

## Genetic variability of Tropical Milking Criollo cattle of Mexico estimated from genealogy information<sup>□</sup>

*Variabilidad genética de la raza bovina criolla Lechero Tropical de México estimada a partir de información genealógica*

*Variabilidade genética da raça bovina crioula Leiteiro Tropical do México estimada a partir de informação genealógica*

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### Abstract

**Background:** Genetic variability of populations is essential for their genetic conservation and improvement, and genealogy analysis is a useful tool to estimate it. **Objective:** To determine genetic variability and inbreeding levels in Tropical Milking Criollo (LT). **Methods:** This study analyzed the genealogy of LT using the ENDOG software. Registration certificates of 3,427 LT animals born between 1945 and 2013, and from 608 born between 1950 and 2013 were used. Two populations were defined: the first one with all registered animals (PLT), and the second with nucleus animals (PCP). **Results:** Estimates for PLT and PCP were: founders 890, 114; ancestors 855, 102; effective founders 111, 43; effective ancestors 72, 26, and effective population size 68.1 and 64.6, respectively. Inbreeding coefficients were 4.32 and 3.48% for the highest genetic integrity index; and the average relatedness (*AR*) coefficients were 1.19 and 5.55 for PLT and PCP, respectively. Genealogy depth was shallow in both populations, with full equivalent generations of 2.00 and 3.53. Global generation intervals were about seven years. **Conclusions:** The LT population is not endangered and its genetic improvement program should continue.

**Keywords:** Cattle populations, conservation, hot climates, originating genes, pedigree analysis.

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## Resumen

**Antecedentes:** la variabilidad genética de las poblaciones es esencial para su conservación y mejora genética, y el análisis de genealogía es útil para estimarla. **Objetivo:** determinar la variabilidad genética y los niveles de consanguinidad en la raza criolla Lechero Tropical (LT). **Métodos:** el estudio analizó la genealogía de la raza criolla LT con el programa ENDOG v4.8. Se utilizaron 3.427 registros de animales LT nacidos entre 1945 y 2013, y de 608 nacidos entre 1950 y 2013. Se definieron dos poblaciones, una que incluye todos los animales registrados (PLT) y otra solamente con los animales provenientes del núcleo genético (PCP). **Resultados:** en la PLT y PCP se estimaron animales fundadores 890, 114; ancestros 855, 102; número efectivo de fundadores 111, 43; número efectivo de ancestros 72, 26, y tamaño efectivo de población 68,1, 64,6, respectivamente. Para la categoría más alta de índice de integridad genética, los coeficientes de consanguinidad fueron 4,32 y 3,48%; y el coeficiente medio de relación global ( $AR$ ) fue 1,19 y 5,55 para PLT y PCP, respectivamente. La profundidad de la genealogía en ambas poblaciones fue superficial con generaciones completas equivalentes de 2,00 y 3,53. Los intervalos generacionales globales fueron cercanos a siete años. **Conclusiones:** la población LT no se encuentra en riesgo de extinción y puede continuar con su programa de mejora genética.

**Palabras clave:** *análisis de pedigrí, climas cálidos, conservación, genes originarios, poblaciones ganaderas.*

## Resumo

**Antecedentes:** a variabilidade genética das populações é essencial para sua conservação e melhora genética e a análise da genealogia é útil para estimá-la. **Objetivo:** determinar a variabilidade genética e os níveis de consanguinidade na raça crioula Leiteiro Tropical (LT). **Métodos:** o estudo analisou a genealogia da raça crioula LT com o programa ENDOG v4.8. Foram utilizadas 3.427 registros de animais LT nascidos de 1945 a 2013, e de 608 nascidos entre 1950 e 2013. Foram definidas duas populações, uma que inclui todos os animais registrados (PLT) e outra somente com os animais provenientes do núcleo genético (PCP). **Resultados:** na PLT e PCP foram estimados animais fundadores 890, 114; ascendentes 855, 102; número efetivo de fundadores 111, 43; número efetivo de ascendentes 72, 26 e tamanho efetivo da população 68,1, 64,6, respectivamente. Para a categoria mais alta de índice de integridade genética os coeficientes de consanguinidade foram 4,32 e 3,48%; o coeficiente médio da relação global ( $AR$ ) foi 1,19 e 5,55 para PLT e PCP, respectivamente. A profundidade da genealogia em ambas populações foi superficial com gerações completas equivalentes de 2,00 e 3,53. Os intervalos geracionais globais foram próximos a sete anos. **Conclusões:** a população LT não se encontra em risco de extinção e pode continuar com seu programa de melhoria genética.

**Palavras-chaves:** *análise de pedigree, climas quentes, conservação, genes originários, populações de gado.*

## Introduction

Genetic variability of livestock populations is fading. This is due, in part, to genetic improvement programs (FAO, 1998; Gutiérrez and Goyache, 2005). Boichard *et al.* (1997) mentioned that change in genetic variability across generations could be described by genealogical characterization; and the conservation and consanguinity status of populations can be monitored (Vicente *et al.*, 2012; Pienaar *et al.*, 2015). Genealogical analysis requires the creation and analysis of sorted databases (Gutiérrez and Goyache, 2005); it has been widely used in equines (Vicente *et al.*, 2012; Roos *et al.*, 2015), and canines (Głazewska, 2008; Leroy, 2011). The most important population descriptors are based on the gene probability origin and include the effective number of founders ( $fe$ ) and

ancestors ( $fa$ ), effective population size ( $Ne$ ), genetic integrity index ( $PEC$ ), inbreeding coefficient ( $F$ ), average relatedness ( $AR$ ), genetic conservation index ( $GCI$ ), and generation intervals ( $GI$ ) (Lacy, 1989; Boichard *et al.*, 1997; Gutiérrez *et al.*, 2003).

Tropical Milking cattle of Mexico (LT) is a criollo breed naturalized to hot climates (de Alba, 2011). Due to the small size of its current population -less than 1,000 pure heads (AMCROLET, 2015)- LT is considered endangered (FAO, 2013; 2015). The LT original population developed from cattle brought to the Americas by the Spaniards during the XV century. The current LT population originates from 17 cows and two bulls from Central America brought to Mexico in 1965 (de Alba, 2011). The original herd was disbanded in 2000, from which the initial

selection nucleus of 61 females, 5 males and AI straws from 15 bulls was shaped. Currently, this nucleus is the only source of breeding stock with a recognized genetic improvement program with evaluations for milk production (Santellano-Estrada *et al.*, 2011). A review has been published on LT (Rosendo-Ponce and Becerril-Pérez, 2015). The LT population was divided in six families in a mating scheme of sire rotation to avoid the detrimental effects of inbreeding (Yamada, 1981). The LT has genealogical and production records collected for over 65 years. The Mexican Romosinuano and the Tropical Milking Breeder Association (AMCROLET) is responsible for the LT breed Herd Book. If necessary studies and precautionary measures are not taken, small criollo populations can lose genetic variability, increase inbreeding and become extinct. The aim of this study was to determine the genetic variability and inbreeding levels of the Tropical Milking criollo cattle of Mexico.

## Materials and Methods

A total of 3,427 LT genealogical records between years 1945 and 2013 registered in the AMCROLET's Herd Book (HB) were used. The database was constructed with the individual animal identification, parents, birthdate and sex. Animals with offspring not used for breeding were discarded. Two populations were defined from the HB: i) 3,427 of the LT breed population (PLT), and ii) 608 animals born between 1950 and 2013, ancestors of the current selection nucleus (PCP). Analysis of gene origin is useful for divided populations to assess the selection effects in small populations (Boichard *et al.*, 1997). Animals that did not have at least one known parent were considered founders. Estimates of genetic variability parameters were calculated with the ENDOG v4.8 program (Gutiérrez *et al.*, 2010) as described.

Animals with unknown genealogy are *fe*. It is assumed they are the ancestors' parents and the origin of the current genetic variability in the population; calculated as  $fe = \frac{1}{\sum_{k=1}^f q_k^2}$ , where the sum of  $q_k$  corresponds to the gene proportion of the offspring population provided by each founder ( $k$ ), and  $f$  is the total of alleged founders in the population genealogy. When each founder has the

same contribution ( $1/f$ ), population variability is in equilibrium (Boichard *et al.*, 1996). The minimum of ancestors is *fa* (founders or not), which provided genes into the population and explain all the variability. It is calculated as  $fa = \frac{1}{\sum_{j=1}^a q_j^2}$  the sum of  $q_j$  corresponds to the marginal genetic contribution of the ancestor  $j$ , which is not explained by other ancestors that were previously chosen, and is the total alleged ancestors in the genealogy.

The existing number of effective breeding individuals giving rise to the inbreeding rate is  $Ne$  (Falconer and Mackay, 1996). It is estimated by the inbreeding increase between two consecutive generations ( $\Delta F$ ) in the form  $Ne = 1/2\Delta F$ . The genealogy integrity is the amount of genealogical information of each individual, which can be evaluated with the  $PEC_{ind} = 4C_{males} \times C_{females} / C_{males} + C_{females}$  (MacCluer *et al.*, 1983), where the paternal and maternal line contributions are  $C_{males}$  and  $C_{females}$ , respectively, calculated as  $c = \frac{1}{d} \sum_{i=1}^d a_i$ , where  $a_i$  is the proportion of current ancestors in the generation  $i$ , and  $d$  is total generations. The genealogy depth of each individual is determined by the number of *complete generations* ( $CG$ ), which indicates the farthest generation in which all the ancestors are known, the *maximum number of generations* ( $MG$ ) between the individual and its oldest known ancestor, and the number of *equivalent full generations* ( $EG$ ) calculated by the sum of the coefficients  $(1/2)^n$ , where  $n$  is the number of generations separating the individual from each known ancestor (Goyache *et al.*, 2003).

The probability that two haplotypes at any locus sampled at random from all loci in the genome are identical by descendant is  $F$  (Curik *et al.*, 2014). The increase of inbreeding ( $\Delta F$ ) per generation is calculated as  $\Delta F = \frac{F_t - F_{t-1}}{1 - F_{t-1}}$ , where  $F_t$  and  $F_{t-1}$  are the average inbreeding in generation  $t$ , and the previous. The coefficient average in the corresponding row of the additive relationship matrix ( $A$ ) for each individual is  $AR$  (Gutiérrez and Goyache, 2005). The allele percentage of the current population coming from the initial one is  $GCI$ , which is calculated as  $GCI = \frac{1}{\sum_{i=0}^f p_i^2}$ , where  $p_i$  is the founder's gene proportion,  $i$  in one animal's genealogy. The time elapsed to replace one generation with the next one is  $GI$ .

## Results

The animals that made up the databases are shown in Table 1. In the PLT 458 bulls and 2,969 cows were registered, and 59.8 and 50.1% of these animals were breeding animals. The PCP genealogy had 219 bulls and 389 cows, of which 60.2 and 78.1% were chosen to mate. The PLT had 41.2% of the animals with both parents unknown, while in the PCP only 13.3%, while animals with offspring accounted for 51.4 and 71.7% for each population. The number of offspring per sire in the PCP was reduced to more than half of the PLT, and the number of cows increased by almost 12%. The sire/dam ratio, and the maximum number of offspring per sire, and per dam were 43.4%, and 23 and 5 in the PLT, and 18.3%, and 65 and 8 in the PCP.

**Table 1.** Number of animals included in the databases of all registered animals (PLT), and in the selection nucleus (PCP) of the Tropical Milking Criollo cattle.

Animals	PLT	PCP
Database	3,427	608
Both parents known	2,013	461
Both parents unknown	1,055	81
Sire unknown	230	42
Dam unknown	129	24
Base population (animals with at least one parent unknown)	1,414	147
Males with progeny	274	132
Females with progeny	1,489	304
Offspring per sire	7.79	3.73
Offspring per dam	1.51	1.69

The gene origin parameters were estimated to determine genetic variability (Table 2). The founders ( $f$ ) and alleged ancestors ( $a$ ) in the PLT were lower

**Table 2.** Descriptors of gene origin in all registered animals (PLT) and in the selection nucleus (PCP) of Tropical Milking Criollo cattle.

Parameter	PLT	PCP
Founders assumed ( $f$ )	890	114
Effective number of founders ( $fe$ )	111	43
Ancestors assumed ( $a$ )	855	102
Effective number of ancestors ( $fa$ )	72	26
Effective population size ( $Ne$ )	68.1	64.6
Inbreeding coefficient ( $F$ , %)	1.07	2.14
Average relatedness (AR, %)	1.19	5.55

than 900, of which only  $fe/f= 12.5$  and  $fa/a= 8.4\%$  were effective, in the PCP  $f$  and  $a$  barely exceeded 100, although still in reduced proportion, and  $fe/f= 37.7$  and  $fa/a= 25.5\%$ , three times bigger than in the PLT. The small  $fe$  indicates more frequent use of some sires, one of them had 46 descendants. The  $fe$  was reduced in the PCP (38%) compared to the PLT. The  $fa/fe$  indexes calculated were 0.64 and 0.60 in PLT and PCP.

The  $Ne$  of PLT was similar to that of PCP and both exceeded the 50 threshold. Approximately 70% of the PLT had limited genealogical information and appears in the categories under 0.4 of  $PEC$ ; only 10% occurred in the category above 0.8. All the categories of the inbreeding coefficient  $F$  had wide ranges, and tended to increase as the  $PEC$  category did. The estimated  $F$  of 4.32% of the highest  $PEC$  category is the most appropriate estimate of homozygosity in the PLT. Although only 22.5% of the animals belonged to the top two categories of 0.60 of  $PEC$ , more than 80% of inbred animals occurred there, in the PLT 22.5% of the animals showed some consanguinity (Table 3).

In the PCP, the increased occurrence of almost 40% of the animals was presented in the superior category of 0.8 of  $PEC$ , which had an  $F$  of 3.48% (Table 4).

**Table 3.** Estimated inbreeding coefficient ( $F$ ) by genealogy integrity index classes ( $PEC$ ) of all registered animals (PLT) of Tropical Milking Criollo cattle.

PEC		Animals		F (%)		Animals with F (%) > 0		
Interval	Mean	No.	%	Interval	Mean	Interval	No.	%
0.000 - 0.239	0.02	1520	44.4	0.0 - 16.80	0.04	0.31 - 16.80	17	2.2
0.240 - 0.399	0.31	850	24.8	0.0 - 27.12	0.26	0.07 - 27.12	32	4.2
0.400 - 0.599	0.52	287	8.4	0.0 - 25.00	2.32	0.05 - 25.00	75	9.7
0.600 - 0.799	0.70	427	12.5	0.0 - 37.50	2.96	0.02 - 37.50	326	42.2
0.800 - 1.000	0.88	343	10.0	0.0 - 29.49	4.32	0.07 - 29.49	322	41.7
Total	0.49	3427	100.1				772	100.0

**Table 4.** Estimated inbreeding coefficients ( $F$ ) by genealogy integrity index classes ( $PEC$ ) of the selection nucleus ( $PCP$ ) of Tropical Milking Criollo cattle.

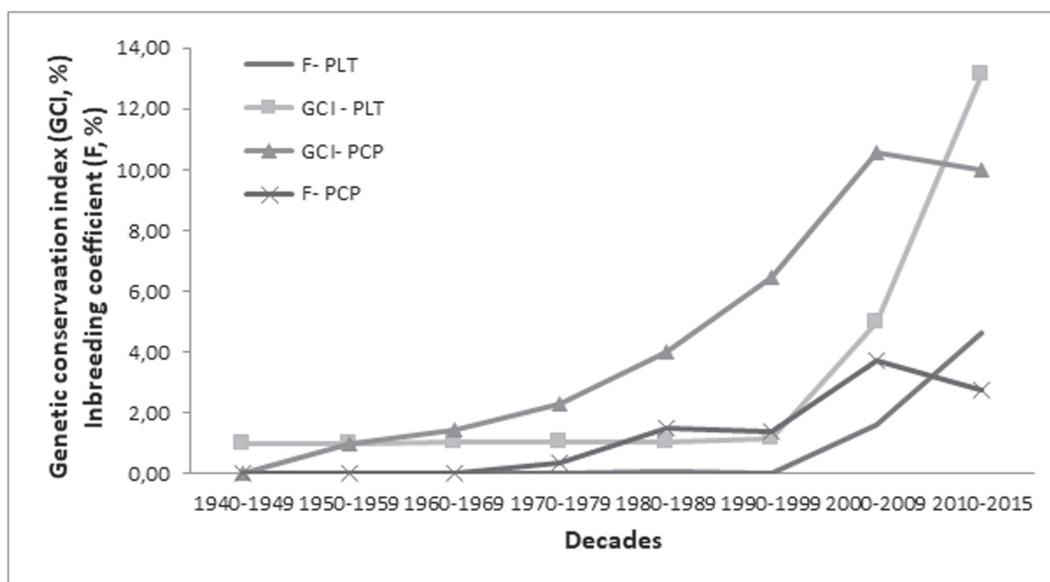
PEC		Animals		F (%)		Animals with F (%) > 0		
Interval	Mean	No.	%	Interval	Mean	Interval	No.	%
0.000 - 0.239	0.02	159	26.2	0.0 - 12.82	1.15	0.04 - 12.82	76	22.8
0.240 - 0.399	0.30	59	9.7	0.0 - 6.38	0.78	0.53 - 6.38	20	6.0
0.400 - 0.599	0.52	45	7.4	0.0 - 8.82	0.67	0.92 - 8.82	11	3.3
0.600 - 0.799	0.71	107	17.6	0.0 - 15.88	1.88	0.07 - 15.88	52	15.6
0.800 - 1.000	0.92	238	39.1	0.0 - 26.09	3.48	0.20 - 26.09	175	52.4
Total	0.56	608	100.0				334	100.1

However, 55% of the animals are considered with some consanguinity; out of these, more than 50% occurred in the top category of  $PEC$ . In the  $PCP$ , the percentage of animals in the two lowest categories of  $PEC$  was medium (36%). The  $F$  and  $AR$  estimated in the whole populations were less than 1.2 in  $PLT$ , which doubled and quadrupled in  $PCP$  (Table 2). Emphasis should be placed on avoiding matings between closely related animals, as the  $PCP$  population is small and has high  $AR$ .

For genealogy depth, the  $PLT$  was incomplete in all generations, with  $CG=0.95$ ,  $MG=4.64$  and  $EG=2.00$ , with an  $F$  increase of 0.24% between an individual and its farthest known ancestor by generation. The two recent generations and 68 animals described the

current  $PLT$  variability, the increase in consanguinity between these generations was 0.73%. The genealogy depth of  $PCP$  was better with  $CG=1.82$ ,  $MG=7.04$  and  $EG=3.53$ , and an inbreeding increase of 0.23% between an individual and its farthest known ancestor; the number of generations that explained the genetic variability was 3.5 with an average increase of inbreeding of 0.77% per generation.

Over time, the  $GCI$  appears to be related to  $F$  changes (Figure 1). In the  $PLT$  and  $PCP$  there are similar trends between  $GCI$  and  $F$ ; however, both estimators in the  $PCP$  showed a tendency to decrease. The estimated  $GCI$  of the  $PCP$  with  $PEC$  higher than 0.8 was 3.08%, indicating a reduction in the gene conservation of the original population.

**Figure 1.** Genetic conservation index ( $GCI$ , %) and inbreeding coefficient ( $F$ , %) through decades in all registered animals ( $PLT$ ), and in the selection nucleus ( $PCP$ ) of Tropical Milking Criollo cattle.

The *GIs* were shorter than seven years in both populations; the shortest were observed in the paternal line, except in the father-son line in PCP. The biggest difference between the two populations was in the mother-son line, 8.4 months (Table 5).

**Table 5.** Generation interval (years) for complete populations with register certificate (PLT) and in the selection nucleus (PCP) of Tropical Milking Criollo cattle.

Pathway	PLT	PCP
Father/son	7.2 ± 3.6	7.0 ± 3.4
Father/daughter	6.3 ± 3.2	6.6 ± 3.3
Mother/son	7.4 ± 4.5	6.7 ± 3.6
Mother/daughter	7.0 ± 3.8	7.3 ± 3.8
Parent/offspring	6.8 ± 3.6	6.9 ± 3.6

## Discussion

The difference of *f* and *fe* indicates the loss of genetic variability in the original PLT population, as a result of the unequal contributions of the founders by preferential use of some parents (Boichard *et al.*, 1997); this decrease also occurred in the PCP to a lesser extent. The *fe* (38%) decrease in the PCP is due to a limited number of individuals coming from the PLT (Cervantes *et al.*, 2009). In an ideal population *f* equals *fe* and this in turn *fa* (Kliman *et al.*, 2008). The genetic variability could be reduced by the effect of the genetic drift by its small size (Lacy, 1989). The *fe/f* ratio estimated in the PLT was 0.13, which indicates the effects of selection. In the PCP the ratio was 0.38, higher than in Colombian creole (criollo) breeds such as Costeño Con Cuernos (CCC, 0.23), Sanmartinero (SM, 0.21), and Romosinuano (Romo, 0.12), but lower than in Blanco Orejinegro (BON, 0.50) (Martínez *et al.*, 2008). The larger estimate in the PCP nucleus is related to the selection program for dams of bulls within each of the six families. The relatively high number of ancestors regarding founders in both populations indicates a shallow genealogy and a short founder - last generation period. The *fa* is useful for detecting recent significant changes in the breeding improvement strategy (Goyache *et al.*, 2003). The existence of bottlenecks can be assessed by the ratio *fa/fe*. These indexes were greater than 0.6 in both populations and indicate that these bottlenecks have not been narrow (Boichard *et al.*, 1997). Similar estimates were obtained in the CCC (0.66), BON

(0.65), and Limonero (0.60) breeds, and lower in SM (0.53), and Romo (0.34) (Martínez *et al.*, 2008; Villasmil-Ontiveros *et al.*, 2008). Close to one *fa/fe* values are common in populations oriented to genetic conservation (Korrida *et al.*, 2013).

A *Ne* of at least 50 animals per generation leads to an *F* increase of 1% per generation. The PLT *Ne*= 68.1 would not be endangered (FAO, 1998, 2013; Falconer and MacKay, 1996) nor the PCP *Ne*= 64.6. The *Ne* of the PCP was higher than in other populations with *per se* genetic conservation programs; such as bull fight cattle populations (55.5 and 44.5), Reyna breed ( $\leq 46$ ) and Moroccan Houbara Bustard ( $>40$ ) (Corrales *et al.*, 2010; Domínguez *et al.*, 2010; Korrida *et al.*, 2013). Some small-size dairy populations have shown that genetic improvement may involve the loss of genetic variability (Boichard *et al.*, 1996); *Ne* is particularly sensitive to unequal number of females and males in the population (Kliman *et al.*, 2008). As the genealogy integrity influences *F* and *GCI*, only genealogies with *PEC* index  $>0.8$  are reliable for estimating these parameters (MacCluer *et al.*, 1983; Corrales *et al.*, 2010). The inbreeding estimated was 3.48% in the PCP, with 8.5% animals with *F* higher than 6.25%. As for the genealogical information kept within the generations, the PCP was slightly lower than the values of *CG*= 2.1, *MG*= 8.2, and *EG*= 4.5 estimated for double-purpose Slovenian spotted cattle (Hazuchová *et al.*, 2012a); the highest *MG*= 6.9 estimated in Colombian criollos was similar to the one estimated in the PCP, although the maximum *EG*= 4.8 in Romo was higher; Romo had the largest population size and genealogical depth, in relation to other Colombian breeds (*GE* interval 3.1-3.8) (Martínez *et al.*, 2008).

The estimated interval of AR in Spanish native breeds was 0.1-1.7% (Gutiérrez *et al.*, 2003). The AR is useful for predicting inbreeding in the long run (Gutiérrez *et al.*, 2010). The *GCI* for the PCP population was 3.73%, similar to 4.18% estimated in the dual-purpose Slovak bull population (Hazuchová *et al.*, 2012b). However, animals with high *F* are not those with the greatest *GCI*; this index assumes that the genetic goal is to preserve and retain the full allele range that the initial population has (Gutiérrez *et al.*, 2010). Through time, the matings made it possible for an individual to have information from more than

one of the founders so the GCI increased (Figure 1), as has happened with the population of Slovak bulls (Hazuchová *et al.*, 2012b). This difference indicates that in the PCP selection nucleus, rotational mating has decreased the tendency of F, and the selection of animals with better milk yields has decreased GCI; a different situation in the PLT, where the family rotation mating has been blurred. The generation intervals (GI) for the PLT were similar to those of Reyna (6.9) and SM (6.8), although higher than other Colombian criollos, BON (4.5), CCC (5.4) and Romo (5.7) (Corrales *et al.*, 2010; Martínez *et al.*, 2008). The GI through the mother-son line was the longest in the PLT; in the PCP selection nucleus, genetic improvement is carried out through the dams of bulls having the highest genetic values for milk production (Rosendo-Ponce and Becerril-Pérez, 2015). The GIs through the paternal line were shorter than those through the maternal line, which agrees with previous studies (Corrales *et al.*, 2010, Martínez *et al.*, 2008). The father-daughter line was the shortest; the cattlemen retain all females born in their farms. To shorten the GI by the paternal and maternal lines, sires must be replaced every two-three years and reproductive life of heifers starts at a younger age. The overall GI in the PLT was  $6.8 \pm 3.9$  years higher than in Brangus, Santa Gertrudis and Saler (Delgadillo, 2014), but similar to ring fight cattle (Domínguez *et al.*, 2010). The differences found in the GI can be attributed to variations in age at puberty and first parturition; as well as to the tropical fluctuating environmental conditions in years. The mating of bulls and females that had better genetic evaluation for milk production was favored. The loss of genetic variability happens by generation; thus, as GI shortens, the annual genetic variation loss can be accelerated.

Apparently, the Tropical Milking population (PLT) is not currently endangered. Its effective population size allows for continued genetic improvement through selection for milk production, avoiding matings between related individuals. Mating of sires and females with better genetic evaluation for milk production in the selection nucleus (PCP) has led to differences between the estimates of the effective founder number and the effective ancestor number. Trends toward increasing the inbreeding coefficient and the genetic conservation index were found; from

year 2000 they decreased in the PCP. Genealogy integrity in the PLT could influence the obtained estimates, reflecting higher inbreeding levels and reduced effective population size.

### Conflicts of interest

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

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