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Development of multiplex microsatellite sets for noninvasive population genetic study of the endangered Tatra chamois

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Abstract. The only autochthonous population of Tatra chamois (*Rupicapra rupicapra tatrica*) occurs in the Tatra Mountains (northern Slovakia and southern Poland). Another population has been introduced to the Low Tatra Mts., while Alpine chamois (*R. r. rupicapra*) has been introduced to the neighbouring mountain ranges, Veľká Fatra and Slovenský raj. All these populations have undergone intensive bottlenecks. Any resulting low genetic variability would mean that only few genetic markers could be used for population genetic studies due to prevailing monomorphism. We tested 65 markers previously used in chamois or other Caprinae species, from which 20 most suitable loci for noninvasive genetic study of the Tatra chamois were selected. These polymorphic loci were used for optimisation of three multiplex sets and revealed a mean number of alleles of 2.1 and mean expected heterozygosity of 0.331 for the Tatra population. Low genetic diversity was also observed in the Low Tatra population while slightly higher values were obtained for Alpine chamois population in Slovenský raj. We subsequently assessed the amplification success rate for noninvasively obtained samples (faeces), which ranged from 85.1% to 92.7% for particular loci. The developed polymorphic microsatellite sets provide a unique tool for population genetic study of the endangered Tatra chamois, even when using noninvasive sampling, and is also suitable for Alpine chamois.

Key words: noninvasive genetic sampling, amplification success rate, ungulates

Introduction

Chamois (*Rupicapra* spp.) are mountain ungulates distributed over Southern and Central Europe, the Balkans, Turkey and the Caucasus (Shackleton 1997). As they inhabit only higher altitudes, their distribution is markedly fragmented and thus gene flow between populations is restricted. Under such conditions, populations within particular mountain regions may differentiate through mutation, selection and genetic drift (Slatkin 1987, Frankham et al. 2002). Chamois occurring in the Tatra Mountains (northern Slovakia and southern Poland) have been recognized as a separate subspecies, *Rupicapra rupicapra tatrica*, based on their morphological characteristics (Blahout 1972). Only one autochthonous population of Tatra chamois now exists, and this has been evolving

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separately from other chamois populations for at least 10000 years (Pérez et al. 2002, Jamrozy 2006). The population, therefore, can be regarded as an evolutionary significant unit (Crandall et al. 2000).

During the first half of the 20th century, this population underwent two significant bottlenecks, each time declining from around 1000-1500 to 200-300 individuals. Relatively low numbers were found over most of the second half of the century, with a further drop to ca. 200 individuals at the end of the century (Jurdíková 2000, Janiga & Zámečníková 2002, Jamrozy 2006). Consequently, in 2001, a conservation action plan was begun with the aim of saving the Tatra chamois from extinction (Koreň et al. 2001). Strong protection has led to an increase in the Tatra population, with recent estimate of ca. 700 individuals. Tatra chamois nevertheless remain classified as critically endangered in the IUCN Red List of Threatened Species (Aulagnier et al. 2008), even if the species as a whole is not considered endangered in Europe (Temple & Terry 2009).

As a consequence of the dramatic decrease in Tatra chamois abundance in the second half of 1960s, a 'reserve' population of this endangered subspecies was established in the Low Tatra Mts. of Slovakia in the 1970s (Radúch & Karč 1983). At present, the Low Tatra population has ca. 100 individuals (P. Bačkor, S. Ondruš pers. comm.). Nevertheless, in the early 1960s, i.e. prior to recognition of the Tatra chamois as a separate subspecies, Alpine chamois (*R. r. rupicapra*) were introduced into the Veľká Fatra and Slovenský raj Mts. of Slovakia. As these mountain ranges are geographically close to the Low Tatra Mts., hybridisation between the two subspecies may have occurred.

The disturbance of vulnerable Tatra chamois populations is highly undesirable, making it almost impossible to obtain a sufficient number of tissue samples. In such cases, noninvasive genetic sampling (e.g. Kohn & Wayne 1997, Taberlet et al. 1999) remains the only feasible method for studying genetic composition of populations. However, as PCR amplification of DNA extracted from noninvasively obtained material is still rather problematic (e.g. Pompanon et al. 2005, Waits & Paetkau 2005, Hájková et al. 2006, Beja-Pereira et al. 2009), the selection of markers with appropriate parameters is one of the most important factors affecting the success of analysis. Although a higher number of analysed loci reveals a more accurate description of genetic variability and structure, with noninvasive samples, a higher number of genotyped loci has the disadvantage of an increased risk of genotyping errors and higher costs (Waits & Leberg 2000). It is essential, therefore, that the most informative markers are identified. Furthermore, the length of amplified loci may also be important as shorter loci tend to be amplified with a higher success rate (Sefc et al. 2003, Buchan et al. 2005, Broquet et al. 2007). Lastly, DNA quantity is often very limited in noninvasive samples. If multiplex sets are developed, the study requires less DNA and time- and financial costs decrease (Skrbinšek et al. 2010).

In this study, we attempt to ascertain appropriate microsatellite markers in order to produce multiplex sets for the population genetic study of the Tatra chamois using noninvasive samples. Due to the risk of hybridisation with introduced Alpine chamois, the markers were also tested on this subspecies.

Material and Methods

Sixty-five microsatellite loci (Table 1 and 2) that had previously been used in other studies of chamois or other Caprinae species (e.g. Pérez et al. 2002, Maudet et al. 2004a, An et al. 2005, Cassar et al. 2007, Glowatzki-Mullis et al. 2008) were tested for amplification. Tissue samples were used in the tests, Tatra chamois samples being obtained from natural deaths and those of Alpine chamois from legal hunting. Singleplex PCRs were performed on a Mastercycler ep gradient S (Eppendorf) using a mixture of 1 μL of PCR buffer with 25 mM Mg^{2+}, 200 μM of each dNTP, 3.2 μg of BSA, 0.2 U of HotMaster Taq DNA Polymerase (Eppendorf), 0.25 μM of each primer (forward ones fluorescently labelled), 1 μL of extracted DNA and ddH_2O to a volume of 10 μL in each reaction. Cycling conditions consisted of an initial denaturation at 94°C for 2 min, followed by 38 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s and extension at 65°C for 60 s, and a final extension at 65°C for 10 min. The gradient of annealing temperatures was tested when necessary. PCR products were checked on 1.5% agarose gel by electrophoresis and, when a clear band was present, the products were electrophoresed on an ABI 3130 Genetic Analyzer with GeneScan 500 LIZ Size Standard and analysed using GENEMAPPER 3.7 (Applied Biosystems).

First, the microsatellite markers were tested using three Tatra and four Alpine chamois tissue samples. Loci that proved polymorphic in initial testing were subsequently genotyped using a more extensive set of 11, 5 and 22 samples from the Tatra, Low Tatra and Slovenský raj populations, respectively. Number of alleles (A), allelic richness (AR) and expected (H_E) and observed (H_O) heterozygosity were then calculated in order to characterise the polymorphism of the markers. The allelic richness for particular loci and populations was

computed using the rarefaction procedure implemented in FSTAT 2.9.3 (Goudet 2001), expected (unbiased estimate according to Nei 1978) and observed heterozygosity was calculated using GENETIX 4.05.2 (Belkhir et al. 1996–2004). Hardy-Weinberg equilibrium for particular loci in populations was tested using GENEPOP 3.4 (Raymond & Rousset 1995). We corrected for multiple testing with QVALUE software (Storey 2002) using the false discovery rate (FDR) approach (Benjamini & Hochberg 1995). Frequency of null alleles was computed using FREENA software (Chapuis & Estoup 2007).

Based on (i) the polymorphism rate of the locus, especially in the Tatra chamois populations, (ii) length of the amplified fragment, (iii) frequency of null alleles, and (iv) previous use of the marker in other studies of European chamois populations, the most suitable loci for noninvasive genotyping were selected. For all the populations studied, genotypic linkage disequilibrium between the selected microsatellites was tested using exact tests based on the Markov chain method implemented in GENEPOP 3.4. The FDR correction for multiple testing was done using QVALUE software. Unbiased probability of identity ($PI_{unbiased}$, corrected for small sample size) and probability of identity for siblings (PI_{sibs}) were calculated for selected loci using software GIMLET 1.3.3 (Valière 2002).

Three multiplex sets were designed from the selected loci and in addition, an ungulate species based SRY marker was included into the second multiplex set to enable sex identification (Wilson & White 1998); the microsatellite loci serving as a positive control of successful amplification. For all three sets, PCR was performed using the Qiagen Multiplex PCR Kit (Qiagen). Each reaction contained 5 μL of Multiplex PCR Master Mix, 1 μL of Q-Solution, primers at various concentrations (Table 2), 1 μL of extracted DNA and ddH_2O to a volume of 10 μL. Cycling conditions for the first set were as follows: an initial activation step at 95°C for 15 min, followed by 10 cycles of touch down PCR at 94°C for 30 s, 59°C−0.2°C per cycle for 90 s and 72°C for 60 s, followed by 28 cycles at 94°C for 30 s, 57°C for 90 s and 72°C for 60 s, with a final extension at 60°C for 30 min. The cycling conditions were the same for each set except annealing temperatures, i.e. 59°C−0.4°C per cycle for 90 s (10 ×) and 55°C for 90 s (28 ×) for the second set and 63°C−0.8°C per cycle for 90 s (10 ×) and 55°C for 90 s (28 ×) for the third set. The PCR products were electrophoresed on the ABI 3130 Genetic Analyzer and analysed as described above. Polymorphism of the multiplex sets was assessed based on 17, 5 and 22 tissue samples from the Tatra, Low Tatra and Slovenský raj Mts., respectively.

Subsequently, 347 samples of faeces obtained from specimens from all the Slovak chamois populations were analysed in order to assess the amplification success rate using the three multiplex sets. Very fresh faeces were collected in cold weather, usually from snow. These were either stored in 96% ethanol and put into a cool-box in the field and then into a freezer as soon as possible, or placed straight into silica gel and stored at room temperature. DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen) with washing of the pellet surface in the ASL buffer (Qiagen) for ca. 50 min as the first step. The PCR protocols, cycling conditions and fragment analysis were the same as those described for multiplex PCR of tissue samples, with 2 μL of extracted DNA used in the reaction instead of 1 μL. Two PCRs per sample and per multiplex set were performed, representing 670–694 PCR reactions for each multiplexed microsatellite locus. Subsequently the amplification success rate was assessed as a proportion of positive PCRs from all the PCRs performed. In this study, we did not aim at construction of consensus genotypes (based on multiple tubes approach, Taberlet et al. 1996), thus we were not able to assess the frequency of genotyping errors (i.e. allelic dropout and false alleles). Instead, we implemented comparison of two PCRs for every particular sample and locus as another indicator of reliability of the faecal DNA amplification using our protocols. Only the samples with both PCRs positive were taken into account, i.e. 276–321 PCR (552–642 allele) pairs were compared for particular loci and proportion of matching alleles was subsequently assessed. Furthermore, 'homozygotes' (i.e. samples that were genotyped as a homozygote for the identical allele in both PCR repetitions) were excluded and the proportion of matching alleles was computed from only the remaining ('heterozygote') PCRs, which represented 90–410 allele pairs for particular loci.

When using faecal DNA, we were not able to clearly amplify two of the loci, NRAMP1 and SRCRSP06, with any of the sets. For this reason, we excluded SRCRSP06 from faecal DNA analysis and NRAMP1 (the more informative locus) was amplified separately using the same PCR protocol and cycling conditions as for the initial testing of microsatellites (Eppendorf HotMaster Taq DNA Polymerase, annealing temperature 52°C), with only the amount of DNA extract used differing, i.e. 2 or 3 μL of faecal DNA in a reaction. Nevertheless, due to considerably lower success rate achieved using this protocol when compared to the

Table 1. *List of 45 microsatellite markers tested in this study but not selected for multiplex sets. n = number of tissue samples; A = number of alleles; AR = allelic richness (based on n = 5); H_E = expected heterozygosity – unbiased estimate according to Nei (1978); H_O = observed heterozygosity; Ref = reference. After the reference, the species/domesticated form for which the locus was developed is noted. All polymorphic loci were in Hardy-Weinberg equilibrium in all populations when p-values were corrected for multiple testing (FDR correction in QVALUE software).*

	Tatra chamois						Alpine chamois				
	Tatra Mts. (n = 11)			Low Tatra Mts. (n = 5)			Slovenský raj Mts. (n = 22)				
Locus	A/AR	H_E	H_O	A/AR	H_E	H_O	A/AR	H_E	H_O	Size (bp)	Ref
BM203	Monomorphic			Monomorphic			2/2.00	0.474	0.546	215–230	1)
BM848	2/2.00	0.524	0.636	3/3.00	0.511	0.600	Monomorphic			232–238	1)
BM1329	Monomorphic			Monomorphic			2/1.95	0.359	0.273	165–174	1)
BM1818	Monomorphic			Monomorphic			2/2.00	0.507	0.546	258–269	1)
BM4505	2/2.00	0.507	0.455	2/2.00	0.467	0.200	2/2.00	0.507	0.546	245–264	1)
HSC	Monomorphic			2/2.00	0.200	0.200	2/2.00	0.485	0.409	268–272	2)
ILSTS005	Monomorphic			Monomorphic			2/1.74	0.206	0.136	166–172	3)
ILSTS019	2/1.86	0.247	0.273	Monomorphic			2/2.00	0.474	0.364	168–191	4)
INFG = OarKP6	Monomorphic			Monomorphic			2/1.98	0.426	0.500	199–205	5)
INRA003	Monomorphic			Monomorphic			2/2.00	0.511	0.500	185–187	6)
INRA005	Monomorphic			Monomorphic			2/1.23	0.046	0.046	156–160	7)
INRA023*	2/2.00	0.525	0.375	2/2.00	0.467	0.600	Monomorphic			198–202	8)
INRA049	Monomorphic			Monomorphic			2/1.99	0.444	0.455	164–166	7)
McM218	2/2.00	0.520	0.546	3/3.00	0.600	0.600	3/2.92	0.639	0.773	278–298	9)
OarFCB11	Monomorphic			3/3.00	0.378	0.400	Monomorphic			156–160	10)
SRCRSP01	Monomorphic			Monomorphic			2/2.00	0.485	0.409	126–128	11)
SRCRSP08	2/1.99	0.416	0.364	2/2.00	0.533	0.400	3/2.94	0.661	0.546	225–243	12)
SY3A	2/1.99	0.416	0.364	2/2.00	0.533	0.400	Monomorphic			296–298	13)
SY12A	Monomorphic			2/2.00	0.200	0.200	2/1.55	0.130	0.136	189–193	13)
SY84B	Monomorphic			Monomorphic			2/1.93	0.333	0.318	215–223	13)
SY93	2/1.86	0.247	0.273	Monomorphic			2/1.98	0.426	0.409	86–90	13)
BM757	Monomorphic										1)
BMC1009	No PCR product										1)
HEL1	Non-specific PCR products										14)
ILSTS011	Monomorphic										15)
INRA036	No PCR product										7)
INRA040	Monomorphic										7)
INRA063**	Monomorphic on gel										7)
MAF65	No PCR product										16)
MAF70	Non-specific PCR products										17)
MB026	Monomorphic										18)
McM527	Monomorphic										19)
OarAE119	Monomorphic										20)
OarCP20	No PCR product										21)
OarCP34	Monomorphic										22)
SRCRSP12	No PCR product										23)
SRCRSP14	No PCR product										23)
SRCRSP15	No PCR product										23)
SRCRSP23	Non-specific PCR products										24)
SY3B	Non-specific PCR products										13)
SY12B	Monomorphic										13)
SY17	Monomorphic										13)
SY48	Non-specific PCR products										13)
SY50	Monomorphic										13)
SY242	Monomorphic										13)

** INRA023: corrected sequence of reverse primer as referred on http://projects.roslin.ac.uk/cdiv/markers.html (CaDBase, Roslin Institute, Edinburgh). ** INRA063: analysed for polymorphism only on 4% agarose gel.*

1) Bishop et al. 1994, cattle; 2) Blattman & Beh 1992, domestic sheep; 3) Brezinsky et al. 1993a, cattle; 4) Kemp et al. 1993, cattle; 5) Paterson & Crawford 2000, domestic sheep; 6) Vaiman et al. 1992, cattle; 7) Vaiman et al. 1994a, cattle; 8) Vaiman et al. 1994b, cattle; 9) Hulme et al. 1996, domestic sheep; 10) Buchanan & Crawford 1993, domestic sheep; 11) Arevalo et al. 1994, domestic goat; 12) Bhebhe et al. 1994, domestic goat; 13) An et al. 2005, Korean goral; 14) Kaukinen & Varvio 1993, cattle; 15) Brezinsky et al. 1993b, cattle; 16) Buchanan et al. 1992, domestic sheep; 17) Buchanan & Crawford 1992a, domestic sheep; 18) Cassar et al. 2007, cattle; 19) Hulme et al. 1994, domestic sheep; 20) Penty et al. 1993, domestic sheep; 21) Ede et al. 1995a, domestic sheep; 22) Ede et al. 1995b, domestic sheep; 23) Kogi et al. 1995, domestic goat; 24) Yeh et al. 1997, domestic goat.

multiplexed loci, the Qiagen Multiplex PCR Kit was later used also for the singleplex PCR of NRAMP1. The PCR protocol was the same as that described for multiplex sets, using 0.25 μM of each primer and 2 μL of faecal DNA extract. Cycling conditions differed from those described for the first multiplex set only during the annealing steps, i.e. touch down at 59°C−0.5°C per cycle for 90 s (10 ×) and 54°C for 90 s (28 ×). In total, 346 and 342 PCRs were analysed using the first ('Eppendorf HotMaster Taq DNA Polymerase') and the second ('Qiagen Multiplex PCR Kit') protocol, respectively. The amplification success rate was calculated for each protocol.

Results

From the 65 tested microsatellites, 41 loci proved polymorphic in at least one of studied populations (Table 1 and 2). Twenty-one loci (32.3%) were polymorphic in both Tatra chamois populations and 16 (24.6%) in all three populations. None of the loci deviated from Hardy-Weinberg equilibrium in any of the populations after FDR correction for multiple testing. Following the above described criteria, 20 loci deemed most suitable for noninvasive genetic analyses of population structure of Slovak chamois were selected (Table 2). No evidence of linkage disequilibrium was observed between any of the loci analysed, i.e. no pair of loci remained significant after FDR correction. Multi-loci $PI_{unbiased}$ for all 20 loci was 3.101×10^{-7}, 1.696×10^{-10} and 4.335×10^{-10} in Tatra, Low Tatra and Slovenský raj population, respectively, while multi-loci PI_{sibs} was 1.088×10^{-3}, 1.669×10^{-3} and 5.645×10^{-5} in the particular populations. Based on these calculations, nine, ten and seven most informative loci should be sufficient to find less than 1% of individuals with identical genotype in the Tatra, Low Tatra and Slovenský raj population, respectively, even if the individuals are closely related (siblings).

Analysis of tissue samples using three multiplex sets of selected loci revealed low genetic diversity in all the Slovak chamois populations studied (Table 2). Mean number of alleles and mean allelic richness calculated for the lowest sample size (n = 5) were 2.1 and 1.88 in the Tatra population, 2.0 and 1.95 in the Low Tatra population, and 2.4 and 2.25 in the Slovenský raj population, respectively. Expected heterozygosity was also lower in endemic Tatra chamois (0.331 in the Tatra Mts. and 0.341 in the Low Tatra Mts.) as compared to Alpine chamois in the Slovenský raj Mts. (0.460). Genetic identification of sex (using SRY marker) was consistent with morphological assessments, which were undertaken after legal hunting or, if possible, after finding of carcasses.

Success rate of faecal DNA amplification was 90.7%, ranging from 85.1% to 92.7% for particular loci, and was 91.5%, 91.5% and 88.4% for the first, second, and third set, respectively. The first and second PCR of individual faecal samples matched in 98.8% of the total number of alleles and the proportion of matching alleles ranged from 97.1% to 99.8% for particular loci. When 'homozygotes' were excluded, the proportion of matching alleles remained very high. The first and second PCR of 'heterozygous loci' matched in 97.1% of alleles and the values for the particular loci ranged from 94.5% to 99.7%, with the exception of locus SY58 which reached only 88.8%. Amplification success rate of 36.7% was detected in singleplex PCR of NRAMP1 locus using the 'Eppendorf HotMaster Taq DNA Polymerase' protocol. Application of the Qiagen Multiplex PCR Kit increased the proportion of positive PCRs to 91.5%.

Discussion

Low genetic variability was found in all the studied Slovak chamois populations, with lower values in both autochthonous and introduced population of Tatra chamois when compared to the Slovenský raj population of Alpine chamois. This result was obtained despite the fact that polymorphism in the Tatra chamois was one of the criteria for microsatellite selection, and that the Slovenský raj population was established through the introduction of a very low number of individuals. Slightly higher values for Tatra chamois, especially for the Low Tatra population, were found in the study of Crestanello et al. (2009), ascertaining $H_E = 0.33$ and 0.41 and $A = 2.18$ and 2.82 in the Tatra and Low Tatra population, respectively, when six and nine samples from the populations were analysed. This probably resulted from the different microsatellite markers used as well as different, and rather small, number of individuals analysed in those two studies.

Low genetic diversity is obviously not unusual in populations of highly endangered mountain ungulates that have suffered dramatic declines in their demographic history. Lorenzini (2005) observed a similarly low level of polymorphism (only 10 polymorphic microsatellites from 60 tested, all but one with only two alleles, although with slightly higher heterozygosity values) in Apennine chamois (*R. pyrenaica ornata*), which became nearly extinct in the late 1940s (less than 100 animals). Very low genetic variability was also recorded for alpine populations of ibex (*Capra ibex*) by Maudet et al. (2002), who rank their results (mean H_E = 0.29–0.45, but only 0.13

Table 2. *Characteristics of 20 multiplexed microsatellite markers found suitable for noninvasive genetic sampling study of Slovak chamois populations (+ ungulate species based SRY marker). n = number of tissue samples; A = number of alleles; AR = allelic richness (based on n = 5); H_E = expected heterozygosity – unbiased estimate according to Nei (1978); H_O = observed heterozygosity; Set = multiplex set in which the locus was included; FC = final concentration of primers in multiplex PCR; Dye = fluorescent dye of forward primer. After the reference, the species/domesticated form for which the locus was developed is noted – sheep = domestic sheep, goat = domestic goat, goral = Korean goral. NRAMP1 and SRCRSP06 loci were only amplified in the third multiplex set when tissue samples were analysed; for analyses of faeces, NRAMP1 was amplified in singleplex PCR and SRCRSP06 was not used. All loci were in Hardy-Weinberg equilibrium in all populations when p-values were corrected for multiple testing (FDR correction in QVALUE software).*

	Tatra chamois						Alpine chamois							
	Tatra Mts. (n = 17)			Low Tatra Mts. (n = 5)			Slovenský raj (n = 22)							
Locus	A/AR	H_E	H_O	A/AR	H_E	H_O	A/AR	H_E	H_O	Set	FC (μM)	Dye	Size (bp)	Reference
BM1258	2/1.77	0.214	0.118	2/2.00	0.200	0.200	3/2.74	0.517	0.455	3	0.08	VIC	111–124	Bishop et al. 1994, cattle
BOBT24	2/2.00	0.499	0.471	2/2.00	0.467	0.200	3/2.89	0.630	0.455	2	0.25	VIC	161–174	Buitkamp et al. 1996, cattle
CSSM66	3/2.94	0.656	0.765	3/3.00	0.600	0.600	2/2.00	0.495	0.455	1	0.20	FAM	193–227	Barendse et al. 1994, cattle
ETH10	2/2.00	0.487	0.529	2/2.00	0.200	0.200	2/1.90	0.304	0.273	1	0.20	NED	206–208	Toldo et al. 1993, cattle
ETH225	2/2.00	0.513	0.471	2/2.00	0.533	0.400	3/2.86	0.635	0.682	3	0.10	FAM	142–150	Steffen et al. 1993, cattle
ILSTS030	3/2.62	0.437	0.471	2/2.00	0.533	0.800	3/2.73	0.563	0.546	3	0.50	PET	160–169	Kemp et al. 1995, cattle
INRA121	2/2.00	0.508	0.412	2/2.00	0.467	0.600	1/1.00	0	0	2	0.10	FAM	109–111	Vaiman et al. 1994a, cattle
MAF214	2/2.00	0.499	0.706	2/2.00	0.467	0.200	3/2.88	0.621	0.455	3	0.80	PET	214–252	Buchanan & Crawford 1992b, sheep
NRAMP1	1/1.00	0	0	2/2.00	0.356	0.400	3/2.39	0.487	0.409	3	0.30	VIC	195–213	Matthews & Crawford 1998, sheep
OarFCB20	2/1.51	0.114	0.118	2/2.00	0.200	0.200	3/2.69	0.513	0.636	1	0.80	PET	81–101	Buchanan et al. 1994, sheep
OarFCB304	4/2.25	0.271	0.294	1/1.00	0	0	3/2.74	0.595	0.455	1	0.10	FAM	127–148	Buchanan & Crawford 1993, sheep
SRCRSP05	2/1.66	0.166	0.177	1/1.00	0	0	2/2.00	0.502	0.500	1	0.15	VIC	163–169	Arevalo et al. 1994, goat
SRCRSP06	2/1.77	0.214	0.235	2/2.00	0.200	0.200	1/1.00	0	0	3	0.60	PET	140–142	Bhebhe et al. 1994, goat
SRCRSP09	2/1.98	0.401	0.529	2/2.00	0.556	0.600	2/1.55	0.130	0.136	3	0.30	NED	124–132	Bhebhe et al. 1994, goat
SRCRSP11	2/2.00	0.471	0.471	2/2.00	0.356	0.400	2/2.00	0.474	0.364	2	0.90	PET	114–120	Kogi et al. 1995, goat
SY58	1/1.00	0	0	2/2.00	0.356	0.400	3/2.81	0.622	0.500	1	0.15	PET	199–203	An et al. 2005, goral
SY84	1/1.00	0	0	2/2.00	0.200	0.200	2/2.00	0.495	0.546	1	0.70	NED	172–176	An et al. 2005, goral
SY259	2/1.29	0.059	0.059	2/2.00	0.200	0.200	2/1.99	0.460	0.500	2	0.10	FAM	157–163	An et al. 2005, goral
SY434	3/2.92	0.628	0.647	2/2.00	0.467	0.600	3/2.89	0.647	0.682	2	0.30	NED	84–100	An et al. 2005, goral
TGLA53	2/2.00	0.487	0.177	2/2.00	0.467	0.600	2/2.00	0.502	0.682	2	0.40	VIC	139–143	Barendse et al. 1994, cattle
SRY	-	-	-	-	-	-	-	-	-	2	0.15	PET	180	
mean*	2/1.88	0.331	0.332	2/1.95	0.341	0.350	2/2.25	0.460	0.436					

** Mean values were calculated including monomorphic loci.*

when monomorphic loci were included) amongst the lowest reported from microsatellites in mammal species. These results all highlight the significance of demographic history (especially population bottlenecks and prolonged periods at low numbers) in affecting genetic variability within a population.

We reached a very high amplification success rate (90.7%) when faecal DNA was analysed, which, in general, is not typical for noninvasive genetic sampling. However, also other noninvasive studies of ungulates report very high success rate. Flagstad et al. (1999) analysed reindeer (*Rangifer tarandus*) and domestic sheep (*Ovis aries*) faecal samples with 95% and 96.5% PCR success rate, respectively. Further, 99% and 95% of Corsican mouflon (*Ovis musimon*) and Alpine ibex (*Capra ibex*) genotyping repetitions, respectively, provided correct genotype when winter samples were examined (Maudet et al. 2004b). Even in Central Africa (Gabon), where climate enhances quick DNA degradation, forest ungulate faeces of ≥ 50 pg/μL DNA concentration were analysed with 83% amplification success or greater (Soto-Calderón et al. 2009). Although in some other mammals, e.g. in Asian elephant (*Elephas maximus*), very high PCR success was achieved (99.6%, Fernando et al. 2003), usually in the non-ungulate species the success is lower (for overview of amplification success rate in extensive set of mammal species see Broquet et al. (2007) and Hájková et al. (2009).

Many factors influence quality and quantity of target DNA in a sample as well as our ability to utilise this DNA during the analyses (e.g. Waits & Paetkau 2005, Broquet et al. 2007). In ungulates, that produce pellet-like faeces, this can be an important factor for high success of the analyses, as it is possible to wash surface of the pellet and thus utilise only outer part of the sample, which seems to be very efficient (Flagstad et al. 1999, Wehausen et al. 2004, Stenglein et al. 2010). Also in the study of Fernando et al. (2003) the outer parts of elephants' dungs were collected by scraping of the surface and used for analysis. Together with the other factors that have strong impact on PCR success, we found that also chemicals, not only those used for sample storage or DNA extraction (e.g. Piggott & Taylor 2003), but also PCR chemicals may affect the success rate significantly. In our study, the use of Qiagen Multiplex PCR Kit increased PCR success in NRAMP1 locus two and a half times.

High proportion of matching alleles between two PCR repetitions in faecal samples – 97.1% (when 'homozygotes' were excluded) – may indicate future low allelic dropout in our data. This is in concordance with high amplification success rate achieved. Locus SY58 showed lower proportion (88.8%) of matching alleles than other loci, thus higher frequency of allelic dropout is expected in this case. This might be an effect of higher length of the locus (Buchan et al. 2005); another possible reason is mutation in primer sequence of one/some of the alleles and thus lower frequency of its/their amplification in faecal DNA analysis (Okello et al. 2005).

Through the testing of an extended set of microsatellite markers that had previously been used in studies of chamois or other Caprinae, we were able to identify those that showed the highest level of polymorphism in Slovak chamois populations. These proved suitable for studies of population structure and demographic history of the highly endangered Tatra chamois. As we considered amplification fragment length through a marker selection process, we were able to develop a unique tool enabling the study of our target species using noninvasive genetic sampling, the only feasible method for large scale analyses of population structure, e.g. using a landscape genetic approach. The usability of our sets in faecal samples was confirmed also through achievement of high amplification success rate.

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