Isolation, characterization and PCR multiplexing of polymorphic microsatellite markers in the edible dormouse, *Glis glis*

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Abstract

We isolated and characterized 10 dinucleotide microsatellite loci in the edible dormouse, *Glis glis* (Linnaeus). Four multiplex panels were developed. Loci were amplified in samples from two geographically distant populations (Torgny in Belgium and Montseny in Spain). All loci were polymorphic in Spain but four were monomorphic in Belgium. Individuals from Belgium and Spain exhibited an average allelic diversity of 1.9 and 3.3 and an observed heterozygosity ranging from 0.08 to 0.47 and from 0.04 to 0.72, respectively.

Keywords: dormouse, *Glis*, microsatellite, multiplex, rodentia

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The edible dormouse, *Glis glis* (Linnaeus, 1766), is an arboreal and nocturnal inhabitant of deciduous and mixed forests in central Europe (Storch 1978). It is rare in many parts of its distribution particularly in the north where it is of conservation concern (Krystufek 1999). Here we report a set of novel polymorphic microsatellites that will be useful to describe the levels of genetic diversity and differentiation within and between populations, thus providing an initial genetic framework useful to identify conservation priorities and develop management strategies. We optimized these loci into four polymerase chain reaction (PCR) multiplexes.

Microsatellite screening was performed following A. Estoup and J. Turgeon (personal communication, see http://www.inapg.inra.fr/dsa/microsat/microsat.htm for detailed protocol). Genomic DNA was extracted from a single individual and digested using *Rsa*I restriction enzyme (Promega). A 500–900-bp fraction of the digested DNA was selected, purified and ligated to Rsa-21 and Rsa-25 linkers (Rsa-21 (5′–3′) CTCTTGCTTACGCGTGGACTA; Rsa-25 (5′–3′) phosphate-TAGTCCACGCGTAAGCAAGAG). The enrichment procedure followed the protocol from Kijas *et al*. (1994), based on streptavidin-coated magnetic particles (Magnesphere, Promega), with slight modifications. Both 5′–biotinylated $(TC)_{10}$ and $(TG)_{10}$ oligonucleotides were used as probes. Enrichment products were then amplified with Rsa-21, purified and ligated into pGEM-T Easy Vector (Promega) then transformed into *Escherichia coli* competent cells (JM109, Promega). A total of 2103 recombinant clones were transferred on Hybond-N nylon membranes (Amersham). Colonies were hybridized at 46.5 C with the mixture of oligonucleotide probes $(TC)_{10}$ and $(TG)_{10}$ labelled with the DIG oligonucleotide tailing kit (Roche). A total of 435 positives clones were detected, 96 of which were sequenced using a BigDye terminator (Applied Biosystems) sequencing kit on an ABI 310 (Applied Biosystems) automated sequencer. A total of 20 unique sequences containing clear repeated motifs were appropriate for designing primers. We designed pairs of primers using the Primer Designer Software (version 2.0, Scientific & Educational Software 1990, 1991).

The 20 selected loci were first tested using monolocus PCRs. Ten of them provided good quality and polymorphic amplification products and were thus retained for multiplex PCRs. The forward primer of each locus was 5′-end labelled with a fluorescent dye (FAM, HEX, NED). Based on size limitations and amplification specificity, we distributed the 10 microsatellites in four sets: set A (*Gg9*, *Gg13*, *Gg3*), set B (*Gg12*, *Gg14*, *Gg8*), set C (*Gg15*, *Gg11*) and set D (*Gg7*, *Gg16*) (Table 1). Amplification of the four PCRs was performed on a Mastercycler ep-gradient-S (Eppendorf) using the Multiplex PCR kit (QIAGEN). The multiplex PCRs were conducted following Gauffre *et al*. (2007). All multiplex PCRs started with an initial activation at 95 C for 15 min, followed by 35 cycles (set A and set D) or 30 cycles (set B and set C) of 30 s at 94 C, annealing at 58 C (set A) or 63

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Table 1 Characteristics of 10 microsatellite loci from *Glis glis* with primer sequences, size of cloned allele, number of alleles (A), size range of PCR product, expected (H_E) and observed (H_O) heterozygosities with deviation from Hardy–Weinberg proportions (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) and null allele frequency (NAF) calculated by FreeNA software

Locus Accession no.	Primer sequence (5′–3′) includes fluro-label dye	Repeat motif	Size (bp)	Belgium ($n = 36$)				Spain ($n = 17$)			
				A	Size range	H_E/H_O	NAF	A	Size range	H_E/H_O	NAF
Gg9 AM930996	F NED-GTTCAAATGTTTAGAGACTC R-GTAAAGATGCTGATGAAGG	$(TG)_{18}$	189	2	188–194	0.21/0.22	0	3	194–200	0.25/0.16	0.169
Gg13 AM930997	F FAM-TCTATCCCTTGATCCTAG R-CATGAAATGGGACTTCTTC	$(TG)_{20}$	191	4	188–194	0.16/0.17	0	4	188–196	0.74/0.55	0.061
Gg3 AM930998	F FAM-CACAACAGCCCATCATAC R-GATGGACGCATAATAATCG	$(CA)_{20}$	239	1	235	—	0.001	4	233–239	0.68/0.55	0.015
Gg12 AM930999	F HEX-CACAGCCCATCATTGACAG R-GTATTAGTTAGGGGTTTCCAGA	$(CA)_{17}$	119	1	118	—	0.001	2	116–118	0.54/0.42	0.066
Gg14 AM931000	F FAM-ATTTGGCTGGAGGTATAACTTG R-GGGTGTGAGGGGATGAAA	$(CA)_{20}$	141	2	138–140	0.28/0.22	0.099	2	138–140	0.32/0.17	0.135
Gg8 AM931001	F NED-CTATCGGGGACGGGTTTTG R-CTCCTGTTTCTTGCCAATTCC	$(CA)_{18}$	205	1	211	—	0.001	3	207–213	0.44/0.50	0
Gg15 AM931002	F HEX-CCCCTTCTCAAATTGTCTC R-GAATAAATAGGACTGGGGGT	$(TG)_{21}$	146	1	145	—	0.001	4	141–147	0.74/0.67	0.062
Gg11 AM931003	F HEX-GCCAGTGTGGTAACTTAG R-GATTCATGTTTGAGCTATTACC	$(CA)_{20}$	258	2	258–260	0.18/0.08*	0.056	5	252–260	0.74/0.39**	0.215
Gg7 AM931004	F HEX-CCACATCTGACCCCAAAC R-GAGGATCACAGGTTGAGGA	$(TG)_{21}$	205	3	202–210	0.50/0.33	0.064	4	202–210	0.66/0.72	0
Gg16 AM931005	F NED-CTTTCCTTCCCTGTTTGTCTC R-GGGGATGGTAGAATGCCTC	$(TG)_{18}$	122	2	127–129	0.44/0.47	0	2	129–133	0.22/0.04**	0.157

— not enough alleles to perform HW test.

(set B, set C and set D) for 90 s, extension at 72 C for 60 s and final extension of 60 C for 30 min. The PCR products were detected using a monocapillary ABI PRISM 310 Genetic Analyser (Applied Biosystems). Null allele frequency (NAF) was determined using the expectation maximum algorithm (EMA) (Dempster *et al*. 1977), and the F_{ST} values were then adjusted accordingly in FreeNA (Chapuis & Estoup 2007).

We tested the four microsatellite sets on 36 *G. glis* trapped in Torgny (southern Belgium) and on 17 *G. glis* trapped in Montseny National Park (northern Spain). All loci were polymorphic in the Spanish populations but four were monomorphic in Belgium (Table 1). Individuals from Spain and Belgium exhibited an average of 3.3 and 1.9 alleles per locus, ranging from two to five and one to four, respectively. The small number of alleles observed in Belgium may be due to genetic drift and inbreeding because these populations are fragmented and small in size. Exact tests performed using Arlequin 2.0 program (Schneider *et al*. 2000) revealed significant deviations from Hardy–Weinberg equilibrium for locus *Gg11* in Belgium and for two loci (*Gg11*, *Gg16*) in Spain. Both loci exhibited a deficit in heterozygotes, indicating the possibility of null alleles (Table 1). The values of NAF were always ≤ 0.2 (except for *Gg11*), indicating that null alleles are not expected to cause significant problems in the analysis (Chapuis & Estoup 2007). Therefore, we only considered locus *Gg11* (NAF = 0.215) to be potentially problematic. When correcting allele frequencies for putative null alleles, we found that the presence of null alleles did not change the outcome of the statistical test of differentiation between two populations (F_{ST} not using EMA = 0.58 and F_{ST} using EMA = 0.57). After Bonferroni correction, no significant linkage disequilibrium was detected using Fisher's exact test conducted in GenePop (Raymond & Rousset 1995). In conclusion, these first microsatellite primers developed for *G. glis* will provide necessary and powerful molecular tools for management and conservation studies in the future.

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Sixteen polymorphic microsatellite markers from *Zizania latifolia* Turcz. (Poaceae)

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Abstract

Sixteen polymorphic microsatellite markers were isolated and identified in *Zizania latifolia* Turcz. (Poaceae), a perennial aquatic plant widespread in Eastern Asia. The microsatellite-enriched library was constructed using the fast isolation by AFLP of sequences containing repeats method. These markers revealed two to 14 alleles, with an average of 5.6 alleles per locus. The observed and expected heterozygosities varied from 0.071 to 0.690 and from 0.174 to 0.812, respectively. These markers will be useful for studying of gene flow and evaluating the genetic diversity of the *Zizania latifolia* population.

Keywords: genetic diversity, microsatellite, wild rice, *Zizania latifolia* Turcz.

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The *Zizania*, also known as 'wild rice', belongs to the tribe Oryzeae. The genus *Zizania* consists of four species, including *Zizania aquatica* L., *Zizania palustris* L., *Zizania texana* Hitchc. and *Zizania latifolia* Turcz. The first three are found throughout North America, known as Canadian wild rice, American wild rice and Texas wild rice, respectively; in contrast, *Z. latifolia* Turcz. is widely spread throughout Eastern Asia (Hass *et al*. 2003; Guo *et al*. 2007). The species *Z. latifolia* Turcz., is a perennial aquatic plant grown in lakes, ponds and wetlands, and its flowing can be abolished by epiphyte *Ustilago esculenta* infection (Guo *et al*. 2007). As a popular aquatic vegetable, *Z. latifolia* has been cultivated for more than 2000 years, and is distributed widely in the eastern and southern areas of China. Although wild populations of *Z. latifolia* are important genetic resources for vegetables and forage, very limited research on population structure and conservation of *Z. latifolia* has been reported. One previous study has assessed the genetic diversity of *Z. latifolia* using *Adh1a* gene sequences (Xu *et al*. 2008), but no microsatellite markers have been reported for *Z. latifolia*. Importantly, microsatellite markers are reliable tools for investigating population structure. Here we report the isolation and characterization of microsatellite markers designed for *Z. latifolia.*

Total genomic DNA was extracted from tender leaves using the cetyltrimethyl ammonium bromide method (Murray & Thompson 1980). The microsatellite-enriched library was constructed following the fast isolation by amplified fragment length polymorpshim (AFLP) of

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