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Genetic diversity and differentiation among Korean-Holstein, Hanwoo, and Uganda-Holstein breeds

G. Bigirwa[1,2], D. Kim[2], O. Acai[1], C. Na[2], J. Oh[2#] & K. Song[2,3#]

[1]College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Uganda
[2]Department of Animal Biotechnology, Jeonbuk National University, Jeonju 54896, Korea
[3] Department of Animal Science, Feeds and Animal Nutrition, Faculty of Agriculture, Ankara University, Ankara, 06110, Turkey

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Abstract

The aim of this research was to assess genetic diversity of Korean-Holstein, Korean Hanwoo, and Ugandan-Holstein dairy cattle. DNA was extracted from either blood or hair of Korean-Holstein (n=74), Korean-Hanwoo (n=75) and Ugandan-Holstein (N=77) using AccuPrep® PCR purification kit. The DNA samples were amplified by multiplex polymerase chain reaction, using GeneTrack™ Hanwoo genotyping kit and assayed using ABI genetic analyser 3130XL. Number of alleles, expected heterozygosity (H_e), observed heterozygosity (H_o), and the polymorphism information content (PIC) were estimated from 10 microsatellite loci in the three breeds. In addition, *F*-statistics for each of the 10 microsatellites in the three cattle breeds were estimated using fstat version 2.9.3.2 computer program. GENETIX (v.4.02) was used to perform factorial correspondence analysis (FCA) from the allele frequencies and multi-locus clustering was done using STRUCTURE analyses. A total of 124 alleles were detected. The number of alleles per locus varied from eight (TGLA126) to 22 (TGLA122), with an overall mean of 12.2. Expected heterozygosity ranged from 0.617 (SPS115) to 0.854 (TGLA53) and averaged 0.761. Observed heterozygosity ranged from 0.6 (SPS115) to 0.859 (TGLA53); and averaged 0.761. The mean PIC was 0.723; and means of the *F*-statistics F_{IT}, F_{ST} and F_{IS} were 0.077, 0.076 and 0.001 respectively. Although FCA revealed clear differentiation of Uganda-Holstein, Korean-Holstein, and Hanwoo, clustering assignments showed genetic admixture between Ugandan dairy cattle (Uganda-Holstein) and Hanwoo. In conclusion, the allelic variation present at the 10 loci was sufficient to categorize these cattle into distinct breed groups.

Keywords: dairy cattle, genetic differentiation, F-statistics, heterozygosity, microsatellite
[#]Corresponding authors: oh5ow@naver.com, kiduk.song@jbnu.ac.kr

Introduction

Genetic improvement of dairy cattle is considered indispensable to increasing milk production and fostering animals' adaptability to farming environments in many countries around the world (Olesen *et al*., 2000; Rischkowsky & Pilling, 2007). For this reason, several breeding programmes have chosen to employ a few high-producing dairy animals as parent stock, which are multiplied with assisted reproductive technologies (Goddard & Hayes, 2007; Schefers & Weigel, 2012). At the same time, the necessity to clearly define breed-specific attributes through genomic studies among cattle populations has been pointed out as a key factor for sustainable dairy genetic improvement programmes (Kim *et al.*, 2017; Tixier-Boichard *et al.*, 2015). Moreover, genomic studies have been used to assess the levels of genetic diversity of cattle breeds, which is necessary for conserving breeds and designing future genetic improvement programmes (Biscarini *et al.*, 2015).

DNA-based tools such as microsatellite analyses have been developed and are now widely used for genetic diversity assessment in dairy breeding programmes (Silva *et al.*, 2014). Additionally, microsatellite markers are used for identification of parental lineages (Erhardt & Weimann, 2007), mapping genes for economically important traits (Goddard & Hayes, 2007; Naicy, 2008), and aiding in marker-assisted selection (MAS) (Oltenacu & Broom, 2010; Schefers & Weigel, 2012). Microsatellites have been commended for use

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in genetic diversity studies because of their accuracy, versatility, abundancy and being spread over the entire genome (Agung *et al.,* 2019; Erhardt & Weimann, 2007).

Worldwide, the Holstein-Friesian is regarded as the highest milk producing breed of cattle (Oltenacu & Broom, 2010; Robinson *et al.,* 2011). In Uganda, dairy cattle constitute only 6-7% of the national herd, and dairy genetic improvement has been implemented through crossbreeding with imported Holstein semen and embryos from foreign countries (Mugisha *et al.*, 2014). In particular, the major dairy cattle breed in Uganda, which is also referred to as 'Uganda-Holstein', has been produced through crossbreeding Ankole with Holstein cattle. This programme has been implemented over the last 50 years (Galukande, 2010; Kim *et al.,* 2017). However, this approach has not been supported with modern genomic tools to assess the genetic diversity of bred dairy cattle in Uganda and the introduced exotic dairy breeds. As part of the Ugandan dairy genetic improvement programme, the Korean-dairy breeding programme was launched in 2014 to improve dairy cattle production by using germplasm from Korean-Holstein. In this study, the authors used microsatellite markers to assess the genetic diversity of dairy cattle in Uganda in relation to Korean-Holstein and Korean-Hanwoo.

Materials and Methods

This study was conducted after a review by the Ethical Committee of the School of Veterinary Medicine and Animal Resources, Makerere University (Reference: SVARREC/10/2018), and approved for compliance with animal use standards. Approximately 50 tail-hairs were gathered and pulled firmly from the skin to secure hair-roots.

DNA was extracted from hair samples of Korean-Holstein (n=74), Hanwoo (n=75) and Uganda-Holstein (n=77) using AccuPrep® PCR Purification Kit (Bioneer, SK). Ten microsatellites (Table 1), which are recommended by the International Society for Animal Genetics for parentage assessment in cattle were used. Microsatellites were amplified in multiplex PCR reactions using GeneTrack^{TM} Hanwoo genotype kit (TNT Research, Republic of Korea) according to the manufacturer's instructions. The resultant fluorescent labelled PCR products were separated in the ABI genetic analyser 3500XL, as per the manufacturer's instructions. The number of alleles, observed heterozygosity (H_o) and expected heterozygosity (H_e), and polymorphism information content (PIC) were calculated for each microsatellite marker using the microsatellite tool kit (Park, 2001). Wright's F-statistics (F_{IS}, F_{IT} and F_{ST}) were calculated for each locus across the three breed populations using the fstat version 2.9.3.2 computer program (Goudet, 2002). GENETIX (version 4.02) program was used to perform factorial correspondence analysis (Belkhir *et al*., 2001). Clustering assignments of the three breeds were obtained by using STRUCTURE version 2.3.4 (Pritchard *et al*., 2000).

Results

The number of alleles, H_E, H_O and PIC for the three cattle breed populations are shown in Table 2. A total of 124 alleles were detected at 10 microsatellite loci across the three breeds. The number of alleles at each locus varied between eight (TGLA126) and 22 (TGLA122), with an overall mean allele number of 12.2. Expected heterozygosity ranged between 0.617 (SPS115) and 0.854 (TGLA53) with an average of 0.761. Observed heterozygosity varied between 0.6 (SPS115) and 0.859 (TGLA53), with a mean of 0.761. The mean PIC was 0.723.

Microsatellite polymorphism results for individual cattle breed are shown in Table 3. The Uganda-Holstein showed the greatest overall H_E (0.799) and the Korean-Holstein had the lowest H_E (0.717). Observed heterozygosity was greatest in Hanwoo (0.811) and lowest in Korean-Holstein.

The estimates of PIC and *F*-statistics (F_{IS}, F_{IT} and F_{ST}) are shown in Table 4. Overall, the mean PIC was higher in Uganda-Holstein than in Korea-Holstein and Hanwoo. Moreover, PIC results from all microsatellite loci were still higher in Uganda-Holstein than in Korea-Holstein and Hanwoo. The total inbreeding estimates per locus (F_{IT}) ranged from 0.003 (ETH10) to 0.214 (ETH225) across the three breed populations, with the overall mean of 0.077. The lowest and highest within-population inbreeding estimate values (F_{IS}) were -0.005 (TGLA52) and 0.088 (ETH225), respectively. Genetic differentiation (F_{ST}) was observed in all markers, with the highest estimate in ETH3 marker.

Table 1 Microsatellite primers used to assess genetic diversity in Korean-Holstein, Korean Hanwoo and Uganda-Holstein

Microsatellite marker	Size range (bp)	Primer sequence (5'-3')	Reference
BM2113	125-143	Forward: GCT GCC TTC TAC CAA ATA CCC Reverse: CTT CCT GAG AGA AGC AAC ACC	Bishop *et al.,* 1994
ETH10	210-226	Forward: GTT CAG GAC TGG CCC TGC TAA CA Reverse: CCT CCA GCC CAC TTT CTC TTC TC	Toldo *et al.,* 1993
ETH225	140-156	Forward: GAT CAC CTT GCC ACT ATT TCC T Reverse: ACA TGA CAG CCA GCT GCT ACT	Toldo *et al.,* 1993
ETH3	117-129	Forward: GAACCTGCCTCTCCTGCATTGG Reverse: ACTCTGCCTGTGGCCAAGTAGG	Toldo *et al.,* 1993
INRA023	197-223	Forward: GAG TAG AGC TAC AAG ATA AAC TTC Reverse: TAA CTA CAG GGT GTT AGA TGA ACT C	Vaiman *et al.,* 1992
SPS115	234-252	Forward: AAA GTG ACA CAA CAG CTT CTC CAG Reverse: AAC GAG TGT CCT AGT TTG GCT GTG	Bishop *et al.,* 1994
TGLA122	130-193	Forward: CCC TCC TCC AGG TAA ATC AGC Reverse: AAT CAC ATG GCA AAT AAG TAC ATA	Barendse *et al.,* 1992
TGLA126	104-133	Forward: CTA ATT TAG AAT GAG AGA GGC TTC T Reverse: TTG GTC TCT ATT CTC TGA ATA TTC C	Barendse *et al.,* 1992
TGLA227	64-115	Forward: CGA ATT CCA AAT CTG TTA ATT TGC T Reverse: ACA GAC AGA AAC TCA ATG AAA GCA	Barendse *et al.*, 1992
TGLA53	144-178	Forward: GCTTTCAGAAATAGTTTGCATTCA Reverse: ATCTTCACATGATATTACAGCAGA	Barendse *et al.,* 1992

Table 2 Estimates of expected and observed heterozygosity and polymorphism information content for 10 microsatellite loci that were assessed in Korean-Holstein, Hanwoo, Uganda-Holstein

Locus	No. of Alleles	H_e	H_o	PIC
BM2113	11	0.789	0.818	0.751
ETH10	9	0.775	0.801	0.739
ETH225	9	0.744	0.679	0.705
ETH3	11	0.702	0.682	0.655
INRA23	11	0.793	0.800	0.755
SPS115	9	0.617	0.600	0.578
TGLA122	22	0.828	0.850	0.803
TGLA126	8	0.652	0.693	0.585
TGLA227	15	0.853	0.822	0.829
TGLA53	17	0.854	0.859	0.832
Overall Mean	12.2	0.761	0.761	0.723

H_e: expected heterozygosity, H_o: observed heterozygosity, PIC: polymorphism information content

Table 3 Expected and observed heterozygosity and *F*-statistics for 10 loci from Korean-Holstein, Hanwoo, Uganda-Holstein

Microsatellite marker	UH		KH		HAN		F_{IT}	F_{ST}	F_{IS}
	H_e	H_o	H_e	H_o	H_e	H_o			
BM2113	0.867	0.844	0.731	0.811	0.769	0.800	0.030	0.064	-0.036
ETH10	0.817	0.753	0.719	0.757	0.788	0.893	0.003	0.035	-0.033
ETH225	0.850	0.688	0.746	0.671	0.637	0.680	0.214	0.138	0.088
ETH3	0.694	0.584	0.692	0.622	0.721	0.840	0.194	0.169	0.030
INRA23	0.836	0.789	0.758	0.797	0.786	0.813	0.044	0.052	-0.009
SPS115	0.605	0.595	0.547	0.500	0.698	0.707	0.063	0.038	0.026
TGLA122	0.816	0.779	0.826	0.838	0.843	0.933	0.060	0.084	-0.026
TGLA126	0.751	0.805	0.529	0.568	0.676	0.707	0.018	0.076	-0.063
TGLA227	0.889	0.857	0.808	0.743	0.862	0.867	0.089	0.055	0.036
TGLA53	0.872	0.818	0.816	0.892	0.874	0.867	0.034	0.038	-0.005
Overall Mean	0.799	0.751	0.717	0.719	0.765	0.811	0.077	0.076	0.001

UH: Uganda-Holstein, KH: Korea-Holstein, HAN: Hanwoo, H_e: expected heterozygosity, H_o: observed heterozygosity, F_{IT}: total inbreeding estimate, F_{ST}: measurement of population differentiation, F_{IS}: within-population inbreeding estimate.

Table 4 Polymorphism information content of 10 microsatellite loci in Korean-Holstein, Hanwoo, and Uganda-Holstein

Microsatellite Marker	Polymorphism information content		
	Uganda-Holstein	Korea-Holstein	Hanwoo
BM2113	0.847	0.678	0.727
ETH10	0.792	0.677	0.752
ETH225	0.826	0.701	0.590
ETH3	0.667	0.630	0.667
INRA23	0.809	0.709	0.747
SPS115	0.579	0.512	0.645
TGLA122	0.796	0.796	0.817
TGLA126	0.714	0.414	0.629
TGLA227	0.873	0.776	0.839
TGLA53	0.856	0.785	0.855
Overall mean	0.776	0.668	0.727

The factorial correspondence analysis of allele frequencies for the 10 microsatellite loci revealed the very clear separation between the Uganda-Holstein, Korean-Holstein and Hanwoo (Figure 1). The FCA plot shows 62% of total variance was accounted for by Axis 1, and 32% was explained by Axis 2, which in large part separated Uganda-Holstein from Korean-Holstein and Hanwoo. Multi-locus clustering structure of the three populations is shown in Figure 2. The breeds were clearly separated into three distinct groups.

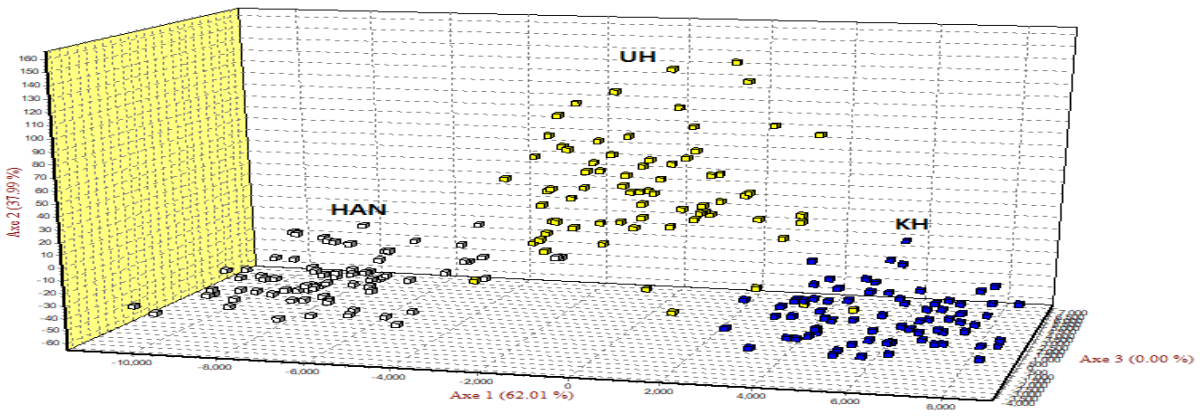

Figure 1 Factorial correspondence analysis of allele frequencies from 10 microsatellite loci in Korean-Holstein, Hanwoo, Uganda-Holstein

HAN: Hanwoo, UH: Uganda-Holstein, KH: Korean-Holstein

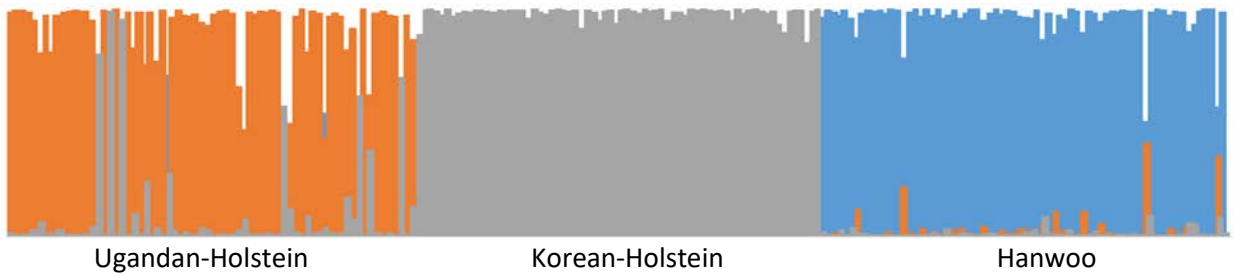

Figure 2 Clustering assignment with *k*=3 of Ugandan Holstein, Korean Holstein and Hanwoo samples

Each of the 226 animals is represented by a thin vertical line that is divided into segments of height proportional to the population represented and colour, which corresponds to the founder population

Discussion

The number of microsatellite alleles at each locus has been used to assess genetic diversity and between-breed relationships (Egito *et al.*, 2007; Mateus *et al.*, 2004; Suh *et al.*, 2014; Stevanovic *et al.*, 2010). The overall mean allele number per locus was comparable with previous results, which varied from 5 to 16 (Egito *et al.*, 2007; Freeman *et al.*, 2004; Mateus *et al.*, 2004; Stevanovic *et al.*, 2010; Suh *et al.*, 2014, Zhao *et al.,* 2017). Additionally, it has been suggested that individual microsatellite markers that are used to assess genetic diversity should have at least four alleles in order to generate reliable information (Barker, 1994; Shi *et al.*, 2010). Here, the number of alleles per locus satisfies this recommendation. Although 30 loci are recommended for genetic diversity studies in cattle (Erhardt & Weimann, 2007), the use of 10 microsatellite markers in this study was able to differentiate the three breeds of cattle adequately. Similarly, proven genetic diversity studies among cattle breeds have been undertaken using 10 microsatellites (Zhou *et al.*, 2005; Cervini *et al.*, 2006).

In general, the levels of heterozygosity (H_e and H_o) that were observed in this study are similar to previous reports (Agung *et al.*, 2019; Egito *et al.*, 2007; Freeman *et al.*, 2004; Suh *et al.,* 2014; Zhao *et al.,* 2017). Given that these observed levels of heterozygosity were greater than 0.5, this indicates that the 10 microsatellite markers are suitable for genetic diversity studies in the three dairy breeds (Sheriff & Alemayehu, 2017).

The estimates of H_e and H_o in Hanwoo and Korean-Holstein that were observed in this study were similar to or slightly greater than those reported previously for the two breeds (Hanwoo: H_e = 0.713 to 0.740 and H_o = 0.68 to 0.7442; and Korean-Holstein: H_e = 0.701 to 0.7361 and H_o = 0.715 to 0.753) (Kim *et al.,* 2002, Shi *et al.,* 2010, Suh *et al.,* 2014)**.** Any perceived discrepancy in H_e and H_o among studies could be as a result of differences in the numbers of animals and in the laboratory settings, which could lead to

genotyping errors (Egito *et al.,* 2007; Suh *et al.,* 2014). The Korean Holstein group that was characterized in this study was found to be less heterozygous than the Hanwoo cattle, in agreement with Choi *et al.* (2012). Compared with the Hanwoo and Korean-Holstein, the value of H_e in Uganda-Holstein was greater than H_o for nine of the microsatellites (Table 3). This observed greater H_e compared with H_o in Uganda-Holstein could be attributed to haphazard and unregulated breeding practices among Uganda dairy cattle herds (Mugisha *et al.,* 2014). In the present study, a low estimate of genetic differentiation (F_{ST}=0.076) between the studied breeds was identified, indicating that the three cattle breed populations could have close proximity and share the same ancestry. These populations are all taurine breeds and could have common alleles in their genomes. An inbreeding coefficient (F_{IS}) of 1% identified in this study could be an indication of limited inbreeding within the three populations. Moreover, there was a significant shortfall in the total inbreeding estimates (mean F_{IT}=0.077) across the three populations. However, the indices of *F*-statistics (F_{IS}, F_{ST} and F_{IT}) that were obtained in the current study population were equivalent to those reported among taurine breeds (Suh *et al.,* 2014; Mateus *et al.,* 2004). The PIC values for the 10 markers in this study ranged between 0.578 (SPS115) and 0.832 (TGLA53) (Table 4), suggesting that the microsatellite markers used in this study were informative.

The clear separation between the Uganda-Holstein, Korean-Holstein and Hanwoo that is revealed in the FCA (Figure 1) suggests that these breeds are distinct. A previous study on the genetic structure of Korean native breeds revealed similar findings for Hanwoo and Korean-Holstein (Suh *et al.,* 2014). In this study, the Ugandan-Holstein was clearly separated from Hanwoo and Korean-Holstein. The differences in the level of alleles that are present in animals have been shown to contribute to distinction between breeds (Egito *et al.,* 2007; Mateus *et al.,* 2004). The results from the current study therefore suggest that each of the breeds has distinct genetic characteristics, which can be utilized when designing conservation and breeding programmes. A previous study using single nucleotide polymorphisms (SNPs) revealed genetic heterogeneity and shared ancestry between Ankole cattle and other taurine breeds, including Hanwoo (Kim *et al.,* 2017). Most dairy cattle in Uganda are products of crossbreeding Holstein with Ankole cattle (Galukande, 2010; Kim *et al*., 2017).

Conclusion

From the present study, all 10 markers displayed a significant abundance of heterozygosity and were effective in determining the genetic diversity of the three breeds. The alleles identified in this study were able to separate the breeds into three distinct clusters, that is, Korean-Holstein, Uganda-Holstein and Hanwoo. Further comparative studies on historical, cultural, animal phenotype and environmental aspects regarding breeding programmes in these breeds are recommended.

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Authors' Contributions

KDS, JOA and JDO designed the study, GB collected the blood samples and isolated the DNA. GB, DHK, JDO and CSN performed the molecular genetic and statistical analyses. All authors contributed to manuscript writing.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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