Comparison of Microsatellite and Single Nucleotide Polymorphism Markers for the Genetic Analysis of a Galloway Cattle Population

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Highly informative genetic markers are essential for efficient management of cattle populations, as well as for food safety. After a decade of domination by microsatellite markers, a new type of genetic marker, single nucleotide polymorphism (SNP), has recently appeared on the scene. In the present study, the exclusion power of both kinds of markers with regards to individual identification and parental analysis was directly compared in a Galloway cattle population. Seventeen bovine microsatellites were distributed in three incremental marker sets (10, 14 and 17 microsatellite markers) and used for cattle genotyping. A set of 43 bovine SNP was used for genotyping the same cattle population. The accuracy of both kinds of markers in individual identification was evaluated using probability of identity estimations. These were 2.4×10^{-8} for the 10 microsatellite set, 2.3×10^{-11} for the 14 microsatellite set, and 1.4×10^{-13} for the 17 microsatellite marker set. For the 43 SNP markers, the estimated probability of identity was 5.3×10^{-11}. The exclusion power of both kinds of markers in parental analysis was evaluated using paternity exclusion estimations, and, in addition to this, by estimation of the parental exclusion probability in 18 Galloway family trios. Paternity exclusion was estimated to be over 99% for microsatellites, and approx. 98% for SNP. Both, microsatellite and SNP sets of markers showed similar parental exclusion probabilities.

Key words: Microsatellite, Single Nucleotide Polymorphism, Exclusion Power

Introduction

The development of highly informative genetic markers is critical for individual identification and parental control in cattle, and, therefore, essential for traceability and efficient management of cattle populations. Extremely robust and competent methods are required for the analysis of large numbers of samples. Microsatellites are highly polymorphic DNA markers suitable for such studies. The co-dominant Mendelian-inherited microsatellite markers are currently well established and successfully employed in cattle (Glowatzki-Mullis *et al.*, 1995; Heyen *et al.*, 1997; Bredbacka and Koskinen, 1999; Schnabel *et al.*, 2000). Microsatellites have been the most widely used genetic markers due to their ease of use and analysis, and to the high degree of information provided by the large number of alleles per locus (Baumung *et al.*, 2004). Nevertheless, single nucleotide polymorphism (SNP), the most recent tool for studying DNA sequence variation, has some promising advantages over microsatellite markers, such as high-throughput automated analysis and genetic stability in mammals (Kruglyak, 1997; Landegren *et al.*, 1998; Krawczak, 1999; Nielsen, 2000). The SNP markers have gained high popularity, even though they are only bi-allelic co-dominant markers (Vignal *et al.*, 2002). Recently, two different SNP marker sets were reported by Heaton *et al.* (2002) and Werner *et al.* (2004), for animal identification and parentage testing in American and European beef cattle.

Simulations predict that at least two to six times more SNPs will be necessary to achieve the same resolution as microsatellites when used for individual identification and the study of parentage assessment and relatedness (reviewed by Morin *et al.*, 2004). In order to test this prediction in a practical case, we aimed in the present study to compare the exclusion power of both kinds of markers in individual identification and parental analysis

when genotyping the same Galloway cattle population. Moreover, breeders' records of Galloway animals were available, which were necessary to perform parentage assessment in Galloway family trios, and obtain statistical calculations thereof.

Material and Methods

DNA source and genetic markers

Blood samples of 218 Galloway animals were obtained from four different German farms, including breeders' records for these animals (*e.g.* sex, date of birth, parental information, and identification number from ear tag). Genomic DNA was extracted following standard protocols from Macherey-Nagel (Düren, Germany). The animals were genotyped using both microsatellite and SNP markers. Seventeen bovine microsatellites, recommended by the International Society for Animal Genetics (ISAG, http://www.isag.org.uk), were used for cattle genotyping (see Table I). A set of 43 bovine SNP (see Table II), partially based on the marker set reported by Werner *et al.* (2004), was used as well for cattle genotyping.

Microsatellite PCR conditions

The microsatellite primer pairs were distributed in two multiplex-PCR (see Table I). The 20 μl total volume PCR mix comprised 100 ng genomic DNA, dNTPs each at 400 μM, 1 × PCR buffer [10 mM tris(hydroxymethyl)aminomethane (Tris-)-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$], fluorescent-labeled primers from 0.05 μM to 1 μM, 2.5 units of AmpliTaq Gold® Polymerase (Applied Biosystems Division, Perkin-Elmer, Foster City, CA, USA). The temperature profile was: initial denaturation at 95 °C for 20 min; this was followed by 32 cycles of: denaturation at 95 °C for 1 min 30 s; primer annealing at 58 °C for 1 min 30 s; and elongation at 72 °C for 1 min 30 s. Final extension was at 72 °C for 10 min.

Microsatellite fragment analysis

Capillary electrophoresis was performed in an ABI PRISM Genetic Analyzer 3100 (Applied Biosystems Division, Perkin-Elmer) according to the manufacturer's recommendations. Genotyping data were analyzed with GeneScan® version 3.1 Software (Applied Biosystems Division, Perkin-Elmer), then imported into Genotyper® version 3.1 NT Software (Applied Biosystems Division, Perkin-Elmer), and sized according to the internal lane size standard (GENESCAN® 400 HD [ROX], Applied Biosystems Division, Perkin-Elmer). Allele calling was performed according to ISAG bovine microsatellite allelic nomenclature of the 2001–2002 Cattle DNA Comparison Test.

SNP PCR conditions and primer extension reactions

The SNP primer pairs were amplified in a number of different multiplex-PCR reactions. Multi-

Table I. Microsatellite marker sets, loci, chromosomal location, fluorescent dye and distribution in multiplex PCR.

	Locus	Chromosome	Dye*	Multiplex PCR
10 Marker set	*BM1824*	1	6FAM	A
	BM2113	2	6FAM	A
	ETH10	5	NED	A
	ETH225	9	NED	A
	INRA023	3	HEX	B
	SPS115	15	6FAM	A
	TGLA122	21	NED	B
	TGLA126	20	6FAM	B
	TGLA227	18	6FAM	A
	TGLA53	7	6FAM	B
14 Marker set[a]	*BM1818*	23	6FAM	B
	CYP21	23	HEX	A
	MGTG4B	4	HEX	B
	SPS113	10	HEX	A
17 Marker set[b]	*AGLA293*	5	NED	B
	TGLA48	7	6FAM	B
	TGLA57	7	HEX	A

* Fluorescent label used with forward primer.
[a] In addition to the 10 microsatellite marker set.
[b] In addition to the 14 microsatellite marker set.

plex-PCR and primer extension reactions were carried out for all assays in a 384-well microtiter plate. The 5 μl total volume PCR mix comprised 50 ng genomic DNA, 10 μM of each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.5 μM of each primer and 0.5 units of AmpliTaq (Applied Biosystems Division, Perkin-Elmer). The temperature profile was: initial denaturation at 94 °C for 3 min; then 30 cycles of: denaturation at 95 °C for 30 s; primer annealing at 60 °C for 1 min; and elongation at 72 °C for 1 min. Final extension was at 72 °C for 10 min. After the PCR reaction, excess primers, dNTPs and salts were removed using a modified DNA purification system with magnetic beads (Macherey-Nagel). Purified PCR products were used for primer extension reactions in a total volume of 5 μl containing 200 μM of each dNTP/ddNTP, 20 pmol of the appropriate extension primer, and 0.5 units Thermosequenase (Amersham Pharmacia Biotech Europe GmbH, Freiburg, Germany). Allele specific extension products were purified with the magnetic bead system mentioned above and eluted in 5 μl buffer.

SNP mass spectrometry

Matrix solution (0.5 μl of an aqueous solution of 8 mg/ml 3-hydroxypicolinic acid and 2 mg/ml dibasic ammonium citrate) was pipetted on an AnchorChip sample target (anchor size 400 μm, Bruker Daltonics GmbH, Bremen, Germany) which was then dried at room temperature. Analyte DNA (0.5 μl) was added to the dried matrix spots, and the target was dried again at room temperature. The target was introduced into the source of an AutoFlex mass spectrometer with a SCOUT MTP ion source (Bruker Daltonics), operated in linear mode with 19 kV and 16.9 kV for the conversion dynode and the sample target, respectively. Mass spectrometry MALDI-TOF was performed in fully automated mode using the XACQ Software (Bruker Daltonics) in combination with the AutoXecute automation package (Bruker Daltonics). Determination of genotypes from mass spectra was carried out using the GenoTools SNP manager (Bruker Daltonics).

Biometric estimations derived from cattle genotyping

Calculations of observed heterozygosity and polymorphism information content (PIC; Botstein *et al.*, 1980) for the Galloway population were derived from the microsatellite genotyping data based on allele frequencies estimated using the Microsoft® Excel workbook template PowerStatsV12 (Tereba, 1999). Allelic frequencies derived from SNP genotyping data were obtained using the GenoTools SNP software mentioned above. Observed heterozygosity was defined as the number of heterozygotes divided by the sample size. Unbiased expected heterozygosity (Nei, 1987) was obtained from allele frequencies assuming Hardy-Weinberg equilibrium. Observed and expected heterozygosity obtained for each SNP and microsatellite marker are listed in Tables II and III, respectively. In case of the SNP method, 10 SNP markers from the 43 SNP marker set used in the present study were not heterozygous in Galloway cattle (see Table II).

Calculation of probability of identity (PI)

The PI estimations (see Table IV) were based on allele frequencies and were calculated for unrelated animals by using the API-CALC 1.0 computer program (Ayres and Overall, 2004). Multilocus PI values were obtained by multiplying single-locus PI values, assuming independence of microsatellite as well as SNP loci.

Statistical calculations in parental analysis

Estimations of paternity exclusion (PE) were obtained according to Brenner and Morris (1990). Cumulative paternity exclusion for the applied marker sets (see Table IV) was calculated as:

$$PE_{\text{cumulative}} = 1 - \prod_{i=1}^{n} PE_i$$

where PE_i is the paternity exclusion of marker i and n the total number of markers.

The exclusion power of microsatellite and SNP markers for parentage assessment was evaluated using cumulative parental exclusion probability (PEP) estimations in 18 Galloway family trios, which were defined as such from the breeders' pedigree records. Values of PEP were calculated according to three different situations: in PEP1, there is an exclusion of a parent-offspring relationship, where the genotypes for one parent and offspring are given (Garber and Morris, 1983; Chakraborty *et al.*, 1988; Jamieson and Taylor, 1997), *e.g.* father or mother genotype missing; in PEP2, there is an exclusion of one parent, where the genotypes for both parents and offspring are given

Table II. The 43 SNP marker set selected for individual identification and parentage analysis in Galloway breed with estimates of allele frequencies, expected and observed heterozygosity.

Locus identifier	GenBank accession no.	Chromosome	Allele 1	Allele 2	Estimated frequency of allele 1	EH[a]	OH[b]
417_16	AF440365	4	G	A	0.41	0.48	0.46
423_24	AF440366	10	G	A	0.06	0.11	0.17
421_10	AF440368	1	C	G	0.69	0.43	0.43
16_2	AF440369	7	G	A	NA	–	–
425_2	AF440371	9	A	G	NA	–	–
431_A2	AF440372	5	G	A	0.57	0.49	0.48
437	AF440374	14	C	A	0.12	0.21	0.25
448_67	AF440377	2	T	C	0.91	0.16	0.18
463_67	AF440379	17	C	T	0.39	0.48	0.48
486_67	AF440380	3	C	T	0.90	0.18	0.18
487_67	AF440381	14	G	A	NA	–	–
CAC	AF440382	4	C	G	0.09	0.16	0.19
013.sp6	AJ496635	6	T	C	0.91	0.16	0.20
018.sp6	AJ496636	3	C	T	0.71	0.41	0.36
022.t7	AJ496762	16	G	A	NA	–	–
039.t7	AJ496765	19	T	C	0.17	0.28	0.36
045.t7	AJ496766	NS	G	A	0.89	0.20	0.17
048.sp6	AJ496767	21	T	G	0.08	0.15	0.18
055.t7	AJ496768	11	G	T	0.88	0.15	0.21
058.t7	AJ496771	NS	G	A	NA	–	–
092.t7	AJ496778	NS	G	A	0.37	0.47	0.48
099.sp6	AJ496779	NS	A	G	0.48	0.50	0.48
107.sp6	AJ496780	21	C	G	NA	–	–
118.t7	AJ496782	12	A	G	NA	–	–
140.sp6	AJ496785	8	C	T	NA	–	–
073.sp6	AJ496787	NS	T	C	0.86	0.24	0.31
105.sp6	AJ496789	18	T	C	0.20	0.32	0.32
BULGE102	AJ505153	NS	G	A	0.67	0.44	0.52
BULGE114	AJ505154	NS	C	T	0.84	0.27	0.27
BULGE113	AJ505155	20	C	T	0.37	0.47	0.49
BULGE122	AJ505156	NS	A	G	0.43	0.49	0.42
BULGE100	AJ505157	24	G	T	0.19	0.31	0.31
BULGE104	AJ505158	NS	A	G	0.31	0.43	0.48
BULGE105	AJ505159	16	A	G	NA	–	–
BULGE101	AJ505160	9	C	T	0.56	0.49	0.39
BULGE128	AJ505161	23	G	C	0.31	0.43	0.32
BULGE119	AJ506186	NS	G	A	NA	–	–
BULGE121	AJ506187	NS	T	C	0.23	0.35	0.32
BULGE108	AJ506784	NS	C	T	0.34	0.45	0.39
BULGE110	AJ506785	NS	C	T	0.41	0.48	0.21
077.t7	AJ506786	1	G	A	0.52	0.50	0.44
TIGR_TC2908	BM089822	NS	A	G	0.51	0.50	0.50
TIGR_TC1921	CF762929	NS	C	T	0.37	0.47	0.47

[a] EH, expected heterozygosity.
[b] OH, observed heterozygosity.
NS, not specified.
NA, not applicable in Galloway breed (no heterozygosity observed).

(Jamieson, 1965; Chakraborty *et al.*, 1988; Jamieson and Taylor, 1997), *e.g.* familiar paternity case; in PEP3, there is an exclusion of both parents, where genotypes for parents and offspring are given (Grundel and Reetz, 1981; Jamieson and Taylor, 1997), *e.g.* a changeling.

Results and Discussion

Microsatellites and SNP are used for a diversity of scientific studies and commercial tasks in cattle, such as linkage mapping (Grosse *et al.*, 1999), genetic diversity and differentiation (Hanslik *et al.*, 2000; Cañón *et al.*, 2001; Maudet *et al.*, 2002; Ibea-

Table III. Polymorphism and allele-frequency-based biometric estimations for bovine microsatellites in Galloway breed.

Locus	Number of alleles	EH[a]	OH[b]	PIC[c]
AGLA293	6	0.67	0.70	0.61
BM1818	4	0.46	0.46	0.37
BM1824	4	0.64	0.61	0.57
BM2113	5	0.57	0.52	0.52
CYP21	8	0.77	0.76	0.74
ETH10	5	0.53	0.55	0.45
ETH225	6	0.76	0.79	0.72
INRA023	7	0.76	0.78	0.72
MGTG4B	6	0.65	0.65	0.61
SPS113	6	0.66	0.64	0.60
SPS115	6	0.76	0.75	0.72
TGLA122	7	0.47	0.45	0.44
TGLA126	4	0.65	0.72	0.58
TGLA227	8	0.84	0.89	0.82
TGLA48	3	0.57	0.59	0.49
TGLA53	8	0.49	0.48	0.47
TGLA57	6	0.71	0.75	0.67

[a] EH, expected heterozygosity.
[b] OH, observed heterozygosity.
[c] PIC, polymorphism information content.

gha-Awemu and Erhardt, 2005), and individual identification and kinship investigation (Glowatzki-Mullis *et al.*, 1995; Heyen *et al.*, 1997; Schnabel *et al.*, 2000; Heaton *et al.*, 2002). Simulation-based studies show that genetic analysis requires a large number of SNP markers relative to microsatellite markers (reviewed by Morin *et al.*, 2004). In the present study, we have attempted to determine which of both methods more accurately identifies individuals and family relationships, in the practical case of genotyping a cattle population.

One measure of the utility of a genetic system is the PI, which is the probability that two randomly chosen individuals in a population have identical genotypes. For the PI estimations (see Table IV), the 33 heterozygous SNP markers in Galloway cattle showed approx. similar power to the 14 microsatellite marker set. The power to identify individuals depends mainly on the number of independent markers and their heterozygosity rather than on the number of alleles per locus (Miller *et al.*, 2002).

Table IV. Multi-locus PI and cumulative PE estimations for the marker sets applied in Galloway genotyping.

	Microsatellite			SNP
	10 marker set	14 marker set	17 marker set	43 marker set
PI	2.4×10^{-8}	2.3×10^{-11}	1.4×10^{-13}	5.3×10^{-11}
PE	0.9968	0.9994	0.9999	0.9772

The PE is a measure of the ability of a certain marker to identify genetic paternity, excluding all other candidates. Cumulative PE estimations (see Table IV) showed higher exclusion power for the microsatellite marker sets, which was expected due to the higher information content (*i.e.* heterozygosity, allele frequencies) of multi-allelic microsatellites over that of the bi-allelic SNP. The values of cumulative PE estimations increased with increasing numbers of microsatellite markers, *e.g.* 14 and 17 microsatellite marker sets. In contrast, 10 SNP markers from the 43 SNP marker set used in the present study contributed nothing to the cumulative PE, since no heterozygosity for those markers was observed in the Galloway population. For this reason, the value of the cumulative PE estimation for the SNP marker set was significantly lower.

The PEP is a measurement of the probability for a correct parentage assessment, and it is expressed in powers of the allele frequencies. Average cumulative PEP estimations of 18 Galloway family trios obtained when using microsatellite and SNP marker sets are listed in Table V. In case of the microsatellite method, the average cumulative PEP estimations were substantially higher when using expanded marker sets, *e.g.* the 14 and the 17 microsatellite marker sets. However, the estimates for correct parental assessment derived from our study showed similar exclusion power for both microsatellite and SNP methods. The values of the PEP calculations depended directly on the allelic frequency distribution of each marker within the studied population.

The median for the number of ISAG recommended microsatellites used in cattle genotyping is 12 loci (Baumung *et al.*, 2004). Since our study was part of a major project involving the recommendation of a microsatellite-based system for commercial work in German cattle, the 10 microsatellite marker set represents an optimized set of markers for that purpose (López Herráez, 2005). This marker set demonstrated its efficiency in individual identification and in parental analysis. In the case of the SNP method, the selection of highly informative SNP markers results in a significant

Table V. Average cumulative PEP estimations in parental assessment of 18 Galloway family trios when using 10, 14 and 17 microsatellite and 43 SNP marker sets.

	18 Galloway family trios			
	Microsatellite			SNP
	10 marker set	14 marker set	17 marker set	43 marker set
PEP1	0.9457	0.9890	0.9946	0.9497
PEP2a[a]	0.9950	0.9997	0.9999	0.9929
PEP2b[b]	0.9995	0.9999	0.9999	0.9944
PEP3	0.9999	0.9999	0.9999	0.9999

[a] Cumulative PEP2a, exclusion of father, where genotypes for parents and offspring are given.
[b] Cumulative PEP2b, exclusion of mother, where genotypes for parents and offspring are given.

increase in power of identification compared with unselected SNP markers and, therefore, the ability of such markers for identification of family relationships is improved (Heaton *et al.*, 2002). During the development of efficient SNP-based marker systems it is critical to consider that SNP informativity may vary significantly between populations (Krawczak, 1999), as occurred with the informativity of the 43 SNP marker set used in the present study in Galloway cattle when compared to German Holstein, Fleckvieh and Braunvieh cattle populations (Werner *et al.*, 2004). For this reason, a large number of studies is required to develop a SNP marker set suitable for use in different cattle breeds and populations.

The choice of method for genotyping depends on many criteria. From the geneticist's point of view, the genotyping procedure should be as simple, robust, and inexpensive as possible, since generating vast amounts of genotype data is often necessary. From the statistician's point of view, the accuracy of each type of analysis may depend on a few key characteristics, such as information content, neutrality, map positions or genetic independence of the markers. The SNP markers have promising advantages over microsatellite markers, such as high-throughput automated analysis, lower mutation rates and lower genotyping costs (Landegren *et al.*, 1998; Nielsen 2000; Morin *et al.*, 2004). For microsatellites, there is a standard procedure for genotyping involving PCR and size determination of the amplified fragment by gel electrophoresis. For SNP genotyping, there is no standard method for analysis, and many techniques are available (summarized by Landegren *et al.*, 1998; Vignal *et al.*, 2002). In conclusion, both microsatellite and SNP analysis are similarly suited for cattle genotyping. Thus either method can be used for genotyping though the choice of method will have to be made according to the purpose of the study and the equipment available.

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