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Linkage disequilibrium, population stratification and patterns of ancestry in Simmental cattle

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RESUMEN

El uso masivo de pocos toros en programas de inseminación artificial afecta la composición y la estructura genética de una población. El objetivo de este estudio fue estimar la diversidad genética, estratificación genética, patrones de ancestría y el desequilibrio de ligamiento en ganado Simmental. Una muestra de 233 animales genotipados con 30106 marcadores tipo polimorfismo de nucleótido simple (SNP) fue usada. Los patrones de subdivisión genética y ancestría fueron estimados a través de análisis de componentes principales y probabilidades de asignación a grupos genéticos. El desequilibrio de ligamiento fue estimado como el cuadrado del coeficiente de correlación entre los SNP. El análisis por componentes principales no mostró patrones de subdivisión genética dentro de la población Simmental de Colombia. Sin embargo, el análisis de ancestría identificó una subdivisión genética de de tres grupos. Un valor de 0.3 para desequilibrio de ligamiento fue encontrado a una distancia de 33 kb. Los resultados permiten evidenciar el cambio en la estructura genética de una población debido al uso e importación de material genético proveniente de poblaciones que difieren en objetivos de cría y criterios de selección.

Desequilibrio de ligamiento, estratificación y patrones de ancestría en ganado Simmental

SUMMARY

The massive use of a few bulls in artificial insemination programs affects the structure and genetic composition of a population. The aim of this study was to estimate the genetic diversity, population stratification, patterns of ancestry and linkage disequilibrium in Simmental cattle. A sample of 233 genotyped animals with 30.106 single nucleotide polymorphism (SNP) was used. Principal components analysis and probabilistic assignments were performed to estimate patterns of genetic subdivision and ancestry. Linkage disequilibrium was estimated as the square of the genetic correlation coefficient between SNP´s. The principal components analysis did not show genetic subdivision patterns within the population. However, the analysis of ancestry showed a genetic subdivision of three groups. A value of 0.3 for linkage disequilibrium was found at a distance of 33 kb. Results indicate that imported genetic material from populations with different breeding goals and selection criteria could contribute to changes on genetic structure.

ADDITIONAL KEYWORDS
Admixture.
Crossbreeding.
Genetic diversity.
Genomics.
Principal component analysis.

PALABRAS CLAVES
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Cruzamiento.
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INTRODUCTION

Population stratification, demographic history, genetic drift, recombination rate and selection affect the composition and genetic structure of a population (Qanbari et al. 2010, p. 352). The description of the genetic composition and structure of a population are essential for a breeding program and can be used to reflect the history of the breeding system and the geographical subdivision patterns (Makina et al. 2014, p. 2; Jasielczuk et al. 2016, p. 57).

The linkage disequilibrium (LD) and the population stratification are important methodologies to identify genes associated with quantitative traits, selection signatures and to know the number of SNP´s needed to implement a genomic selection program (Khatkar et al. 2007, p. 2; Sargolzaei et al. 2008, p. 2106; Bohmanova et al. 2010, p. 1; Mouresan et al. 2017 p.59-60). The LD pattern has been related to genetic structure population parameters such as effective population size and genetic variability in conservation genetics programs (Khanyile et al. 2015, p. 7). However, the results from commercial breeding programs have been contradictory. Karimi et al. (2016, p. 11) found changes in genetic structure and high levels of genetic diversity within breeds that had geographic isolations and a high genetic flow with foreign populations. On the other hand, populations in similar conditions reported a low level of genetic variability within breeds due to the strong selection and the use of a few bulls (Makina et al. 2014, p. 4).

In Colombia, about 90% of the registered Simmental cattle births come from European and American bulls. The use of a few bulls through artificial insemination and geographic isolations within countries can generate differences in allele frequencies between and within populations (Decker et al. 2014, p. 2; Amaya et al. 2019, p. 2). The latter could contribute to a possible differentiation regarding the original populations in their phenotypic performance, structure and population stratification patterns (Gibbs et al. 2016, p. 530). Additionally, successful genomic selection schemes are based on describing more accurately estimates related to population and quantitative genetics. This can be accomplished by the availability of a sufficient number of SNP´s that ensure the persistence of LD (Neves et al. 2015, p. 100; Cañas-Álvarez et al. 2016, p. 2780). Therefore, the aim of this study was to know the genetic diversity, the level of population stratification, the extent of the LD and the patterns of ancestry in Colombian Simmental cattle.

MATERIAL AND METHODS

In this study, approval by an ethics committee was not necessary because all records used came from an existing database and did not involve experiments or procedures with the animals.

Genotyping and quality control

A sample of 233 animals were genotyped from the genomic selection program of Colombian Simmental cattle using the GeenSeek Genomic Profiler-LD chip (GGP Bovine LD v4). This chip contains 30106 SNP´s. Animals and SNPs with a genotyping rate <90%, SNP´s whose minor allele frequency (MAF) had a frequency <0.05 and SNP´s with statistical significance for the Hardy-Weinberg equilibrium test <0.001 were removed using the software PLINK (Purcell et al. 2007, p. 71). The final analysis for genetic diversity and LD included 23820 autosomal SNP´s and with a known position.

Genetic diversity

The degree of heterozygosity and inbreeding coefficient were calculated with the algorithms implemented in the software PLINK (Purcell et al. 2007, p. 97). The MAF´s were estimated after quality control was carried out, and was later tabulated as the proportion of SNP´s in different frequency categories.

Linkage disequilibrium

The LD measurements used the square of the genetic correlation coefficient () mean and were calculated in PLINK (Purcell et al. 2007, p. 71) from all possible correlations among SNP´s. The estimates were calculated within each chromosome and with a maximum distance of 10 Mb. The average values between distances from <1 kb to 150 kb, with intervals of 1 kb were estimated to plot the decay of the LD. SNP´s with a MAF> 0.2, autosomal and with a known position were used for this analysis.

Population stratification and ancestry

Genotyped samples with the Illumina BovineSNP50 chip from other populations were obtained from the Dryad Digital Repository (Decker et al. 2014, doi:10.5061/dryad.th092) in order to compare other breeds with Colombian Simmental cattle. Common SNP´s between Illumina BovineSNP50 and GeenSeek Genomic Profiler-LD were extracted for all breeds. A final number of 9116 common autosomal SNP´s among the chips were used for population stratification and ancestry analysis. The breeds included in this study were American Simmental (n = 20), Holstein (n = 20), Braunvieh (n = 20), Brown Swiss (n = 12) and Gelbvieh (n = 20). Colombian population was examined in three ways as follows: five random samples (n = 20), founder animals (n = 20) and the entire population (n = 233) in order to discard sample size effects on principal components analysis (PCA) results.

The PCA was estimated from the differences between the allelic frequencies of the SNP´s between and within the populations. To evaluate the proportions of ancestry, analysis assumed different numbers of possible ancestral populations. These were carried out with Colombian Simmental population including K values from 1 to 5 to know the mixing patterns in the population. Analysis were also done including all populations with K values from 1 to 10. ADMIXTURE 1.3 program (Alexander et al. 2009, p. 1) was used to identify individual ancestries and the best number of ancestral populations.

RESULTS

The **Figure 1** shows the distribution of MAF for 23820 SNP´s. The observed heterozygosity was 0.389 ± 0.153 and the expected heterozygosity was 0.380 ± 0.122. The estimated inbreeding average was 0.002. The highest proportion of MAF was found in the frequency range from ≥ 0.4 to ≤0.5, including 28% of the total SNP´s. The lowest proportion of SNP´s was found in the frequency range ≥0.01 and <0.1, including 11% of the total SNP´s.

The **Figure 2** shows PCA results. The six breeds evaluated were clearly differentiated by the first three principal components. The breed that was most distant from the Simmental population according to the first two components was Holstein, which also showed a higher variability in its stratification compared to the other breeds. Braunvieh, Brown Swiss and Gelbvied showed smaller distances compared to the other breeds in the first two components. The Simmental cattle populations of Colombia and the United States did not show a possible stratification or subdivision within populations. The first and third components showed higher homogeneity within Colombian individuals and with the Simmental population evaluated by Decker et al. (2014, doi:10.5061/dryad.th092).

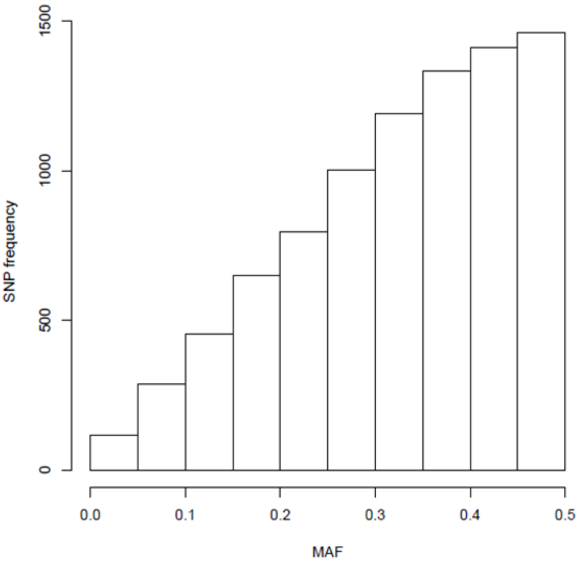

Figure 1. Frequency distribution of minor allele frequency in Simmental cattle. (Distribución de alelos de menor frecuencia en ganado Simmental)

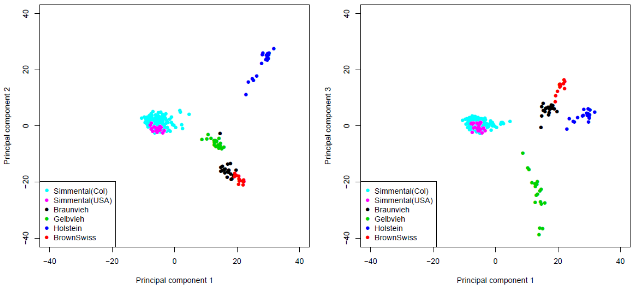

Figure 2. Stratification analysis for six bovine populations including Simmental animals from the United States (USA) and Colombia (Col). (Análisis de estratificación para seis poblaciones bovinas incluyendo animales Simmental de Estados Unidos (USA) Y Colombia (Col)).

The probabilistic assignment analysis for the Colombian Simmental population identified that the number of populations (K) was 3 (**Figure 3**) and for the analysis including all breeds, the analysis identified a K = 7 (**Figure 4**). For the K = 3 analysis, 2.5% of the animals had a high probabilistic assignment (>80%) of belonging to one of the populations, 24.5% of the population had a probabilistic assignment ranging from 60 to 80% and the 73 % of the population had more uniform probabilistic distributions between 25 and 35%. For K = 7, the most differentiated population was Brahman in which all the animals had a probability higher than 85% of being in the same cluster. Simmental (USA), Braunvieh, Gelbvieh, Holstein, and BrownSwiss populations had a probability higher than 60% to belong to one of the estimated groups.

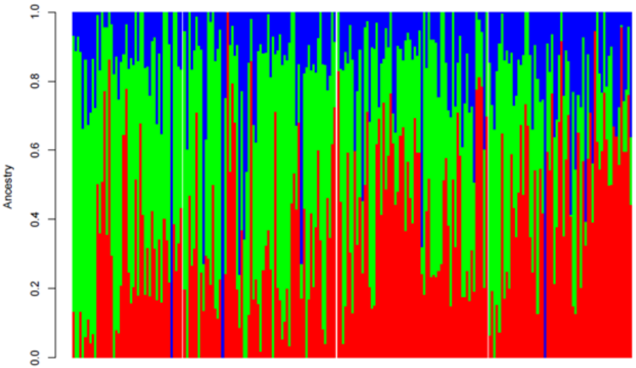

Figure 3. Probabilistic assignation for Colombian Simmental individuals belonging to one of the three estimated genetic groups (Green: European origin, Red: American origin, Blue: Colombian origin) (Asignación probabilística para animales Simmental a uno de los tres grupos genéticos estimados para individuos Simmental en Colombia (Verde: origen europeo, Rojo: origen americano, Azul: origen Colombiano)).

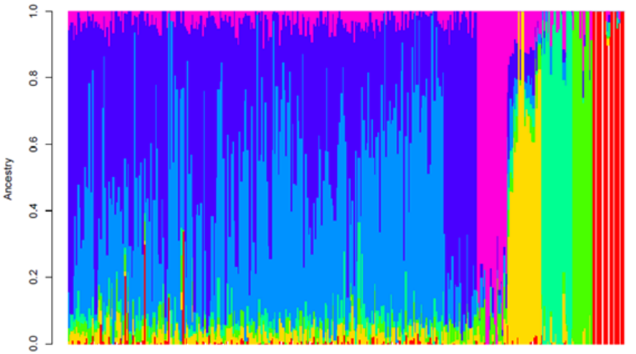

Figure 4. Probabilistic assignment for individuals from six breeds (Red: Brahman, intense green: BrownSwiss, pale green: Holstein, yellow: Gelbvieh, pink: Braunvieh, dark blue: American Simmental, light blue: European Simmental) (Asignación probabilística para individuos de seis razas (rojo: Brahman, verde intenso: BrownSwiss, verde pálido: Holstein, amarillo: Gelbvieh, rosa: Braunvieh, azul oscuro: Simmental americano, azul claro: Simmental europeo)).

The Simmental population born in Colombia with parents from the United States or Canada had an average probability of 75% to belong to the same group of Simmental animals studied by Decker *et al.* (2014, doi:10.5061/dryad.th092). Animals with parents from Europe had an average probability of 27% of belonging to the genetic American Simmental group and animals with parents born in Colombia had an average probability of 49% of belonging to the American Simmental population.

The number of paired estimates depends on the MAF criteria. The number of comparisons ranged from 83183 to 52776 when using MAF values of 0.05 and 0.2, respectively. The mean and the standard deviation for the LD with MAF values of 0.05, 0.1 and 0.2 were 0.288 ± 0.216, 0.287 ± 0.210 and 0.291 ± 0.208, respectively. The **Figure 5** shows the decrease and extension of the LD values. The value decreased when the SNP´s distance increased. When analysing the LD fall as a function of the physical distance between the SNP´s with a MAF > 0.2, the average value at a distance of <5 kb was 0.68. The mean value at distances less than 25 and 50 kb was 0.48 and 0.38, respectively. values lower than 0.3 were found from a distance of 33 kb.

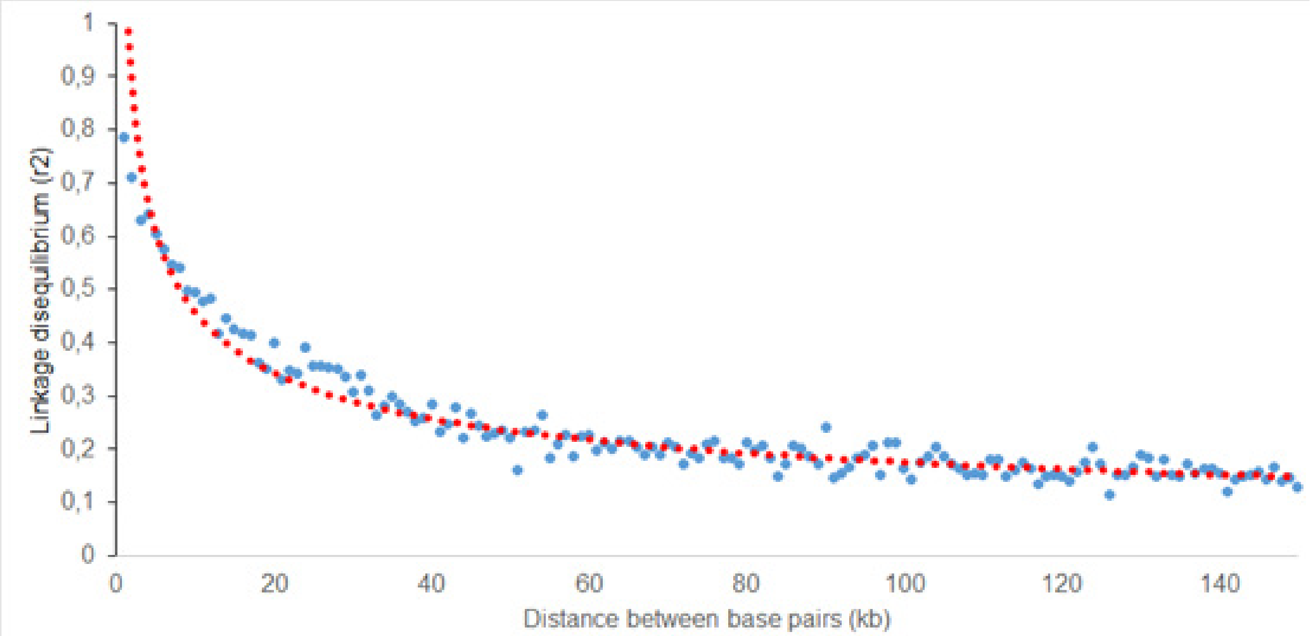

Figure 5. Decay and extension of linkage disequilibrium in Colombian Simmental cattle (red dots: trend values of the linkage disequilibrium decay, blue dots: observed values of the linkage disequilibrium decay) (Caída del desequilibrio de ligamiento en ganado Simmental de Colombia (puntos rojos: valores tendencia de la caída del desequilibrio de ligamiento, puntos azules: valores observados de la caída del desequilibrio de ligamiento)).

DISCUSSION

The Simmental population showed at least 50% of the SNPs with a MAF higher than 0.2. This agrees with Lin *et al.* (2010, p. 285), who also found in six *Bos taurus* breeds more than 50% of SNPs with a MAF > 0.2. In contrast, three *Bos indicus* breeds in the same study had 20% of SNPs with a MAF > 0.2. This trend may be associated with a higher implementation of breeding programs and stronger selection intensities in Bos taurus compared to *Bos indicus* populations, which causes higher number of SNPs in intermediate frequencies. Although Colombian Simmental population does not have an official breeding program, importing genetic material from selected populations in Europe and North America could explain that the distribution of MAFs is similar to results in improved populations (Edel *et al.* 2011, p. 152).

The observed and expected heterozygosity were higher than other studies in *Bos taurus* breeds. In local Angus and Holstein populations, the expected heterozygosity was 0.31 ± 0.18 and 0.31 ± 0.16, respectively (Makina *et al.* 2014, p. 4). Higher values and similar to this study were reported in Simmental cattle in Poland, with an expected heterozygosity of 0.37 ± 0.12 (Jasielczuk *et al.* 2016, p. 58). Although heterozygosity depends on factors such as selection, mutation and genetic drift, the highest value observed in Colombian Simmental population may be due to farmers own decisions and different selection criteria according to geographic or market conditions, which leads to higher diversity and mixture of genetic material from different populations. For example, Carruthers *et al.* (2011, p. 84) evaluated heterozygosity levels in Angus populations from different countries. The expected international heterozygosity mean was 0.40. However, in countries like Canada where the breed has been subject to genetic improvement and high selection intensity, the expected heterozygosity value was 0.26. The highest diversity found in Colombia can be caused by the genetic dependence from Europe and North America, where Simmental livestock populations have opposite selection criteria.

Although the Simmental population in Colombia is not a composite breed, the mixture of populations with different breeding objectives can contribute to the higher level of heterozygosity. Composite populations or those originated from the mixture of two populations with different selection criteria reported higher heterozygosity values (0.34) compared to populations originated from a single population (0.28) (Porto-Neto *et al.* 2014, p. 4). In breeds with high selection intensities such as Holstein, Karimi *et al.* (2016, p. 3) found similar values with a heterozygosity value of 0.36. However, those samples were obtained from different Holstein populations across the world. In Simmental, samples of animals from different geographical regions could also explain the high heterozygosity found. The inbreeding values consulted were higher than those reported for other Simmental populations. Marras *et al.* (2015, p. 6) reported an inbreeding average of 0.074 for a dual-purpose Simmental population. Nonetheless, for Colombian Simmental cattle, a low inbreeding level was expected due to the high heterozygosity values, the use of beef and milk foreign bulls that differ from their origins and relationships.

In contrast to the PCA results, the analysis of ancestry allowed visualizing the existence of three and two ancestral populations for Simmental in the K = 3 and K = 7 analysis, respectively. However, in the PCA, stratification signals were in agreement with the population of the United States evaluated by Decker *et al.* (2014). Furthermore, the probabilistic assignment showed that the Colombian population cannot be considered as pure. A population of Simmental cattle in Asia evaluated could not be considered pure either, although the most appropriate K value was 2, and the probabilistic assignments to the cluster with the highest participation were high (Agung *et al.* 2016, p. 181).

The probabilities assigned to the Colombian population were related to the genetic origin of each animal, which was given by the genetic line from which they were offspring. Wu *et al.* (2014, p. 2) found a similar stratification in a Simmental population and was associated with the geographic origin of the reference population and the genetic lines used in the analysis. Fan *et al.* (2015, p. 4) also found in Simmental the presence of subpopulations and a relationship between structure and geographical origin. In indicine breeds, the influence of the geographic effect and breeding farmer practices has also been identified on the genetic structure of populations (Uzzaman *et al.* 2014, p. 1384). For Colombia, this process was more evident due to the lack of selection criteria and the fact of mixing beef and dairy lines according to the geographical conditions. Mckay *et al.* (2007, p. 4) suggested that the stratification within breeds may indicate that direct or indirect selection for different productive purposes leads to a differentiation of the genome. This could have greater importance for countries as Colombia where Simmental population has small values in the effective size and where the breeding programs are based on the massive use of a few foreign bulls.

The LD decreased with an increase in the physical distance of the SNP´s (**Figure 5**). The influence of the MAF (> 0.05, > 0.1 and > 0.2) on the decrease of the LD was explored. However, it did not show large differences in at short distances <20 kb or larger distances as 100 kb. Jasielczuk *et al.* (2016, p. 60) found values of at distances <25 kb and this increased to 0.42 when SNP´s with MAF>0.2 were evaluated. Similarly, the mean of in a population of Simmental beef cattle in China was 0.3 at a distance <25 kb (Zhu *et al.* 2013, p. 775). Although these populations are subject to selection, it is possible that the low global values are because selection occurs in different traits and populations. The above mentionated can generate a higher diversity and in turn, a lower correlation between SNP´s, with low LD values at short distances and a reduced number of fixed regions in the genome.

In incidine breeds, Espigolan *et al.* (2013, p. 4) found lower values of 0.2 and 0.11 at distances of 30 and 100 kb, respectively. These differences between *Bos taurus* and *Bos indicus* have been documented (Pérez *et al.* 2014, p. 6) and can partially be explained due to differences in the allelic frequencies of the SNP´s, where the *Bos taurus* breeds generally have high proportions of SNP´s with MAF > 0.2. Although low values for LD were expected in the population of Colombia, the between SNP´s was higher compared to the incidine breeds consulted, possibly due to high selection intensities in foreign populations that contribute with genetic material in Colombian Simmental population.

The results can also be very different within breeds. In a Holstein population of the United States, values of > 0.8 were shown at distances of 73.4 Kb (Kim & Kirkpatrick. 2009, p. 282). Another study in Holstein in North America reported an average of > 0.55 at a distance <100 Kb (Sargolzaei *et al.* 2008, p. 2111). Contrarily, Qanbari *et al.* (2010, p. 349) found in Germany Holstein population values of 0.29 at a distance of 50 Kb, showing differences between subpopulations that can be given by different factors such as selection, migration and genetic drift. Therefore, the LD in Colombian Simmental cattle could increase if breeders decide to implement an independent breeding program for dairy, beef and dual-purpose Simmental lines.

An important alternative for countries with small genotyped populations is the incorporation of genotyped animals from other countries. One of the most crucial requirements for this strategy is to guarantee the LD phase through all the populations (Roos *et al.* 2008, p. 1511; Mouresan *et al.* 2017, p. 60). The high genetic connectivity of European and American Simmental population with Colombia could be an opportunity to increase the genotyped population and accuracy of genomic estimates. Likewise, the presence of three subpopulations in this study must be considered into account in genome-wide association studies and the values suggest that genotyping animals must be at least with 50000 SNPs to obtain reliable genomic association estimations.

CONCLUSIONS

The intensive use of artificial insemination and the simultaneous use of European and North American bulls could be the major causes of the the high levels estimated of genetic diversity. This reveals opportunities to implement selection schemes into the the Colombian Simmental population. The results of this study also confirm the effect that interbreeding practice among two subpopulations with opposite breeding objectives can have on the changes in the genetic structure. Although genetic diversity in Colombian Simmental population was higher than in other studies, the genetic progress could be lower due to the lack of a local defined breeding program, especially when farmers are mating animals from milk, beef and dual-purpose Simmental lines. Finally, the population assignment probabilities estimated in this study can be a criteria to decide designing a breeding program that considers different breeding objectives and genetic lines according to local needs and farmers preferences to produce milk and beef in Colombian local conditions.

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