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TECHNICAL NOTE

New fourteen highly polymorphic STR markers developed and characterized from edible dormouse (*Glis glis*)

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Abstract Fourteen new polymorphic di- and di-tetra complex microsatellite loci were isolated and characterized from the edible dormouse (*Glis glis*). The markers were tested on 427 individuals representing populations from Croatia and Poland. All loci were polymorphic, with the number of alleles per locus ranged from 4 to 31, with a mean of 10. Observed and expected heterozygosities ranged from 0.208–0.957 to 0.228–0.907, respectively. No evidence of linkage disequilibrium between loci has been found. These highly polymorphic markers could provide a powerful tool for detailed genetic studies of this endangered species.

Keywords Microsatellites · Polymorphic loci · Endangered species · Multiplex PCR · Edible dormice

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The edible dormouse (*Glis glis*) (Rodentia: Gliridae) is largely widespread in western, central, and southeastern Europe and western Asia (Storch 1978). This hibernating arboreal rodent is especially influenced by habitat fragmentation and human activities. In Poland, where edible dormouse is endangered and protected by law (Pucek and Jurczyszyn 2001), some efforts have been made to develop management plans for *Glis glis* that include monitoring, protection, and reintroduction of this species. All these activities could be monitored and helped by the use of polymorphic DNA markers. Despite the efforts that were done to develop microsatellite markers for this species (Hürner et al. 2009; Dabert et al. 2009; Segelbacher et al. 2010), still there was a need of new markers due to a relatively low level of polymorphism of the published loci, which displayed no more than 7 alleles per locus. Here we present new fourteen polymorphic microsatellite markers for dormice, including four loci exhibiting from 10 to 31 alleles per locus.

Total genomic DNA for this study was extracted from a liver tissue of the edible dormouse specimen died during hibernation in the Zoological Garden in Poznan, 1997. After digestion with *Hae*III and *Alu*I (NEB, New England Biolabs, USA) DNA was treated with MBN nuclease (NEB) and FastAP alkaline phosphatase (Fermentas, Lithuania). Then, column-purified DNA fragments were ligated to the double-stranded SNX linkers as described by Hamilton et al. (1999). Ligation success was monitored by PCR with SNX forward primer. Ligation mixture was denatured at 95 °C for 5 min and hybridized to 5′-biotinylated $(CA)_{12}$ and $(CT)_{12}$ oli-gonucleotides at 60 °C for 1 min, and then incubated in rotation at 60 °C for 15 min with streptavidin-coated paramagnetic beads (Dynabeads, Life Technologies, USA). Repeat-enriched fragments were PCR amplified using SNX forward primer and cloned into pCR4-TOPO vector using TOPO TA Cloning Kit (Life Technologies). After

screening, 96 positive clones were sequenced on an ABI 3130XL DNA analyzer (Applied Biosystems). After excluding clones with <15 repeats, we found 27 non-redundant microsatellite sequences flanked by regions sufficient to design primers. However, among primer sets developed for these loci, only 14 gave specific PCR products and/or were polymorphic in 15 tested specimens representing Croatian and Polish populations (Table 1).

The microsatellites were tested for polymorphism on 427 individuals representing one population from Croatia (48) and several populations from Poland (329). PCR amplifications with fluorescently labeled primers were carried out in 6 µl reactions containing Type-it Microsatellite Kit (Qiagen, Germany), 0.2 µM each of primers, and 10–100 ng of template DNA using program as follows: 95 °C for 5 min, 30 cycles of 95 °C for 30 s, annealing temperature (see Table 1) for 90 s, 72 °C for 30 s, and final extension at 65 °C for 45 min. M13-tailed loci (Oetting et al. 1995) were amplified at the same conditions, except the concentration of primers, which was 0.15 µM for forward primer, 0.25 µM for fluorescently labeled M13 primer, and 0.4 µM for reverse primer. We found that four loci (*23pilch*, *24pilch*, *30pilch*, and *36pilch*) could be amplified in a multiplex PCR under the conditions as described above. The amplified alleles were separated on an ABI PRISM 3130XL with Genescan 600LIZ size standard and scored in GeneMapper 4 (Applied Biosystems).

Hardy–Weinberg equilibrium (HWE) and genotypic disequilibrium between pairs of loci were tested using GENEPOP 4.0 (Rousset 2008). Number of alleles, and expected and observed heterozygosities were estimated in GENALEX 6 (Peakall and Smouse 2006). Each locus was tested for null allele frequencies using Micro-Checker (van Oosterhout et al. 2004). The number of alleles per

Table 1 Characterization and amplification conditions of 14 STR markers developed for edible dormice

Locus name/ GenBank Acc.	5′-Label	F/R	Primer sequence (5′–3′)	Repeat motif	Size range (bp)	T_a (°C)
7pilch	6FAM	F	CACCTGTAGTCCCCATACTTAG	$(CA)_{22}$	244–252	60
JX976185		R	TACTTCTGAGCTATACCCCTAGC			
20pilch	VIC	F	CAACAGATGAATGGATAAAG	$(TC)_{11}AT(CA)_{18}$	109–124	55
JX976186		R	TCTTCTTTATGGGTGTGTAG			
23pilch	NED	F	CGAAGAGGAGAGTTTATCAA	$(CA)_{20}$	185–193	60
JX976187		R	CTGGCAACCCTAGTAATCAT			
24pilch	VIC	F	TAACATAACCCACATACGGC	$(CA)_{19}$	255–261	60
JX976188		R	CAAGCAAGGCAGTAGAACAT			
26pilch	NED	F	TCACGCAGCACTACTCACAAT	$(CA)_{20}$	141–151	55
JX976189		R	TAGTATTTCTTTGTGTGTGTGT			
28pilch	NED	F	CCAGGAGAGGTAGAATGCC	$(CA)_{22}$	94–108	60
JX976190		R	GCTACTGCCAGAATGACACA			
30pilch	6FAM	F	AGAGAAGAAGGGCAGACACA	$(CA)_{23}$	97–115	60
JX976191		R	TAGGTATTGGGTCTCTGGTG			
31pilch	VIC	F	ATGGTTCCCTGGTTCTCTCT	$(CT)_{18}TT(CT)_5$	288–298	50
JX976192		R	AGTATTTGTCCTTCTGTGCC			
36pilch	PET	F	AACTTTCACCACCCTGCTCA	$(CT)_5TT(CT)_{19}$	182–206	60
JX976193		R	CAACTTGGAGATAGAATGTC			
37pilch	PET	F	CTAGAGAGACCCTGTTTCAA	$(CA)_{10}GA(CA)_3GA(CA)_3$ GA$(CA)_{15}$	206–230	55
JX976194		R	ACTTTCCTTTCTCTTCAGCA			
47pilch	PET	F	AGTCACTCCTACACAAACAT	$(GC)_5ACAC(GC)_4(CA)_{15}$	132–142	50
JX976195		R	GGATCATTTAACTAACAGCG			
48pilch	M13tail-VIC	F	GTCTTTCTCTCTCTCCATTTATTTCC	$(CTTT)_{12}(CT)_{22}$	233–289	60
JX976196		R	AGAGACAAAGAGAAGGAGGGA			
49pilch	M13tail-PET	F	CGCCTGATTCATTCAGACTGGA	$(CTTT)_{15}(N)_{95}(CTTT)_{11}$ $(CT)_{13}$	248–320	55
JX976197		R	AAAAAGAAGAAAGAAAGAGAGAG			
50pilch	M13tail-6FAM	F	AGCATGGTTATAGGAATTAG	$(CT)_{14}$	87–93	55
JX976198		R	ACAGAGAAACAGAGAGGGAC			

Locus name and GenBank Accession nos, 5′-labeling of PCR primer, forward (F), reverse (R), primer sequences, repeat motif, size range of observed allelic variation, optimal annealing temperature (T_a)

Table 2 Statistical parameters of 14 STR markers in dormouse populations

Population	Total		Poland		Croatia				
Locus name	N	Na	N	Na	N	Na	Ho/He	*P*-values (HWE)	NAF
7pilch	423	5	376	5	47	4	0.596/0.576	0.982	0.0819
20pilch	425	10	377	9	48	5	0.771/0.673	0.899	0.0000
23pilch	425	5	377	5	48	5	0.479/0.493	0.763	0.0090
24pilch	424	4	376	4	48	4	0.208/0.228	0.813	0.0157
26pilch	422	6	374	6	48	5	0.375/0.342	0.005*	0.0000
28pilch	415	8	375	6	40	4	0.250/0.305	0.365	0.3816
30pilch	425	6	377	5	48	5	0.375/0.389	0.427	0.0100
31pilch	411	6	370	4	41	5	0.268/0.265	0.474	0.3385
36pilch	425	12	377	12	48	9	0.938/0.819	0.883	0.0000
37pilch	425	9	378	9	47	5	0.426/0.574	0.833	0.1552**
47pilch	423	6	376	6	47	6	0.404/0.535	0.790	0.1507**
48pilch	426	26	378	22	48	12	0.896/0.878	0.242	0.0000
49pilch	424	31	377	19	47	20	0.957/0.907	0.227	0.0324
50pilch	361	4	313	3	48	2	0.354/0.489	0.056	0.0908

N, sample sizes; Na, number of alleles; Ho/He, observed/expected heterozygosities; P, probability values for exact tests of HWE; NAF, null allele frequencies

* significant deviations from HWE with confidence interval 95 %

** significant evidence of null allele with 95 % confidence interval

locus in all analyzed specimens ranged from 4 to 31 (Table 2), with a mean of 10. This relatively high level of polymorphism was found in both Polish and Croatian populations (a mean of 8 and 7 alleles per locus, respectively). The remaining statistical parameters were tested on the Croatian population only, because it showed a more equilibrated structure than strongly isolated populations from Poland. We found no evidence of linkage disequilibrium between loci. In two loci, *37pilch* and *47pilch*, significant evidences for null alleles were found with 95 % confidence interval. Only one locus (*26pilch*) demonstrated significant deviations from HWE in the sampled population (P value = 0.005), and exhibited deficit in heterozygotes. This deviation from expectations could be a result of insufficient sample size of the tested Croatian population.

These new highly polymorphic microsatellite loci in combination with previously published markers (Hürner et al. 2009; Dabert et al. 2009; Segelbacher et al. 2010) could provide a powerful tool for detailed genetic studies of this endangered species.

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References

Dabert M, Jarmolowski A, Jurczyszyn M (2009) New polymorphic microsatellite loci developed and characterized from edible dormouse (*Glis glis*). Conserv Genet 10:2029–2031. doi:10.1007/s10592-009-9886-0

Hamilton MB, Pincus EL, Di Fiore A, Fleischer RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques 27:500–507

Hürner H, Martin JF, Ibas AR, Arrizabalaga A, Michaux JR (2009) Isolation, characterization and PCR multiplexing of polymorphic microsatellite markers in the edible dormouse *Glis glis*. Mol Ecol Resour 9(3):885–887. doi:10.1111/j.1755-0998.2008.02365.x

Oetting WS, Lee HK, Flanders DJ, Wiesner GL, Sellers TA, King RA (1995) Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. Genomics 30:450–458

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295. doi:10.1111/j.1471-8286.2005.01155.x

Pucek Z, Jurczyszyn M (2001) *Glis glis* (Linne, 1766) Fat dormouse (edible dormouse). In: Glowaciński Z (ed) Polish red data book of animals. PWRL, Warszawa, pp 79–81

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour 8:103–106. doi:10.1111/j.1471-8286.2007.01931.x

Segelbacher G, Tomiuk J, Heinz T, Kun JFJ, Weis-Dootz T, Fietz J (2010) Permanent genetic resources added to the molecular ecology resources database 1 February 2010–31 March 2010. Mol Ecol Resour 10(4):751–754. doi:10.1111/j.1755-0998.2010.02871.x

Storch G (1978) *Glis glis* (Linnaeus, 1766)—Siebenschläfer. In: Niethammer J, Krapp F (eds) Handbuch der Säugetiere Europas. Akademische Verlagsgeselschaft, Wiesbaden, pp 243–258

van Oosterhout C, Hutchison WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535. doi:10.1111/j.1471-8286.2004.00684.x