Microsatellites with variation and heredity applicable to genetic studies of Norwegian red deer (Cervus elaphus atlanticus)

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Description: In Norwegian red deer (Cervus elaphus atlanticus), few microsatellites show variation [1,2]. Here, we describe genetic variation and probability of parentage exclusion of 25 microsatellites. Pedigreed families were used to verify Mendelian inheritance.

Sampling and PCR: We genotyped 93 wild red deer from different locations in Norway with 25 microsatellites (Table 1), as well as 32 calves and their 26 known mothers from two red deer farms with 21 of these microsatellites. Genomic DNA was isolated from muscle tissue collected from the wild red deer (Qiagen, Dneasy Kit; VWR International, Oslo, Norway) and from 10 plucked hairs from the farmed red deer (12-h standard proteinase K). Polymerase chain reaction and electrophoresis conditions are described elsewhere [1,2]. Allele frequency, observed heterozygosity (H_0) and average probabilities of parentage exclusion with no known parents (pE_1) and one known parent (pE_2) were estimated with Cervus 2.0,[3] which also simulated the expected proportion of solved parentages given various numbers of candidate parents and a genotyping error rate. Apparent non-inheritance of parental null alleles can result in offspring–parent mismatches [4,5] and was used as an indication of null alleles.

Mendelian inheritance: Of the 25 microsatellites we tested, RT1 and ETH225, which are in linkage with BM4208 and BM757, respectively [6], and NVHRT16 were excluded because of low variation. NVHRT34 was excluded because of troublesome PCR with hair isolates. For the remaining 21 loci, proofreading of apparent mismatches revealed 16 genotyping errors among 1218 electromorphs. Null alleles were indicated for nine mismatches in McM104 and two in NVHRT21 after repeated PCR and re-isolation of DNA from homozygous individuals. For McM104, six of the exceptions were repeated misinheritances of two hinds (three cohorts) and two exceptions were in a grandmother–mother–calf relationship. For NVHRT21, nine wild red deer with good products for other microsatellites appeared blank after repeated genotyping.

Genetic variation: Among 2303 electromorphs, most of the 25 microsatellites showed low genetic variation but there were differences among loci (Table 1). In parentage studies, marker variation is closely connected to exclusionary power [3,7], and the 19 microsatellites with verified Mendelian inheritance had combined exclusion probabilities of 0.9879 (pE1) and 0.9998 (pE2). Simulations showed that with a genotyping error rate of 0.01 and a 95% confidence level for parental assignment, >80% of parentages were solved for up to 1000 candidate parents with one known

parent, while the maximum with no known parent was 50 candidate parents. This indicated that our battery is suitable for parentage analysis in relatively large populations when one parent is known, which is usual in most studies of captive deer, but is limited to much smaller populations when no parents are known, which is often the case in field studies of wild populations.

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Table 1: The allele number, allele size range, observed heterozygosity (H0), probability of parentage exclusion with no parents known (pE1) or one parent known (pE2) for 25 microsatellites screened in Norwegian red deer (Cervus elaphus atlanticus, n ¼ 93) with linkage groups (LG) according to Slate et al.8 and multiplexes indicated (Plex).

Locus[1]	Reference	Alleles	Size (bp)	H_0	pE_1	pE_2	LG	Plex
McM58	Hulme et al.[9]	6	166–182	0.72	0.395	0.535		A
FCB304	Buchanan & Crawford [10]	6	126–144	0.72	0.357	0.490	24	A
BM4208	Bishop et al.[11]	5	148–162	0.70	0.314	0.526	**26**	–
BM888	Bishop et al. [11]	9	202–233	0.70	0.348	0.434	12	B
FCB193	Buchanan & Crawford [10]	6	100–124	0.70	0.268	0.418	5	A
NVHRT48	Røed & Midthjell [2]	5	80–103	0.62	0.252	0.348		–
OarCP26	Ede et al.[12]	3	125–149	0.59	0.205	0.310	18	–
BM5004	Bishop et al. [11]	4	132–138	0.57	0.178	0.329		B
NVHRT73	Røed & Midthjell [2]	5	203–227	0.56	0.167	0.375		C
RT7	Wilson et al. [13]	4	214–222	0.55	0.220	0.338		–
CSSM003	Moore et al. [14]	4	202–218	0.49	0.178	0.259		D
RT5	Wilson et al. [13]	3	149–166	0.46	0.119	0.270	15	E
RT6	Wilson et al. [13]	3	96–104	0.44	0.131	0.258	19	E
CSSM066	Barendse et al. [16]	4	171–187	0.43	0.114	0.329	21	F
BM4107	Bishop et al.[11]	3	156–168	0.42	0.192	0.239	25	B
SR-CRSP10	Bhebhe et al. [17]	3	196–204	0.42	0.125	0.288		D
BMC1009	Bishop et al.[11]	4	271–293	0.37	0.137	0.159	3	–
BM757	Bishop et al.[11]	2	163–175	0.33	0.079	0.112	**28**	–
BM203	Bishop et al.[11]	3	212–224	0.27	0.031	0.243	32	B
McM104	Smith et al. [15]	7	102–120	0.47	0.204	0.347		–
NVHRT21	Røed & Midthjell [2]	4	151–175	0.16	0.096	0.241		G
NVHRT34	Røed & Midthjell [2]	3	134–138	0.52	0.161	0.284	14	C
RT1	Wilson et al. [13]	3	211–222	0.53	0.146	0.535	**26**	E
ETH225	Bishop et al. [11]	4	136–154	0.23	0.027	0.112	**28**	F
NVHRT16	Røed & Midthjell [2]	2	152–154	0.11	0.019	0.087		G

1 Mendelian inheritance was verified in 19 of 21 loci using family material. The 19 microsatellites above the line are recommended for use in parentage determination.
Bold numbers show linked loci [8].

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