Small Ruminant Research 206 (2022) 106575

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Technical note

Development of a standardized multiplex SSR kit for genotyping both goats and sheep

M.C. Spanoghe [a,*,1], H.R.V. Whannou [b,1], C. Dekuijper [c], L.H. Dossa [b], D. Lanterbecq [a,c,d]

[a] Laboratory of Biotechnology and Applied Biology, Haute Ecole Provinciale de Hainaut-CONDORCET, 11 rue Paul Pastur, 7800, Ath, Hainaut, Belgium
[b] Ecole des Sciences et Techniques de Production Animale, Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, 03 BP 2819, Cotonou, Benin
[c] Centre pour l'Agronomie et l'Agro-Industrie de la Province de Hainaut (CARAH), 11 rue Paul Pastur, 7800, Ath, Hainaut, Belgium
[d] Hainaut-Analyses, 55 Bd Sainctelette, 7000, Mons, Belgium

ARTICLE INFO

Keywords:
Animal breeding
Genetic diversity
Genotyping
Multiplex
Small ruminant
SSR

ABSTRACT

In this technical note, we report the development of a reliable standardized protocol based on the multiplex SSR-PCR approach for genotyping the two small ruminant species, goats and sheep. The method is based on PCR conditions optimization allowing 12 informative microsatellites (SSR) markers to be co-amplified in two multiplex sets of 6 SSR markers, followed by a co-migration into one run onto a genetic analyzer. The performance of the assay was tested on a total of 207 goats and 197 sheep, originating from different breeds and localities sampled mainly in Benin, but also to lesser extent in Belgium. The optimized assay showed that the 12 selected markers were suited to amplify all the loci distributed between the two species with valuable genetic information, specific-species allelic patterns and similar polymorphism information content. The validated loci were polymorphic with high allele numbers for the two species, ranging from 6 to 21 for goats and 7 to 15 for sheep, with average 10.58 alleles per locus for goats and 11.33 for sheep, respectively. The Polymorphic Information Content (PIC) of selected markers averaged a value of 0.712 for goats and 0.751 for sheep. The differentiation of SSR alleles at an intraspecific resolution level allowed different heterotic groups to be detected, such as geographical or breeding groups. The newly developed genotyping kit could be successfully applied in diverse genetic variability studies and relatedness analyses, as well as for population structure assessment, parentage determination and traceability concerns, within and among sheep and goat species.

1. Introduction

As part of the breeding program enhancement for indigenous goats and sheep in Benin, a preliminary genetic diversity survey based on microsatellite markers (SSR) approach was considered on the current population. However, a literature review highlighted the lack of consensual multiplex PCR protocols suited for genotyping both species with valuable genetic information and similar polymorphism information content. Apart from studies already conducted by the same teams and mostly based on the same set of SSR markers (Álvarez et al., 2005; Legaz et al., 2008; Álvarez et al., 2009; Traoré et al., 2009; Álvarez et al., 2012; Traoré et al., 2012), we noted that most of the time the SSR markers currently used and mentioned in the literature varied from one study to another (e.g., Luikart et al., 1999; Dixit et al., 2012; Awobajo et al., 2015 for goats, and Pramod et al., 2009; Hepsibha et al., 2014; Ocampo et al., 2016; Al-Atiyat et al., 2018; Dossybayev et al., 2019 for sheep). Moreover, it seems unclear whether those markers could amplify in once the loci for the two species, and if they could achieve the same performance for all breeds. Preliminary tests performed by our team indicated that some SSR markers could be polymorphic for one species while being monomorphic (or even no amplification) for the other. The same observation can be found in the literature, whereas some SSR markers present a good discriminating score for one species but not for the other (e.g., Álvarez et al., 2009; Traoré et al., 2009). In addition, most of the published studies used a simplex PCR genotyping protocol, which lead to time-consuming, costly and laborious efforts, despite the number of individuals to be analyzed. In the present note, we describe an efficient technical procedure based on multiplexing 12 highly informative SSR loci that were specifically developed to implement genetic fingerprinting for both sheep and goat individuals in a single output data file.

* Corresponding author.
E-mail addresses: martin.spanoghe@condorcet.be (M.C. Spanoghe), habib.whannou@fsa.uac.bj (H.R.V. Whannou).
[1] Equal contribution.

https://doi.org/10.1016/j.smallrumres.2021.106575
Received 5 July 2021; Received in revised form 27 October 2021; Accepted 1 November 2021
Available online 3 November 2021

Table 1
Number of analyzed individuals (N) among the two predominant goat and sheep breeds of Benin and Belgium.

	Goat		Sheep	
Benin				
Breed	*Sahelian*	*Djallonke*	*Sahelian*	*Djallonke*
N	66	68	69	64
Belgium				
Breed	*Saanen*	*Alpine*	*Ardennais roux*	*Laitier belge*
N	35	38	32	32
Total	207		197	

2. Materials and methods

2.1. Sampling and DNA isolation

The study material consisted of a panel of 207 goats (*Capra hircus*) and 197 sheep (*Ovis aries*) individuals, originated from different areas of Benin (134 goats; 133 sheep) and Belgium (73 goats; 64 sheep). The introduction of Belgian individuals into the study panel initially constituted of Beninese individuals aimed to test the performance of the genotyping kit regardless of both the herd origin and breeds. Sampling of individuals was conducted on four distinct breeds per species (i.e., two per nation), and in distinct localities, to avoid a bias due to family ties (Table 1). Breeds were selected based on their national predominance and individuals based on their genetic purity according to the breeding information provided by the respective breeders. Genomic DNA isolation was performed from around 30 rooted hairs (i.e., containing bulb) per individual using DNeasy Blood & Tissue kits as per manufacturer's protocol (Qiagen, Venlo, Netherlands). DNA extracts were quantified using a ND-3300 NanoDrop spectrofluorimeter (Thermo Scientific; Waltham, MA, USA).

2.2. Multiplex SSR kit development

Twelve SSR markers were selected from the literature based on the following criteria: (i) homogeneous repartition on the chromosomes; (ii) optimal amplification and resolution; (iii) ability to detect high rates of polymorphism for both goats and sheep; (iv) adequacy of observed fragment sizes with those reported in selected studies (Sodhi et al., 2006; Traoré et al., 2009; Pramod et al., 2009; Missohou et al., 2011; Awobajo et al., 2015; Sharma et al., 2016; Ocampo et al., 2016; Dossybayev et al., 2019); (v) absence of outlier loci effects (Negrini et al., 2012); and (vi) suitability to be used in a multiplexed PCR reaction, according to the step-by-step protocol of Henegariu et al. (1997) and the FAO guidelines (FAO, 2011). The 12 SSR loci were distributed in two multiplex sets of 6 SSR markers (Table 2). The 5′ end of the forward primer of each pair was labeled with a fluorescent dye (6-FAM, VIC, NED or PET dyes; Table 2). The methodological strategy of a minimum number of markers for high information was chosen as the most efficient in terms of cost-effectiveness criteria.

The PCR amplifications were carried out on a Biosystems™ SimpliAmp Thermal Cycler (Applied Biosystems, Waltham, MA USA) using a Kapa2G Fast Multiplex Mix (Kapa Biosystems, Boston USA) in a total reaction volume of 25 µl contained 1× Kapa2G Fast Multiplex Mix, 0.1–0.2 µM of each primer (Thermo Fisher Scientific, Waltham, MA USA), and 15 ng of template DNA. The PCR cycling parameters were as follows: initial denaturing step of 3 min at 95 °C, then 30 cycles of 15 s at 95 °C, 30 s at 54 °C (T_a), 30 s at 72 °C, followed by a hold step of 1 min at 72 °C for final extension. After amplification, 0.75 µl fraction of each multiplex PCR products were pooled and then transferred into 13.5 µl HiDi (Applied Biosystems) containing 2.5 % of GeneScan™ 500 LIZ™ dye Size Standard (Applied Biosystems). The PCR products were run on a SeqStudio Genetic Analyzer System (Applied Biosystems) following standard run-module parameters. Estimations of fragment lengths of the PCR products were determined using GeneMapper Software 6.0

Table 2
Information about the 12 SSR markers used in the newly developed Multiplex Genotyping Kit, where the SSR Markers Locus, Linkage Group (LG), Multiplex set, Fluorescent Dye Labeling, Number of Alleles (NA), Allele Size Range (Range) and PIC are given from goats and sheep genotyped individuals from Benin and Belgium, and from the combined dataset. The source of the SSR markers is available in the FAO guidelines (FAO, 2011).

					GOATS								SHEEP							
SSR markers locus	LG Goat	LG Sheep	Multiplex set	Dye labeling	BENIN (n = 134)			BELGIUM (n = 73)			BENIN x BELGIUM (n = 207)			BENIN (n = 133)			BELGIUM (n = 64)			BENIN x BELGIUM (n = 197)		
					NA	Range	PIC	NA	Range	PIC	NA	Range	PIC	NA	Range	PIC	NA	Range	PIC	NA	Range	PIC
ILSTS11	14	9			8	266–282	0.429	6	266–280	0.740	9	266–282	0.619	6	268–282	0.596	7	268–282	0.693	7	268–282	0.652
ILSTS5	10	7		6-FAM	4	184–192	0.192	5	184–197	0.481	6	184–197	0.311	10	194–221	0.675	6	198–221	0.618	11	194–221	0.682
MAF065	5	5			18	118–183	0.782	11	118–157	0.727	21	118–183	0.781	10	120–141	0.714	7	122–138	0.746	10	120–141	0.743
MCM527	5 - 7	5	1		6	153–168	0.674	7	153–170	0.721	7	153–170	0.751	8	160–178	0.740	7	162–182	0.624	10	160–182	0.731
SCRSP9	12	12		PET	12	117–147	0.798	10	117–143	0.737	13	117–147	0.837	6	111–123	0.620	6	111–127	0.541	8	111–127	0.653
TCRVB6	10	10			13	222–253	0.641	13	222–253	0.834	16	222–253	0.773	13	226–269	0.738	8	226–255	0.822	13	226–269	0.836
INRA023	3	3			11	195–218	0.778	8	197–216	0.624	12	195–218	0.777	14	197–222	0.823	9	201–220	0.821	14	197–222	0.852
OARFCB20	2	2		VIC	7	99–111	0.760	6	99–109	0.520	7	99–111	0.733	13	94–123	0.798	10	90–121	0.803	14	90–123	0.821
OARFCB48	17	17	2		7	153–169	0.755	10	151–169	0.801	10	151–169	0.820	14	143–171	0.802	10	143–173	0.838	15	143–173	0.846
BM8125	17	17			9	111–127	0.750	7	107–125	0.592	10	107–127	0.706	7	115–127	0.659	6	113–123	0.605	8	113–127	0.645
CSRD247	14	14		NED	9	220–245	0.769	8	232–245	0.671	9	220–245	0.814	10	216–256	0.568	7	216–238	0.765	12	216–256	0.728
INRA063	18	14			6	174–184	0.582	6	172–184	0.642	7	172–184	0.627	12	172–208	0.783	10	172–208	0.734	14	172–208	0.824
				mean	9.17		0.659	8.08		0.674	10.58		0.712	10.25		0.710	7.75		0.718	11.33		0.751

Locus	GOATS	SHEEP
ILSTS11	266 268 270 272 274 276 278 280 282	268 270 274 276 278 280 282
ILSTS5	184 188 190 192 195 197	194 198 200 202 203 204 206 208 210 215 221
MAF065	118 120 122 124 126 128 130 132 134 136 138 145 147 152 157 159 169 171 177 179 183	120 122 126 128 130 132 134 136 138 141
MCM527	153 155 162 164 166 168 170	160 162 164 166 168 170 172 174 178 182
SCRSP9	117 121 123 125 127 130 132 134 136 138 141 143 147	111 115 117 119 121 123 125 127
TCRVB6	222 224 226 228 230 232 234 236 238 240 242 244 246 248 251 253	226 234 236 240 242 244 251 253 255 257 259 267 269
INRA023	195 197 199 201 205 206 208 210 212 214 216 218	197 199 201 203 205 206 208 210 212 214 216 218 220 222
OARFCB20	99 101 103 105 107 109 111	90 94 97 99 101 103 105 107 109 111 113 115 121 123
OARFCB48	151 153 155 157 159 161 163 165 167 169	143 145 147 149 151 153 155 157 159 161 163 165 167 171 173
BM8125	107 111 113 115 117 119 121 123 125 127	113 115 117 119 121 123 125 127
CSRD247	220 232 234 236 238 240 241 243 245	216 220 224 226 228 230 232 234 236 238 241 256
INRA063	172 174 176 178 180 182 184	172 174 176 178 180 182 184 186 190 197 201 203 206 208

Fig. 1. Alleles detected in the total pool of all genotyped individuals (n = 404) grouped per species and Dye labeling (6-FAM; PET; VIC; NED). The common alleles were highlighted in grey.

(Applied Biosystems) and used to build a multilocus genotyping dataset for all individuals. Number of alleles (Na) and Polymorphic Information Content (PIC) were computed for each marker using Cervus software v 3.0 (Kalinowski et al., 2007). PIC was calculated as PIC = $1 - \sum P_i^2$, where P_i is the frequency of the i^{th} allele detected in the entire pool, according to the Nei's statistic (1973).

2.3. Analysis data and kit performance assessment

The performance of the multiplex SSR genotyping kit in detecting allelic polymorphism between and within the two species was assessed on the basis of three levels of the dataset; 1) the entire pool of genotyped individuals (i.e., goats and sheep combined); 2) the pool within each species (i.e., all origins combined); and 3) the pool of each species and localities (i.e., all breeds combined).

Genotypic data was then analyzed based on a Discriminant Analysis of Principal Components (DAPC) (Jombart et al., 2010) using R software (R Development Core Team, 2010) with the R package *adegenet* version 2.1.4 (Jombart, 2008). DAPC was applied with prior knowledge for data organization, a first DAPC based on the inter-species clustering (i.e., goats and sheep from Benin and Belgium), a second on the intra-species clustering (i.e., breeds from Benin and Belgium) for goats and a third on the intra-species clustering for sheep. A cross validation function (Xval.dapc) was used to define the correct number of Principal Components (PCs) to be retained for each analysis, as the lowest root mean square error according to R *adegenet* procedure. Discriminant Analyses was then conducted using n discriminant Eigenvalues, where $n = number\ of\ a\ priori\ groups - 1$. The resulting clusters were plotted in a scatterplot of the first and second linear discriminants of DAPC, with individuals as points and inertial ellipses around predefined groups.

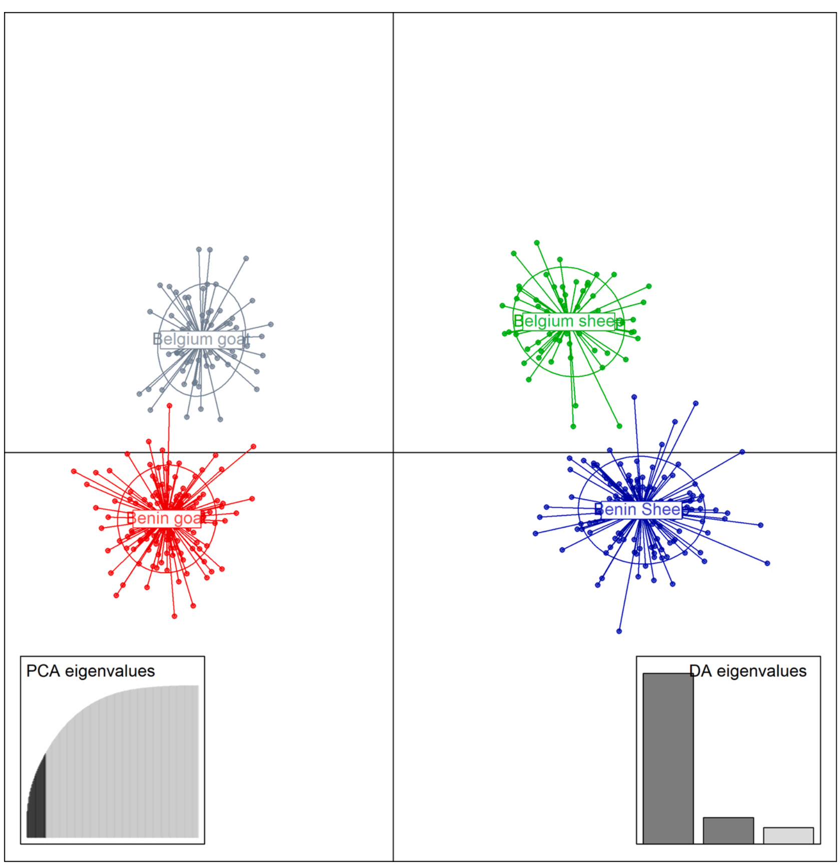

Fig. 2. Discriminant analysis of principal components (DAPC) for a total of 404 genotyped individuals, distributed in 207 goats and 197 sheep, using the R package *adegenet* version 2.1.4 in R software. The axes correspond to the first two Linear Discriminants (LD). Each ellipse represents a species cluster by locality (goats from Benin, goats from Belgium, sheep from Benin, and sheep from Belgium) and each dot represents an individual.

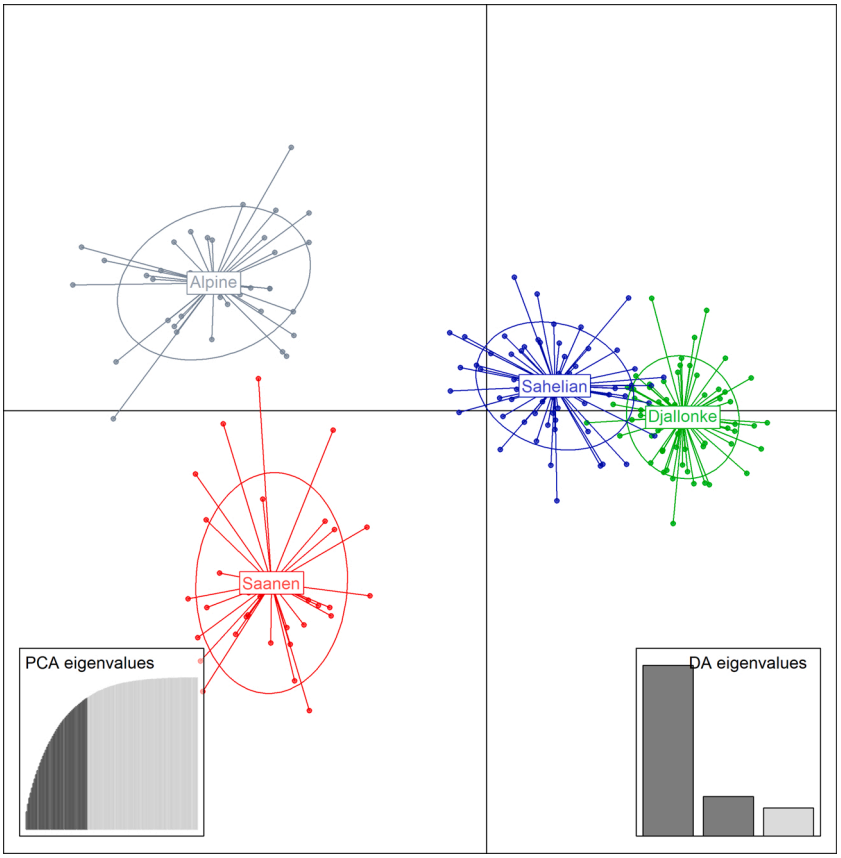

Fig. 3. Discriminant analysis of principal components (DAPC) for 207 genotyped goats, using the R package *adegenet* version 2.1.4 in R software. The axes correspond to the first two Linear Discriminants (LD). Each ellipse represents a goat breed cluster (Djallonke, Sahelian, Saanen, and Alpine) and each dot represents an individual.

3. Results and discussion

3.1. Assessment of the multiplex SSR genotyping kit performance

The performance of the multiplex SSR genotyping kit in detecting allelic polymorphism between and within the two species was assessed based on three levels of the dataset:

1) Entire pool of individuals

A total of 181 alleles were detected in the entire pool (i.e., goats and sheep; n = 404), whose 45 (24.8 %) alleles were specific to goats and 54 (29.8 %) to sheep, meaning that 82 alleles (45.3 % of the total alleles of all accessions) were shared by the two species (Fig. 1). Only the marker ILSTS5 did not produce any shared allele between the two species, whereas all other 11 markers shared between 31.2 % (SCRSP9) and 80.0 % (BM8125) of common alleles (Fig. 1).

2) Pool within each species

A total of 127 and 136 alleles were detected in the pool of goats (n = 207) and sheep (n = 197) with a mean number of alleles per locus of 10.58 and 11.33, respectively. The PIC index averaged values of 0.712 and 0.751 for goats and sheep pool, respectively (Table 2).

3) Pool of each species and localities

Within the goat's dataset, a total of 110 alleles were detected in samples from Benin (n = 134) and 97 in those from Belgium (n = 73). Thirty alleles (23.6 %) were specific to Benin and 17 alleles (13.4 %) to Belgium, while 80 alleles (63.0 % of the total number of alleles in goats) were shared by the goats from Benin and Belgium. The PIC index of goats averaged values of 0.659 for Benin and 0.674 for Belgium, respectively (Table 2). Within the sheep dataset, a total of 123 alleles were detected in samples from Benin (n = 133) and 93 in those from Belgium (n = 64). Forty-three (31.6 %) alleles were specific to Benin and 13 alleles (9.56 %) to Belgium, while 80 alleles (58.8 % of the total alleles of sheep) were shared by the sheep from Benin and Belgium. The PIC index of sheep averaged values of 0.710 for Benin and 0.718 for Belgium, respectively (Table 2).

3.2. DAPC analyses

DAPC analyses were applied on the entire pool of genotyped individuals (n = 404) to infer the relationships at inter- and intra-species resolutions. DAPC analysis conducted at inter-species clustering resolution retained only 20 first PCs (about 55 % of total variance conserved) and three discriminant eigenvalues. Clear separation between the two species with no overlap event was visualized in the scatterplot with respect to the first Linear Discriminant (LD1) (Fig. 2). Separation was also observed according to the localities (Benin or Belgium) with respect to LD2. DAPC analyses conducted at intra-specific clustering resolution retained 45 first PCs (about 85 % of total variance conserved) and three discriminant eigenvalues for both goats (Fig. 3) and sheep (Fig. 4). For both cases, the different breeding groups could distinctly be visualized in the scatterplot by means of different origins (Benin, Belgium) as well as of breeds, with respect to LD1 and LD2. It should be noted that for the two species, the Beninese breeds appeared much closer to each other than the Belgian breeds.

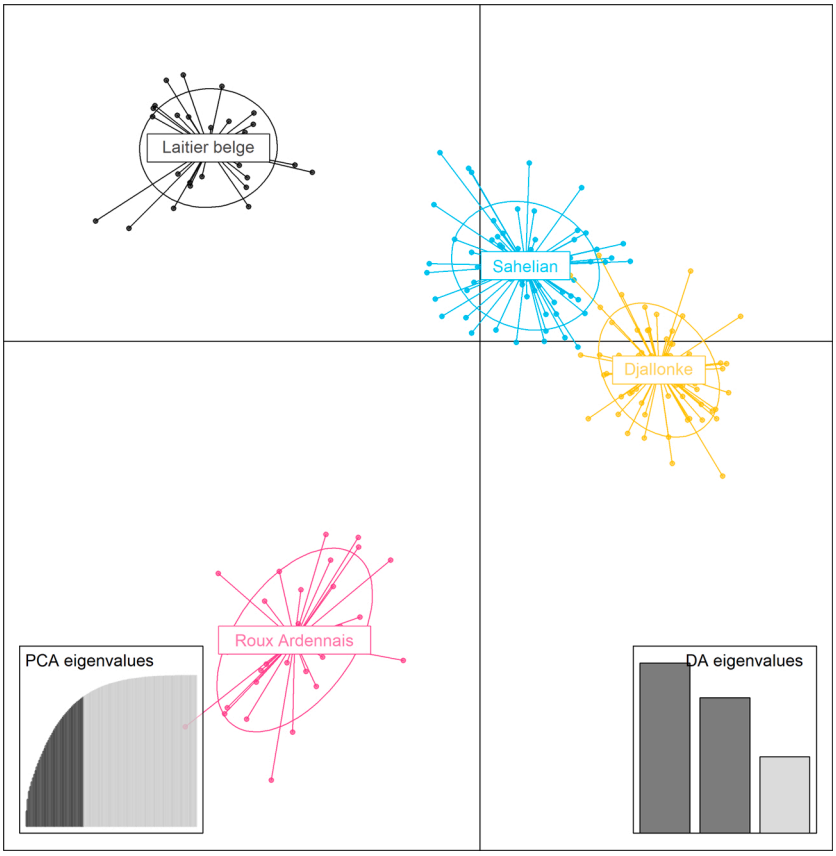

Fig. 4. Discriminant analysis of principal components (DAPC) for 197 genotyped sheep, using the R package *adegenet* version 2.1.4 in R software. The axes correspond to the first two Linear Discriminants (LD). Each ellipse represents a sheep breed cluster (Djallonke, Sahelian, Laitier belge, and Ardennais roux) and each dot represents an individual.

4. Conclusion

Measurements applied on all markers parameters indicated that the multiplex SSR genotyping kit provided comparable performances in revealing valuable rates of polymorphism, both within the two species (goats and sheep) and the two countries of origin (Benin and Belgium). Despite its low number of SSR markers, the newly developed genotyping kit, in conjunction with the use of appropriate multivariate analyses, demonstrated to be a powerful tool for detecting heterotic groups within the populations of the two small ruminant species. The methodological strategy of using a minimum number of markers with a resulting high level of information has proven to be a good choice, and remain a suitable alternative compared to the more efficient (but also a lot more expensive) SNP chips. The kit therefore meets the expectations of researchers and breeders who do not necessarily have access to more advanced technologies using more abundant markers, as is often the case in developing countries. For instance, its use is of further interest to circumvent the difficulties inherent to the rough definition of livestock breeds encountered in some regions of the world, such in West Africa, which is often referred to a sociocultural concept, to geographic locations or based on unstandardized phenotyping method (FAO, 2012; Dossa et al., 2007; Whannou et al., 2021a, b).

Thereby, the newly developed genotyping kit constitutes a valuable standardized tool for carrying out diverse genetic studies among goat and sheep species, such as genetic diversity assessments, admixture measurements, traceability concerns as well as parentage determination. In addition, our kit is exploitable for conducting the assessment of the population structure, as a prerequisite for further genome-wide association studies, regarding the selection strategies to adopt in the breeding program, as the one recently started in Benin (PRD/ARES/2018 Project, 2018).

Funding

This work is financially supported by the Government of Belgium through the Académie de Recherche et d'Enseignement supérieur (ARES). ARES-PRD Project (Amélioration des systèmes traditionnels d'élevage de petits ruminants (ovins et caprins) dans un contexte de mutation environnementale et sociétale au Bénin).

Acknowledgments

The authors gratefully acknowledged ARES for the financial support for the PRD/ARES/2018 research project, Dominique Demblon, Claire Billion and Claire Avril (Haute-Ecole CONDORCET) for coordinating the research actions and implementing the enriching collaboration between the different actors of the project. We fully acknowledged all the breeders, especially Alain Vryghem, Vincent Delobel, Johan Van Ryckeghem, Fabienne Hausmann and Christian Mulders, Thierry Mulders, Leatitia Peeters, and Pascale Cuvelier (ARSIA), for providing samples and for their availability, as well as anonymous reviewers for their critical and constructive comments on the earlier version of the manuscript. We would like also to thank Justine Dendoncker, Celia Mabille, Patty Calers, and Morena Vetturini for their help in DNA extractions.

References

Al-Atiyat, R.M., Aljumaah, R.S., Alshaikh, M.A., Abudabos, A.M., 2018. Microsatellite-based genetic structure and diversity of local Arabian sheep breeds. Front. Genet. 9, 408. https://doi.org/10.3389/fgene.2018.00408.

Álvarez, I., Gutiérrez, J.P., Royo, L.J., Fernández, I., Gómez, E., Arranz, J.J., Goyache, F., 2005. Testing the usefulness of the molecular coancestry information to assess genetic relationships in livestock using a set of Spanish sheep breeds. J. Anim. Sci. 83, 737–744. https://doi.org/10.2527/2005.834737x.

Álvarez, I., Traoré, A., Tamboura, H.H., Kaboré, A., Royo, L.J., Fernández, I., Ouédraogo-Sanou, G., Sawadogo, L., Goyache, F., 2009. Microsatellite analysis characterizes Burkina Faso as a genetic contact zone between Sahelian and Djallonké sheep. Anim. Biotechnol. Bull. 20, 47–57. https://doi.org/10.1080/10495390902786926.

Álvarez, I., Capote, J., Traoré, A., Fonseca, N., Pérez, K., Cuervo, M., Fernández, I., Goyache, F., 2012. Genetic relationships of the Cuban hair sheep inferred from microsatellite polymorphism. Small Rumin. Res. 104, 89–93. https://doi.org/10.1016/j.smallrumres.2011.10.025.

ARES-PRD Project, 2018. Amélioration des systèmes traditionnels d'élevage de petits ruminants (ovins et caprins) dans un contexte de mutation environnementale et sociétale au Bénin. https://www.ares-ac.be/fr/cooperation-au-developpement/pays-projets/projets-dans-le-monde/item/150-prd-amelioration-des-systemes-traditionnels-d-elevage-de-petits-ruminants-ovins-et-caprins-dans-un-contexte-de-mutation-environnementale-et-societale-au-benin.

Awobajo, O.K., Salako, A.E., Osaiyuwu, O.H., 2015. Analysis of genetic structure of Nigerian West African Dwarf goats by microsatellite markers. Small Rumin. Res. 133, 112–117. https://doi.org/10.1016/j.smallrumres.2015.09.006.

Dixit, S.P., Verma, N.K., Aggarwal, R.A.K., Vyas, M.K., Rana, J., Sharma, A., 2012. Genetic diversity and relationship among Indian goat breeds based on microsatellite markers. Small Rumin. Res. 105, 38–45. https://doi.org/10.1016/j.smallrumres.2011.11.026.

Dossa, L.H., Wollny, C., Gauly, M., 2007. Spatial variation in goat populations from Benin as revealed by multivariate analysis of morphological traits. Small Rumin. Res. 73, 150–159. https://doi.org/10.1016/j.smallrumres.2007.01.003.

Dossybayev, K., Orazymbetova, Z., Mussayeva, A., Naruya Saitou, N., Zhapbasov, R., Makhatov, B., Bekmanov, B., 2019. Genetic diversity of different breeds of Kazakh sheep using microsatellite analysis. Arch. Anim. Breed. 62, 305–312. https://doi.org/10.5194/aab-62-305-2019.

FAO, 2011. Molecular Genetic Characterization of Animal Genetic Resources. FAO Animal Production and Health Guidelines. No. 9. Rome.

FAO, 2012. Phenotypic Characterization of Animal Genetic Resources. FAO Animal Production and Health Guidelines No. 11. available at. FAO, Rome. http://www.fao.org/docrep/015/i2686e/i2686e00.pdf.

Henegariu, O., Heerema, N.A., Dlouhy, S.R., Vance, G.H., Vogt, P.H., 1997. Multiplex PCR: critical parameters and step-by-step protocol. Biotechniques 23, 504–511. https://doi.org/10.2144/97233rr01.

Hepsibha, P., Karthickeyan, S.M.K., Guru, V., 2014. Microsatellite marker based assessment of genetic structure of Coimbatore breed of sheep (*Ovies aries*) in Tamil Nadu. Indian J. Biotechnol. 13, 203–206.

Jombart, T., 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129.

Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 11, 94. https://doi.org/10.1186/1471-2156-11-94.

Kalinowski, S.T., Taper, M.L., Marshall, T.C., 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16, 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x.

Legaz, E., Álvarez, I., Royo, L.J., Fernández, I., Gutiérrez, J.P., Goyache, F., 2008. Genetic relationships between Spanish Assaf (Assaf.E) and Spanish native dairy sheep breeds. Small Rumin. Res. 80, 39–44. https://doi.org/10.1016/j.smallrumres.2008.09.001.

Luikart, G., Biju-Duval, M.P., Ertugrul, O., Zagdsuren, Y., Maudet, C., Taberlet, P., 1999. Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (*Capra hircus*). Anim. Genet. 30, 431–438. https://doi.org/10.1046/j.1365-2052.1999.00545.x.

Missohou, A., Poutya, M.R., Nenonene, A., Dayo, G.-K., Ayssiwede, S.B., Talaki, E., Issa, Y., Fané, A., 2011. Genetic diversity and differentiation in nine West African local goat breeds assessed via microsatellite polymorphism. Small Rumin. Res. 99, 20–24. https://doi.org/10.1016/j.smallrumres.2011.04.005.

Negrini, R., D'Andrea, M., Crepaldi, P., Colli, L., Nicoloso, L., Guastella, A.M., Sechi, T., Bordonaro, S., Ajmone-Marsan, P., Pilla, F., 2012. Effect of microsatellite outliers on the genetic structure of eight Italian goat breeds. Small Rumin. Res. 103, 99–107. https://doi.org/10.1016/j.smallrumres.2011.08.006.

Nei, M., 1973. Analyses of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. 70, 3321–3323. https://doi.org/10.1073/pnas.70.12.3321.

Ocampo, R., Cardona, H., Martínez, R., 2016. Genetic diversity of Colombian sheep by microsatellite markers. Chil. J. Agric. Res. 76, 40–47. https://doi.org/10.4067/S0718-58392016000100006.

Pramod, S., Kumarasamy, P., Rosalyn Mary Chandra, A., Sridevi, P., Rahumathulla, P.S., 2009. Molecular characterization of vembur sheep (*Ovis aries*) of south India based on microsatellites. Indian J. Sci. Technol. 2, 55–58. https://doi.org/10.17485/ijst/2009/v2i11.9.

R Development Core Team, 2010. R: A Language and Environment for Statistical Computing. ISBN 3-900051-07-0, URL. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org./.

Sharma, R., Kumar, B., Arora, R., Ahlawat, S., Mishra, A.K., Tantia, M.S., 2016. Genetic diversity estimates point to immediate efforts for conserving the endangered Tibetan sheep of India. Meta Gene 8, 14–20. https://doi.org/10.1016/j.mgene.2016.01.002.

Sodhi, M., Mukesh, M., Bhatia, S., 2006. Characterizing Nali and Chokla sheep differentiation with microsatellite markers. Small Rumin. Res. 65, 185–192. https://doi.org/10.1016/j.smallrumres.2005.04.032.

Traoré, A., Álvarez, I., Tamboura, H.H., Fernández, I., Kaboré, A., Royo, L.J., Gutiérrez, J.P., Sangaré, M., Ouédraogo-Sanou, G., Toguyeni, A., Sawadogo, L., Goyache, F., 2009. Genetic characterisation of Burkina Faso goats using microsatellite polymorphism. Livest. Sci. 123, 322–328. https://doi.org/10.1016/j.livsci.2008.11.005.

Traoré, A., Álvarez, I., Fernández, I., Pérez-Pardal, L., Kaboré, A., Ouédraogo-Sanou, G. M.S., Zaré, Y., Tamboura, H.H., Goyache, F., 2012. Ascertaining gene flow patterns in livestock populations of developing countries: a case study in Burkina Faso goat. BMC Genet. 13, 35. https://doi.org/10.1186/1471-2156-13-35.

Whannou, H.R.V., Afatondji, U.C., Demblon, D., Houinato, R.B.M., Dossa, L.H., 2021a. Morphological variability and geographical distribution of goat populations from Benin (West Africa). Genet. Resour. 2 (S1) https://doi.org/10.46265/genresj.QULD1720 p46.

Whannou, H.R.V., Afatondji, U.C., Ahozonlin, M.C., Spanoghe, M., Lanterbecq, D., Demblon, D., Houinato, M.R.B., Dossa, L.H., 2021b. Morphological variability within the indigenous sheep population of Benin. PLoS One 16 (10), e0258761. https://doi.org/10.1371/journal.pone.0258761.