Robustness Of A Microsatellite Based Marker Panel For Parentage Verification In South African Angora Goats

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Introduction

Parentage analyses have an important role in genetic evaluation of farm animals, where both correct and complete pedigrees are an essential component (Dodds *et al.* (2005)). Under practical farming conditions correct recording of the sire and the progeny is often limited, especially where multi-sire systems are applied. In Angora goats there are several limitations to correct recording such as group mating, does with poor mothering ability and extensive pasture based systems (Bolormaa *et al.* (2008)). Similar limitations are experienced by South African Angora goat producers that prevent the effective use of estimated breeding values.

Since the genome mapping of most farm animals including goats, numerous DNA-markers have become available for application in DNA-based parentage analyses. For goats two panels of microsatellite markers have been recommended by ISAG during 2001/2002 (http://www.isag.org.uk/ISAG/all02_PVpanels_LPCGH.doc) and an updated panel in 2005 http://www.isag.org.uk/journal/comparisonguide.asp-). These markers have been used in several studies for parentage verification in goats (Luikart *et al.* (1999); Ganai & Yadav (2005); Glowatzki-Mullis *et al.* (2007); Bolormaa *et al.* (2008); Friedrich, (2009)). Microsatellite markers are widely used for parentage analyses due to their availability, high polymorphism and informativeness as co-dominant markers (Webster & Reichart (2005)). In order to compile a panel of microsatellite markers for parentage analyses, the polymorphic nature of the loci, expected heterozygosity and deviation from HWE needs consideration. Further prerequisites reported by MacAvoy *et al.* (2008) for suitability of microsatellite markers include the ease of PCR amplification, allele scoring and the absence of null alleles. Finally the power of the marker is evaluated on the Exclusion probability (Fan *et al.* (2008)). These parameters are all dependent on the size of the population in which they were measured. In this study a panel of microsatellite markers was evaluated in various populations, differing in size, to verify the accuracy and efficiency of the panel.

Material and methods

The Angora goat database in South Africa currently consists of genotypic and phenotypic data contributed by breeders for the development of a reference population (Visser & Van Marle-Köster (2009)). For this study 1067 animals from 4 different herds were selected. The animals originally belonged to 12 different families, all consisting of sires and half-sib offspring. All animals were genotyped with 96 markers, which were previously screened for ease of PCR amplification, ease of scoring, polymorphicity and genome coverage. For this study, four scenarios were proposed with consecutively less animals, as described in Table 1.

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The best performing 28 markers were selected based on the number of alleles (≥ 8) and PIC values (≥ 0.70) and evaluated in the data set consisting of the four scenarios, with total number of animals varying from 1067 to 54 animals. Cervus 3.0 (Marshall (1998)) was used to perform the statistical analyses, including allele frequencies, polymorphic information content (PIC), deviation from HWE, observed and expected heterozygosity (H_O and H_E) and Null allele frequencies (F_{Null}) for each microsatellite marker. Combined Exclusion probabilities (CPE) for the different scenarios were also estimated.

Table 1: Structure of herds for the four scenarios

	Scenario 1	**Scenario 2**	**Scenario 3**	**Scenario 4**
Herd 1				
Family 1	108	108	108	54
Family 2	106	106		
Family 3	82	82		
Family 4	104	104		
Herd 2	105			
Herd 3	213			
Herd 4	349			
Total	**1067**	**400**	**108**	**54**

Results and discussion

The results indicated that the size of the population affects the performance of the markers in parentage analyses. All markers were highly polymorphic with the number of alleles varying between 8 and 27 when evaluated in 1064 animals. The average number of alleles decreased from 12.14 to 6.93 as the population size decreased to 54 (Table 2). Average PIC values followed the same trend, with a decrease from 0.74 (1067 animals) to 0.63 (54 animals). The drop in PIC varied significantly between markers, with 4 markers (*MCM58, BM3517, HEL11* and *SRCRSP05*) decreasing to below 0.55, while 2 markers (*INRA011* and *INRA006*) maintained values of above 0.75.

The ten best markers were selected for each scenario based on the parameters in Table 2. These markers were compared and four markers performed consistently well in all scenarios. Markers were tested for HWE in the total population and 14 of the 28 deviated from HWE with a homozygote deficiency. The 4 best performing markers (*BM415, BM4208, INRA 006* and *INRA011*) remained in the top 10 of all markers, irrespective of population size and these were also in HWE. Markers *BM1258, BM3517, BMS2782* and *MCM58* were consistently poor performers over all scenarios and deviated from HWE. It has been shown that markers not in HWE may result in incorrect assignment and should be excluded (Bolormaa *et al.* (2008)).

If the complete set of 28 markers is used for calculation, the CPE remained above 0.997 for all scenarios. However, a cost-effective panel is required for parentage verification. Therefore the CPE was estimated for the 10 best-performing markers in Scenario 1 and 4,

with a decrease in CPE from 0.999 to 0.978. In contrast, the CPE decreases if the best ten markers from Scenario 4 (0.990) are applied in Scenario 1 (0.995).

Table 2: Individual marker parameters (n alleles, Observed and Expected Heterozygosity and PIC) in four scenarios

	Scenario 1				Scenario 2				Scenario 3				Scenario 4			
Locus	**n**	**HObs**	**HExp**	**PIC**	**n**	**HObs**	**HExp**	**PIC**	**n**	**HObs**	**HExp**	**PIC**	**n**	**HObs**	**HExp**	**PIC**
BM1258	**10**	**0.73**	**0.74**	**0.7**	**9**	**0.74**	**0.71**	**0.65**	**9**	**0.69**	**0.65**	**0.58**	**7**	**0.7**	**0.65**	**0.58**
BM1818	9	0.76	0.76	0.73	9	0.79	0.76	0.73	6	0.74	0.65	0.58	6	0.69	0.65	0.59
BM3517	**14**	**0.72**	**0.74**	**0.71**	**12**	**0.67**	**0.68**	**0.64**	**11**	**0.54**	**0.45**	**0.42**	**8**	**0.49**	**0.41**	**0.39**
BM415	*10*	*0.85*	*0.83*	*0.81*	*9*	*0.84*	*0.82*	*0.79*	*6*	*0.89*	*0.78*	*0.74*	*6*	*0.88*	*0.78*	*0.73*
BM4208	*12*	*0.84*	*0.84*	*0.82*	*11*	*0.87*	*0.83*	*0.81*	*10*	*0.82*	*0.77*	*0.74*	*9*	*0.76*	*0.75*	*0.71*
BM6526	17	0.68	0.76	0.72	12	0.6	0.74	0.69	10	0.81	0.72	0.68	5	0.63	0.7	0.63
BMS0712	9	0.74	0.75	0.71	9	0.78	0.75	0.71	7	0.75	0.71	0.66	6	0.73	0.69	0.63
BMS0745	13	0.81	0.76	0.73	9	0.88	0.75	0.71	8	0.99	0.78	0.74	6	0.97	0.77	0.72
BMS2782	**12**	**0.77**	**0.75**	**0.71**	**9**	**0.71**	**0.64**	**0.6**	**8**	**0.75**	**0.57**	**0.54**	**7**	**0.78**	**0.59**	**0.55**
DRBP1	8	1	0.74	0.71	8	1	0.74	0.71	8	1	0.79	0.73	7	1	0.79	0.71
HEL11	14	0.67	0.76	0.74	11	0.63	0.78	0.74	8	0.53	0.61	0.53	5	0.42	0.62	0.52
ILSTS058	12	0.75	0.8	0.76	9	0.77	0.78	0.75	9	0.7	0.67	0.6	7	0.74	0.64	0.56
INRA006	*11*	*0.77*	*0.78*	*0.75*	*10*	*0.88*	*0.83*	*0.81*	*8*	*0.99*	*0.81*	*0.77*	*7*	*1*	*0.8*	*0.76*
INRA011	*27*	*0.77*	*0.78*	*0.76*	*16*	*0.88*	*0.82*	*0.8*	*13*	*0.93*	*0.79*	*0.76*	*11*	*0.91*	*0.79*	*0.76*
INRA206	10	0.8	0.81	0.78	8	0.87	0.82	0.79	6	0.88	0.69	0.64	6	0.83	0.66	0.61
LSCV25	11	0.72	0.79	0.76	9	0.7	0.75	0.7	9	0.8	0.73	0.69	8	0.83	0.76	0.7
MAF050	9	0.74	0.75	0.71	9	0.74	0.73	0.69	9	0.76	0.71	0.66	7	0.77	0.73	0.67
MAF214	16	0.65	0.76	0.73	12	0.69	0.72	0.67	10	0.98	0.82	0.79	5	1	0.78	0.71
MCM58	**18**	**0.73**	**0.75**	**0.72**	**13**	**0.69**	**0.7**	**0.67**	**10**	**0.6**	**0.5**	**0.48**	**7**	**0.57**	**0.47**	**0.44**
OARCP34	10	0.74	0.74	0.71	8	0.65	0.63	0.58	6	0.71	0.68	0.62	5	0.67	0.63	0.55
OARCP73	16	0.83	0.83	0.81	13	0.79	0.76	0.73	12	0.72	0.7	0.65	7	0.68	0.66	0.6
OARFCB48	8	0.8	0.79	0.76	6	0.84	0.75	0.71	6	0.8	0.59	0.55	6	0.8	0.6	0.55
OARHH35	10	0.78	0.78	0.75	9	0.82	0.79	0.77	9	0.8	0.75	0.71	9	0.8	0.76	0.72
OLADRB	15	0.77	0.77	0.75	12	0.8	0.79	0.76	9	0.87	0.74	0.7	7	0.87	0.78	0.73
SRCRSP05	9	0.77	0.77	0.74	7	0.8	0.75	0.72	7	0.73	0.57	0.54	7	0.76	0.58	0.54
SRCRSP10	11	0.77	0.75	0.72	9	0.8	0.75	0.71	8	0.74	0.57	0.54	8	0.79	0.6	0.56
SRCRSP24	10	0.75	0.77	0.74	8	0.83	0.78	0.75	7	0.77	0.73	0.69	7	0.79	0.74	0.69
TGLA179	9	0.84	0.81	0.79	9	0.84	0.81	0.79	9	0.81	0.75	0.71	8	0.79	0.74	0.7
Ave	**12.1**	**0.77**	**0.77**	**0.74**	**9.82**	**0.78**	**0.76**	**0.72**	**8.5**	**0.79**	**0.69**	**0.64**	**6.9**	**0.77**	**0.68**	**0.63**

Bold: Worst performers in all scenarios
Italics: Best performers in all scenarios

From these results it is clear that the number of animals in the database or family to be tested will also influence the selection of the final panel of markers, as some markers tend to

perform more consistently as the population size increased. Markers should be tested in a reference population of reasonable size in order to verify individual parameters.

Conclusion

A larger database with Angora goats typed for the desired markers for parentage verification will improve the power of the final panel to be applied for parentage testing of South African Angora goats. This paper emphasizes the necessity of compiling a parentage verification panel based on screening in the largest possible population group.

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