Electronic Journal of Biotechnology 18 (2015) 116–121

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

The power of 28 microsatellite markers for parentage testing in sheep

Raed M. Al-Atiyat *

Animal Science Department, SRSNR, University of New England, NSW, Australia

A R T I C L E I N F O

Article history:
Received 8 October 2014
Accepted 3 December 2014
Available online 24 January 2015

Keywords:
Merino sheep
Paternity verification
Pedigree records
SSR markers

A B S T R A C T

Background: In sheep breeding, there are situations where relationships recorded at the farm among pedigrees such as parent-offspring, full-sibs or half-sibs need to be tested. A panel of 28 microsatellite (MST) markers was tested to provide accurate pedigree information and resolve the common problem of significant error in pedigree records in Merino sheep. Three different flocks of Australian Merino sheep were investigated. A private farm flock represents a flock with no record availability. Two other flocks were maintained under good managements of full keeping records and being selected for high and low parasite resistances.
Results: In the studied panel, eight MSTs provided an average of Polymorphic Information content (PIC) equal to 0.65 or more in order to be sufficient to make an accurate and successful DNA-based parentage analysis. The panel of twenty-eight MST loci was obviously sufficient for providing 100% accurate pedigree and genotyping data. DNA-based pedigree records were constructed and all significant pedigree record errors were eliminated.
Conclusions: These results were used for further study of population genetic parameters such as recombination and haplotyping which heavily based on pedigree information. Nevertheless MST based parentage testing is still available and affordable in most countries and for each farmer with reasonable cost in comparison with fast growing SNP based parentage technologies.

© 2015 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V. All rights reserved.

1. Introduction

In sheep breeding, there are situations where relationships recorded at the farm among pedigrees such as parent-offspring, full-sibs or half-sibs need to be tested. Many studies have shown that the accuracy of pedigree recording is still deficient and the amount of error in pedigree records made in the field is high [1,2]. Revealing pedigree errors is a fundamental step in animal breeding in order to obtain accurate values of heritability and estimated breeding values (EBVs).

Significant pedigree record errors seem to be a common problem in sheep populations and show a lack of accurate pedigree information that reduces the genetic progress of the populations whenever these records are used [1,3]. It has been found in Merino sheep incorrect pedigree record information for 9, 15 and 24% of singles, twins and triplets, respectively [3]. The incorrectness of ewe pedigree recording was usually due to ewes failing to keep their litter together, or lamb desertion, whereas the error in ram pedigree records was due to wrong recording of lambs at weaning or other times as progeny of particular ram mated to ewes in one paddock [3].

Genotyping of DNA using one or more of the genetic markers has become the most common procedure for paternity testing and pedigree inferences in human and livestock species. Many highly polymorphic MSTs have been reported and these MST loci have alleles that are often in the 70–250 bp range [4,5]. Rosa et al. [2] used panel of MST in order to evaluate their use in paternity testing of Brazilian sheep. They have shown that this MST panel was successful for paternity inference in randomly chosen animals. Crawford et al. [1] studied the reliability of pedigrees of five sheep flocks using protein polymorphisms and MST markers and found that pedigree error ranged from 0.31 to 5%. Barnett et al. [3] found that the overall proportion of Australian Merino lambs with incorrect pedigrees was about 10% using MST markers. In the same study it was also found that the proportion of error was 9.9% of single lambs, 15.2% of twins and 3.9% in ram pedigree records. Furthermore, Parsons et al. [6] reported that DNA-derived pedigree using MST markers could be successfully applied in the Australian Merino Sheep. A panel of MSTs for establishing parentage analysis in Australian Merino sheep was developed and used. The panel comprised sixteen MST markers which were highly polymorphic; about half of them had heterozygosity in excess of 80% [7]. The panel was extensively tested in Australian Merino sheep, giving accurate parentage assignment to unambiguously candidate parents even when they were highly related. It was estimated that DNA-based parentage as a pedigree system would almost eliminate any type of error because it has been reported that pedigree analysis

* Present address: Animal Production Department, King Saud University, Kingdom of Saudi Arabia.
E-mail addresses: raedatiyat@yahoo.com, raedatiyat@gmail.com.
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2015.01.001
0717-3458/© 2015 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V. All rights reserved.

using MSTs was very close to 100% accurate [2]. Many and different MST panels were recommended and utilized for parentage testing in sheep [8]. In this study, a panel of 28 MSTs for establishing parentage in Australian Merino sheep was tested for validation.

2. Materials and methods

2.1. Sheep

Three different populations of Australian Merino sheep were investigated. A private farm flock represents a control population (CR). Ewes were self-replacing and superior fine wool rams were purchased. Two other populations were flocks maintained by the Commonwealth Scientific and Industrial Research Organization (CSIRO, NSW). One of these flocks was selected for low parasite resistance (LR) and the other for high parasite resistance (HR). Both flocks originated from the same initial population and have been totally separated from each other and other Merino sheep populations since 1976 [9]. The numbers of sampled sheep in the studied populations are shown in Table 1.

2.2. Sampling and DNA extraction

Tissue samples were taken from sheep ears. The samples were digested overnight at 55°C in 0.5 mL digestion buffer with 200 μg proteinase K. Following digestion, genomic DNA was extracted from the tissue using phenol/chloroform extraction protocol [10]. After extraction, DNA pellet was dried for 30 min in a 37°C incubator, resuspended in 100 μL TE buffer and then incubated at 55°C for 5 min to aid solubilization. DNA quantified and purified into 10 ng per mL.

Table 1
Average number of alleles, H_e, H_o, and *PIC* at twenty-eight microsatellite loci in the three studied populations.

Flocks	Individuals no.				Alleles no.	H_e	H_o
	Total	Rams	Ewes	Progeny			
CR	198	21	79	98	9.93	0.739	0.764
LR	155	5	71	79	8.39	0.718	0.724
HR	176	3	81	92	7.64	0.726	0.752

Locus	*PIC*		
	CR	LR	HR
CSRD2108	0.601	0.616	0.668
MCM58	0.866	0.793	0.807
MCM147	0.820	0.877	0.842
INRA040	0.634	0.322	0.489
CSRD2105	0.781	0.607	0.657
OARHH30	0.451	0.541	0.542
ILSTS030	0.587	0.492	0.569
CSRD254	0.729	0.591	0.614
MCM512	0.842	0.691	0.683
MCM218	0.781	0.811	0.811
MCM53	0.714	0.712	0.567
MCMA14	0.699	0.738	0.667
OARAE101	0.677	0.672	0.645
OARHH55	0.665	0.660	0.699
BM143	0.745	0.806	0.769
CSRD2129	0.766	0.709	0.803
MCMA10	0.781	0.744	0.636
CSRD240	0.486	0.403	0.641
MCM152	0.687	0.632	0.558
CSRD247	0.611	0.741	0.815
MCM104	0.764	0.795	0.747
MCM159	0.811	0.827	0.721
MCM38	0.536	0.652	0.725
MCMA36	0.717	0.675	0.528
MCM373	0.789	0.795	0.818
CSRD2148	0.733	0.804	0.696
MCM136	0.685	0.687	0.720
MCMA7	0.778	0.716	0.765
Mean	0.705	0.682	0.686

2.3. Microsatellite genotyping

All studied sheep were genotyped for 28 microsatellite (MST) markers located on different chromosomes (Table 2). For the MST markers interrogated by the automated genotyping approach, analysis was performed using an ABI's 373XL sequencer [11]. A panel of twenty-eight MST markers from the ovine, caprine and bovine genome was used to genotype DNA (Table 2). The panel of MST markers was designed, developed and used as a part of an automated progeny testing system used in sheep lineage analysis at the McMaster Laboratory-CSIRO, Prospect-Sydney, Australia [7]. The MST marker panels were grouped in four sets of fluorescent-labeled primers. In sets one to three, five primer pairs were used in each set for multiplex amplifications. Set four consisted of seven primer pairs. Forward and reverse primers in sets one to three were end-labeled with 6-carboxyfluorescein (6-FAM; blue), tetrachloro-6-carboxyfluorescein (TET; green), or hexachloro-6-carboxyfluorescein (HEX; yellow), respectively. In set four, primers were labeled with only one [7]. The size standard GX-350-6-carboxytetramethylrhodamine (GX-350 TAMRA; red) was used. Each MST panel was used individually in four PCR reactions.

PCR reactions of 10 μL were performed in 384-well microlitre PCR plates. The volume and concentration of PCR reagents used in the automated genotyping experiments were 3 μL of 10 ng/μL genomic DNA, 1 μL of 4 mM primer mix, 0.8 μL of 25 mM $MgCl_2$, 1 μL of 2 mM 4dNTP's, 1 μL of 10× Taq polymerase buffer, 0.1 μL of 5 U/μL Taq polymerase and 3.1 μL of Sterile milliQdH_2O. Master mixes for each of four MST sets were prepared individually. Sample DNA was loaded into the wells of the PCR plate and then 7 μL of master mix was added. The plate was then placed onto a PTC-200 programmable Thermal Controller (MJ Research, Inc.) using the following cycling parameters; initial denaturation at 95°C for 2 min, denaturation 94°C for 45 s, annealing 57°C for 45 s, extension 72°C for 60 s, and final extension 72°C for 7 min. Initial denaturation and final extension were performed for one cycle, whereas denaturation, annealing and extension were repeated for 30 cycles. The PCR products for panels one to three were co-loaded in each well and panel four was loaded in a separate well into the gel.

2.4. Statistical analysis

The CERVUS was also used for parentage analysis. The CERVUS [12] is designed for large-scale parentage analysis using autosomal and co-dominant loci. For each offspring tested, the parentage analysis module calculates likelihood ratio (LOD) scores for each candidate parent, finds the two most likely parents and calculates the corresponding Delta score. The final step is to evaluate the confidence of the Delta score using the appropriate criteria. Different modules were determined and then utilized to calculate the allele number, expected (H_e) and observed (H_o) heterozygosities and polymorphic information content (*PIC*), probability of exclusion (*PE*), Hardy Weinberg Chi-square statistics and null allele frequency at each locus. LOD score measures the likelihood that the candidate parent is the true parent divided by the likelihood that the candidate parent is not the true parent. For each offspring, a new score was then calculated called the Delta score (Δ), which is calculated as the difference in LOD scores between the first and second most likely candidate parents. Then when using real data in parentage analysis, any most likely candidate parent with Δ score exceeding the critical Δ score for 95% confidence was simulated and awarded parentage with 95% confidence.

3. Results

3.1. Allele frequency and polymorphism

A total of 519 individuals from the three studied populations were genotyped for 28 microsatellite loci distributed across the

Download English Version:

https://daneshyari.com/en/article/200556

Download Persian Version:

https://daneshyari.com/article/200556

Daneshyari.com