HO			0.60	0.74	0.76		0.74	0.60	0.54		0.74	0.44		
	HE			0.63	0.73	0.76		0.77	0.61	0.58		0.77	0.44		
Eastern Slovakia (n=16) (Straka *et al.* 2011)	HO			0.63	0.38	0.81		0.69	0.50	0.67		0.40	1.00		
	HE			0.65	0.58	0.77		0.61	0.48	0.64		0.49	0.80		
Western Slovakia (n=57) (Graban *et al.* 2013)	HO			0.35		0.81			0.81						
	HE			0.49		0.70			0.50						
Romania (n=109) (Straka *et al.* 2011)	HO			0.76	0.81	0.71		0.78	0.79	0.62		0.74	0.76		
	HE			0.75	0.82	0.73		0.79	0.84	0.67		0.80	0.80		
Romania (n=16) (Zachos *et al.* 2008)	HO		0.60	0.56	0.67	0.75			0.75			0.81			
	HE		0.80	0.76	0.87	0.77			0.83			0.84			
Grevena Greece (n=49) (Karamanlidis *et al.* 2010)	HO				0.76	0.63		0.76				0.81			
	HE				0.80	0.76		0.68				0.80			
Italy (n=17) (Zachos *et al.* 2008)	HO		0.24	0.56	0.71	0.35			0.50			0.29			
	HE		0.21	0.54	0.47	0.54			0.48			0.38			
Abruzzo Apennines (n=30)(Lorenzini *et al.* 2004)	HO	0.50	0.21	0.47	0.40	0.57	0.35		0.29			0.32	0.45		
	HE	0.52	0.19	0.51	0.48	0.50	0.36		0.34			0.36	0.51		
Cantabriam Moutains Spain Eastern subpopulation (n=8) (Pérez *et al.* 2009)	HO		0.38	0.00	0.00	0.38		0.5	0.00		0.00	0.25	0.13		
	HE		0.30	0.00	0.00	0.30		0.38	0.00		0.00	0.22	0.49		
Cantabriam Moutains Spain Western Subpopulation (n=39) (Pérez *et al.* 2009)	HO		0.64	0.13	0.41	0.00		0.69	0.64		0.00	0.49	0.26		
	HE		0.51	0.17	0.50	0.00		0.65	0.74		0.00	0.45	0.29		
Hokkaido Japan (n=38) (Itoh *et al.* 2009)	HO		0.74	0.68	0.16	0.66	0.29	0.26	0.79	0.50		0.71	0.47		
	HE		0.73	0.77	0.15	0.6	0.28	0.38	0.69	0.53		0.66	0.54		
Deosai National Park Pakistan (n=28) (Bellemain *et al.* 2007)	HO		0.50	0.52	0.52	0.68	0.76	0.68	0.58		0.04		0.12		
	HE		0.49	0.47	0.49	0.64	0.60	0.66	0.77		0.04		0.28		
Prudhoe Bay Region Alaska (n=36) (Cronin *et al.* 1999)	HO	0.71	0.75	0.80	0.74	0.81	0.61	0.68	0.66	0.76	0.61	0.76	0.65		

Table 3. Comparison of the expected (HE) and observed (HO) heterozygosities of selected Brown bear populations around the world (n – number of investigated samples).

Locality/Locus		Mu05	Mu09	Mu10	Mu15	Mu23	Mu26	Mu50	Mu51	Mu59	Mu61	Mu64
Scandinavia NN (n=29) (Waits *et al.* 2000)	HO	0.67		0.80	0.50	0.67		0.67	0.67	0.63	0.60	
	HE	0.75		0.74	0.51	0.71		0.69	0.75	0.80	0.50	
Scandinavia NS (n=108) (Waits *et al.* 2000)	HO	0.68		0.72	0.54	0.66		0.64	0.75	0.85	0.49	
	HE	0.72		0.76	0.51	0.70		0.69	0.75	0.84	0.47	
Scandinavia M (n=88) (Waits *et al.* 2000)	HO	0.67		0.78	0.43	0.84		0.74	0.79	0.81	0.63	
	HE	0.65		0.72	0.47	0.82		0.78	0.75	0.83	0.64	
Scandinavia S (n=155) (Waits *et al.* 2000)	HO	0.66		0.82	0.69	0.76		0.78	0.81	0.82	0.55	
	HE	0.62		0.80	0.65	0.69		0.74	0.76	0.78	0.55	
Scandinavia - south 1985-1987 (n=22) (Tallmon *et al.* 2004)	HO	0.64		0.77	0.82	0.77		0.73	0.96	0.82	0.55	
	HE	0.62		0.81	0.68	0.74		0.74	0.77	0.78	0.56	
Scandinavia - south 2000-2002 (n=127) (Tallmon *et al.* 2004)	HO	0.65		0.76	0.65	0.72		0.64	0.75	0.74	0.53	
	HE	0.61		0.79	0.65	0.70		0.65	0.76	0.75	0.54	
Norwegian South (n=62) (Eiken *et al.* 2009)	HO	0.71	0.89	0.74	0.59	0.63		0.76	0.81	0.75		
	HE	0.68	0.79	0.78	0.59	0.65		0.78	0.80	0.74		
Norwegian Northeastern (n=75)(Eiken *et al.* 2009)	HO	0.89	0.91	0.72	0.79	0.67		0.75	0.77	0.82		
	HE	0.83	0.86	0.80	0.74	0.65		0.83	0.81	0.86		
Norwegian Middle (n=43) (Eiken *et al.* 2009)	HO	0.81	0.93	0.79	0.61	0.74		0.81	0.74	0.83		
	HE	0.73	0.83	0.77	0.65	0.80		0.72	0.73	0.83		
Norwegian Northwestern (n = 26)(Eiken *et al.* 2009)	HO	0.73	0.69	0.75	0.39	0.83		0.86	0.83	0.65		
	HE	0.73	0.66	0.68	0.37	0.75		0.77	0.80	0.68		
Malá Fatra Slovakia (n=23) (Janiga *et al.* 2006)	HO						0.65					0.74
	HE						0.44					0.46
Northern Slovakia (Straka *et al.* 2011)	HO			0.81		0.80		0.76	0.52	0.70		
	HE			0.76		0.78		0.75	0.74	0.76		
Central Slovakia (Straka *et al.* 2011)	HO			0.78		0.79		0.72	0.73	0.73		
	HE			0.75		0.80		0.65	0.82	0.72		
Eastern Slovakia (Straka *et al.* 2011)	HO			0.67		0.50		0.69	0.62	1.00		
	HE			0.58		0.75		0.58	0.72	0.77		
Western Slovakia (n=57) (Graban *et al.* 2013)	HO						0.14	0.61	0.77			0.67
	HE						0.50	0.59	0.56			0.66
Romania (Straka *et al.* 2011)	HO			0.76		0.78		0.80	0.72	0.83		
	HE			0.83		0.81		0.82	0.78	0.89		
Romania (Zachos *et al.* 2008)	HO				0.88				0.81	0.69		
	HE				0.81				0.79	0.86		
Grevena Greece (n=49) (Karamanlidis *et al.* 2010)	HO							0.80		0.88		
	HE							0.73		0.80		

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Italy (Zachos *et al.* 2008)	HO				0.09				0.67	0.53			
	HE				0.18				0.58	0.76			
Abruzzo Apennines (Lorenzini *et al.* 2004)	HO	0.38						0.40		0.48			
	HE	0.52						0.47		0.50			
Cantabriam Moutains Spain Eastern subpopulation (n=8) (Pérez *et al.* 2009)	HO	0.75	0.75	0.50		0.88		0.00	0.63	0.00	0.00	0.00
	HE	0.63	0.50	0.50		0.63		0.00	0.49	0.00	0.00	0.00
Cantabriam Moutains Spain Western Subpopulation (n=39) (Pérez *et al.* 2009)	HO	0.56	0.51	0.28		0.72		0.69	0.38	0.59	0.49	0.33
	HE	0.58	0.66	0.42		0.65		0.67	0.37	0.56	0.44	0.46
Hokkaido Japan (n=38) (Itoh *et al.* 2009)	HO	0.63	0.42	0.61	0.29	0.74		0.84	0.74	0.84	0.76	0.74
	HE	0.70	0.55	0.50	0.49	0.76		0.77	0.71	0.65	0.62	0.63
Deosai National Park Pakistan (n=63) (Bellemain *et al.* 2007)	HO			0.50	0.56	0.89		0.57	0.50	0.86		
	HE			0.66	0.53	0.77		0.54	0.43	0.83		
Prudhoe Bay Region Alaska (n=36) (Cronin *et al.* 1999)	HO							0.79		0.48		

Table 4. Comparison of the expected (HE) and observed (HO) heterozygosities of selected Brown bear populations around the world (n – number of investigated samples).

ranges of 137-155 and 170-184, which each differ by 18 and 14 basepairs (bp), respectively. This indicates that a large portion of the original allelic variability, partly still present in the recovered Scandinavian population, might have been lost by random sorting of alleles in the small, isolated population of the Apennine brown bear (Lorenzini *et al.* 2004). Microsatellite variation is significantly correlated also with allozyme variation. If both allozymes and microsatellites are neutral markers in a mutation-drift balance, then it is expected that genetic variation at these two marker types should be highly correlated. However, the correlation can be weakened by various demographic factors. For example, populations that are highly inbred or have low effective size are more likely to have lower degrees of heterozygosity, and it is unlikely that the microsatellite and allozyme datasets are based on the same population within each species (Neff and Gross 2001). If such a 'false allele' occurs in a homozygous individual, then this individual can be recorded as a heterozygote, and if it occurs in a heterozygous individual, then the presence of three 'alleles' will allow the detection of the error. These artefacts generating false alleles are easily confused with sporadic contaminations. They generally occur in less than 5% of the PCRs (Taberlet *et al.* 1996), but should not be disregarded as they can lead to erroneous genotypes (Taberlet and Luikart 1999).

Comparison of individuals between different areas

Comparison of individual DNA-profiles between different laboratories require the data to be standardized (Aarnes *et al.* 2009). New calibration keys were determined in order to make the genotypes from Norwegian bears comparable with the whole Swedish bear database. Changes in observed and expected heterozygotes and heterozygote excess have been used to estimate effective immigration in Brown bear populations (Swenson *et al.* 2011, Tallmon *et al.* 2004). Comparisons are based on Mu10, Mu23, Mu50, Mu51, Mu59 and G10L loci. The difference between single alleles of the Mu59 marker was not consistent, therefore the calibration key for this marker is uncertain. (Aarnes *et al.* 2009). To design a population genetics broad global study we can select a set of loci used in the majority of the described studies. This selected group of Brown bear loci can consist of G1P, G10L, Mu10, Mu23, Mu50, Mu51 loci and also frequently used Mu59 marker.

Microsatellite alleles of similar length are more likely to be related by descent than alleles of different length, and there will be an inherent 'temporal memory' in the allelic distance data that can be incorporated into the estimate of the inbreeding coefficient (Hansson and Westerberg 2002). When two loci are very close together on a chromosome, they may not assort independently and will be transmitted to offspring as a pair. Even if loci are not linked physically on a chromosome, they can be functionally related or under selection to be transmitted as a pair. Hence the more accurate term gametic disequilibrium is starting to replace the term linkage disequilibrium (Selkoe and Toonen 2006). While functional linkage would be unusual for microsatellite loci, microsatellites can be clustered in the genome (Bachtrog 1999).

Division animals into 'family clusters' based on microsatellites

Acquired data evaluation and in particular in finding answers to the question of individuals distribution on the site led them to propose procedures for comparation of microsatellite data was processed using our original Java script software methods. Two methods were used Neighbor-joining and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) for construction of clusters graphic presentation based on microsatellites data processing (Graban *et al.* 2013).

The Neighbor-joining method is proposed for reconstructing phylogenetic trees from evolutionary distance data. The principle of this method is to find pairs of operational taxonomic units (OTUs). Provides not only the topology but also the branch lengths of the final tree. A pair of 'neighbors' is a pair of animals connected through a single interior node in an unrooted, bifurcating tree (Saitou and Nei 1987).

UPGMA is a simple agglomerative or hierarchical clustering method often used for the creation of phenetic trees (phenograms). UPGMA assumes a constant rate of similarity between animals. UPGMA was initially designed for use in protein electrophoresis studies, but is currently most often used to produce guide trees for more sophisticated phylogenetic reconstruction algorithms (Graban *et al.* 2013).

This two methods are also usefull for construction of dendrograms based on microsatellites data profiles, 'DNA fingerprints'. Animals are consequently divided into 'family' clusters based on degree of relatedness between individual microsatellite profiles (Fig. 1.). Animals comparation divided into dendrograms with GPS data of non-invasive sampling locations allows to find movement strategy of population. Dendrograms of bear' microsatellites' families are very effective for identification of migration individuals bearing new alleles.

Conclusions

Microsatellite DNA sequences are rapidly becoming the dominant source of nuclear genetic markers for a wide range of applications, from genome mapping to forensic testing to population studies. If misinterpretation is to be avoided, it is vital that we understand fully the way in which microsatellite sequences evolve. The microsatellite applications range from the estimation of the spatial relationships between chromosome segments to the elucidation of temporal relationships between origins of species and genera (Chambers and MacAvoy 2000). Microsatellite length mutations are often modeled using the generalized stepwise mutation process, which is a type of random walk. If this model is sufficiently accurate, one can estimate the coalescence time between alleles of a locus after a mathematical transformation of the allele lengths. When large-scale microsatellite genotyping first became possible, there was substantial interest in using this approach to make inferences about time and demography, but that interest has waned because it has not been possible to empirically validate the clock by comparing it with data in which the mutation process is well understood (Sun *et al.* 2009).

Research of bear activities focuses on acquisition of useful population parameters subsequently used for effective protection. Conservationists and wildlife management in the selected protected areas around Slovakia focus their activity on obtaining data on the distribution of individuals, population size, sex and social structure, home range and activity patterns, population trends and migration to other orographic units. A reliable identification of individuals based on the optimal number of loci also allows to investigate kinship of the studied animals. Three basic marker systems are available for genetic studies: 1) uniparental mitochondrial DNA (mtDNA), which characterise maternal lineages, 2) biparental autosomal markers such as microsatellites which characterise the combined history of femal and male lineages, 3) and uniparental sex chromosome markers, like Y-chromosome microsatellites, which characterise paternal lineages (Manel *et al.* 2003, Tammeleht 2010). Microsatellites have been a very useful tool in population genetic studies because they are neutral, codominant, biparentally inherited and fairly abundant and well-dispersed throughout the genome (Tautz *et al.* 1986, Weber and May 1989, Manel *et al.* 2003). Population genomics involves sampling many loci across the genome, in addition to the sampling of many individuals from each population. It also uses many loci from functional genes with known map locations. An important reason to sample many loci is to increase power to identify 'outlier' loci that might be under selection (Vitalis *et al.* 2001, Manel *et al.* 2003).

Authors of the selected studies described above focus interest on the construction of individual microsatellite profiles. In the study of the 24 microsatellite markers previously reported by Paetkau and Strobeck (1994), Paetkau *et al.* (1995) and Taberlet *et al.* (1997) large range of microsatellite loci the identification of animals is more efficient. Seven loci were sufficient to identify individuals, and 12 or 15 loci could be used to assign parentage (Itoh *et al.* 2009). However, the two laboratories have minor differences in methods and equipment, and e. g electrophoretic conditions may affect mobility of DNA fragments and size determination of microsatellite alleles. Thus, normalisation of allele sizes using a set of size standards will be required to perform interlaboratory comparisons (Aarnes *et al.* 2009, Budowle *et al.* 2005).

Comparisons of individual DNA-profiles between different laboratories require that the data be standardised. New calibration keys were determined in order to make the genotypes from Norwegian bears comparable with the whole Swedish bear database. Comparisons are based on Mu10, Mu23, Mu50, Mu51, Mu59 and G10L loci. The difference between single alleles of the Mu59 marker was not consistent, therefore the calibration key for this marker is uncertain (Aarnes *et al.* 2009).

For design of population genetics continental or global study we can select a set of loci used in the majority of described studies. This selected group of Brown bear loci consist of G1P, G10L, Mu10, Mu23, Mu50, Mu51 loci and also the frequently used Mu59 marker. In conclusion, numbers of loci

used in different studies were between 4 and 20. It is clear that higher number of selected loci results in better population-genetic results if used on an adequate number of samples. In the study of Andreassen *et al.* (2012) were to perform the recommended validation tests on thirteen dinucleotide microsatellite markers (G1A, G10B, G1D, G10L, MU05, MU09, MU10, MU15, MU23, MU26, MU50, MU51, MU59) commonly used for bear population management and conservation genetics. The validations tests could aid in the selection of markers for a forensic DNA profiling system for the Brown bear in Northern Europe. The validation tests included species specificity testing, measurements of sensitivity as well as measurements of precision, stutter and heterozygote balance. Selected common alleles from all STR loci were sequenced to explore the allelic size variation at the sequence level.

Microsatellite profiles can be also helpful for construction 'family' clusters dendrograms.

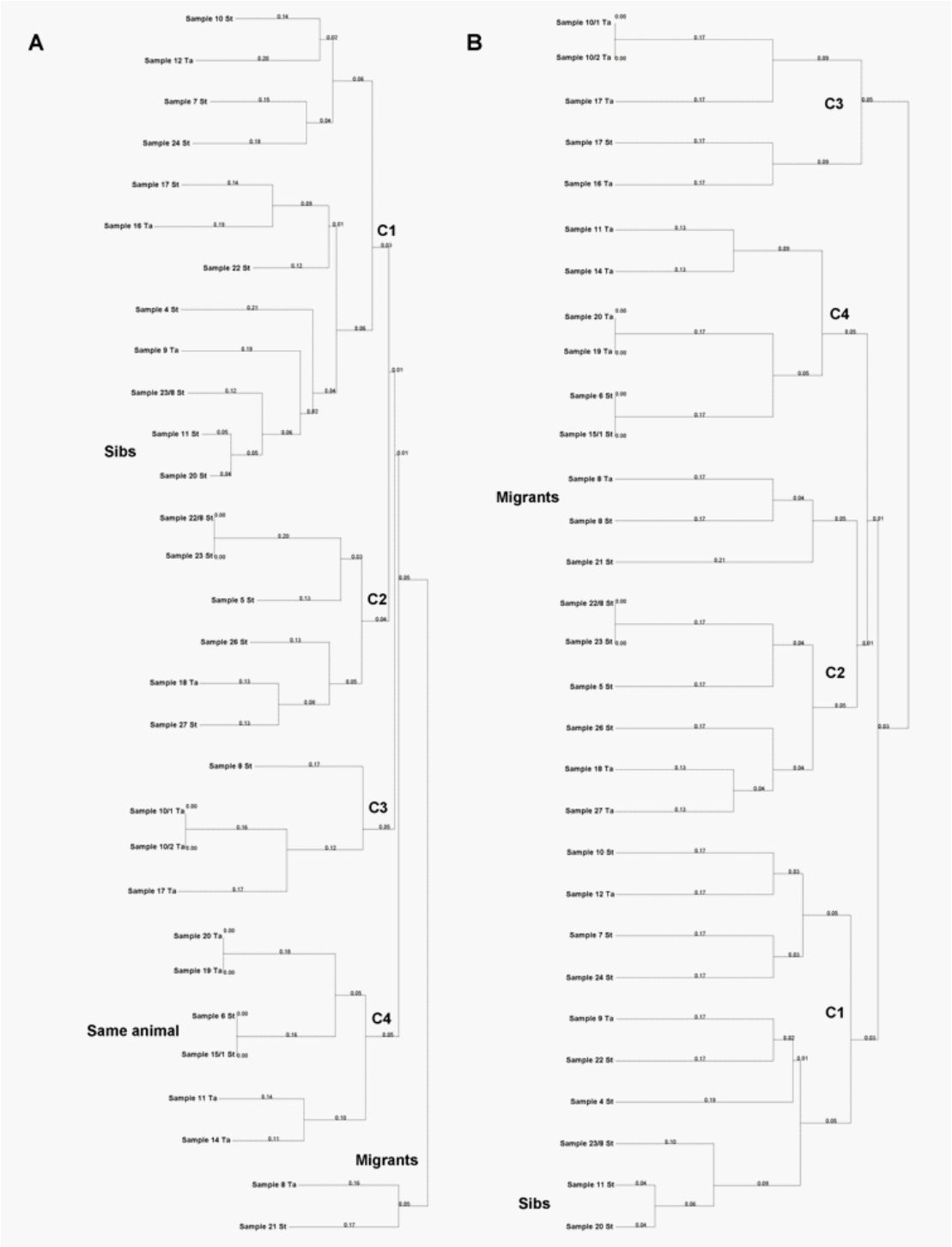

Fig. 1. Comparation of two methods (NJ - A and UPGMA - B) for dendrograms creation based on microsatellites profiles. More effective is Neighbor-Joining method with exact distribution of animal into clusters (C1 - C4) with selection of specific alellic combinations (Migrants). UPGMA method offer not so exact creation of clusters or 'microsatellites families'. Localities of samples colection: Ta - High Tatras (Slovakia), St - Stražovske vrchy Mountains (Slovakia).

On the geographical level we can find interesting genetic connections between different localities. In this context it is possible to use microsatellite data not only to obtain a population genetic characteristics but also for the evaluation of the individuals distribution in the area. GPS parameters of non-invasively obtained samples enable to obtain an overview of the bears movement and also genetic connection weith other localities in the relation to migration. On the basis of the relevant comparisons we can frame our own research activities in broader regional context as a part of comprehensive global mapping of Brown bear microsatellite profiles.

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