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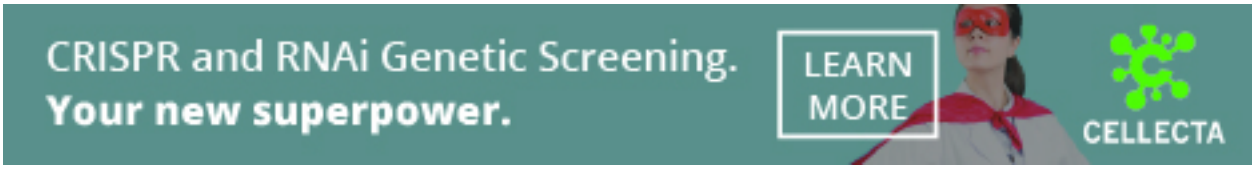

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LETTERS

Mutations in Sheep Microsatellites

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During the construction of a primary linkage map of the sheep genome, a total of 46,225 parent–offspring transfer of microsatellite alleles were examined through the genotyping of the AgResearch International Mapping Flock. Any observations of mutations were thoroughly rechecked, including examination of new DNA samples, to yield five bona fide mutations from the study. This gave us an observed spontaneous mutation rate for microsatellites in sheep of $1.1 \pm 0.5 \times 10^{-4}$ mutations/gamete per locus. Untransformed lymphocytes were used as the source of DNA. Two of the mutations could have happened at either meiosis or during mitotic growth of the male germ cells. The other three mutations probably occurred early in the mitotic generation of the female germ cells. All of the mutations consisted of a 2-bp insertion and occurred in individuals heterozygous at the mutant locus.

Microsatellites are regions of the genome that contain simple tandem repeats with variation occurring in the number of repeats within the region (Tautz 1989; Weber and May 1989). By amplifying the repeat region using the polymerase chain reaction (PCR) and determining the size of the product using DNA sequencing gels, the markers are easily characterized. Because of this and their high variability microsatellites have become the markers of choice for many genetic studies of eukaryotic organisms. As well as generating genetic linkage maps, microsatellites are used extensively in studies of population and behavior and in defining evolutionary relationships between closely related species, strains, and races within a species. It is therefore important to obtain reliable estimates of microsatellite mutation rates if genetic distances are to be calculated accurately and we are to use allele frequency changes as a measure of evolutionary distance.

Microsatellite mutation rates are difficult to measure because they are rare events that are often hard to confirm. In many cases the "mutation" could be due to a sample misidentification or an error in recording the pedigree. The AgResearch International Mapping Flock, a series of nine interrelated three-generation sheep pedigrees containing a total of 127 individuals, was genotyped with 246 markers (Crawford et al. 1995), removing any doubt about its pedigree record. This paper describes five bona fide mutations discovered during the construction of the sheep genetic linkage map and the subsequent estimate of the ovine microsatellite mutation rate. The five mutations are all of the same type, suggesting a common mechanism for microsatellite evolution.

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RESULTS

The recently published sheep genetic linkage map (Crawford et al. 1995) was generated from the linkage analysis of 246 polymorphic markers. Of these markers 215 were microsatellites genotyped at the AgResearch Molecular Biology Unit. All of the microsatellites used had dinucleotide (mostly AC) repeats. It is this set of genotypes, in which a total of 46,225 alleles were characterized and scored, that was screened for mutations. All observations of possible mutations were thoroughly rechecked, including reamplification of the alleles using a different DNA sample from the same individual.

Eleven putative mutations were rechecked to yield the five mutations illustrated in Figures 1-3. An arrowhead indicates the mutant alleles. All five mutations were 2-base-pair (bp) insertions. The sheep DNA used as template was purified from untransformed leukocytes so there was no possiblity that the mutation occurred during any transformation process, a common occurrence with human DNA samples (Weber and Wong 1993; Banchs et al. 1994).

Two of the mutations (MAF4 and BM1227) were sire-derived and could have happened at either meiosis or during mitotic growth of the male

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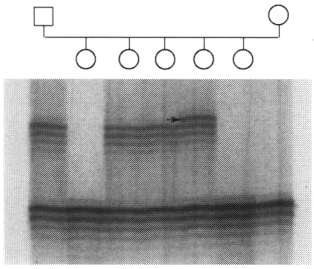

Figure 1 Amplification of microsatellite MAF4 in part of pedigree F of the AgResearch International Mapping flock. The pedigree structure is illustrated above the microsatellite alleles. The mutant allele is indicated with an arrow.

germ-line cells. It is possible the mutations in these individuals were somatic and not in the germ line. As there were no offspring from these individuals, this possibility cannot be tested. Examination of the linkage mapping data (Crawford et al. 1995) showed that crossovers were not present in regions flanking the mutant loci (data not shown). Recombination is therefore unlikely to have contributed to the cause of the mutations. The other three mutations (BM4513) were dam-derived and almost certainly occurred early in the mitotic generation of the female germ-line cells. They provide a good example of germ-line mosaicism.

An average of 215 parent–offspring transfer

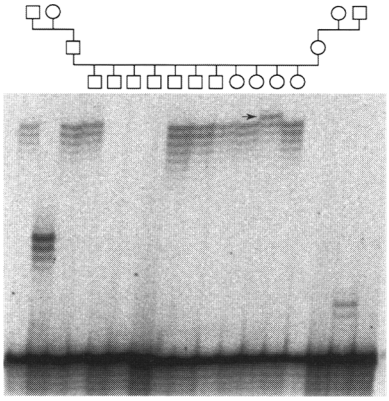

Figure 2 Amplification of microsatellite BM1227 in pedigree D of the AgResearch International Mapping flock. The pedigree structure is illustrated above the microsatellite alleles. The mutant allele is indicated with an arrow. The very dark lower band is an allele. It is our observation that the smaller of the two microsatellite alleles is often amplified significantly more, especially when there is a large difference in the size of the alleles.

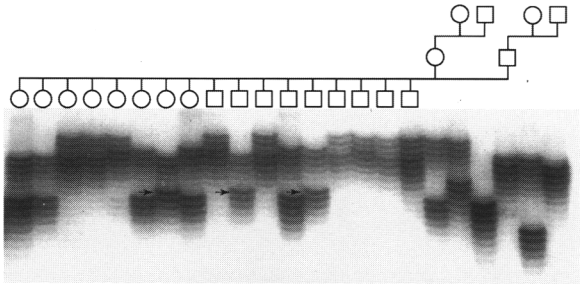

Figure 3 Amplification of microsatellite BM4513 in pedigree F of the AgResearch International Mapping flock. The pedigree structure is illustrated above the microsatellite alleles. The mutant alleles are indicated with arrows.

of alleles were examined for each of the 215 microsatellite markers used to generate the linkage map. This gives us an observed spontaneous mutation rate for microsatellites in sheep of $1.1 \pm 0.5 \times 10^{-4}$ mutations/gamete per locus.

DISCUSSION

Estimates of the mutation rate of microsatellites have been made in other mammals, but this is the first for sheep. The rate is within the range found for humans, mice, and pigs, the only other species where the microsatellite mutation rate has been determined (see Table 1).

The mutation rate is probably an underestimate, as some mutations would not be detected because the mutated allele may have been converted to another allele existing in the family and consistent with the pedigree. This would indicate the wrong origin for the inherited allele and alter the ratio of recombinants to nonrecombinants but would not show up as a mutation that disobeyed Mendel's rules. Weber and Wong (1993) estimate that approximately one mutation in five will not be detected as a violation of Mendel's rules. Assuming that the same estimate holds for sheep, the likely absolute sheep mutation rate for dinucleotide microsatellites will be $1.4 \pm 0.6 \times 10^{-4}$ mutations/gamete/locus.

The estimate should also be regarded as an average because it is likely that the more polymorphic markers have a higher mutation rate than the less polymorphic mutations. The three loci in which mutations were discovered have an average polymorphic information content (PIC) of 0.79, which is significantly higher ($P > 0.05$;

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Table 1. A Comparison of Mutation Rate Estimates for Microsatellites in Mammals

Species	Mutation rate	Reference
Human	4.5×10^{-4}	Kwiatkowski et al. 1992
Human	2.2×10^{-4}	Petrukhin et al. 1993
Human	1.0×10^{-3}[a]	Weissenbach et al. 1992
Human (dinucleotide)	5.6×10^{-4}	Weber and Wong 1993
Human (tetranucleotide)	2.1×10^{-3}	Weber and Wong 1993
Mouse (Ckmm/105)[b]	1.2×10^{-4}	Dallas 1992
Mouse (Gfap/150)[b]	4.7×10^{-4}	Dallas 1992
Pig	7.0×10^{-5}	Ellegren 1995
Sheep	1.3×10^{-4}	This study

[a]This is probably an overestimate due to mutations in the lymphoblastoid cell lines used as a source of human DNA (Banchs et al. 1994).
[b]The mouse estimates are for individual microsatellite loci rather than an estimate of the overall mutation rate.

Student's *t*-test) than the average PIC (0.61) of all the microsatellites examined.

An interesting feature of these results is that all the mutations involve a 2-bp increase in allele size. This finding is similar to that found by Weber and Wong (1993) in humans where 8 out of 15 confirmed mutations were found to be insertions of a single repeat unit. Clearly this is the most commonly occurring type of mutation among microsatellites and may provide a clue as to the likely mechanism.

It is also interesting that three of the five mutations were the result of a mutation occurring during the mitotic growth of the germ-line cells (Fig. 3) rather than at meiosis. Similar germ-line mosaicism has been observed in other species (Weber and Wong 1993) and suggests that mutations occurring during mitosis of germ-line cells is an important source of mutations. The ratio of female- to male-derived germ-line mutations was found to be 4:15 in humans (Weber and Wong 1993). In sheep we found one mutation in females (which gave us three mutant gametes) and two in males.

If one assumes that most mutations occur during the mitotic growth of germ-line cells rather than during meiosis, the higher number of mutations in male gametes can be explained by the greater number of mitotic divisions male germ-line cells undergo, thereby increasing their chances of mutation. The large number of mutations found in transformed lymphocyte cell lines (Banchs et al. 1994) also supports the idea that mitosis rather than meiosis is important in the generation of microsatellite mutations.

Given the small number of mutations discovered, it will be some time before solid conclusions can be made about the mechanism of microsatellite mutation, but a tentative conclusion, that the majority of mutations are insertions of a single-repeat element during mitosis, is warranted.

METHODS

Animals and Markers

The AgResearch International Mapping Flock comprises nine three-generation pedigrees. Five breeds contributed to the pedigrees: Texel, Coopworth, Perendale, Romney, and Merino. The different breed crosses were used to maximize the heterozygosity of the F1 generation. The four different F1 sires had a common Texel sire and different Coopworth dams. The nine F1 dams all had different Perendale–Coopworth cross dams and one of three Merino–Romney cross sires. The third generation, established from two rounds of multiple ovulation and embryo transfer, varied from seven to 17 individuals. A total of 127 animals were genotyped. All members of the International Mapping Flock have now been typed extensively using over 500 blood and DNA markers with no evidence for pedigree errors.

Genotyping

Our method of microsatellite analysis involves end-labeling (P^{33}) one of the primers prior to amplification and has been documented fully previously (Crawford et al. 1991). A complete list of all the microsatellite makers used in this study including the primer sequences and reference to the original citation can be found in Crawford et al. (1995).

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