

Evaluation of the Romosinuano cattle population structure in Mexico using pedigree analysis

Evaluación de la estructura poblacional de bovinos Romosinuano en México mediante el análisis de pedigrí

Avaliação da estrutura populacional de bovinos Romosinuano no México usando análise de pedigree

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Abstract

Background: Romosinuano cattle breed in Mexico has endured isolation and it is necessary to characterize it in order to facilitate sustainable genetic management. **Objective:** To assess the evolution of the structure and genetic diversity of the Romosinuano breed in Mexico, through pedigree analysis. **Methods:** Pedigree data was obtained from Asociación Mexicana de Criadores de Ganado Romosinuano y Lechero Tropical (AMCROLET). The ENDOG program (4.8 version) was used to analyze two datasets, one that includes upgrading from F_1 animals (UP) and the other with only straight-bred cattle (SP). For both datasets, three reference populations were defined: 1998-2003 (RP1), 2004-2009 (RP2), and 2010-2017 (RP3). The pedigree included 3,432 animals in UP and 1,518 in SP. Demographic parameters were: Generation interval (GI), equivalent number of generations (EG), pedigree completeness index (PCI), and gene flow among herds. Genetic parameters were: Inbreeding (F) and average relatedness (AR) coefficients, effective population size (N_{ec}), effective number of founders and ancestors, and number of founder genome equivalents. **Results:** The GI varied from 6.10 to 6.54 for UP, and from 6.47 to 7.16 yr for SP. The EG of the UP and SP improved $>63\%$ from RP1 to RP3. The PCI increased over time. No nucleus or isolated herds were found. For RP3, F and AR reached 2.08 and 5.12% in the UP, and 2.55 and 5.94% in the SP. For RP3, N_{ec} was 57 in the UP and 45 in the SP. Genetic diversity losses were attributed mainly ($>66\%$) to genetic drift, except for RP3 in the SP (44%). **Conclusions:** A reduction of the genetic diversity has been occurring after the Romosinuano breed association was established in Mexico, and this is mainly due to random loss of genes.

Keywords: *effective population size; gene flow; genetic diversity; genetic drift; generation interval; inbreeding; pedigree; population structure; probability of gene origin; Romosinuano cattle.*

Resumen

Antecedentes: La raza bovina Romosinuano ha estado prácticamente aislada en México y requiere ser caracterizada para un manejo genético sostenible. **Objetivo:** Evaluar la evolución de la estructura y diversidad genética de la raza Romosinuano en México, mediante el análisis del pedigrí. **Métodos:** Los datos genealógicos provinieron de la Asociación Mexicana de Criadores de Ganado Romosinuano y Lechero Tropical (AMCROLET). Los análisis se realizaron con el programa ENDOG (versión 4.8) para dos bases de datos, una que incluyó animales en cruzamiento absorbente (UP) a partir de F_1 y la otra con sólo animales puros (SP). Para ambas bases de datos se definieron tres poblaciones de referencia: 1998-2003 (RP1), 2004-2009 (RP2), y 2010-2017 (RP3). El pedigrí incluyó 3.432 animales en la UP y 1.518 en la SP. Los parámetros demográficos fueron: intervalo generacional (GI), número de generaciones equivalentes (EG), índice de completitud del pedigrí (PCI), y flujo de genes entre hatos. Los parámetros genéticos fueron: coeficientes de consanguinidad (F) y de relación genética aditiva (AR), tamaño efectivo de la población (N_{ec}), número efectivo de fundadores y ancestros, y número equivalente de genomas fundadores. **Resultados:** El GI varió de 6,10 a 6,54 para la UP, y de 6,47 a 7,16 años para la SP. El EG de la UP y la SP mejoró $>63\%$, de RP1 a RP3. El PCI aumentó a través de los años, pero más para la SP que para la UP. No se encontraron hatos núcleo o aislados. Para RP3, F y AR alcanzaron 2,08 y 5,12% en la UP, y 2,55 y 5,94% en la SP. Para RP3, N_{ec} fue 57 en la UP y 45 en la SP. Más de 66% de las pérdidas en diversidad genética se debieron a deriva genética, excepto para RP3 en la UP (44%). **Conclusiones:** una reducción de la diversidad genética ha estado ocurriendo después de que se formó la asociación de criadores de ganado Romosinuano en México, y es debida principalmente a pérdidas aleatorias de genes.

Palabras clave: *consanguinidad; deriva genética; diversidad genética; estructura poblacional; flujo de genes; ganado Romosinuano; intervalo generacional; pedigrí; probabilidad de origen del gen; tamaño efectivo de población.*

Resumo

Antecedentes: A raça bovina Romosinuano tem estado praticamente isolada no México e precisa ser caracterizada para um manejo genético sustentável. **Objetivo:** Avaliar a evolução da estrutura e diversidade genética da raça Romosinuano no México, através da análise de pedigree. **Métodos:** Os dados genealógicos vieram da Associação Mexicana de Criadores de Ganado Romosinuano y Lechero Tropical (AMCROLET). As análises foram feitas com o programa ENDOG (versão 4.8) para duas bases de dados, uma que incluiu animais em cruzamento absorbente (UP) a partir da F_1 e a outra base de dados somente com animais puros (SP). Para ambas bases de dados foram definidas três populações de referência: 1998-2003 (RP1), 2004-2009 (RP2) e 2010-2017 (RP3). O pedigree incluiu 3.432 animais na UP e 1.518 na SP. Os parâmetros demográficos foram: intervalo entre gerações (GI), número de gerações equivalentes (EG), índice de completude do pedigree (PCI), e fluxo de genes entre rebanhos. Os parâmetros genéticos foram: coeficiente de consanguinidade (F) e da relação genética aditiva

(AR), tamanho efetivo da população (N_{ec}), número efetivo de fundadores e ancestrais, e número equivalente de genomas fundadores. **Resultados:** O GI variou de 6,10 a 6,54 para a UP, e de 6,47 a 7,16 anos para a SP. EG da UP e a SP melhorou >63%, de RP1 a RP3. O PCI aumentou ao longo dos anos, mas mais para a SP do que para o UP. Não se encontraram rebanhos núcleo ou isolados. Para RP3, F e AR alcançaram 2,08 e 5,12% na UP, e 2,55 e 5,94% na SP. Para RP3, N_{ec} foi 57 na UP e 45 na SP. Mais de 66% das perdas em diversidade genética foram ocasionadas pela deriva genética, exceto para RP3 no UP (44%). **Conclusões:** Depois que a associação da raça Romosinuano foi estabelecida no México, tem ocorrido uma redução da diversidade genética, principalmente devido a perdas aleatórias de genes.

Palavras-chave: consanguinidade; deriva genética; diversidade genética; estrutura populacional; fluxo de genes; intervalo entre gerações; pedigree; probabilidade de origem do gene; Romosinuano; tamanho efetivo da população.

Introduction

Romosinuano, a Creole breed developed in Colombia, is based on cattle that arrived from the Iberian Peninsula five centuries ago. According to De Alba (2011), Romosinuano germplasm entered into México as semen. Semen from Colombia was first exported to North Carolina, USA, in the middle of the 20th century. Then, semen was exported to Venezuela in 1982. A research herd was founded in Turrialba, Costa Rica, in 1955 using sires and heifers imported from North Carolina. Subsequently Romosinuano embryos were imported by Florida State University, from Venezuela. The Mexican herd was developed from germplasm of Turrialba, and later from genetics of Florida State University.

Several breeders in the Mexican tropics have used Romosinuano due to ease of management, longevity (Carroll *et al.*, 2011; 2012), fertility (Riley *et al.*, 2007), tolerance to heat, humidity (Scharf *et al.*, 2010), and to the toxic effects of endophytes commonly present in tall fescue (Riley *et al.*, 2016). The Asociación Mexicana de Criadores de Ganado Romosinuano y Lechero Tropical (AMCROLET) was established in 1998 (De Alba, 2011), and it is responsible for the herd-book and the performance records. Recently, the first Romosinuano genetic evaluation in Mexico was carried out (AMCROLET, 2016), which would allow breeders to identify superior breeding stock for genetic improvement. However, the selection of breeding stock may result in a smaller population size and in higher inbreeding (Bernardes *et al.*, 2016). This is even

more important considering that the Mexican herd of Romosinuano is relatively isolated due to sanitary restrictions that would not allow import genetic material directly from Colombia (SAGARPA, 2017).

The main goal of conservation programs is to control the effects of genetic drift in order to maintain the genetic variability of the population and to keep a low rate of inbreeding (Jamieson and Allendorf *et al.*, 2012; Hammerly *et al.*, 2013; Toro *et al.*, 2014). Genetic variability works similar to an 'insurance' for the population to face potential environmental and marketing changes; at the same time, it reduces the unfavorable effects on performance due to inbreeding (Burrow *et al.*, 1993; Santana *et al.*, 2012). The studies on genetic variability of populations have been conducted using pedigree analysis or genetic markers. Pedigree information provides a useful tool for conservation programs to maintain genetic variability and minimize inbreeding (Pinheiro *et al.*, 2013; Santana *et al.*, 2016; Sheikhlou and Abbasi *et al.*, 2016).

Considering the potential of Romosinuano cattle to improve beef production under tropical conditions, the availability of predicted breeding values, and the fact that it is a breed with a small population size and relatively isolated, it is imperative to characterize the structure of this population and evaluate changes in its genetic variability. Therefore, the objective of this study was to assess the evolution of the structure and genetic diversity of Romosinuano breed in Mexico through pedigree analysis.

Materials and methods

Description of the data

Pedigree data (identification of animal, sire and dam, sex, birth date and herd) was obtained from AMCROLET. The database included 4,809 animals born between 1953 and 2017, belonging to 39 herds located mainly in the Southeast region of Mexico.

Given that the Romosinuano population has been developing from upgrading, pedigree analyses were performed for two datasets: one that includes upgrading from F_1 animals (UP; $n = 3,432$) and the other with only straightbred cattle (SP; $n = 1,518$); thus, SP is included in UP. For both datasets, three reference populations were defined: 1998-2003 (RP1), 2004-2009 (RP2), and 2010-2017 (RP3) to describe the time-trend of pedigree parameters for approximately three generation intervals after AMCROLET was created.

Pedigree analyses

The analyses of genealogical information were carried out using ENDOG version 4.8 (Gutiérrez and Goyache, 2005).

Demographic parameters

Generation interval (GI). The GI (average age of the parents when their replacements were born) was obtained for the four gametic paths (sire-son, sire-daughter, dam-son, dam-daughter).

Pedigree completeness level. To evaluate the level of integrity of the pedigree, the number of complete (CG), maximum (MG) and equivalent (EG) generations were calculated. The CG implies that the 2^n ancestors of an animal are known, where n is the number of fully traced generations, whereas MG counts the number of generations that separate the animal from

the oldest ancestor (Gutiérrez y Goyache *et al.*, 2005). For a given animal, EG was calculated as the sum of $(1/2)^n$ coefficients of all known ancestors, where n is the number of generations separating the individual to each known ancestor (Maignel *et al.*, 1996). The percentage of known ancestors during the last three generations was calculated, a period that encompasses the time from AMCROLET formation. Finally, the pedigree completeness index (PCI; MacCluer *et al.*, 1983) was generated for each generation.

Gene flow among herds. The role of each herd was evaluated according to the contribution of the sires to the population (Vassallo *et al.*, 1986). Herds were grouped as: a) nucleus, if breeders only use their own sires, which may also be sent to other herds; b) multiplier, when breeders use sires from other herds and provide them to other herds; c) commercial, if breeders use sires from other herds, but do not deliver sires; and d) isolated, when breeders only use own sires and do not provide them to other herds.

Genetic parameters

Inbreeding (F) and average relatedness coefficients (AR). The F was calculated using the algorithm of Meuwissen and Luo *et al.* (1992), assuming unrelated and noninbred founders. For each animal, AR was defined as the probability that an allele randomly chosen in the population belongs to a particular animal (Gutiérrez and Goyache *et al.*, 2005). The AR for a particular animal is equivalent to the average of the coefficients in the row of the numerator relationship matrix.

Effective population size (N_e). The N_e was estimated using two methods. The first was based on the individual increase in inbreeding (ΔF_i) and was computed as follows (Gutiérrez *et al.*, 2009):

$$\Delta F_i = 1 - \sqrt[t-1]{1 - F_i}$$

where, t is the number of equivalent generations. The N_e was calculated from the average of the inbreeding rate of the n animals included, as .

$$\overline{N_e} = \frac{1}{2\Delta F}$$

The second method was based on increases in coancestry (Δc_{jk}) between any pair of individuals j and k (Cervantes *et al.*, 2011), as:

$$\Delta c_{jk} = 1 - \frac{g_j + g_k}{2} \sqrt{1 - c_{jk}}$$

where, c_{jk} is the inbreeding of a progeny from j and k , and g_j and g_k are the discrete equivalent generation for the parents. Then, N_e was estimated by averaging the rate of coancestry (N_{ec}) for all pairs of the individuals as.

$$\overline{N_{ec}} = \frac{1}{2\Delta c}$$

Probability of gene origin. To assess the amount and kind of genetic diversity losses in the populations, the number of founders (f), effective number of founders (f_e), effective number of ancestors (f_a), and founder genome equivalents (f_g) were obtained. The f_e counts founders with unknown genetic relationships with other animals in the pedigree, except to its descendant (Lacy, 1989). Preservation of genetic diversity of founders toward the studied population may be evaluated by its contributions, that must add up to one (Boichard *et al.*, 1997).

The f_e is the number of equally contributing founders that would be expected to produce the same amount of genetic diversity as observed in the population under study (Lacy, 1989), and was calculated as:

$$f_e = \frac{1}{\sum_{k=1}^f (p_k^2)}$$

where, p_k is the probability of gene origin of founder k to the reference population. The f_a is the minimum number of ancestors (founders or not) needed to explain the full genetic diversity of the population (Boichard *et al.*, 1997), and it was obtained as:

$$f_a = \frac{1}{\sum_{i=1}^a (p_i^2)}$$

where, p_i is the marginal contribution of the i^{th} ancestor (genetic contribution not yet explained by other ancestor chosen before), and a is the number of influential ancestors (i.e. ancestors with non-zero marginal contributions). Additionally, based on the marginal contributions, the number of ancestors explaining 50% of the genes in the population and the contribution of the main ancestor were obtained.

The f_g was calculated as the inverse of twice the average coancestry of individuals in the predefined reference population. This parameter accounts for all causes of loss of genetic diversity in the population (Caballero and Toro *et al.*, 2000).

The effective number of non-founders (f_n) accounts only for the genetic drift effects in non-founder generations (Caballero and Toro *et al.*, 2000):

$$\frac{1}{f_g} = \frac{1}{f_e} + \frac{1}{f_n}$$

Thus, the f_g has two components, f_e that accounts for the contributions of founders to the actual population, and f_n which accounts for the contribution of non-founders, and is accumulated over generations. Based on these parameters, the genetic diversity (GD) in the reference population relative to the base population was obtained as (Lacy, 1989, 1995):

Therefore, $1 - GD$ represents the loss of genetic diversity in the population since the

founder generation. The loss of genetic diversity because of unequal contributions of founders was estimated as $1 - GD^*$, where (Caballero and Toro *et al.*, 2000):

Finally, the difference between GD and GD^* accounts for the loss of genetic diversity due to genetic drift accumulated over the non-founder generations (Caballero and Toro, 2000), and it can be expressed as the inverse of twice the number of non-founders (Lacy, 1995).

Results

Demographic parameters

For the UP, average GI was similar from RP1 to RP3 (6.51 to 6.54 yr); however, for the SP these values tended to decrease from 7.16 in RP1 to 6.57 yr in RP3 (Table 1). Among parent-offspring pathways, the GI for path sire-son were larger and tended to decrease from RP1 to RP3 in both the UP and the SP, and were half to one year longer for the SP than the UP.

Table 1. Generation intervals (yr) for the upgraded and straightbred Romosinuano and their respective reference populations (RP1, RP2, and RP3).

| Breeding paths | Upgraded (UP) | | | Straightbred (SP) | | |
|------------------|---------------|------|------|-------------------|------|------|
| | RP1 | RP2 | RP3 | RP1 | RP2 | RP3 |
| Sire-son | 8.41 | 7.81 | 6.97 | 8.98 | 8.20 | 7.96 |
| Sire-daughter | 6.73 | 5.83 | 6.12 | 7.53 | 5.93 | 6.38 |
| Dam-son | 5.84 | 7.02 | 6.79 | 5.87 | 6.73 | 6.35 |
| Dam-daughter | 5.68 | 6.83 | 6.79 | 5.59 | 6.55 | 6.44 |
| Parent-offspring | 6.41 | 6.42 | 6.54 | 7.16 | 6.47 | 6.57 |

Generation intervals = average age of the parents when their replacements are born; RP1 = 1998-2003; RP2 = 2004-2009; RP3 = 2010-2017.

Table 2. Number of maximum (MG), complete (CG) and equivalent (EG) generations traced for the upgraded and straightbred Romosinuano and their respective reference populations (RP1, RP2, and RP3).

| Generations | Upgraded (UP) | | | Straightbred (SP) | | |
|-------------|---------------|------|------|-------------------|------|------|
| | RP1 | RP2 | RP3 | RP1 | RP2 | RP3 |
| MG | 5.34 | 7.24 | 9.02 | 5.15 | 7.07 | 8.80 |
| CG | 0.82 | 1.07 | 1.17 | 0.47 | 0.96 | 1.56 |
| EG | 2.10 | 2.89 | 3.43 | 1.75 | 2.54 | 3.34 |

RP1 = 1998-2003; RP2 = 2004-2009; RP3 = 2010-2017.

The level of completeness of the UP and the SP pedigrees improved from RP1 to RP3 more than 69, 43 and 63% for MG, CG and EG, respectively, after herd-book recording started (Table 2).

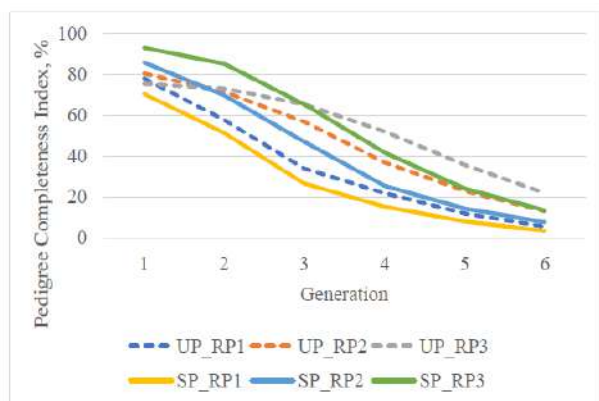
The EG reached 3.43 and 3.34 yr for the UP and the SP in RP3. The percentage of known ancestors improved during the recent 20 yr and for the UP averaged 76, 73 and 66% for the generations of parents, grandparents and great-grandparents, respectively, and those values for the SP were 93, 85 and 65% (Table 3); thus, the improvement was higher for the SP.

Table 3. Percentage of known ancestors by generation for the upgraded and straightbred Romosinuano and their respective reference populations (RP1, RP2, and RP3).

| Generation | Upgraded (UP) | | | Straightbred (SP) | | |
|--------------------|---------------|-------|-------|-------------------|-------|-------|
| | RP1 | RP2 | RP3 | RP1 | RP2 | RP3 |
| Parents | 78.00 | 80.50 | 75.50 | 70.50 | 85.50 | 93.00 |
| Grandparents | 57.75 | 71.50 | 73.00 | 51.25 | 70.00 | 85.00 |
| Great-grandparents | 33.88 | 56.75 | 65.50 | 26.50 | 46.88 | 65.25 |

RP1 = 1998-2003; RP2 = 2004-2009; RP3 = 2010-2017.

Considering various generations, there was a steady improvement of the PCI during the recent 20 yr (Figure 1). Including three generations back, the PCI of RP3 were similar, 65.3 and 65.2% for the UP and the SP, but it increased to 75.4 and 93.1%, respectively, when only one generation was considered.

**Figure 1.** Pedigree completeness index for the upgraded (UP) and straightbred (SP) Romosinuano and their respective reference populations (RP1, 1998-2003; RP2, 2004-2009; and RP3, 2010-2017) for generations 1 to 6.

Mostly multiplier herds were found in this Romosinuano population (67 and 77% in the UP and the SP, respectively). Commercial herds in the UP were about twice as many as those in the SP, while no nucleus was found (Table 4).

Table 4. Classification of herds according to the origin and use of sires for the upgraded (UP) and straightbred (SP) Romosinuano populations.

| Item | UFS | UOS | SS | UP | | SP | |
|------------|-----|-----|-----|-----|-----|-----|-----|
| | | | | NoH | PFS | NoH | PFS |
| Nucleus | No | Yes | Yes | 0 | 0 | 0 | 0 |
| Multiplier | Yes | Yes | Yes | 15 | 91 | 12 | 88 |
| Multiplier | Yes | No | Yes | 11 | 100 | 12 | 100 |
| Commercial | Yes | Yes | No | 0 | 0 | 0 | 0 |
| Commercial | Yes | No | No | 13 | 100 | 7 | 100 |
| Isolated | No | Yes | No | 0 | 0 | 0 | 0 |

UFS = use foreign sires; UOS = use own sires; SS= sell sires; NoH = number of herds; PFS = percent of foreign sires.

As previously indicated, development of Mexican Romosinuano population was based mainly on germplasm importation from a herd in Turrialba, Costa Rica (herd 75), which is depicted in Figure 2. Regarding the UP, some herds (e.g. herd 12) included a large number of dams in upgrading to Romosinuano. In the SP, the genetic representation of herd 75 is declining, whereas that of Mexican herds are increasing (e.g. herd 40; 9.7 to 23.5% from RP1 to RP3, respectively).

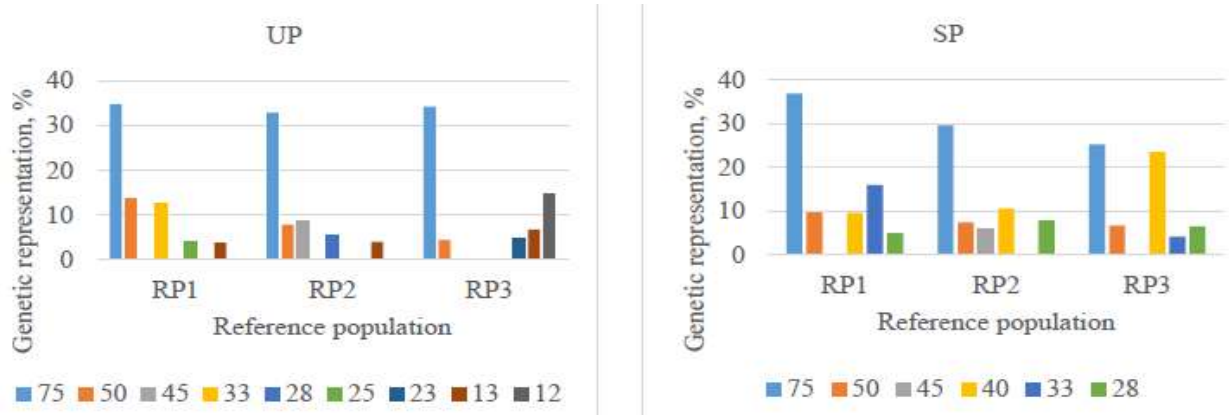


Figure 2. Main founder herds genetically represented (%) in RP1 (1998-2003), RP2 (2004-2009), and RP3 (2010-2017) in upgraded (UP) and straightbred (SP) Romosinuano populations.

Genetic parameters

Generally, all inbreeding statistics increased from RP1 to RP3 (Table 5). Considering RP3,

all inbreeding statistics were higher in the SP than the UP, except for the percentage of average inbreeding in inbred animals.

Table 5. Inbreeding statistics (%) for upgraded and straightbred Romosinuano and their respective reference population (RP1, RP2, and RP3).

| Item | Upgraded (UP) | | | Straightbred (SP) | | |
|------|---------------|-------|-------|-------------------|-------|-------|
| | RP1 | RP2 | RP3 | RP1 | RP2 | RP3 |
| IA | 34.52 | 44.57 | 42.07 | 23.08 | 49.49 | 65.83 |
| AR | 4.26 | 5.07 | 5.12 | 4.38 | 5.56 | 5.94 |
| IP | 0.97 | 2.17 | 2.08 | 0.65 | 1.99 | 2.55 |
| IF | 2.82 | 4.88 | 4.94 | 2.80 | 4.01 | 3.87 |

RP1 = 1998-2003; RP2 = 2004-2009; RP3 = 2010-2017; IA = inbred animals; AR = average relatedness; IP = average inbreeding in the population; IF = average inbreeding in inbred animals.

Table 6. Average inbreeding coefficients (*F*), inbred animals and relatedness coefficients (*AR*) for upgraded and straightbred Romosinuano populations, by complete generations.

| Generation | N | <i>F</i> (%) | Inbred animals (%) | <i>F</i> in inbred animals (%) | <i>AR</i> (%) |
|-------------------------------------|-------|--------------|--------------------|--------------------------------|---------------|
| <i>Upgraded population (UP)</i> | | | | | |
| 0 | 1,610 | 0.00 | 0.00 | 0.00 | 3.21 |
| 1 | 702 | 1.61 | 51.14 | 3.14 | 4.86 |
| 2 | 739 | 3.59 | 78.21 | 4.59 | 6.18 |
| 3 | 341 | 5.52 | 100.00 | 5.52 | 7.23 |
| 4 | 40 | 6.65 | 100.00 | 6.65 | 8.57 |
| <i>Straightbred population (SP)</i> | | | | | |
| 0 | 474 | 0.00 | 0.00 | 0.00 | 3.28 |
| 1 | 538 | 1.91 | 58.18 | 3.29 | 5.68 |
| 2 | 436 | 3.46 | 80.73 | 4.28 | 6.79 |
| 3 | 70 | 3.68 | 100.00 | 3.68 | 7.20 |

When inbreeding statistics were obtained for complete generations in the UP and the SP (Table 6) the average F reached 6.7 and 3.7%, respectively, and all animals were inbred in the most recent generations (3 and 4 for the UP and 3 for the SP). The AR has been increasing every complete generation from 3.2 and 3.3% to 8.6 and 7.2% for the UP and the SP, respectively.

Even when both N_e and N_{ec} were larger for the UP than the SP, for N_e there was a reduction of these sizes in RP2 and a subsequent increase to reach sizes of 79 and 48 in RP3 (Table 7).

Estimates of N_{ec} were smaller than N_e and increased over the recent 20 yr for the UP and the SP, reaching in RP3 57 and 45, respectively

Table 7. Realized effective population size (N_e) and effective population size from an increase in coancestry (N_{ec}) for the upgraded and straightbred Romosinuano and their respective reference populations (RP1, RP2, and RP3).

| Item | RP1 | RP2 | RP3 |
|---------------------|-------|-------|-------|
| <i>Upgraded</i> | | | |
| N_e | 61.99 | 49.08 | 78.51 |
| N_{ec} | 39.49 | 44.92 | 56.53 |
| <i>Straightbred</i> | | | |
| N_e | 46.19 | 34.93 | 47.68 |
| N_{ec} | 35.32 | 38.45 | 44.81 |

RP1 = 1998-2003; RP2 = 2004-2009; RP3 = 2010-2017.

The parameters describing the probabilities of gene origin for the UP and the SP and their respective reference populations (RP1, RP2, and RP3) are shown in Table 8. The f in the UP were larger than in the SP and increased from RP1 (273) to RP3 (827). Although f_e remained approximately constant across reference populations the ratio f_e/f for the UP decreased from RP1 (22.0%) to RP3 (7.4%) whereas those ratios for the SP were larger and varied from

20.3 to 32.2%. During the last 20 years, f_a in the SP decreased 16.7% from RP1 to RP3 and f_g had a reduction of 17.6 and 30.1% for the UP and the SP, respectively. The f_a represented about 50% of the f_e . The f_g represented 27 and 33% of f_e for the UP and the SP, respectively. From RP1 to RP3 there was a reduction of the proportion of founder genomes that have been retained in the reference populations (f_g/f), from 7.3 to 2.0% in the UP and from 10.6 to 7.4% in the SP.

Table 8. Probability of gene origin parameters for the upgraded and straightbred Romosinuano and their respective reference populations (RP1, RP2, and RP3).

| Item | Upgraded (UP) | | | Straightbred (SP) | | |
|-------------|---------------|------|------|-------------------|------|------|
| | RP1 | RP2 | RP3 | RP1 | RP2 | RP3 |
| F | 273 | 527 | 827 | 183 | 251 | 184 |
| f_e | 60 | 56 | 61 | 59 | 51 | 50 |
| f_a | 31 | 29 | 33 | 30 | 24 | 25 |
| f_g | 20.0 | 16.7 | 16.5 | 19.3 | 15.4 | 13.5 |
| C_{max} % | 11.0 | 9.9 | 8.6 | 9.8 | 10.3 | 9.9 |
| N_{50} | 12 | 10 | 11 | 11 | 9 | 9 |

RP1 = 1998-2003; RP2 = 2004-2009; RP3 = 2010-2017; f = number of founders; f_e = effective number of founders; f_a = effective number of ancestors; f_g = equivalent number of founder genomes; C_{max} = contribution of the main ancestor; N_{50} = number of ancestors explaining 50% of the gene pool.

Total losses of genetic variability from RP1 to RP3 increased from 2.5 to 3.0% in the UP and from 2.6 to 3.7% in the SP (Figure 3).

More than 66% of the losses on genetic diversity were accounted for genetic drift, except for RP3 in the SP (44%).

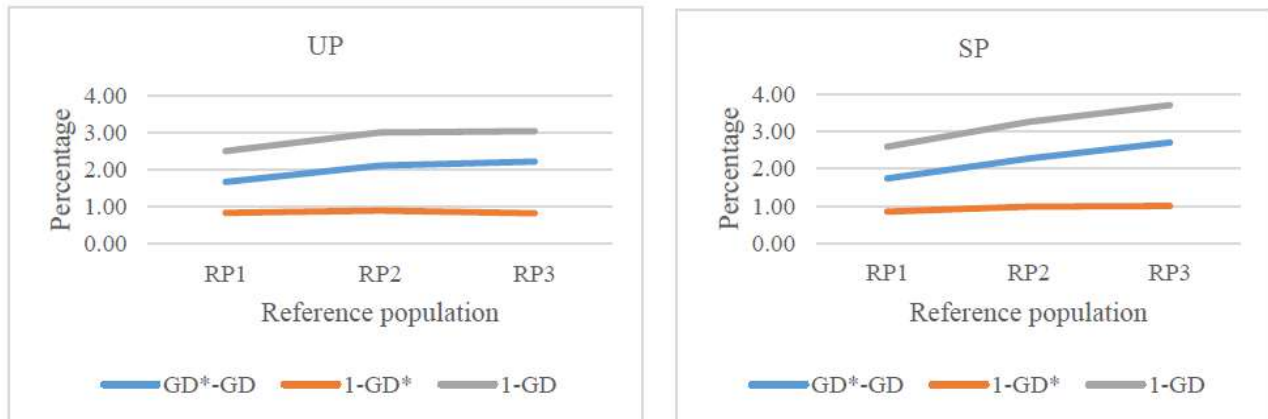


Figure 3. Losses (%) of genetic diversity due to genetic drift (GD*-GD), unequal founder contribution (1-GD*), and both reasons (1-GD) since the founder generation in upgraded (UP) and straightbred (SP) Romosinuano reference populations (RP1, 1998-2003; RP2, 2004-2009; and RP3, 2010-2017).

The main ancestor contributed 8.6 to 11.0, and 9.8 to 10.3% of the genes in the UP and the SP, respectively. The numbers of ancestors that explained half of the genetic variability of the populations ranged from 10 to 12 in the UP, and 9 to 11 in the SP, with a decreasing trend from RP1 to RP3.

Discussion

The average GI observed in the present study for the different reference populations (6.41 to 6.54 yr for UP and 6.47 to 7.16 yr for SP; Table 1) were larger than those of Colombian creole breeds such as Costeño con Cuernos (CCC, 5.4 yr), Blanco Orejinegro (BON, 4.7 yr) and Romosinuano (ROM, 5.7 yr), but similar to Sanmartinero (SM, 6.8 yr) (Martínez *et al.*, 2008), and to Brazilian Lageana Crioula cattle breed (6.41 yr; Pezzini *et al.*, 2018). Gutiérrez *et al.* (2003) estimated an interval of GI in eight autochthonous Spanish cattle breeds (Alistana, Asturiana de la Montaña, Asturiana de los Valles, Avileña–Negra Ibérica, Bruna dels Pirineus, Morucha, Pirenaica and Sayaguesa) of 3.7 to 6.1 yr. However, compared to the present study, larger GI were reported in Kumamoto (9.4 yr) and Kouchi (10.4 yr)

Japanese Brown cattle breeds (Honda *et al.*, 2006). Differences of GI among breeds may be the result of variations in calving age, fluctuating environmental conditions through the years in the tropic (Rosendo *et al.*, 2018), number of years that sires and dams are used, and breeding objectives; for example, large GI are convenient in conservation breeding programs.

Regarding the integrity of the pedigree, the EG of the present study increased during the recent 20 yr and reached in RP3, 3.43 for UP and 3.34 for SP (Table 2). Those estimates were smaller than those for Romosinuano (4.8), SM (3.8) and CCC (3.7) Colombian creole breeds (Martínez *et al.*, 2008) but higher than the interval 0.81 to 2.97 in eight Spanish autochthonous breeds (Gutiérrez *et al.*, 2003), the EG in BON (3.1; Martínez *et al.*, 2008), and the estimate in Lageana Crioula cattle breed (0.77, Pezzini *et al.*, 2018). The PCI is an important measure of the quality of inbreeding estimates from the pedigree since PCI represents a harmonic mean of parental genetic contributions and equals zero if any parent is unknown regardless of how deep and complete is the pedigree for the other parent. Similarly, inbreeding can be estimated only if information for both parents is known

(Battagin *et al.*, 2010; Onogi *et al.*, 2017). Estimates of PCI in RP3 from the present study (65.3 for UP and 65.2% for SP, and considering three generations back, and 75.4 and 93.1% considering one generation back; Figure 1) were lower than the PCI (90 and 69% in 2009 considering 2 and 5 generations, respectively) observed by Battagin *et al.* (2010) in Burlina native Italian cattle, and those reported by Santana *et al.* (2014) for Brazilian Gir cattle (99.8, 96.3 and 92.8% for generations 1, 2 and 3, respectively). The observed differences of EG and PCI among studies could be the result of variations in the number of generations traced and the quality of pedigree recording.

The three different ways to evaluate the degree of pedigree completeness (number of MG, CG and EG; percentage of known ancestors up to three generations; and PCI) indicated that for the UP and the SP the integrity of the pedigree was improved during the latest 20 yr. It is clear that an important progress has occurred after AMCROLET was established; however, these alternative measures suggest that greater efforts are needed in the recording of genealogical data to improve the accuracy of inbreeding parameters in this population (Boichard *et al.*, 1997).

Given that Romosinuano breed was developed in Colombia and later introduced into Mexico, no nucleus herds were found. The high proportion of multiplier herds found in the present study indicates that the population is growing. Similar results were reported by Silva *et al.* (2016). Since the demand for Romosinuano germplasm in Mexico is increasing, commercial herds in the UP were about twice as many as those in the SP. Mexican herds (like herd 40, Figure 3) are having an increasing genetic representation in the population. Thus, it is beneficial if breeders share germplasm from various herds to avoid short-term significant increases in inbreeding, with the consequent reduction in animal performance (Burrow, 1993; Santana *et al.*, 2012). Germplasm used

in the UP matings mainly comes from the SP; thus, it is important to compare results of the SP and RP3 with other studies. The percentage of inbred animals in SP (65.8%) was higher than the range 5.8 to 30.8% in Colombian creole breeds (Martínez *et al.*, 2008), and the interval 2 to 32% in Spanish autochthonous breeds (Gutiérrez *et al.*, 2003), but lower than in Alentejana Portuguese (nearly 80%; Carolino and Gama, 2007), and in Burlina Italian cattle (>80%; Battagin *et al.*, 2010). Possible causes of these differences among studies could be the pedigree completeness, monitoring of matings to avoid inbreeding, and gene flow among herds. Even though averages of F for the Romosinuano breed in Mexico have been increasing during the last 20 yr, they are still low (2.5 and 3.9% for all and inbred animals, respectively; Table 5). Lower values (<1.41% for the reference population, and <3.21% for inbred animals) were estimated for various breeds by Martínez *et al.* (2008), except for Romosinuano that had 3.1 and 4.0%, respectively. These differences may be due mainly to variations in effective population sizes, integrity level of the pedigree, and efficacy to prevent inbreeding. The AR was high (5.9%) as compared to the interval 2.0 to 3.9% reported by Martínez *et al.* (2008) in four Colombian breeds, and the range 0.10 to 1.70% by Gutiérrez *et al.* (2003) in eight Spanish breeds. The size of AR is important to predict the F of following generations since, under random mating, F of the progeny will be half of the AR between parents. For RP3, in the SP the expected F in the following generation would be still low at about 3%. However, these estimates may be biased downward because of incomplete pedigree data since pedigree recording in the Romosinuano Herd-Book was formally carried out after AMCROLET was created (1998).

The average N_{ec} and N_e reached 45 and 48 in RP3 SP, respectively (Table 7). The N_e estimates from the present study are within the range (21 to 123) published by Gutiérrez *et al.* (2003). Larger values of N_e were reported in Romosinuano (66), BON (260), and CCC (143) Colombian breeds, except for SM (27) (Martínez

et al., 2008), in Tabapuã (411; Bernardes *et al.*, 2016), and in Brazilian Gir cattle (94; Santana *et al.*, 2014). Estimates of N_{ec} were smaller than N_e and increased over the last 20 yr. Smaller values of N_{ec} than N_e were also reported by Santana *et al.* (2012) for Marchigiana (98 vs 140) and Bonsmara (55 vs 325) cattle breeds. However, Santana *et al.* (2014) found higher N_{ec} than N_e in Brazilian Gir (166 vs 94). As indicated by Cervantes *et al.* (2011), the differences between N_e and N_{ec} are mainly due to population substructures (caused by mating policies, breeding goals or geographical distances) that affect the increase in F , while the increase in coancestry practically does not change. For RP3 in the SP, the size of N_{ec} (45) was lower than the limit (>50) recommended for conservation of genetic resources to have rates of inbreeding per generation smaller than 1.0% (FAO, 1998).

In the present study, there was an uncommon relationship between N_e and F , since there was a trend on both parameters to increase over time (Tables 5 and 7). There is a direct relationship between N_e and F . Additionally, we expect that as N_e increases, F decreases. However, sometimes this does not happen, since F depends not only on the N_e , but also on the number of equivalent generations (t). Other authors have reported this situation; for example, Rosendo *et al.* (2018) published N_e of 68.1 for the total and 64.6 for the selection nucleus Tropical Milking Criollo cattle, but corresponding F were 1.07 and 2.14.

Boichard *et al.* (1997) indicated that F trend, although commonly used to quantify the rate of genetic drift, has some limitations; particularly when the pedigree completeness is low. The analysis of probabilities of gene origin is suggested as a complementary approach. In the present study, the f_g/f_a ratio indicated that part of the initial genetic variability was lost due to unbalanced gene contributions of founders to the reference populations (Boichard *et al.*, 1997). Additionally, the f_a represented about 50% of the f_g , which results from bottlenecks during the development of the population. Estimates of f_g for RP3 in the UP and the SP (Table 8)

indicate that the amount of genetic diversity can be generated by 17 and 14 unrelated founders, respectively (Lacy, 1989; Ballou and Lacy, 1995).

Both, genetic drift and unbalanced founder contributions of genes to the reference populations were responsible for the reduction of genetic diversity, and the former accounted for >66% of the total losses, except for the SP most recent reference population (44%). Honda *et al.* (2006) also reported that the decay in genetic diversity in Kumamoto and Kouchi sub-breeds of Japanese Brown cattle was due mainly to accumulated genetic drift (>78%, except for Kumamoto, 16%, in 2000 reference population; estimated by the authors of the present study).

The numbers of ancestors that explained 50% of the genetic variability of Romosinuano in the present study (9 to 12) were similar to Lageana Crioula breed (10; Pezzini *et al.*, 2018), Romosinuano (8) and CCC (12), but smaller than SM (22) and BON (22) Colombian breeds (Martínez *et al.*, 2008), Marchigiana (13) and Bonsmara (41) (Santana *et al.*, 2012), and Gir (38; Santana *et al.*, 2014).

The estimates of demographic and genetic parameters suggest that the Romosinuano population in Mexico is on the borderline of risk status. One of the purposes of conservation is to maintain, as much as possible, the genetic diversity present in the founder population. Among the alternatives to maintain high levels of genetic diversity in the population, the most efficient has been to minimize the average pairwise coancestries every generation and by definition to maximize f_g (Ballou and Lacy, 1995; Lacy 1995; Caballero and Toro, 2000). Therefore, it is important that Romosinuano breeders contribute to reduce losses of genetic variability through matings designed to minimize average pairwise coancestries and a more balanced use of sires.

In conclusion, reduction of the genetic diversity has been occurring since the Romosinuano breed association was established in Mexico, mainly due to random losses of genes. Even though no nucleus herds were found in this study, there was a tendency to concentrate the use of germplasm from a reduced number of herds; therefore, it is important to diversify the sources of genetic material for future matings. Monitoring genetic parameters and defining matings aimed to minimize pairwise coancestries are suggested to increase the effective population size and thus, to reduce the inbreeding rate per generation. Efforts are needed to improve the level of completeness of the pedigree and the accuracy of estimates of demographic and genetic parameters.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

This study was designed and directed by Rafael Núñez Domínguez, Ph.D. and Rodolfo Ramírez Valverde, Ph.D. Part of the study was presented as a Professional Thesis by Ricardo E. Martínez Rocha, M.C. All co-authors were closely involved in the collection, analysis and interpretation of data as well as in the writing and decision to submit the work for publication.

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