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PRIMER NOTE

Isolation of 39 polymorphic microsatellite loci and the development of a fluorescently labelled marker set for the Eurasian badger (*Meles meles*) (Carnivora: Mustelidae)

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Abstract

We have isolated 78 microsatellite loci from the Eurasian badger (*Meles meles*). Of the 52 loci characterized, 39 were found to be polymorphic. A fluorescently labelled primer set was developed to enable individual-specific 17-locus genotypes to be obtained efficiently.

Keywords: badger, DNA profile, fluorescent set, Meles, microsatellite, Mustelidae

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British badgers (*Meles meles*) form stable social groups (Neal & Cheeseman 1996). As badgers occupy underground setts during the day and forage alone nocturnally, studying their breeding behaviour is extremely difficult. To enable investigation of relatedness, maternity and paternity, and so understand the social structure and mating system in this species, we have characterized 39 polymorphic microsatellite loci and developed a 17-locus fluorescently labelled primer set.

Blood was collected from individual badgers inhabiting Woodchester Park, Gloucestershire, England. Genomic DNA was extracted from blood using proteinase K and phenol:chloroform:isoamyl alcohol (Sambrook *et al*. 1989). Badgers have been implicated in the transmission of bovine tuberculosis. Therefore to reduce the possibility of human infection, the transfer of blood tissue was handled in a Level 3 containment laboratory (Advisory Committee on Dangerous Pathogens 1995) and blood samples were incubated at 95 °C for 30 min (Rubin 1991), after the proteinase K digestion had been performed.

Two unenriched microsatellite libraries were constructed. For the first library (A), genomic DNA from one male badger was digested with *Rsa*I, *Alu*I and *Hae*III (Advanced Biotechnologies, Abgene) and the 300–600-bp fraction was ligated to *Sma*I-digested CIP-dephosphorylated pUC18 (Stratagene, La Jolly, CA, USA). For the second library (B), genomic DNA (pooled from three male badgers, from three different social groups) was digested with *Mbo*I (Advanced Biotechnologies) and the 250–1150-bp fragments were ligated to *Bam*HI-digested SAP-dephosphorylated pBluescript II (Stratagene).

For both libraries, transformant colonies were screened with $(CAGT)_n$, $(GACT)_n$ (Amersham Pharmacia Biotech) and $(TTTC.AAAG)_n$ oligonucleotides [prepared as for $(CATG.GTAC)_n$ in Armour *et al*. 1994] radiolabelled with [α^{32}P]-dCTP.

In total, 238 positive clones were sequenced using an ABI 373 DNA Sequencer (Applied Biosystems). Seventy-eight clones were sequenced in both directions to create consensus sequence files. Sequences were found to be unique within each separate library (A or B) using GENEJOCKEY software (Biosoft). After submission to the EMBL database (accession numbers: AJ230687–97, AJ230699–725, AJ293349–85, AJ293387–89), all 78 sequences were checked for duplicates within and between libraries A and B, and confirmed to be unique using BLASTN 2.2.4 software (Altschul *et al*. 1997).

We designed primer pairs for the 78 loci using PRIMER3 software (Rozen & Skaletsky 2000). Each primer pair was used to polymerase chain reaction (PCR)-amplify a panel of 10–33 unrelated badgers, with each individual belonging to a different social group (Table 1). PCR profiles are provided in Table 1. PCR amplification was performed in a Hybaid Touchdown thermal cycler (Thermo Hybaid).

The 17 loci displaying the highest number of alleles with complementary size ranges were selected for the creation

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Table 1 Polymorphic microsatellite loci in the Eurasian badger, *Meles meles*

(a) Seventeen loci for which primers were fluorescently labelled and optimized for use on an ABI 377 DNA Sequencer in two gel-loading sets‡

Locus name	EMBL record, library (A/B) (= gel loading set) and clone name	Repeat motif (5′–3′)†	Primer sequences (5′–3′) (and 5′ primer label or 'pigtail', which is underlined)	Primer T_m (°C)	PCR profile T_a used (°C)	$MgCl_2$ conc. (mM)	Dil.	*N*	*A*‡	Exp. allele size (bp)†	Obs. allele size range (bp)‡	H_E	H_O
*Mel*101	AJ293349	$(CA)_{17}$	F: GTTTCTT-ACGGTCCACCAATGATGAAT	60	64–50	1.5	1 : 7	15	4	114	120–136	0.57	0.40
	A, BAD04D		R: *6-FAM*-CACAAATGGGAAGGTGTCCT	60									
*Mel*102	AJ293353	$(GT)_{20}$	F: GTTTCTT-CTATAATGGAAGGTGGGTTGA	57	64–50	1.5	1 : 20	24	4	187	193–199	0.69	0.71
	A, CGBA53		R: *6-FAM*-ACACGGATTTAACGCCTACG	60									
*Mel*103	AJ293356	$(AC)_{20}$	F: GTTTCTT-CCCTGAAAGGCTATTGGGTA	59	64–50	1.5	1 : 20	25	4	249	255–263	0.63	0.64
	A, CGBA79		R: *6-FAM*-GGCTGATGCAGTTAGTCTGG	58									
*Mel*104	AJ293352	$(CA)_{17}$	F: GTTTCTT-CCTTGTGAACTCACTGCAAC	57	64–50	2.5	1 : 13	29	8	306	315–331	0.80	0.59
	A, CGBA37		R: *6-FAM*-TACACTGACACCCTCAAGTCC	58									
*Mel*105	AJ293350	$(GT)_6G(GT)_{16}$	F: GTTTCTT-GATATTCCCCTCCCACCACT	60	64–50	1.5	1 : 13	26	8	129	136–150	0.86	0.81
	A, BAD05A		R: *TET*-CTCCAAGGGATCCTGGAACT	60									
*Mel*106	AJ293355	$(CA)_{21}$	F: GTTTCTT-CTGAAGCCAAATCCACTGAG	58	64–50	1.5	1 : 20	25	4	211	220–226	0.66	0.68
	A, CGBA78		R: *TET*-GCCACACTGGTGCCCTAAG	62									
*Mel*107	AJ293359	$(GT)_{22}$	F: GTTTCTT-CAAGATCTCCGCAATTCTCC	60	64–50	1.5	1 : 13	19	3	280	284–288	0.36	0.37
	A, CGBA103		R: *TET*-AACCCTAAATGTCTGTCAGTGG	58									
*Mel*108	AJ293354	$(CA)_{13}$	F: GTTTCTT-GTCTGGAGCCCCATGTTG	60	64–50	2.5	1 : 13	20	2	313	322–326	0.26	0.30
	A, CGBA71		R: *TET*-TCTTTGGAATGGAAGTTAATGG	58									
*Mel*109	AJ293357	$(GT)_{21}$	F: GTTTCTT-TGCCAATTAAGTGTCACGGT	59	64–50	1.5	1 : 6	25	5	122	106–129	0.73	0.56
	A, CGBA98		R: *HEX*-ATGTTTCCAGTTCTCAGAGGC	58									
*Mel*110	AJ293360	$(GT)_{25}$	F: GTTTCTT-CATGTTTGCCATTGGAAGG	60	64–50	2.5	1 : 5	10	5	326	324–334	0.73	0.70
	A, CGBA110		R: *HEX*-GCCAGTGCTTGAAATAAAGTAG	56									
*Mel*111	AJ230692	$(CA)_{15}$	F*: *6-FAM*-TGCATACAGCTCCCTGAAAG	59	64–50	1.5	1 : 9	24	3	126	130–138	0.66	0.58
	B, B10E08		R*: GTTTCTT-GTGGTAGATGCTGGGATAGTG	57									
*Mel*112	AJ230700	$(CA)_7CG(CA)_{20}$	F: GTTTCTT-GATCAAGTCCCACATTGCG	60	64–50	2.5	1 : 6	21	5	414	418–430	0.74	0.48
	B, B17D09		R: *6-FAM*-AAGGTCCATCCATGGTGTTG	61									
*Mel*113	AJ230713	$(CA)_{18}$	F: *HEX*-ATAGTTTGGGTTATTTTCTGGG	56	64–50	1.5	1 : 9	20	4	120	120–130	0.43	0.55
	B, B28B03.1		R: GTTTCTT-TTGAGAGGAAAGACCCTACG	57									
*Mel*114	AJ230695	$(CA)_{15}$	F: GTTTCTT-TGCTGAGAGTAGAGTGAACATG	56	57	1.5	1 : 9	22	4	222	231–237	0.74	0.68
	B, B12C07		R: *HEX*-AGAAGTGACAGAGATGAAGATAAAC	55									
*Mel*115	AJ230703	$(TTTTC)_3(TTTC)_{15}$	F*: GTTTCTT-GATCAGTGCCTTCTGGTGAG	58	64–50	2.5	1 : 6	28	7	342	330–351	0.79	0.71
	B, B19D10		R*: *HEX*-TCCTGAGTCTGCATAACTAGCC	59									
*Mel*116	AJ293351	$(TG)_{15}$	F: *NED*-AATAATTGTCAAGTCAATCACCG	58	64–50	1.5	1 : 9	29	6	107	113–135	0.62	0.45
	A, CGBA13		R: GTTTCTT-CCCATTCCCTTAGAAAGCAC	59									
*Mel*117	AJ293358	$(CA)_{17}$	F: *NED*-TTATCTGAGCCAACTTGTGAC	55	57	1.5	1 : 6	24	4	184	174–193	0.69	0.71
	A, CGBA102		R: GTTTCTT-CCACTCACCATCTCATCTGG	59									

Table 1 *Continued*
(b) Characteristics of an additional 22 loci

Locus name	EMBL record, library (A/B) and clone name	Repeat motif (5′–3′)†	Primer sequences (5′–3′)	Primer T_m (°C)	T_a used (°C)	$MgCl_2$ conc. (mM)	*n*	*A*§	Exp. allele size (bp)†	H_E	H_O
*Mel*118	AJ230706	$(CA)_{14}$	F*: ATGTCACTGAGAGTCAAATGC	55	55	1.0	9	3	238	0.50	0.44
	B, B21C08		R*: CAAGTTCCATGTTGGGC	56							
*Mel*119	AJ230714	$(TG)_4(TA)_2(CA)_{14}CG(CA)_3C(CA)_2$	F: AACACCCTCTAGCTCCATCC	58	58	1.0	10	3	179	0.59	0.50
	B, B28G05		R: GAAAGAGCCCACATGTCCAC	61							
*Mel*120	AJ230701	$(GA)_{15}$	F: ATGGCTTCTCTCACATCAGC	58	58	1.0	10	4	197	0.68	0.80
	B, B17D12		R: GACACCTAACCAACTGAGCC	57							
*Mel*121	AJ230688	$(CA)_{13}$	F*: TGATGATGACCAAGAACTTAATG	57	57	1.0	7	2	245	0.50	0.43
	B, B04B07		R*: AACCCTATGGAATTAGGAAGC	56							
*Mel*122	AJ230691	$(CA)_{14}$	F: ACTCCCCAAGGAGGTCTAAG	57	56	1.0	7	2	260	0.14	0.14
	B, B08H04		R: ACACCTCTCCCTGTTCCTAG	56							
*Mel*123	AJ230697	$(CA)_{14}$	F: AGTGGAAGCAGGGGCTTG	61	60	1.0	9	2	232	0.21	0.00
	B, B12F03		R: TCCTTCTGAACGGATGATTTC	59							
*Mel*124	AJ230712	$(CA)_{16}$	F*: TGGACATTTACCAGAGCACATC	60	60	1.0	9	2	138	0.29	0.33
	B, B27B04.2		R*: CTCCACCACATGTGTCCAAC	60							
*Mel*125	AJ230702	$(CT)_8$ and $(CA)_{15}$	F*: CCATTTTACTTGAGAGTTGATTCC	58	58	1.0	2	2	195	0.50	0.50
	B, B17H02		R*: ACCACGAGGATTGACATGAG	59							
*Mel*126	AJ293370	$(TG)_{21}$	F: GTGATGTCAATAGCAAGGTTCA	58	55	1.5	10	3	158	0.64	0.50
	A, CGBA99		R: ACACTACCAGAATACCCTAAGCG	59							
*Mel*127	AJ293368	$(TG)_{15}TC(TG)_7$	F: GCATACCCTCTGGGTCCATC	62	55	1.5	10	3	184	0.63	0.70
	A, CGBA45		R: CAAATGATCTGTATTCCCACTG	57							
*Mel*128	AJ293369	$(CA)_{23}$	F: ATCCAAACATGAAGCCCG	59	55	1.5	9	2	206	0.37	0.22
	A, CGBA47		R: TTACAGGTAGGCTCTGAGAAGG	58							
*Mel*129	AJ293366	$(GC)_6(AC)_{17}$	F: TTCACATAAAGGAGCACGCA	60	55	1.5	10	5	213	0.72	1.00
	A, CGBA28		R: GAATGGGACGTCTTGAGGTT	59							
*Mel*130	AJ293364	$(CA)_{18}$	F: GAGGACCACACGACTGCG	62	55	1.5	10	4	296	0.70	0.50
	A, CGBA17		R: AAAGCGCCAGAGCACCTAGA	62							
*Mel*131	AJ293367	$(TCCC)_5(TC)_4$ and $(GT)_{14}$	F: AAAATCCTGCCTACGTGTGG	60	55	1.5	10	3	116	0.20	0.20
	A, CGBA35		R: GATACAGCATGAAATAGCACTAGG	57							
*Mel*132	AJ293361	$(CA)_{17}$	F: TGATGCAATGCCCACAAC	60	55	1.5	10	2	139	0.40	0.30
	A, BAD06A		R: GGGGGATGCATGTAATCTTG	60							
*Mel*133	AJ293363	$(GA)_{16}$	F: AATGGAATCAAGTGCCTCCT	59	55	1.5	10	2	196	0.27	0.30
	A, CGBA6		R: GAAATTCAAACTCATGTCAGGT	56							
*Mel*134	AJ293365	$(AC)_{19}$	F: GGCTCATCTCATGCTCCTTC	60	55	1.5	10	2	266	0.52	0.30
	A, CGBA19		R: CCATGGGCTGTGGATGATT	62							
*Mel*135	AJ293371	$(GT)_{16}$	F: TCCCTGTTGTCAAACATTGC	60	55	1.5	10	2	131	0.27	0.10
	A, CGBA100		R: TGGGCAGAGGATCTGAGTAG	58							
*Mel*136	AJ293372	$(CA)_{19}$	F: CCCAAACTGAACTGACAAGA	56	55	1.5	8	2	234	0.40	0.50
	A, CGBA101		R: TCAATAACCCACAACTTTCG	56							

Table 1 *Continued*

Locus name	EMBL record, library (A/B) and clone name	Repeat motif (5′–3′)†	Primer sequences (5′–3′)	Primer T_m (°C)	T_a used (°C)	$MgCl_2$ conc. (mM)	*n*	*A*§	Exp. allele size (bp)†	H_E	H_O
*Mel*137	AJ293373	$(GT)_{18}$	F: AGGCTCGGTTCTATTCACCA	60	55	1.5	10	2	120	0.48	0.50
	A, CGBA111		R: AACTAGGGCAAGAAGAAAGG	55							
*Mel*138	AJ293375	$(AC)_{19}$	F: AAGTAGAAATGTAATGTGAGGCA	56	55	1.5	10	3	243	0.42	0.50
	A, CGBA115		R: GCTTAACCAAATGAGGCACC	60							
*Mel*139	AJ230694	$(CA)_{19}$	F: TGAATGGATAAAGAAGATGTGGTG	60	58	1.0	7	2	200	0.44	0.57
	B, B11H08		R: CTCTGCTCAGTGGAGAGCCTG	63							

*Primers designed from reverse complement of sequence on EMBL database.
†From original sequenced clone.
‡Scored on an ABI 377 DNA Sequencer after running in two gel loading groups: Group 1, *Mel*101–110 (from library A) was run as an ABI-C-set and group 2, *Mel*111–117 (from library B) was run as an ABI-D-set.
§PCR products were scored on (40-cm long) 4 or 6% denaturing polyacrylamide gels by end-labelling one primer with [α^{33}P]-dCTP using polynucleotide kinase (Amersham Pharmacia Biotech) (library A) or by staining with silver (Promega; Bassam *et al*. 1991) (library B).
PCR profiles.
Library A loci: 95 °C 1 min; 55 °C, 1 min; 72 °C, 1 min for 30 cycles.
Library B loci: 96 °C for 2 min for 1 cycle then 96 °C, 1 min; T_a °C, 30 s; 72 °C, 30 s for 30 cycles.
Fluorescent-labelled loci *Mel*101–*Mel*117: Touchdown PCR program, 94 °C for 3 min; then 94 °C for 15 s, 64–50 °C (dropping 2 °C every 2 cycles) for 20 s, 72 °C for 30 s; 35 cycles; followed by 72 °C for 10 min, except for *Mel*114 and *Mel*117, which were amplified as previously but with an annealing temperature of 57 °C. The 5′ end of one primer in each pair was labelled with a fluorescent phosphoramidite and the 'pigtail' sequence GTTTCTT was added to the other primer, to reduce noise from variable adenylation during the PCR (Brownstein *et al*. 1996).
T_m, melting temperature of primer (without modifications) as calculated using PRIMER version 3 (Rozen & Skaletsky 2000); T_a, annealing temperature for unlabelled and labelled primers; $MgCl_2$ conc., magnesium chloride concentration for unlabelled and labelled primers; Dil., dilution of individual fluorescent PCR product:total multiplexed gel load; *n*, number of unrelated individuals tested; *A*, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity.

Table 2 Cross-utility of our Eurasian badger *Meles meles* microsatellite loci in other carnivores

Family	Species	Number of loci amplifying/ loci tested	Number of loci polymorphic/ loci amplifying	Number of alleles/ number of unrelated individuals tested (*n*)	Reference
Mustelidae	Eurasian otter, *Lutra lutra*	5/12	2/5	*Mel*101 = 2/9 *Mel*104 = 1/9 *Mel*108 = 2/9 *Mel*109 = 1/9 *Mel*110 = 2/9	J.F. Dallas (personal communication)
Mustelidae	Stoat, *Mustela erminea*	11/17	2/11 (*n* = 1)	—	This study
Mustelidae	American mink, *Mustela vision*	9/17	6/9	—	This study
Herpestidae	Meerkat, *Suricata suricata*	0/21	0/0	—	Griffin *et al*. 2001
Felidae	Cat, *Felis catus*	2/17	2/2	*Mel*106 = 4/6 *Mel*110 = 2/6	This study
Canidae	Dog, *Canis familiaris*	4/17	0/4	*Mel*110 = 1/6 *Mel*112 = 1/6 *Mel*114 = 1/6 *Mel*117 = 1/6	This study
Canidae	Wolf, *Canis lupus*	3/17	1/3 (*n* = 1)	*Mel*112 = 2/1 *Mel*114 = 1/1 *Mel*117 = 1/1	This study
Hyenidae	Spotted hyena, *Crocuta crocuta*	0/17	0/0	—	This study

of a fluorescently labelled primer set (*Mel*101–*Mel*117, Table 1).

Each 10-μL PCR reaction contained 50 ng of genomic DNA, 0.25 μM primers, 0.15 mM of each dNTP, 1.5–2.5 mM $MgCl_2$ (Table 1) and 0.25 units of *Taq* DNA polymerase (Thermoprime^{Plus}, Advanced Biotechnologies) in the manufacturer's buffer [final concentrations: 20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl pH 9.0, 0.01% (w/v) Tween]. PCR products were separated on an ABI 377 DNA Sequencer and analysed using GENESCAN version 3.1 and GENOTYPER version 2.5 software (Table 1).

Of the 52 loci tested, 39 were polymorphic (Table 1) and 13 were monomorphic. Primers were designed for an additional 26 loci but these require further testing. Primer sequences for all 78 loci, including the uncharacterized and monomorphic loci are available from our website http://www.shef.ac.uk/misc/groups/molecol/badgers.html or (for most) from the individual EMBL records (AJ230687–97, AJ230699–725, AJ293349–85, AJ293387–89).

Observed (H_O) and expected heterozygosities (H_E) were calculated using CERVUS version 2.0 (Marshall *et al*. 1998; Table 1). An exact test performed using GENEPOP 3.3 (Raymond & Rousset 1995) found none of the 17 loci (*Mel*101–117) to be in linkage disequilibrium after correcting for multiple tests (Rice 1989).

The low levels of polymorphism, may in part be due to the small number of individuals tested (2–10 individuals for 35 of the 52 loci tested) or alternatively due to a low level of genetic variation in the Woodchester badger population. However, preliminary results from badger populations elsewhere in Britain and continental Europe (unpublished data) suggest that the overall number of alleles may not be significantly greater than shown here.

Despite the low variability of individual markers, we have isolated sufficient loci to develop a set that has successfully provided individual-specific genotypes for the Woodchester Park population. The seven most polymorphic loci were sufficient to distinguish, with 99% certainty, between full siblings in a group of 36 badgers from three Woodchester Park social groups (unpublished data). Seventeen loci (*Mel*101–117) were found to be necessary to determine parentage.

Previous studies have found mustelid microsatellite loci to be of utility in other mustelid species (see web-based appendix: http://www.shef.ac.uk/misc/groups/molecol/badgers.html). Our badger loci have been found to be polymorphic in Eurasian otter (*Lutra lutra*), Stoat, (*Mustela erminea*) and American mink (*Mustela vision*) (Table 2); and we expect them to be polymorphic in other mustelids.

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