

## PERMANENT GENETIC RESOURCES

**A large panel of novel microsatellite markers for the bank vole (*Myodes glareolus*)**

K. RIKALAINEN,\* A. GRAPPUTO,† E. KNOTT\*, E. KOSKELA\* and T. MAPPES\*

\*Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35 (YAC4), FIN-40014 Jyväskylä, Finland,

†Department of Biology, University of Padova, Via Ugo Bassi 58/B, 35121 Padova, Italy

**Abstract**

We describe a set of 66 highly polymorphic microsatellite loci isolated from the bank vole, *Myodes (Clethrionomys) glareolus*. These microsatellites were characterized for a long-term study on periodically fluctuating density of the bank vole population in Central Finland. We detected six to 38 alleles per locus in the population sampled at two different density phases, and the levels of observed and expected heterozygosities varied between 0.17 and 1.00, and between 0.72 and 0.95, respectively. This microsatellite panel serves as an informative tool for population and molecular genetic studies.

*Keywords:* bank vole, microsatellite, *Myodes glareolus*, primer

*Received 8 February 2008; revision accepted 20 March 2008*

The bank vole (*Myodes glareolus*), one of the most common wild mammals in Europe and the carrier host of human-affecting Puumala hantavirus (Brummer-Korvenkontio *et al.* 1980; Vapalahti *et al.* 2003), exhibits periodical population density fluctuations (Hansson *et al.* 2000). High population densities not only facilitate the spread of the hantaviruses (Sauvage *et al.* 2003), but also cause extensive forest damage (Gill 1995) with economical consequences. Since the life history of the bank vole is relatively well defined (Prevot-Juillard *et al.* 1999; Koivula *et al.* 2003; Mappes & Koskela 2004), the species is appropriate for addressing topical questions in evolutionary ecology (e.g. Mappes *et al.* 2008; Oksanen *et al.* 2007; Poikonen *et al.* 2008) as well as in conservation biology (Gerlach & Musolf 2000). Despite the relevance of the bank vole in a variety of biological research, molecular and population genetic studies on this species are lacking.

Bank voles from Konnevesi (62°37'N, 26°20'E), Central Finland, were sampled as discussed elsewhere (mainland areas, see Hakkarainen *et al.* 2007) at low (year 2000,  $N = 20$ ) and high (year 2002,  $N = 20$ ) population density phases. The 20 trapping points were situated independently to avoid family groups. Total genomic DNA for library construction was extracted from tissue samples using QIAGEN chemistry and a Kingfisher processor (Thermo Fisher Scientific).

Microsatellites were isolated using the FIASCO protocol (fast isolation by AFLP of sequences containing repeats, Zane *et al.* 2002) with slight modifications (Grapputo 2006). Enrichment was obtained with four biotinylated oligonucleotide probes, (CA)<sub>22</sub>, (CAG)<sub>11</sub>, (GA)<sub>12</sub> and (CATA)<sub>8</sub> (Tag Copenhagen). Clones containing inserts were detected by blue-white selection and microsatellites of 500 bp and larger were sequenced with BigDye chemistry and visualized with the ABI PRISM 3100 [Applied Biosystems (AB)]. In total, 987 clones were sequenced and primers flanking the microsatellites were designed for 221 of these using PRIMER 3 (Rozen & Skaletsky 2000). Allelic segregation was confirmed with seven individuals of known heredity (parents and five offspring). Altogether, 66 loci suitable for genetic analysis were discovered.

Polymerase chain reaction (PCR) amplifications were performed in 10- $\mu$ L reactions consisting of 1  $\mu$ L (approximately 70 ng) DNA, 0.25 U *Taq* DNA polymerase (Biotools), 1 $\times$  MgCl<sub>2</sub>-free PCR buffer (Biotools), 2 mM each dNTP (Fermentas), 5  $\mu$ M reverse primer, 4.5  $\mu$ M unlabelled forward primer and 0.5  $\mu$ M fluorescence-labelled forward primer (AB) and MgCl<sub>2</sub> at locus-optimized concentrations (Table 1). Thermocycling conditions were 2 min at 94 °C (initial denaturation) and 30 cycles of successive denaturation (45 s, 94 °C), annealing (45 s, 50 °C/55 °C, Table 1) and extension (1 min, 72 °C) stages. Amplified fragments were detected with an ABI PRISM 3100 and scored using GENEMAPPER version 3.7 software (AB).

Correspondence: Kaisa Rikalainen, Fax: +358 14 2602321;

E-mail: akritvan@cc.jyu.fi

**Table 1** Characteristics of 66 microsatellite loci identified in the bank vole, *Myodes (Clethrionomys) glareolus*

Locus name	GenBank accession	Repeat	Primer sequence 5'–3'	Dye	T <sub>a</sub>	MgCl <sub>2</sub> (mM)	Size (bp)	N <sub>a</sub> (P1/P2)	P1 H <sub>O</sub> (H <sub>E</sub> ) P2 H <sub>O</sub> (H <sub>E</sub> )
Cg1A8	EU285376	(CT) <sub>9</sub> N <sub>6</sub> (CT) <sub>12</sub> N <sub>2</sub> (GT) <sub>14</sub>	F: GCACGTGTATGTGATTTGTC R: GAAATGGGAAACAACAAAGA	VIC	50	1.1	234	18/17	0.75 (0.93) 0.75 (0.93)
Cg1E6	EU285377	(GAAA) <sub>14</sub> N <sub>64</sub> (CT) <sub>19</sub> (CA) <sub>12</sub>	F: GGACTGAGAGGAGAGAAAGG R: TAGCTTGTAGGCCATCTT	VIC	50	1.1	280	23/29	0.95 (0.93) 1.00 (0.96)
Cg1E8	EU285378	(CA) <sub>22</sub> N(GA) <sub>25</sub>	F: TATATGCTGCAGGGGAGTAT R: AAGATTCCACCTGTTTTGTG	NED	50	1.5	268	15/15	0.90 (0.89) 0.90 (0.91)
Cg1F11	EU285379	(GT) <sub>14</sub>	F: AATTTCTGTCTTTCCCTTCC R: GAAAAAGGCAACAAAATCTC	PET	55	1.5	166	12/14	0.75 (0.84) 0.90 (0.87)
Cg1G12	EU285380	(GT) <sub>18</sub>	F: GCACAGAACACACTCAAATG R: TTTCTGAAAGCCTTGTCTTTT	NED	55	1.5	222	13/12	0.80 (0.90) 0.85 (0.86)
Cg2A4	EU285381	(GT) <sub>15</sub> (GA) <sub>13</sub>	F: GGACTTGATAGGGGAGAAA R: CTCCTTTCATCCTTGTCTGT	6-FAM	50	1.5	247	17/19	0.75 (0.91) 0.90 (0.93)
Cg2C5	EU285382	(CT) <sub>14</sub>	F: AAAGGCAATGAAAAGAACAC R: GACGCACGGTATAGACACTA	NED	55	1.5	200	13/15	0.85 (0.89) 0.70 (0.90)
Cg2D3	EU285383	(CT) <sub>22</sub> (CA) <sub>13</sub>	F: TCCTTCCTCTTTATTCACCTC R: GAGCAACTAAACGGATTC	PET	50	1.1	185	20/20	0.90 (0.94) 0.84 (0.93)
Cg2D8	EU285384	(CA) <sub>13</sub> N <sub>50</sub> (GATA) <sub>13</sub>	F: TGCAAGTTGTTCTCTTTTCTC R: AGTAAAAAGTGCCAGAATACTA	6-FAM	55	1.5	247	13/13	0.75 (0.90) 0.85 (0.89)
Cg2E2	EU285385	(GT) <sub>22</sub> (GA) <sub>20</sub>	F: CGTGAGTGTATGGAGATGTG R: CAGAAATGGGAAAATACCTG	VIC	55	1.1	153	15/15	0.63 (0.91)* null 0.74 (0.90)
Cg2F2	EU285386	(GT) <sub>23</sub>	F: TGATCTGCTCAGGGATAAAG R: TGTGGGATTAITTCAGTCTT	VIC	55	1.5	171	12/13	0.85 (0.88) 0.90 (0.86)
Cg3A6	EU285387	(GT) <sub>19</sub>	F: CTTCCAGGGAGTGGTAT R: AGTGATTGAGCCTGAGAAG	6-FAM	55	1.1	159	11/11	0.75 (0.82) 0.90 (0.78)
Cg3A8	EU285388	(GT) <sub>21</sub>	F: AATGTGTCATTGGATGTCCT R: TGTCTTCTAAAATCAAATGCT	VIC	50	1.5	236	9/8	0.90 (0.85) 0.70 (0.78)
Cg3D10	EU285390	(GT) <sub>16</sub>	F: TAGCATTAGGGAGGTTGAGA R: CCAGGAGAAATGACACAGAT	VIC	50	1.1	183	7/11	0.50 (0.83) 0.60 (0.85)* null
Cg3D12	EU285391	(CA) <sub>9</sub> N <sub>2</sub> (CA) <sub>21</sub>	F: ATCACAATGGAACCAAGAA R: CAATGGAAAAGAGAACTTGG	PET	55	1.1	178	12/10	0.39 (0.88)* null 0.17 (0.86)* null
Cg3E10	EU285393	(CA) <sub>22</sub>	F: GACAACGCTTCACACTGTC R: GGCAAGGACTAACATATCCA	6-FAM	50	1.1	187	11/14	0.65 (0.85)* 0.80 (0.86)
Cg3E12	EU285394	(CA) <sub>9</sub> N(CA) <sub>9</sub>	F: ATATGACAGACAGCGTGGGA R: AACCTCAACACTGGATCTTG	6-FAM	50	1.1	153	11/9	0.90 (0.85)* 0.65 (0.79)
Cg3E6	EU285392	(CA) <sub>17</sub>	F: ACCAAGCACCAGTAATCAAG R: CCACCTATTTTGTGAACTTTG	VIC	55	1.1	207	11/9	0.30 (0.88)* null 0.40 (0.77)* null
Cg3F12	EU285395	(GT) <sub>16</sub>	F: GCATGACACGTTGACAATAG R: GTCAGAGAGTCTTTGGCTTGT	NED	50	1.1	150	10/9	0.75 (0.80) 0.75 (0.85)
Cg4F9	EU285396	(CA) <sub>20</sub>	F: GGGAAATGACAGGACAATAGA R: CCTAATGCTTCCAAACAGTC	PET	55	1.5	172	10/8	0.80 (0.85) 0.90 (0.84)
Cg5B5	EU285397	(CT) <sub>23</sub> N <sub>2</sub> (CT) <sub>7</sub> N(CA) <sub>10</sub>	F: AGTCCTAAGCCTGAGTGTCA R: GAACAGGCTGTGTTGGAAT	NED	50	1.1	190	16/15	0.90 (0.90) 0.90 (0.89)
Cg5E8	EU285398	(GT) <sub>19</sub> (GA) <sub>21</sub>	F: ATTTTCATAAACGCCTCCTTC R: AGTTCAGTCAAGTGGATCCTG	PET	50	1.5	239	14/14	0.90 (0.92) 0.75 (0.88)
Cg5F11	EU285400	(CA) <sub>24</sub>	F: CAAGATGGACAAGAAGGAAG R: CTGTGAGGGTAAACAGAAGA	6-FAM	50	1.5	236	11/13	0.85 (0.82) 0.95 (0.84)

Table 1 Continued

Locus name	GenBank accession	Repeat	Primer sequence 5'-3'	Dye	$T_a$	MgCl <sub>2</sub> (mM)	Size (bp)	$N_a$ (P1/P2)	P1 $H_O$ ( $H_E$ ) P2 $H_O$ ( $H_E$ )
Cg5F6	EU285399	(CA) <sub>7</sub> N(GA) <sub>24</sub>	F: CCTAGCCTAACTCAGGAAAGT R: GAAGTCTGCATTTGTCAAACA	6-FAM	50	1.5	211	15/14	0.90 (0.89) 0.95 (0.91)
Cg5G6	EU285401	(CA) <sub>22</sub>	F: CTAAGTGGTCCCGTGGTAG R: TGTGAATTTGCATCAAGAGA	NED	55	1.1	180	13/10	0.85 (0.86) 0.95 (0.87)
Cg6A1	EU285402	(CAT) <sub>18</sub>	F: CACTTGGGAGTCAGAGGTAG R: CAGATAAATCTCAACAAAGAGG	PET	55	1.1	202	7/12	0.95 (0.79) 0.95 (0.83)
Cg6C6	EU285403	(CT) <sub>9</sub> N <sub>2</sub> (CT) <sub>17</sub>	F: AAACCTCAGTTCTGAATTAGC R: GGTTAGCGTTTATGGTCATC	6-FAM	50	1.1	191	17/18	0.95 (0.92) 1.00 (0.91)
Cg6D10	EU285404	(CAT) <sub>5</sub> N <sub>9</sub> (CAT) <sub>5</sub> N <sub>9</sub> (CAT) <sub>5</sub>	F: GAGCTCAGGTGAAACAAAG R: GTATTCATCAGGAAGCTGA	6-FAM	50	1.1	288	10/9	0.90 (0.81) 0.90 (0.84)
Cg6G11	EU285405	(CA) <sub>14</sub>	F: TGAAAAGTGAATTTGAAAAGTG R: ACCAATAAATCAGCTGGAAA	NED	50	1.5	151	9/9	0.70 (0.84) 0.75 (0.84)
Cg7C9	EU285406	(CA) <sub>11</sub> N <sub>6</sub> (CA) <sub>10</sub>	F: TTTCTCTGGGACTAAACAGC R: TGTGCATGTCAGTTTCTCTTA	VIC	55	1.1	249	11/12	0.95 (0.86) 1.00 (0.85)
Cg7E5	EU285407	(CAG) <sub>12</sub>	F: GGGTTAGTTAGCTTGCAAAA R: CTAAGCCGAGCTTATTTGA	PET	50	1.5	159	9/12	0.60 (0.76) 0.95 (0.86)
Cg8A5	EU285408	(GGAA) <sub>17</sub>	F: CAGGGACATGAGTTTGATCT R: TTATGCAGCTTCTAGGTGGT	PET	50	1.1	230	18/17	0.90 (0.92) 0.85 (0.92)
Cg8D2	EU285409	(CA) <sub>17</sub>	F: GTTGTCTCCAACTCCATA R: CTGGAAACACCTTCACAGAT	VIC	50	1.1	184	15/9	0.55 (0.90)* null 0.40 (0.82)* null
Cg10A11	EU285410	(GT) <sub>15</sub>	F: TGGGAGATGGTCACATTAGT R: CCCCAAAAGTTGTCTCTAT	6-FAM	55	1.1	230	9/12	0.83 (0.85) 0.74 (0.86)
Cg10D11	EU285411	(CT) <sub>10</sub> N <sub>2</sub> (CT) <sub>12</sub>	F: ATTTCCGGTTGGGTTATTT R: CATGGCTTCAAGTACAGTCC	NED	50	1.1	200	9/8	0.63 (0.85) 0.55 (0.78)
Cg10F6	EU285412	(CA) <sub>8</sub> N <sub>2</sub> (CA) <sub>4</sub>	F: TTGACCAGACACTTTCCTCT R: TTCTAGGTACCTGCCATCTG	6-FAM	50	1.1	169	15/14	0.84 (0.91) 0.90 (0.88)
Cg10H1	EU285413	(GACA) <sub>7</sub>	F: CCATGAACCTCATGACACAAG R: TACTCAGGTGTAAGCCATCC	NED	50	1.5	222	6/7	0.85 (0.74) 0.80 (0.79)
Cg11H5	EU285442	(GA) <sub>6</sub> N <sub>2</sub> (GA) <sub>8</sub>	F: ACAGTCAAGATCCGAGTCAC R: AGAATAGGCAGAGTGAGCTG	VIC	50	1.5	225	15/17	0.90 (0.85) 0.90 (0.87)
Cg12A7	EU285414	(GA) <sub>21</sub>	F: CAGCAACCACATAGTGACTC R: CCTCAGATTTCTCCTTCACA	PET	50	1.5	245	10/8	0.95 (0.86) 0.85 (0.83)
Cg12B9	EU285441	(GA) <sub>21</sub>	F: AGCTGGGGTTACACAGAGA R: GTAGTACATGGGAGACAAGG	PET	55	1.1	240	17/21	0.80 (0.90) 1.00 (0.93)
Cg12E6	EU285415	(GA) <sub>20</sub>	F: ACTGAAGCAGATTTAGGGACT R: TAATGGAAGGAAACGATGG	VIC	55	1.1	191	23/19	0.95 (0.94) 1.00 (0.93)
Cg12H10	EU285416	(CA) <sub>21</sub> (GA) <sub>22</sub>	F: TCAAACGGAAATTTGAGACTT R: TCCACCATAACTTGGAGAAC	6-FAM	50	1.1	219	15/15	0.85 (0.91) 1.00 (0.91)
Cg13B8	EU285417	(CT) <sub>20</sub> (GT) <sub>9</sub>	F: GCCTAATGTTTTCTCTGTGC R: CACATGGAATGAGGTTCTTAC	6-FAM	55	1.1	161	21/24	0.90 (0.93) 1.00 (0.94)
Cg13C12	EU285418	(CT) <sub>21</sub>	F: GCCAAATGTCAGAGTCAGAA R: GCAGCTGGATCTCTATGAAT	PET	55	1.1	197	13/13	0.90 (0.87) 0.90 (0.86)
Cg13D11	EU285419	(CA) <sub>18</sub> N <sub>16</sub> (GA) <sub>18</sub>	F: GGTTTAGCTCTCAAGACAGG R: TCCAATAGTGGCTTCTTAAA	VIC	50	1.5	228	13/15	0.65 (0.89)* null 0.70 (0.92)
Cg13F10	EU285420	(CA) <sub>14</sub> N <sub>12</sub> (GA) <sub>18</sub>	F: GGTTCAGAAAATTTCTCACT R: GATTTCAATACAGTTTAGGC	NED	55	1.5	145	9/12	0.35 (0.82)* null 0.30 (0.84)* null

**Table 1** *Continued*

Locus name	GenBank accession	Repeat	Primer sequence 5'–3'	Dye	$T_a$	MgCl <sub>2</sub> (mM)	Size (bp)	$N_a$ (P1/P2)	P1 $H_O$ ( $H_E$ ) P2 $H_O$ ( $H_E$ )
Cg13F9	EU285421	(GA) <sub>18</sub>	F: TGCTCACACAACTGTGATT R: TAACCGGGGAGTAGAGAAA	VIC	50	1.1	130	8/12	0.75 (0.81) 0.80 (0.84)
Cg13G2	EU285422	(GT) <sub>14</sub>	F: ACGACTTAGAGGGCTAGTTG R: GGAGTGACACAGGAGTTTGT	6-FAM	50	1.1	131	12/11	0.80 (0.87) 0.75 (0.84)
Cg13H9	EU285423	(CA) <sub>7</sub> N <sub>2</sub> (CA) <sub>8</sub> N(GATA) <sub>12</sub>	F: ACATGGTAGTCACTCACATAGA R: GCTTCAACAATTTCAATTCC	VIC	55	1.1	165	13/12	0.95 (0.89) 0.95 (0.89)
Cg14A5	EU285424	(CT) <sub>17</sub>	F: CTCCTTATGTGCATGTGCTA R: GGAGGTTTATTGGGGTATTTC	6-FAM	55	1.1	237	16/20	0.80 (0.91) 0.79 (0.93)
Cg14E1	EU285425	(CT) <sub>20</sub>	F: AATACAGGGCTGAAGTAGCA R: AGATGAGACAGAGCATGACC	PET	55	1.5	197	10/12	0.80 (0.86) 0.80 (0.87)
Cg15F7	EU285426	(CT) <sub>20</sub>	F: ATATTTCCCTGAGGGTGAAC R: GGCTGAGAGATACATTATGGTC	VIC	50	1.1	126	11/12	0.90 (0.83) 0.85 (0.82)
Cg15H8	EU285427	(GA) <sub>20</sub>	F: GCTGGGAAACAGTCTTCTAAC R: GCTTCTAAATTCAGCAGCAC	NED	55	1.1	213	14/14	0.90 (0.90) 0.95 (0.88)
Cg16A3	EU285428	(CT) <sub>22</sub>	F: TAACCTGCCAAGGGTGATAGA R: TCCAAATCTTTTGACCTACCT	6-FAM	55	1.5	135	10/11	0.85 (0.87) 0.90 (0.86)
Cg16D2	EU285429	(CTTT) <sub>13</sub> (CTT) <sub>17</sub>	F: GGTAATAATCAGCACATCACC R: CAGCGGAATTATGATTTGAG	6-FAM	55	1.1	293	21/26	0.90 (0.94) 0.85 (0.95)
Cg16E2	EU285430	(CT) <sub>10</sub> N <sub>2</sub> (CT) <sub>8</sub>	F: CTTATTGACCCACCTACCT R: AATAAAGCCAGGCTGGAATA	NED	50	1.1	109	11/9	1.00 (0.86) 0.90 (0.84)
Cg16E4	EU285431	(GA) <sub>24</sub>	F: CAGGGCTACATAGAGGGACT R: CATTTTCTCCACAGTAATGCTT	PET	50	1.5	196	14/15	0.95 (0.90) 1.00 (0.91)
Cg16E5	EU285432	(CA) <sub>16</sub> (GA) <sub>15</sub>	F: TCTAGGCAAGTCGAGAGTGT R: GGCTTTCTCCAAGACTTTCT	NED	55	1.1	274	14/16	0.95 (0.89) 0.85 (0.89)
Cg16H5	EU285433	(GA) <sub>20</sub>	F: ACTGAGCTCATCAATGTCC R: ATTTGTATTTTTGTGCTGCC	VIC	55	1.1	200	10/9	0.65 (0.85) 0.80 (0.82)
Cg16H6	EU285434	(CT) <sub>18</sub> N <sub>2</sub> (CT) <sub>6</sub>	F: TACCAGCTCTGTTTTCTGCT R: TAACACACCATTGCATATGC	VIC	55	1.5	145	14/16	0.85 (0.88) 0.95 (0.90)
Cg17A7	EU285435	(ATGT) <sub>9</sub>	F: ACATTCAAACCTATGGGACA R: GAAGGCTATTGATCTTGCAC	VIC	50	1.1	238	5/6	0.55 (0.60) 0.65 (0.72)
Cg17C6	EU285436	(CT) <sub>8</sub> (CA) <sub>20</sub>	F: CTCACTCAGAAATGCAGACATC R: GGAAGGAGGACATGAGGTAT	6-FAM	50	1.5	230	16/16	0.90 (0.92) 1.00 (0.92)
Cg17C9	EU285437	(CT) <sub>21</sub>	F: ATTCACCTTTGCATGTTAGG R: AGTTCAGTGAGCGGAACA	VIC	50	1.1	169	12/14	0.90 (0.88) 0.85 (0.89)
Cg17D5	EU285438	(GA) <sub>11</sub> N <sub>2</sub> (GA) <sub>7</sub>	F: TTTTAGCCTCATAAATGGACA R: GCAGTAGATTTTCGCTCACAC	PET	50	1.1	190	20/22	0.95 (0.93) 1.00 (0.93)
Cg17E9	EU285439	(GTAT) <sub>9</sub>	F: AATACTTCCAGTGCTGATGC R: TCAGAACCTGTTCTCTGAC	PET	50	1.1	137	6/7	0.75 (0.79) 0.95 (0.81)
Cg17H7	EU285440	(GA) <sub>7</sub> N <sub>40</sub> (GA) <sub>14</sub>	F: ATGGGGAATCAATATCACTTT R: TTCTCAGTCTCTGTATGCAC	6-FAM	55	1.1	153	7/9	0.44 (0.76) 0.50 (0.84)

For each, we report GenBank Accession no., repeat motif, sequences of forward (F) and reverse (R) primer, fluorescent dye of labelled F primer, PCR conditions [annealing temperature ( $T_a$ ) and MgCl<sub>2</sub> concentration], the product size of sequenced allele, number of alleles ( $N_a$ ), observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity values, significant deviations from Hardy–Weinberg equilibrium (\*), and presence of null alleles (null); P1 and P2 indicate sampling years 2000 and 2002, respectively; ( $N = 20$  individuals/population).

The summary of microsatellite characteristics is reported in Table 1. All the 66 loci were highly polymorphic, having six to 38 alleles per locus. Observed and expected heterozygosity levels were calculated using the program GENALEX version 6 (Peakall & Smouse 2006) and they ranged from 0.17 to 1.00 and 0.72 to 0.95, respectively. Genotypic linkage equilibrium and deviations from Hardy–Weinberg equilibrium were calculated using the online version of GENEPOP (Raymond & Rousset 1995) and *P* values were corrected for multiple comparisons. Significant deviation from Hardy–Weinberg equilibrium was observed at nine loci and significant linkage disequilibrium was found in eight locus pairs (year 2000: Cg3A6 × Cg3A8, Cg10A11 × Cg10D11, Cg6G11 × Cg15H8, Cg3A8 × Cg16E5, Cg8D2 × Cg17D5; year 2002: Cg6D10 × Cg10D11, Cg3D12 × Cg10H1, Cg3A6 × Cg16A3). However, none of the locus pairs indicated linkage disequilibrium in both sampled years. Presence of null alleles at loci deviating from Hardy–Weinberg equilibrium was tested with MICRO-CHECKER version 2.2.3 (van Oosterhout *et al.* 2004).

As our preliminary results suggest, this set of polymorphic microsatellite loci offers a wide repertoire of informative tools for studies on population structure and genetic patterns not easily assessed by other means.

## Acknowledgements

We are grateful to Henni Pietiläinen for technical help in the laboratory. This work was supported financially by the Academy of Finland (Centre of Excellence in Evolutionary Research and projects 100143, 103148, 78777, 115961 to E. Koskela and 118603, 109165, 206091 to T. Mappes).

## References

- Brummer-Korvenkontio M, Vaheri A, Hovi T *et al.* (1980) Nephropathia epidemica: detection of antigen in bank voles and serologic diagnosis of human infection. *Journal of Infectious Diseases*, **141**, 131–134.
- Gerlach G, Musolf K (2000) Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conservation Biology*, **14**, 1066–1074.
- Gill R (1995) A review of damage by mammals in north temperate forests. 2. Small mammals. *Forestry*, **95**, 281–308.
- Grapputo A (2006) Development and characterization of microsatellite markers in the Colorado potato beetle, *Leptinotarsa decemlineata*. *Molecular Ecology Notes*, **6**, 1177–1179.
- Hakkarainen H, Huhta E, Koskela E, Mappes T, Soveri T, Suorsa P (2007) *Eimeria*-parasites are associated with a lowered mother's and offspring's body condition in island and mainland populations of the bank vole. *Parasitology*, **134**, 23–31.
- Hansson L, Jedrzejewska B, Jedrzejewski W (2000) Regional differences in dynamics of bank vole populations in Europe. *Polish Journal of Ecology*, **48**, 163–177.
- Koivula M, Koskela E, Mappes T, Oksanen TA (2003) Cost of reproduction in the wild: manipulation of reproductive effort in the bank vole. *Ecology*, **84**, 398–405.
- Mappes T, Koskela E (2004) Genetic basis of the trade-off between offspring number and quality in the bank vole. *Evolution*, **58**, 645–650.
- Mappes T, Koivula M, Koskela E, Oksanen TA, Savolainen T, Sinervo B (2008) Frequency and density-dependent selection on life-history strategies – a field experiment *PLoS One*, **3**, e1687.
- Oksanen TA, Koivula M, Koskela E, Mappes T (2007) The cost of reproduction induced by body size at birth and breeding density. *Evolution*, **61**, 2822–2831.
- van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.
- Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.
- Poikonen T, Koskela E, Mappes T, Mills S (2008) Infanticide in the evolution of reproductive synchrony: effects on reproductive success. *Evolution*, **62**, 612–621.
- Prevot-Juillard A, Henttonen H, Yoccoz NG, Stenseth NC (1999) Delayed maturation in female bank voles: optimal decision or social constraint? *Journal of Animal Ecology*, **68**, 684–697.
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact test and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Rozen S, Skaletsky H (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawets S, Misener S), pp. 365–386. Humana Press, Totowa, New Jersey.
- Sauvage F, Langlais M, Yoccoz NG, Pontier D (2003) Modelling hantavirus in fluctuating populations of bank voles: the role of indirect transmission on virus persistence. *Journal of Animal Ecology*, **72**, 1–13.
- Vapalahti O, Mustonen J, Lundkvist Å, Henttonen H, Plyusnin A, Vaheri A (2003) Hantavirus infections in Europe. *The Lancet Infectious Diseases*, **3**, 653–661.
- Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. *Molecular Ecology*, **11**, 1–16.