Microsatellite-based estimation of inbreeding level in sheep populations of small effective size

R. Al-Atiyat[#]
Animal Production Department, King Saud University, Kingdom of Saudi Arabia

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Abstract

In sheep populations with small effective population sizes *(N_e),* inbreeding is a major concern because genetic variation has to be maintained. A panel of 28 microsatellite markers was used to measure the inbreeding level in three separate Merino flocks bred for superfine wool (CR), low parasite resistance (LR) or high parasite resistance (HR). The N_e was equal to 71.31, 19.19 and 19.48 in the CR, LR and HR flocks, respectively. Inbreeding levels estimated as inbreeding coefficients (F_{IS}) were 0.019, 0.034, and 0.048 in the CR, LR and HR flocks, respectively. These values are quite low, being lowest in CR. This result is in contrast with the known relationship between small N_e and inbreeding level. The reasons could be the management practices in the CR flock of importing sires and restricting the policy of inbreeding by avoiding the mating between relatives. Thus, despite the small N_e and a very limited number of sires being used in every generation, these breeding practices seemed to be effective in avoiding inbreeding. The results reinforce the usefulness of microsatellite markers as a valuable instrument in various genetic aspects of sheep populations. It is suggested that these observations could be implemented with endangered species and breeds with small N_e, thus improving the effectiveness of correct breeding practices, even without direct measuring of genetic variation in populations.

Keywords: Merino sheep, DNA markers, population size, genetic variation
[#] Corresponding author: raedatiyat@yahoo.com; ralatiyat@ksu.edu.sa
[#] Previous address: Animal Science Department, SRSNR, University of New England, NSW, Australia

Introduction

To avoid high accumulative inbreeding in small domesticated sheep populations, a breeding plan with restricted mating of relatives is recommended that considers reliable and accurate inbreeding measurements. A small sheep population or flock is characterized by a very small number of breeding rams compared with the number of ewes. The number of breeding animals is of prime concern for reasonable accuracy in estimating the effective population size *(N_e),* which determines the genetic properties of a population. In this case, N_e is calculated using the unequal sex ratio formula recommended by Wright (1951). In particular, in evolutionary and conservation genetics, N_e is an important parameter because it influences the rate of inbreeding and loss of genetic variation. For example, when N_e is very small, genetic drift will often be too strong for natural selection to operate efficiently (Frankham, 1995).

Generally, the common breeding scheme follows a close hierarchical system with three basic tiers: nucleus, multipliers and commercials (Massy, 1990). Alternatively, an open nucleus breeding scheme was suggested in which inbreeding would be approximately half that in closed nucleus schemes (James, 1977). Australian Merino sheep are a result of intense selective breeding since their initial introduction to Australia in 1793 (Ryder, 1983). Several open nucleus breeding schemes have been established in the Merino sheep industry in an attempt to maximize rates of genetic gain and minimize inbreeding. As a consequence, flocks of small population sizes were formed for specific environments and breeding requirements (Woolaston, 1990). In different environments some sheep diseases have become more important and in others less important, depending on the climate, the standard of husbandry and the ability of humans to avoid, control and treat the problems created. Breeding for disease resistance in Merino sheep in Australia has attracted the attention of considerable research and development (Woolaston, 1990; Woolaston & Piper, 1996). Some reports described genetic variation in sheep for parasite resistance and procedures for selecting lines of resistant animals for breeding that considered balancing a small population size and inbreeding. In practice,

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lines of Merino sheep selected for increased and decreased resistance to *Haemonchus contortus* were established along with an unselected line (Woolaston & Piper, 1996). So far, these lines are small populations, and as a consequence might be threatened by inbreeding. However, not enough studies describe the level of inbreeding of these lines using DNA genetic markers after many generations of selection. In recent times, microsatellite (MS) DNA markers have been utilized estensively to estimate inbreeding coefficients and its consequences on small Merino sheep populations under different breeding management practices (McKenzie *et al*., 2010; Gowane *et al*., 2013). Microsatellite marker panels have been developed for many purposes in Merino breed, for example, a panel was developed for progeny testing in the Australian Merino sheep (Franklin *et al*., 2000). It provided useful information in cases of complex pedigrees or in the absence of pedigree data (Barnett *et al*., 1999; Li *et al*., 2011; Al-Atiyat 2015). In this study the inbreeding levels of three small Merino flocks were investigated, utilizing the MS markers' genotyping tool.

Materials and Methods

Three Merino sheep populations in New South Wales, Australia, with a low number of individuals in the population, were selected for this study. The first population, superfine wool (CR) had self-replaced dams and imported superior fine wool sires from nearby farms. The second population was selected for high resistance (HR) to *H. contortus* and the third population for low resistance (LR) to *H. contortus* larvae. In 2002 the population breeding structure of rams, ewes and lambs in CR was 22, 5, 5, in LR 94, 118, 188 and in HR, 99, 150 and 221, respectively.

Both HR and LR were maintained as self-replacing populations with one sire age group and five ewe age groups each year. Generally, five sires were used each year. Each sire represented a different paternal half-sib family. There were five sires each in alternate years for both populations. In addition, approximately 20 replacement ewes entered the breeding population each year. This restriction of within-family sire selection was applied in both populations to minimise the rate of inbreeding, so that mate allocation was designed to ensure that mating did not take place between relatives closer than two generations apart, as represented in the breeding scheme in Figure 1.

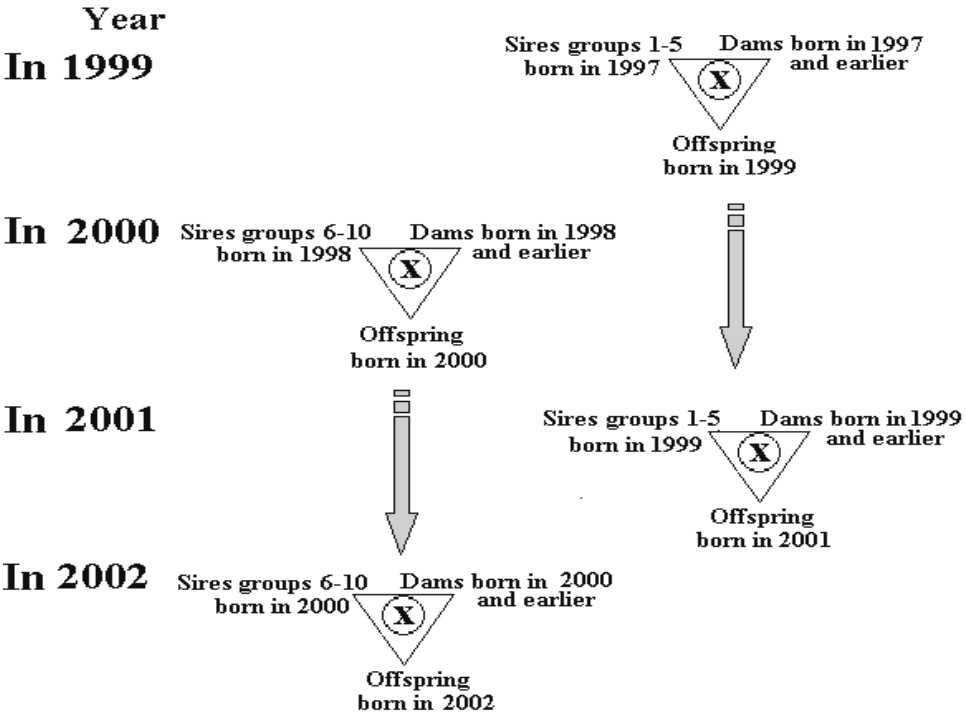

Figure 1 Breeding scheme in the HR and LR sheep populations in 1999 - 2002.

Tissue samples were taken from the ears of the three populations of sheep. The samples were digested overnight at 55 ºC in 0.5 mL digestion buffer with 200 µg proteinase-K. Following digestion,

genomic DNA was extracted from the tissue using the phenol/chloroform extraction protocol (Sambrook *et al*., 1989). The extracted DNA samples were quantified using a NanoDrop spectrophotometer and then made into aliquot DNA samples of 10 ng/µL. The DNA samples were then genotyped for 28 MS markers on different chromosomes (Tables 1a and 1b). A panel of MS markers was designed, developed and used as part of an automated progeny testing system used in sheep lineage analysis at McMaster Laboratory-CSIRO, Prospect, Sydney, Australia (Franklin *et al*., 2000). The PCR reactions of 10 µL were performed with the MS panels into 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 4dNTPs, 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 the 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 at 94 ºC for 45 s, annealing at 57 ºC for 45 s, extension at 72 ºC for 60 s and final extension at 72 ºC 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 using an ABI 373XL sequencer. The amplified fluorescent products were visualized using GENESCAN^{TM} software (Applied Biosystems, 1994).

Table 1a Microsatellite marker names, chromosome location and position, sequences and their PCR reaction requirements

Marker	Chr.	Position (cM)	Species	Primer (5′–3′)	Annealing temp (°C)	$MgCl_2$ (mM)	Size (bp)
CSRD2108	1	86.1	Ovine	**F:**CATGGAATCACAAAGAGTTGGACA **R:**CCTGGTAAGACAGTCAGTATACAA	55	2	117-127
MCM58	1	112.9	Ovine	**F:**CTGGGTCTGTATAAGCACGTCTCC **R:**CAGAACAATAAACGCTAAACCAGAGC	55	1.5	168-204
MCM147	2	39.8	Ovine	**F:**TCCGATGTTAGATGACTTTTGTGC **R:**AGCTGGTATCTGTGTCTGTCATCC	55	2	177-223
INRA040	2	149.9	Bovine	**F:**TCAGTCTGGAGGAGAGAAAAC **R:**CTCTGCCCTGGGGATGATTG	54	2	205
CSRD2105	2	160.2	Ovine	**F:**AGTAGTGGAACCCAGATTGAAACC **R:**CAGGAATTTTACAGGCACAGAATC	55	2	162-190
OARHH30	2	167.4	Ovine	**F:**CTCAGTCTCAACTTTGTTCCTCTATAGC **R:**GAAAGCTAAGGCTGAACATTGTGCCC	55	2	103-117
ILSTS030	2	180.5	Bovine	**F:**CTGCAGTTCTGCATATGTGG **R:**CTTAGACAACAGGGGTTTGG	55	2	140
CSRD254	2	189.2	Ovine	**F:**CTTTAGAACTGGGAAGGACAGTGT **R:**GAGTGAGACAAGACTAAGCAACTA	55	2	68-106
MCM512	2	248.3	Ovine	**F:**CTGAAGTGAAGGAAAGGGGACAC **R:**GGAATTAGAATATCATTCCTTCATCGTG	55	2	68-96
MCM218	4	26.5	Ovine	**F:**GATCCTAGCATCAGTCTCCAGATG **R:**CACTAAAAGCTTATGAAAGTTCCAGC	55	1.8	140-160
MCM53	6	29.7	Ovine	**F:**CATGGAGTTGTAGAGTCAGACATGA **R:**AGCAAAGGTCATGTCAGGTGT	52	3.5	79-103
MCMA14	6	45.0	Ovine	**F:**TGTTTCCTCTTCTCCAAATATC **R:**GCCCTATTAAGCCAATATACAG	52	1.5	196-216
OARAE101	6	49.8	Ovine	**F:**TAAGAAATATATTTGAAAAAACTGATCTCCC **R:**TTCTTATAGATGCACTCAAGCTAGG	60	3	99-123
OARHH55	6	54.6	Ovine	**F:**GTTATTCCATATTCTTTCCTCCATCATAAGC **R:**CCACACAGAGCAACTAAAACCCAGC	55	2	117-155

Table 1b Microsatellite markers' names, chromosome location and position, sequences and their PCR reaction requirements

Marker	Chr.	Position (cM)	Species	Primer (5′–3′)	Annealing temp (°C)	$MgCl_2$ (mM)	Size (bp)
BM143	6	59.0	Bovine	**F:**ACCTGGGAAGCCTCCATATC **R:**CTGCAGGCAGATTCTTTATCG	63	1.5	102-128
CSRD129	8	86.0	Ovine	**F:**CAGCACATTAGTCAGTTTGGCATC **R:**ATAAGGAGAATCTGAAGAGCCAAG	55	2	148-170
MCMA10	9	35.2	Caprine	**F:**GATCTTGTCATCACCAGTTCC **R:**CCCTAAACTTCTGGGCCTTC	52	1.5	104-118
CSRD240	9	80.1	Ovine	**F:**CACATGCACAGCAAAGTGATTCAA **R:**AGGACTGTAAAGCACAGGGAATGA	55	2	124-144
MCM152	13	52.1	Ovine	**F:**CCTAGAAGCCTGGCTAAAATGTG **R:**GGAACTCTCATAGTTTCCCACTCC	55	1.8	128-150
CSRD247	14	25.5	Ovine	**F:**GGACTTGCCAGAACTCTGCAAT **R:**CACTGTGGTTTGTATTAGTCAGG	55	2	220-246
MCM104	14	95.1	Ovine	**F:**TCAGGATACTTTCTCAGAGAATTTGTG **R:**ACCAGTCATTAACTCACAAGGCTG	55	3.5	115-133
MCM159	15	124.4	Ovine	**F:**GATGGTCTTGTTTCTGAATCATTGA **R:**TCAGACAGGACTAAAGCGACTTACA	55	1.8	120-152
MCM38	18	93.4	Ovine	**F:**TGGTGAATGGTGCTCTCATACCAG **R:**CAGCCAGCAGCCTCTAAAGGAC	55	1.5	131-151
MCMA36	20	18.2	Ovine	**F:**TTCATTCCTTAAGGGCTCTG **R:**CTACTGTCTATGGGGTTGGC	55	0.6	230
MCM373	22	82.9	Ovine	**F:**GGGTTTACCAGATGTCTGCTTGT **R:**TATTTGTCCAGCTGGTTGCAG	56	4.5	102-128
CSRD2148	23	31.1	Ovine	**F:**GAGAAGTGGTCAACAGAGGATGAG **R:**TACAGAGAAGCACAAAGAGATGGG	55	0.5	300
MCM136	23	65.6	Ovine	**F:**GCACACACATACACAGAGATGCG **R:**AAAGAGGAAAGGGTTATGTCTGGA	55	2	140-170
MCMA7	25	31.0	Bovine	**F:**ATCAGTCCTTCACAAGGTTG **R:**CCTGTTGCTATGTCATGTTG	52	1.5	240-268

DNA-based pedigree records were constructed based on the panel of 28 MS loci which were sufficient to provide a 100% accurate pedigree, as described in detail by Al-Atiyat (2015). The effective population size (N_e) was then calculated using the unequal sex ratio formula (Wright, 1951):

$$N_e = \frac{4N_f N_m}{(N_f + N_m)}$$

The numbers of sampled animals in the populations were used to calculate N_e for each population. The three populations consisted of different numbers of breeding sires and dams. Therefore, N_e was calculated using this unequal sex ratio formula. Genetic Data Analysis (GDA) software package (version 1d16c, Lewis & Zaykin, 2001) was used to estimate allele number, expected heterozygosity *(H_e)* and observed *(H_o)* heterozygosities and the inbreeding coefficient *(F_{IS})* for each locus, as well as for each population under Hardy-Weinberg equilibrium (HWE). GDA calculates the F_{IS} using the following formula (Weir, 1996):

$F_{IS} = 1 - \frac{n_{Aa}}{2np_A(1-p_A)}$ where n is sample size, p_A is allele frequency, and *Aa* is a heterozygous genotype.

Results and Discussion

The average number of alleles per population was 9.93, 8.39 and 7.64 in the CR, LR and HR populations, respectively (Table 2). The LR and HR populations showed slightly lower numbers of alleles per locus, which probably reflects the closed breeding structure of the flocks. The number of alleles per locus ranged from 18 in LR to four in LR and HR (Table 2). A lower number of alleles was noticed at some loci (45%) for both the LR and HR populations compared with the CR population. Locus *BM143* showed the same number of alleles (6) in the three populations (Table 2). Some loci *(INRA040, OARHH30, ILSTS030, MCM53, OARHH55, MCM104, CSRD2148)* showed similar numbers in the three populations, whereas the others had variable numbers. Levels of H_e at the 28 loci per flock are shown in Table 2. These values were

Table 2 Twenty eight microsatellite markers used in genotyping three Merino sheep populations, their chromosome number, numbers of alleles and expected heterozygosity *(H_e)* under Hardy-Weinberg equilibrium*

Locus	Chromosome	Merino genotypes					
		CR		LR		HR	
		Alleles No.	H_e	Alleles No.	H_e	Alleles No.	H_e
CSRD2108	1	8	0.657	4	0.681	5	0.717
MCM58	1	14	0.880	14	0.815	9	0.831
MCM147	2	16	0.840	18	0.891	11	0.860
INRA040	2	6	0.681	6	0.351*	7	0.521
CSRD2105	2	14	0.800	7	0.646	5	0.711
OARHH30	2	5	0.492	4	0.591	5	0.605*
ILSTS030	2	6	0.652*	4	0.541	6	0.641*
CSRD254	2	15	0.761	9	0.628	8	0.659
MCM512	2	14	0.860	10	0.731	7	0.726
MCM218	4	10	0.808	10	0.836	11	0.811*
MCM53	6	10	0.745	10	0.750	8	0.610
MCMA14	6	13	0.741	9	0.775	8	0.716
OARAE101	6	6	0.723*	4	0.724*	4	0.702*
OARHH55	6	6	0.716*	6	0.711	7	0.743
BM143	6	6	0.781	6	0.833	6	0.801
CSRD2129	8	11	0.795*	8	0.742	10	0.826
MCMA10	9	8	0.808	8	0.780	6	0.690
CSRD240	9	5	0.536	4	0.431*	6	0.703*
MCM152	13	10	0.646	10	0.662*	7	0.601
CSRD247	14	11	0.753*	12	0.773*	11	0.838*
MCM104	14	9	0.798*	9	0.819	8	0.781
MCM159	15	15	0.744	13	0.847	11	0.758
MCM38	18	5	0.615	7	0.694	7	0.763
MCMA36	20	11	0.753	6	0.728*	5	0.605*
MCM373	22	13	0.821	11	0.820	9	0.841
CSRD2148	23	11	0.757	12	0.828	11	0.730
MCM136	23	10	0.730	6	0.729	8	0.752
MCMA7	25	10	0.811	8	0.755	8	0.798*
Mean		9.93	0.739	8.39	0.718	7.64	0.726

CR: superfine wool; LR: low parasite resistance; HR: high parasite resistance.
* Means significant deviations from Hardy-Weinberg proportions at P <0.05.

high and similar in the populations, except for a few cases. These were at *INRA040* locus (0.351) and *CSRD240* locus (0.431) in the LR population and at *OARHH30* locus, 0.492 in the CR population (Table 2). Thus, the three sheep populations expressed a high level of polymorphism.

In the CR, LR and HR populations, N_e was equal to 71.31, 19.19 and 19.48, respectively (Table 3). These results show that N_e was small, especially in the LR and HR populations. Although the N_e of the CR population is apparently much higher than those of LR and HR, it is still considered low. The Food and Agriculture Organization (FAO, 1995) and European Association of Animal Science (EAAP) data (2005) considered N_e of less than 82 and 84, respectively, as critical and endangered levels at which the populations lose diversity. The number of breeding (reproductive) animals (sires and dams) is critical for a reasonable accuracy in estimating N_e. In sheep populations, the number of breeding sires is usually different from the number of breeding dams, which is higher. Sheep populations contain breeding sires and dams of overlapping generations. However, this overlap poses no problems in principle for calculating N_e (Ponzoni, 1997). The number of individuals in a population, census size, is often much larger than the genetically N_e which determines the genetic properties of a population. In particular, in evolutionary and conservation genetics, N_e is an important parameter because it influences the rate of inbreeding and loss of genetic variation. The best methods for estimating effective population size are still under investigation by Cervantes *et al*. (2011), who proposed an estimate of N_e from an increase in co-ancestry. However, the correlation level between the simplest method (number of breeding males and females, which does not require genealogical information) and the proposed co-ancestry ranged from 0.44 to 0.60 according to species and mating method (Leroy *et al*., 2013). Wright's simplest method, used in this study (number of breeding males and females), was utilized by FAO (2011). The recommended N_e by FAO to escape endangerment is at least 82 animals (FAO, 1995). It would be expected that because the N_e of the studied populations is small, inbreeding would increase with successive generations. According to Frankham (1995), the rate of inbreeding depends on N_e, for example, when N_e is very small, genetic drift would often be too strong for natural selection to operate efficiently. Thus, populations with small N_e might exhibit inbreeding depression. Although these three populations had low N_e, they did not show a great deal of inbreeding. As a result of a small N_e in both the LR and HR populations and in the CR population, the expectations might be that the small N_e would lead to more inbreeding.

Table 3 Number of breeding dams *(N_f)* and sires *(N_m)* and effective population size (N_e) for the three populations

Population	N_m	N_f	N_e
CR	22	94	71.31
LR	5	118	19.19
HR	5	188	19.48

CR: superfine wool; LR: low parasite resistance; HR: high parasite resistance (HR).

Table 4 shows the F_{IS} estimated at each locus for each population. The estimated values of F_{IS} have been calculated using genotypic data of progeny in the three populations. The F_{IS} value at each locus was notably varied in the different loci ($-0.253 \leq F_{IS} \leq 0.288$). The size and sign of F_{IS} reflect the deviation from HWEs of the genotypes; such that when F_{IS} is zero the locus is in HWE, and when F_{IS} is positive, there is a deficiency in heterozygotes. A negative F_{IS} value indicates that the level of heterozygosity is higher than its expectation from HWE (Hedrick, 2000). The average F_{IS} in each sheep population of small size was very low (Table 4). Studies have found that small populations exhibit high inbreeding. However, selection performed in these populations against inbred individuals might explain these observations (Frankham, 1995). Some F_{IS} values were high, as for example, locus *CSRD240* showed a value 0.288 for the LR and 0.172 for the HR. This reflects a high frequency of a particular allele of homozygotes among offspring due to homozygosity of some sires. On the other hand, the overall F_{IS} value at a single locus for all studied populations deviated significantly from HWE (Table 4). The F_{IS} values, however, were not consistent as they ranged from −0.156 to 0.148 across loci for the populations, with an average value of −0.022, reflecting the heterozygote excess (outbreeding) in all individuals of these populations for these loci. In other words, the average F_{is} values indicate that individuals in each population are less related than one might expect under a model of random mating or HWE.

Table 4 Inbreeding coefficients *(F_{IS})* in the populations as estimated at individual loci from genotypes of offspring

Locus	Individuals No.	Population					Overall *F**
		CR		LR		HR	
		F_{IS}	Individuals No.	F_{IS}	Individuals No.	F_{IS}	
CSRD2108	99	0.116	77	0.161	92	0.121	0.004
MCM58	97	0.083	77	0.022	92	0.05	−0.024
MCM147	99	0.035	77	−0.018	92	0.017	−0.037
INRA040	99	0.174	77	0.061	92	0.179	0.091
CSRD2105	99	0.004	77	0.011	92	0.002	−0.002
OARHH30	99	−0.194	77	−0.123	92	−0.10	−0.081
ILSTS030	99	−0.137	77	−0.174	92	−0.095	−0.091
CSRD254	99	−0.055	77	0.023	92	0.02	−0.063
MCM512	99	0.077	77	0.125	92	0.144	0.002
MCM218	89	−0.034	57	−0.048	81	−0.061	0.007
MCM53	99	0.06	77	0.088	92	−0.04	−0.040
MCMA14	99	−0.036	77	−0.036	92	−0.042	−0.061
OARAE101	99	0.042	77	−0.025	92	0.051	−0.156
OARHH55	99	−0.147	77	−0.253	92	−0.212	−0.030
BM143	99	−0.001	77	0.007	92	0.078	−0.019
CSRD2129	93	0.055	71	0.119	88	0.039	0.148
MCMA10	92	0.185	77	0.142	92	0.029	0.044
CSRD240	97	0.187	77	0.288	92	0.172	−0.050
MCM152	87	0.151	74	0.087	91	0.252	0.010
CSRD247	90	0.087	77	0.053	92	0.133	0.031
MCM104	89	0.189	77	−0.020	92	0.024	−0.051
MCM159	82	−0.001	70	−0.043	90	−0.041	−0.067
MCM38	87	0.193	69	0.148	86	0.053	−0.052
MCMA36	83	−0.11	76	0.186	88	0.189	0.090
MCM373	85	0.002	75	−0.008	85	0.012	−0.093
CSRD2148	83	0.103	77	−0.001	92	0.034	−0.019
MCM136	81	0.053	77	0.205	92	0.271	0.036
MCMA7	82	−0.036	75	−0.012	89	0.066	−0.035
Mean		0.019		0.034		0.048	−0.022

CR: superfine wool; LR: low parasite resistance; HR: high parasite resistance (HR).
* P <0.0001.

Since N_e was small in the three populations, it was expected that genetic drift might be effective and cause significant loss in genetic diversity. It could also lead to high levels of inbreeding. On the contrary, in each of the three populations the inbreeding levels were low, despite relatively small N_e. This observation is in contrast with the known relationship between small N_e and inbreeding (Frankham, 1995). The data showed that the F_{IS} values were quite low and similar in the three populations, being slightly lower in the CR population, where the immigration rate of sires was high. The management practices in the CR population of importing some sires from other populations could be the reason for avoiding inbreeding, while the only explanation for low inbreeding in the LR and HR populations is the implementation of a strict breeding policy that excludes mating between all but weakly linked relatives. Thus, despite the small N_e and a very limited number of sires used in every generation, the breeding practices in these two populations were effective in

avoiding inbreeding. Similar results were reported when maintaining genetic stability in a control flock of South African Merino sheep (Heydenrych *et al*., 1984). The low N_e in the three populations are equivalent to those found in simulation work by Lewis & Simm (2000). Using simulated pedigree information, they discovered that the F_{IS} was lower than 0.03 per annum. In a real study using pedigree information, F_{IS} was 0.125 in five fully managed, closed French mutton flocks (Huby *et al*., 2003). Furthermore, in a recent study, highly inbred animals (e.g. F_{IS} >0.0625) were reported for pairs of closely related animals (e.g. full- or half-sibs) because of the smaller sample size and relative incompleteness of the pedigree (Li *et al*., 2011).

Although this study indicates low levels of inbreeding in a managed and closed population, it seems that the management practices of avoiding inbreeding were applicable. In addition, using pedigree information might lead to an inaccurate estimation of F_{IS} since it is expected that an error is possible in pedigree records. Nevertheless, in the unmanaged Soay sheep population on St Kilda (an island off the west coast of Scotland), inbreeding was found to be low, calculated data from MS and protein loci (Coltman *et al*., 2003). Recently, selective pressures were found with no effect on survivability of New Zealand Merino (McKenzie *et al*., 2010) and Bharat Merino sheep (Gowane *et al*., 2013) under good breeding management. It is important to mention that MS data are more reliable for accurate estimates of F_{IS} than pedigree information in which a significant error rate has been found by many researchers (Alexander *et al*., 1983; Crawford *et al*., 1993; Barnett *et al*., 1999). In agreement, Hedrick (2013) stated that the higher levels of F_{IS} observed in sheep could possibly be due to smaller population sizes or a higher level of selection pressure, as well as an erroneous estimation approach.

In the present study, F_{IS} was estimated from MS data, indicating accurate and reliable results. Accordingly, these results confirm that the management and selection practices to avoid inbreeding were successful in the three populations.

Conclusion

The data showed that the F_{IS} values were low and slightly similar in the three populations of small N_e, being lowest in the CR population. Despite small N_e and a limited number of sires used in every generation, the breeding practices were effective in avoiding inbreeding and homozygotization. Based on these observations, effective breeding practices can be recommended in order to avoid inbreeding in small-sized populations. N_e is an important parameter in evolutionary and conservation genetics because it influences the rate of inbreeding and loss of genetic variation. The results reinforce the usefulness of MS markers as a valuable instrument for estimating the inbreeding level for sheep populations.

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