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Sequence conservation of microsatellites between *Bos taurus* (cattle), *Capra hircus* (goat) and related species. Examples of use in parentage testing and phylogeny analysis

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A panel of 70 bovine microsatellites was tested for amplification from goat DNA. Forty-three could be successfully amplified by PCR, 20 of which were tested for polymorphism. Three were applied for parentage testing in goat families and their exclusion probability evaluated. Fourteen were cloned and sequenced from goat DNA, and goat and bovine sequences were compared to evaluate interspecific conservation. Correlation between the structure of the dinucleotide repeat and the number of alleles was studied and indicated that interruption(s) in the repeat could explain the difference in the levels of polymorphism between the two species. This study provides a valuable *in vivo* clue to the mechanism generating polymorphism in microsatellites. Sequence conservation was also observed for several microsatellites with two wild species of Bovidae, Nilgaï (*Boselaphus tragocamelus*) and Himalayan Tur (*Capra cylindricornis*), and with one species of Cervidae, the fallow deer (*Cervus dama*).

This study showed that an estimated 40 per cent of the microsatellites isolated from cattle will prove useful to study the caprine genome and to characterize economically important genetic loci in this species. Moreover, bovine microsatellites were shown to constitute very useful tools for the study of genetic diversity of the Artiodactyla.

Keywords: goat, interspecific priming, intraspecific genetic variation, microsatellites, polymorphisms.

Introduction

Ninety-four per cent of all goats are nowadays found in the developing countries of Asia and Africa, and constitute an important source of milk and proteins in these countries. Furthermore, in developed countries, goats are of economic importance and selection of potentially interesting parents at the gene level is already used for milk proteins such as α-S1 casein (Leroux *et al.*, 1990). Therefore, progress in knowledge about the genome of this species is very useful and could bring a rapid improvement of goat breeds and an increase in performance. As very little funding is granted to study the goat genome compared with cattle and sheep, exploitation of genetic markers isolated from these closely related species (given that they can be used in goat) would have a big impact.

The versatility of microsatellite markers (tandem repeat motifs of one to five base pairs), makes them the ideal tools for genetic mapping, as well as valuable tag sequences for physical mapping. Also, their generalized use has allowed an increased knowledge of the genetic map of different vertebrate genomes. Microsatellites have been found throughout eukaryotic organisms, and particularly in different classes of vertebrates (mammals: Cornall *et al.*, 1991; Schlötterer *et al.*, 1991; Ellegren *et al.*, 1992; Weissenbach *et al.*, 1992; birds: Crooijmans *et al.*, 1993; fish: Estoup *et al.*, 1993).

In the past few years, with the development of many new microsatellite markers, bovine and ovine genetic mapping has made rapid progress (Moore *et al.*, 1992; Vaiman *et al.*, 1992, 1994b; Kaukinen & Varvio,

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1993). This is evidenced by the building of the first linkage maps (Barendse *et al.*, 1993a, 1993b), and the finding of markers associated with diseases such as weaver (Georges *et al.*, 1993a), morphological features such as poll (Georges *et al.*, 1993b) or interesting Economic Trait Loci (ETL) such as Booroola (Montgomery *et al.*, 1993). Comparatively, knowledge about the genome of the third economically important Bovidae species, goat, has increased less rapidly. Nevertheless, the apparent chromosomal similarity between cattle, sheep and goat (Hayes *et al.*, 1991) suggests that markers developed in one of the three species could be readily used in the other two. Between cattle and sheep, such a possibility (Kemp *et al.*, 1993b), as well as synteny conservation has been demonstrated (Vaiman *et al.*, 1994a). However, no statistical data on a sufficiently large number of microsatellite markers are available to draw a general conclusion. The purpose of this study is to evaluate a large number of cattle microsatellites to determine the sequence conservation and to explore the possiblity of utilizing these markers in goat.

In this paper, primers derived from 70 bovine microsatellite flanking sequences (Vaiman *et al.*, 1994b), were used to amplify the goat corresponding loci. Forty-one amplification products could be obtained, of which 14 were cloned and sequenced. The possibility of using these microsatellites for goat parentage testing and population polymorphism analysis was evaluated.

Materials and methods

Animals and DNA preparation

Sixty unrelated goats from four different breeds (12 Poitevine, 12 Saanen, 12 Alpine, 12 Guinean, 12 Sahelian, 12 Guadeloupean creolous), were used to estimate the allelic frequencies of each microsatellite. For population studies, the allelic frequencies were estimated from a panel of 30 animals for the Alpine, Saanen and Poitevine breeds and from 12 animals for each of the other breeds. Mendelian codominant transmission of the alleles was ascertained on a family of 15 creolous goats composed of a sire, seven dams and their progeny.

DNA was prepared from 20 mL blood samples collected on EDTA, according to Jeanpierre (1987) or from 200 mL of frozen blood according to Cui *et al.* (1989).

PCR conditions

PCR typing of animals to estimate allelic frequencies was carried out on 100 ng of genomic DNA in a 10 μL reaction with 25 μM dGTP, dCTP, dTTP, 2.5 μM of dATP and 0.1 μL [^{35}S] dATP (Amersham, 1000 Ci/mmol, 10 μCi/μL) with a *Taq* DNA Polymerase kit purchased from Promega (1.5 mM final magnesium concentraton). After a first step of denaturation at 94 °C during 5 min, 30 cycles of amplification were realized (15 s 94 °C, 15 s 55 °C, 20 s 72 °C) using a Perkin-Elmer Cetus 9600 thermocycler. PCR products were then loaded with 5 μL of loading buffer (0.25 per cent Bromophenol Blue, 0.25 per cent Xylene cyanol, in 95 per cent deionised formamide) on a 5 per cent polyacrylamide/7.5 M urea denaturing gel, and run for 2 h at 1500 V. The gels were dried and autoradiographed overnight at room temperature.

Cloning and sequencing of PCR products

After standard PCR procedures using bovine primers, goat amplification products were extracted once with phenol–chloroform, precipitated, phosphorylated with T4 Polynucleotide kinase (Boehringer), extracted again with phenol–chloroform, precipitated and ligated into the *Sma*I site of dephosphorylated PUC 18 using T4 DNA ligase (BRL). Four independent clones from two different animals were sequenced for each microsatellite using an ABI 373A automated sequencer.

Sequence analysis and calculations

Sequence alignments between the sequences of bovine, goat and other species and database searches were realized using the 'gap' and 'fasta' algorithms of the GCG software package, respectively (Devereux *et al.*, 1984). When the difference in the microsatellite size was too large to make an alignment between the two species, the flanking sequences were aligned without including the primers. The similarity coefficients presented in Table 2 were always calculated without the primers and without the dinucleotide repeat.

PIC (Polymorphic Information Content) was calculated in the total number of individuals according to Botstein *et al.* (1980). Exclusion probabilities were calculated according to Hanset (1975).

Results and discussion

Sequence comparison between cattle, goat and other species of Artiodactyla

Table 1 presents a list of the 70 bovine microsatellite markers utilized in this study. All these loci were tested by PCR with goat DNA. The quality of the PCR product was rated by a number between 1 and 4 (1: strong amplification with only one band (or two to account for heterozygous animals) of the expected size;

Table 1 List of bovine microsatellites used in this study

Laboratory number	Amplification quality	Goat sequence realised	Reference
INRA003	1	Yes	Vaiman *et al.* (1992)
INRA005	1	Yes	Vaiman *et al.* (1992)
INRA006	1	Yes	Vaiman *et al.* (1992)
INRA011	1	Yes	Vaiman *et al.* (1992)
INRA016	1	Yes	Vaiman *et al.* (1994b)
INRA023	1	Yes	Vaiman *et al.* (1994a)
INRA031	1		Vaiman *et al.* (1994b)
INRA036	1	Yes	Vaiman *et al.* (1994b)
INRA037	1	Yes	Vaiman *et al.* (1994b)
INRA039	1		Vaiman *et al.* (1994b)
INRA040	1	Yes	Vaiman *et al.* (1994b)
INRA054	1		Vaiman *et al.* (1994b)
INRA061	1		Vaiman *et al.* (1994b)
INRA063	1	Yes	Vaiman *et al.* (1994b)
INRA067	1		Vaiman *et al.* (1994b)
INRA069	1		Vaiman *et al.* (1994b)
INRA071	1		Vaiman *et al.* (1994b)
INRA090	1		Vaiman *et al.* (1994b)
INRA103	1		Vaiman *et al.* (1994b)
INRA128	1	Yes	Vaiman *et al.* (1994b)
ILSTS04	1	Yes	Kemp *et al.* (1993)
ILSTS05	1		Brezinsky *et al.* (1993a)
ILSTS08	1	Yes	Kemp *et al.* (1993a)
ILSTS09	1		Unpublished data
ILSTS11	1		Brezinsky *et al.* (1993c)
ILSTS12	1		Brezinsky *et al.* (1993c)
ILSTS17	1		Kemp *et al.* (1993b)
ILSTS18	1		Guérin *et al.* (1994)
ILSTS19	1		Kemp *et al.* (1993b)
INRA015	2	Yes	Unpublished data
INRA028	2		Vaiman *et al.* (1994b)
INRA032	2		Vaiman *et al.* (1994b)
INRA038	2		Vaiman *et al.* (1994b)
INRA053	2		Vaiman *et al.* (1994b)
INRA055	2		Vaiman *et al.* (1994b)
INRA059	2		Vaiman *et al.* (1994b)
INRA062	2		Vaiman *et al.* (1994b)
INRA074	2		Vaiman *et al.* (1994b)
INRA080	2		Vaiman *et al.* (1994b)
INRA082	2		Vaiman *et al.* (1994b)
INRA084	2		Vaiman *et al.* (1994b)
INRA100	2		Vaiman *et al.* (1994b)
INRA111	2		Vaiman *et al.* (1994b)
INRA025	3		Vaiman *et al.* (1994b)
INRA052	3		Vaiman *et al.* (1994b)
INRA117	3		Vaiman *et al.* (1994b)
ILSTS10	3		Brezinsky *et al.* (1993c)
ETH8	3		Unpublished data
INRA013	4		Vaiman *et al.* (1992)
INRA018	4		Goudarzi *et al.* (1993)
INRA027	4		Vaiman *et al.* (1994b)
INRA030	4		Vaiman *et al.* (1994b)
INRA041	4		Vaiman *et al.* (1994b)
INRA044	4		Vaiman *et al.* (1994b)
INRA046	4		Vaiman *et al.* (1993)
INRA049	4		Vaiman *et al.* (1994b)
INRA050	4		Vaiman *et al.* (1993)
INRA057	4		Vaiman *et al.* (1994b)
INRA064	4		Vaiman *et al.* (1994b)
INRA072	4		Vaiman *et al.* (1994b)
INRA073	4		Vaiman *et al.* (1994b)
INRA079	4		Vaiman *et al.* (1994b)
INRA104	4		Vaiman *et al.* (1994b)
INRA110	4		Vaiman *et al.* (1994b)
INRA130	4		Brezinsky *et al.* (1992)
ILSTS01	4		Brezinsky *et al.* (1993b)
ILSTS06	4		Brezinsky *et al.* (1993c)
ILSTS13	4		Kemp *et al.* (1993b)
ILSTS20	4		Unpublished data
ETH185	4		

2: one prominent band in a relatively weak smear; 3: different bands of various sizes; 4: smear of weak bands or no amplication at all). The counts of loci in decreasing quality of amplification were 29 (41.4 per cent), 14 (20.0 per cent), 5 (7.1 per cent) and 22 (31.4 per cent). A goat PCR product of the expected size could be obtained in 43 cases (61.4 per cent, quality 1 and 2). Peculiarly, primers for INRA015, although designed from the bovine sequence, could give an amplification product with goat DNA but not with bovine.

Fourteen goat microsatellites were cloned after PCR amplification with the bovine primers, and sequenced. Figure 1 shows the sequence comparison between cattle and goat for the microsatellite flanking sequences resulting in an average percentage of similarity of 85.6 per cent (± 12.35 per cent). Most of the sequences were essentially conserved with only some point mutations and small deletions of a few base pairs. Two exceptions are INRA003 and INRA040, for which deletions of 30 and 40 bp in the bovine sequences, respectively, differentiate the two species. These two deleted sequences were compared with the GenBank and EMBL databases, but no significant similarity could be detected with other known DNA fragments.

INRA003, INRA006 and INRA023 were previously cloned and sequenced in sheep (Vaiman *et al.*, 1994a). Between cattle and sheep, INRA006 and INRA023 are well conserved whereas for INRA003, the ovine sequence is very similar to the caprine (Fig. 1, INRA003), including the 30 bp insertion mentioned above that is lacking in the bovine sequence. This is consistent with the hypothesis of a closer phylogenetic relationship between goat and sheep than between cattle and either of these species (Randi *et al.*, 1991).

To extend interspecific sequence comparison, INRA023 was cloned from Nilgaï, classically considered as a close relative of cattle, Himalayan Tur, closely related to goats, and the fallow deer (Cervidae) as a species exterior to the Bovidae family. Comparison of similarity coefficients is summarized in Table 2. Although only one system was considered, results display perfect consistency with published phylogenetic distances in the Order Artiodactyla. The fallow deer is the most distant to other species (0.79 on average). The highest coefficients are found between *Bos* and *Boselaphus* (0.95) and between the two members of the genus *Capra* (0.93) whereas these two subgroups are relatively close (0.92).

Study of polymorphism

Twenty microsatellite loci known by somatic cell mapping to be spread all over the bovine genome (Vaiman *et al.*, 1994b), were chosen for a study of polymorphism in the goat. These loci were selected because they have been extensively used in cattle for population studies (Moazami-Goudarzi *et al.*, 1994). Data on a sufficient panel of animals were obtained for nine loci (Table 3). The number of alleles and PIC observed are indicated in Table 3. Allele sizes were estimated approximately by a parallel run of a M13 mp18 sequencing reaction. No evidence of correlation could be found between the bovine and the caprine PIC or number of alleles. Examples of disparate diversities are given by microsatellites INRA016 and INRA128 (not shown in the table) which were monomorphic in goat (nine and five alleles in cattle, respectively) and microsatellite INRA011, for which more than 20 alleles were visible in goat (only eight alleles were visible in cattle). In all the cases where such differences in polymorphism are observed, sequence analysis suggests that interruption(s) in the dinucleotide repeat in one of the species is correlated with a drop in the number of alleles. The longest uninterrupted repeat in the goat is of only six TG for INRA016, six CA for INRA128 vs. 18 and 14 in cattle, respectively. This result is consistent with a frequent observation of polymorphism loss when using interspecific priming of microsatellites (Kondo *et al.*, 1993). To explain this frequent observation, one can suggest that while the screening in the first species is realized in stringent conditions to isolate exclusively large TG repeats, the extension to another species does not experience the same constraints and may result in shortened or altered TG repeats in this second species, even though the locus is maintained at the homologous chromosomal location.

In one case (INRA011), we observed an increase in the number of alleles in goat. At this locus, the bovine dinucleotide TG repeat is interrupted by a TA whereas in goat this interruption is absent. *In vitro* studies demonstrated that polymerase slippage is probably the predominant mechanism to generate new alleles in simple sequence repeats (Schlötterer & Tautz, 1992). Most probably, interruptions in the stretch of the microsatellite dinucleotide repeat can prevent polymerase slippage. To our knowledge, this study, based on interspecific comparisons of sequences and polymorphism gives one of the first indications that polymerase slippage can favour the creation of new microsatellite alleles in an *in vivo* system. These considerations suggest that interspecific study of microsatellite polymorphisms can give valuable insights into the stability of short repetitive sequences in the genome of mammals. This issue could be important in the interpretation of trinucleotide repeat expansions, correlated with certain human diseases. In at least one case, the expansion of the repeat linked to the disease is correlated with the presence of a perfect run

of CAG repeats whereas individuals bearing an imperfect stretch of repeats (interrupted with CAA triplets) do not undergo triplet expansion (J. L. Mandel, personal communication).

Polymorphism was also extensively tested for three systems (INRA005, INRA006 and INRA023) in six different goat breeds. In Alpine and Saanen the number of animals used to calculate the frequencies

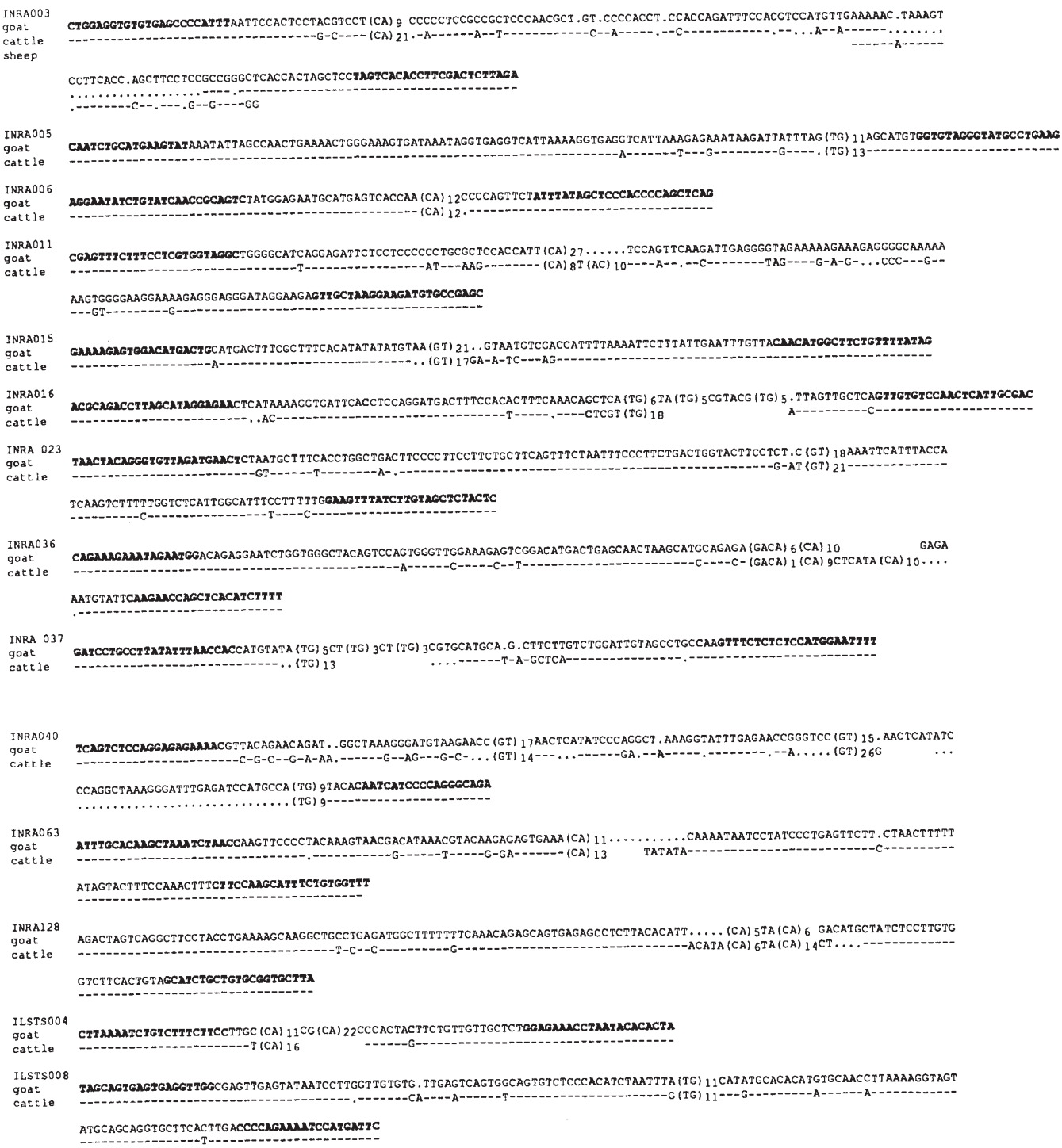


Fig. 1 Alignment of nucleotide sequences between goat (upper sequence) and cattle (lower sequence) for the 14 microsatellites of the study. Primers are indicated in bold characters. Gaps (.) have been placed to maximize the homology. Dashes (-) correspond to nucleotides which are identical between goat and cattle. For microsatellite INRA003, the sheep sequence corresponding to the 30 bp insertion is represented (see text).

Table 2 Coefficients of similarity in INRA023 sequence between different Artiodactyla species

	B. tragocamelus	*B. taurus*	*C. hircus*	*C. cylindricornis*	*Cervus dama*
Boselaphus tragocamelus	1	0.95	0.93	0.93	0.80
Bos taurus		1	0.92	0.92	0.83
Capra hircus			1	0.93	0.76
Capra cylindricornis				1	0.77
Cervus dama					1

Table 3 Comparison of polymorphism between cattle and goat

Laboratory number	Number of alleles in goat	Average size in goat	PIC in goat	Number of alleles in cattle	Average size in cattle	PIC in cattle
INRA003	3	188	0.39	10	200	0.77
INRA005	5	139	0.56	3	150	0.51
INRA006	12	100	0.82	7	105	0.54
INRA011	20	150	0.92	8	200	0.41
INRA023	12	210	0.79	12	220	0.77
INRA036	9	170	0.72	4	170	0.47
INRA040	9	220	0.83	44	205	0.87
INRA063	6	170	0.72	7	180	0.46
ILSTS08	4	180	0.29	4	179	0.57

was above 30. This number was 16, 12, 11 and 10 for the Poitevine, Guadeloupean, Guinean and Sahelian breeds, respectively. The frequencies are reported in Table 4. A dendrogram was tentatively built with these frequencies using the Nei standard distance (Fig. 2), giving a first molecular observation about the proximity of goat breeds. Except for the Sahelian breed, the dendrogram results in data consistent with the geographical repartition of the breeds. Three local French breeds (Alpine, Saanen and Poitevine) show an expected close relationship as with two other breeds (Guadeloupean and Guinean). The unexpected position of the Sahelian breed is probably the result of the low number of individuals (10) used to calculate the allelic frequencies. The tight relationship observed between the Guinean and Guadeloupean breeds is probably explained by the known African origin of the Guadeloupean goat, imported to the French West Indies during the 19th century.

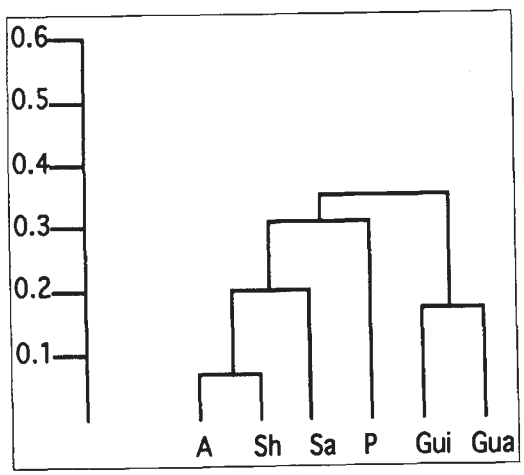
The relatively high number of point mutations differing between cattle and goat is consistent with those described by others for Artiodactyla (Stallings *et al.*, 1991). The phylogenetic divergence between the Bovinae and Caprinae lineage is estimated to have occurred during the early Miocene (14–17 Myr) (Simpson, 1984; Randi *et al.*, 1991; Allard *et al.*, 1992), which allows the calculation of a global divergence rate for neutral nucleotide positions of 0.93 per cent (± 0.23 per cent) Myr^{-1}, higher than the mammalian average of 0.5 per cent (± 0.1 per cent) Myr^{-1} (Wilson *et al.*, 1987) but substantially higher than that found in Cetaceans of 0.09 per cent (± 0.05 per cent) Myr^{-1} (Schlötterer *et al.*, 1991).

Example of use in parentage testing

Three different microsatellites (INRA006, INRA023 and INRA005) were used in parentage control in goat families belonging to three different breeds (Poitevine, Saanen and Alpine; Fig. 3), giving satisfactory results. The exclusion probabilities were calculated according to Hanset (1975), giving 0.75 for INRA006, 0.67 for INRA023 and 0.58 for INRA005. The cumulated exclusion probability was 0.89. As INRA023 and INRA006 are located on the same bovine and ovine chromosomes (BTA3 and OAR1, respectively; Vaiman *et al.*, 1994a), other markers will be necessary to obtain a perfectly reliable exclusion probability. However, preliminary distance calculations on a limited number of goat families suggest that the distance separating the two loci is over 30 cM, confirming mapping data obtained in cattle and in sheep (Vaiman *et al.*, 1994a).

Table 4 Comparison of allelic frequencies in different goat breeds

Laboratory number	Allele	Frequencies in different breeds					
		Alpine	Saanen	Poitevine	Guadeloupean Creolous	Guinean	West African Sahelian
INRA005	1	0.06	0.01	0.16	0.042	0	0
	2	0.29	0.05	0.34	0.541	0.7	0.318
	3	0.15	0.4	0.19	0.042	0	0.091
	4	0.48	0.54	0.31	0.375	0.3	0.591
	5	0.02	0.01	0	0	0	0
INRA006	1	0.01	0.01	0.03	0	0.055	0
	2	0.02	0.01	0.03	0.111	0	0.05
	3	0.14	0.13	0.24	0.111	0.222	0.05
	4	0.32	0.31	0.21	0.056	0.278	0.3
	5	0.06	0.11	0.12	0.056	0.111	0.2
	6	0.25	0.11	0.06	0.611	0.167	0.2
	7	0.14	0.17	0	0	0.167	0.1
	8	0.02	0.01	0.12	0	0	0
	9	0	0	0.15	0	0	0
	10	0.05	0.13	0.06	0.056	0	0.1
	11	0	0.01	0	0	0	0
	12	0	0.01	0	0	0	0
INRA023	1	0	0.01	0	0	0	0
	2	0.1	0.03	0	0	0	0
	3	0.36	0.31	0.65	0.156	0.308	0.167
	4	0.04	0.13	0	0.156	0.038	0.033
	5	0	0	0	0	0	0
	6	0.04	0.02	0	0	0	0.033
	7	0.04	0.03	0	0.032	0	0.3
	8	0.01	0.06	0	0	0	0
	9	0.21	0.24	0.2	0	0.115	0
	10	0.17	0.14	0.15	0.5	0.385	0.233
	11	0.03	0.03	0	0.156	0.115	0.2
	12	0	0	0	0	0.038	0.033



	A	Sa	P	Gua	Gui
Alpine					
Saanen	0.148				
Poitevine	0.227	0.236			
Guadelupean	0.158	0.563	0.587		
Guinean	0.158	0.462	0.284	0.176	
Sahelian	0.080	0.246	0.471	0.274	0.264

Fig. 2 Matrix of genetic distances between six goat breeds using microsatellite allelic frequencies based on the UPGMA method. A dendrogram is drawn according to the data of Table 4.

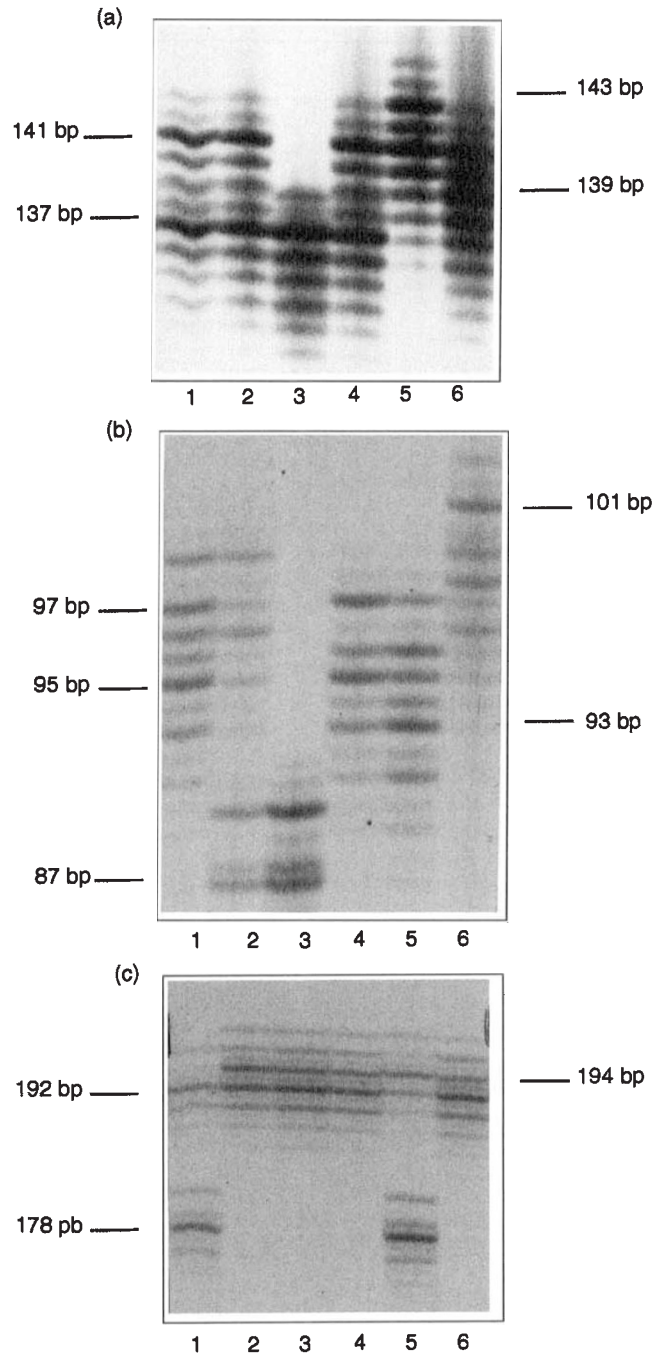


Fig. 3 Parentage analysis for a family of goats using the three microsatellite systems INRA005 (a), INRA006 (b) and INRA023 (c). Lanes 1, 2, 3 correspond to dam, offspring and sire in one putative family and 4, 5, 6 to dam, offspring and sire in another putative family. As revealed by the three systems, filiation is compatible in the first family and incompatible in the second, with a nonparental allele in lane 5 for INRA005 and INRA023, and the absence of the 99 bp allele in lane 5 for INRA006.

In conclusion, the use of bovine microsatellites in the goat seems to be a very powerful and promising approach for providing new tools for genome analysis in this species. As shown above, microsatellites appear particularly useful for establishing registered pedigrees in the goat, which is the first prerequisite to outline selection patterns. Moreover, as selection pressure was less intense in goat than in cattle, improvement of breeds based on genetic markers can probably be more easily achieved in caprine than in bovine breeds. Different biological characteristics of goat (relatively short generation time, lower farming costs, existence of described QTL) make this species an attractive animal model for studying the feasibility of marker-assisted selection schemes and strategies eventually of use in bovine breeding, aside from its own economical interest.

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