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**Research Paper**

# Pedigree analysis of the closed nucleus of Iranian Sangsari sheep

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***ABSTRACT:*** *Genetic diversity in the closed nucleus of Iranian Sangsari sheep was analyzed by quantifying the generation interval, effective population size and the amount of inbreeding using pedigree records of 7311 animals born from 1989 to 2015. Animals born between 2012 and 2015 were selected as a reference population for estimating parameters derived from probability of gene origins. Average generation interval was 4.1 year in the studied period. Mean inbreeding coefficient increased by 0.12% per generation ($P < 0.001$) and average inbreeding coefficient of the animals in the reference population was 1.33%. Realized effective population size estimated from individual increase in coancestry was 96. The effective number of founders, effective number of ancestors and founder genome equivalent of the reference population were 95, 40 and 21.08 respectively. The highest contributing ancestor was a ram with a 7.7% contribution to the reference population. Considering the closed herd with no entry of animals from other herds, the genetic diversity of the population seemed fairly good. However, genetic diversity has been lost compared to the founder population as a result of unbalanced contribution of founders, bottlenecks and genetic drift. Therefore, strategies such as optimum contribution selection is recommended to achieve desired genetic gain while maintaining the genetic diversity at an acceptable level.*

***Keywords:****Effective population size, Inbreeding, Pedigree Analysis, Probability of gene origin.*

## I. INTRODUCTION

The biological diversity of the planet is being depleted rapidly as a consequence of human actions. The World Conservation Union (IUCN), the primary international conservation body, recognizes the crucial need to conserve genetic diversity as one of the three fundamental levels of biodiversity. Genetic diversity may be defined as the "genetic ability to vary", and this is reflected in functional, biochemical, morphological or behavioral dissimilarities that cause differences in reproductive rate, survival or behavior of individuals[1,2]. In livestock populations, loss of genetic diversity generally occurs as a direct consequence of improvement programs, especially due to the increased levels of inbreeding and loss of founder alleles through genetic selection and drift [3]. In these populations, the analysis of a well-recorded pedigree makes it possible to describe genetic diversity of the population by criteria based on probability of identity-by-descend of genes and criteria based on probabilities of gene origin.

Sheep are an important source of meat in Middle East countries. The local sheep had several positive characteristics, including adaptation to the local feed sources, parasite resistance and tasty meat [4]. Iran has various agro-ecological zones from arid to semi–arid, humid to temperate and highland cold types, resulted in the development of more than 27 distinctive breeds of sheep which appear in a variety of size, shapes, types and color. Among them Sangsari is one of the most important meat type sheep breed in center of Iran. Sangsari is fat-tailed and relatively small sized breed of sheep, native and well adapted to Semnan Province, Iran. Sangsari has good fattening performance and meat yield in this breed is about 60% of the weight before slaughter [5]. The selection program of the Sangsari breed is undertaking in order to improving the growth traits of animals at Sangsari breeding station in Damghan-Iran. This breeding station act as a main part of the nucleus-based breeding schemes and superior animals disseminate into local flocks. The objective of this study was to characterize the population structure and genetic diversity of the closed nucleus of Iranian Sangsari Sheep in terms of the inbreeding, effective population size and criteria based on probabilities of gene origin using pedigree information.

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## II. MATERIALSANDMETHODS

**2**.1 DADA

Pedigree records of 7311 animals born from 1989 to 2015 in the research flock of Iranian Sangsari sheep located in Damghan, Iran were used for analysis. For each sheep, the file contains information on individual identification code, sex, dam and sire identification codes and birth date. Animals born between 2012 and 2015 with a pedigree completeness index over 5 generations of at least 0.6 were selected as a reference population in the analysis of probabilities of gene origin. The length of the reference period was chosen such that it represented approximately an entire generation.

### 2.2 Generation Interval

The generation interval was estimated as the average age of parents at the birth of their selected offspring [6]. This parameter was computed for the four possible genetic pathways: ram–son (lmm), ram–daughter (lmf), ewe–son (lfm) and ewe–daughter (lff). Mean generation interval, weighted by the number of animals within each pathway, was subsequently calculated and the overall generation interval was defined as the average of the 4 pathways.

### 2.3 Pedigree completeness

The degree of completeness of pedigree was assessed before analysis. Three parameters were used to characterize the pedigree completeness level of the reference populations. The percentage of known ancestors per generation was calculated for each animal.

The pedigree completeness index (PCI) was estimated as:

$$PCI_{animal} = \frac{2C_{sire}\,C_{dam}}{C_{sire} + C_{dam}} \quad (1)$$

Where Csire and Cdam are contributions from the paternal and maternal lines respectively:

$$C = \frac{1}{d}\sum_{i=1}^{d} a_i \quad (2)$$

Where $a_i$ is the proportion of known ancestors in generation i; and d is the number of generations that is taken into account [7]. In this study, 5 generations are considered (d = 5). This index summarizes the proportion of known ancestors in each ascending generation and quantifies the chance of detecting inbreeding in the pedigree [8]. The number of complete generation equivalents (CGE) was computed for each individual j as:

$$CGE_j = \sum {n_i}/{2^g} \quad (3)$$

where $n_i$ is the number of known ancestors in generation i and $g$ is the number of known generations for individual *j*[9].

### 2.4 Inbreeding AndCoancestry

The coefficient of inbreeding of each animal in the file was computed using the method of Meuwissen and Lou(1992). The average inbreeding coefficients per birth year was computed and annual increases in inbreeding was estimated by linear regression over time. The average coancestry between animals gives an indication about future trends of inbreeding [8]. Hence, the average coancestry among rams, among ewes and between rams and ewes in reference population were estimated.

### 2.5 Probability Of Gene Origin

Founders were defined as the ancestors of the reference population with unknown parents. The founder contribution is defined as the expected proportion of the population's gene pool that has descended from this founder and is equal to the value of the coefficient of relationship between the founder and its descendants [11]. The contribution of a founder to a reference population depended on its use in the past and, therefore, information given by the total number of founders was limited. This limitation is accounted for by calculation of the effective number of founders. Effective number of founders ($f_e$) was estimated as the number of equally contributing founders that would be expected to produce the same genetic diversity in the populations under study [12]. The effective number of founders was computed as:

$$f_e = {1}\Big/{\sum_{k=1}^{f} q_k^2} \quad (4)$$

Where *f* represents the number of founders and qk is the genetic contribution of the *k*th founder to the reference population. However, Boichard et al. (1997) showed that $f_e$ overestimates the effective number of founders if the population has suffered an important bottleneck, and they proposed as an alternative the effective number of ancestors ($f_a$). The $f_a$ is the minimum number of ancestors, not necessarily founders, explaining the

complete genetic diversity of a population. The following equation was used to estimate effective number of ancestors:

$$f_a = 1 \Big/ \sum_{j=1}^{a} q_j^2 \quad (5)$$

Where *qj* is the marginal contribution of an ancestor j; in other words, the genetic contribution made by an ancestor that is not explained by other ancestors chosen previously [12]. Another measurement for genetic diversity of a reference population is the founder genome equivalent ($f_g$), which accounts not only for unbalanced contributions of parents to the next generation (as $f_e$ and $f_a$) and for bottlenecks in pedigrees (as $f_a$) but also for random loss of genes from parents to their offspring [11]. Therefore, $f_g$ expected to be a smaller number than both $f_a$ and $f_e$. Founder genome equivalent estimated from the inverse of twice the average coancestry of the individuals included in a predefined reference population according to Caballero and Toro (2000).

**2.6 Effective population size**

Effective population size (Ne) is a central concept in evolutionary genetics and conservation biology. The effective size of a population is the size of an idealized population that would lose genetic diversity at the same rate as the actual population [14]. Effective population size was estimated using the rate of coancestry between two individuals following Cervantes et al. (2011). The increase in coancestry between any pair of individuals j and k, belonging to different sexes in the reference population can be computed as:

$$\Delta C_{jk} = 1 - \sqrt[\left(\frac{g_j + g_k}{2}\right)]{1 - C_{jk}} \quad (6)$$

In which $C_{jk}$ is the inbreeding of a descendent from both, and $g_j$ and $g_k$ are the complete generation equivalents for the parents. By averaging the increase in pairwise coancestry for all pairs of individuals belonging to different sexes in a reference population we can estimate effective population size as:

$$\bar{N}_{ec} = 1 \Big/ 2\overline{\Delta C} \quad (7)$$

**2.7 Used software**

All parameters were estimated using ENDOG ver. 4.8, except for pedigree completeness index (PCI) of animals that were computed using Eva ver. 1.3.09 [16,17].

## III. RESULTS

**3.1 Generation Interval**

The generation intervals across the alternative selection pathways were $l_{mm}$ = 4.1, $l_{mf}$ = 3.9, $l_{fm}$ = 4.2 and $l_{ff}$ = 4.2 years. Average generation interval was 4.1 years.

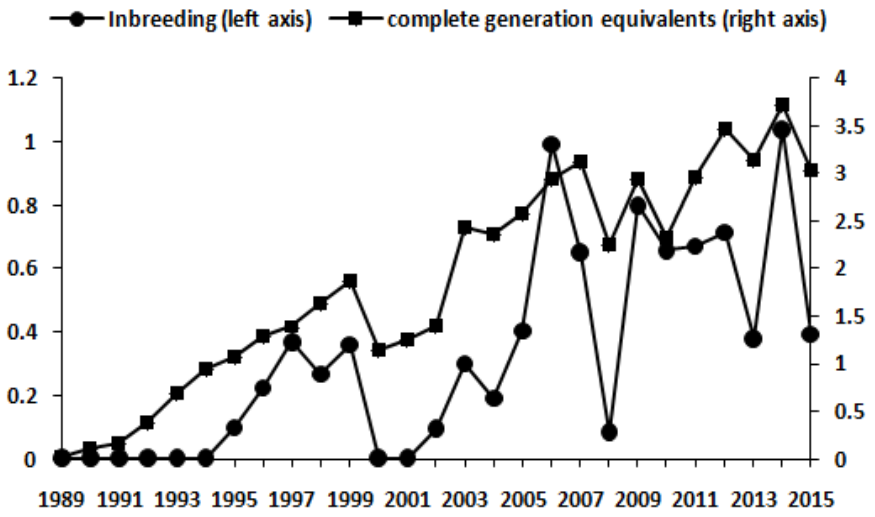
**3.2 Pedigree completeness, Inbreeding and Coancestry**

Average values for the complete generation equivalents, pedigree completeness index (PCI) and the percentage of known ancestors for the first, third, fifth, and seventh generations of the reference population are given in Table 1.

Inbreeding coefficient ranged from 0 to 31.25% with an average of 0.31 and 2.99 for whole pedigree and inbred animals, respectively. Average inbreeding coefficient of animals in the reference population was 1.33%. Trends for the mean inbreeding and complete generation equivalents of animals across years of birth are presented in Fig.1. The fluctuations in average inbreeding of animals are in coincidence with the trend of CGE. Linear regression of inbreeding on birth year resulted in an estimated rate of inbreeding of 0.03% per year ($P < 0.001$). Average coancestry among rams, among ewes and between rams and ewes in the reference population were 2.48, 2.55 and 2.22%, respectively.

**Table 1.** Pedigree completeness level for the reference populations

| | |
|---|---|
| Complete generation equivalents (CGE) | 4.34 |
| Pedigree completeness Index (PCI) | 0.7 |
| Percentage of known ancestors from the: | |
| First generation (parents) | 100 |
| Third generation | 85 |
| Fifth generation | 52 |
| Seventh generation | 6 |




**Figure**

**1.** Evolution of the mean inbreeding and complete generation equivalents of animals by birth year

### 3.3 Probabilities Of Gene Origin

Table 2 presents the parameters derived from the analysis of probability of gene origin in the reference population. Total number of founders for the reference population was 355 animals while a value of 95 was estimated for the effective number of founders ($f_e$), indicating unbalanced contribution of founders to the reference population. The number of ancestors (founders or not) explaining 100, 75, and 50% of the gene pool was 191, 44 and 16 respectively. Marginal genetic contribution of the 10 most influential sires with respective number of offspring is shown in Fig. 2. The marginal genetic contribution of the most popular ancestor to the reference population was approximately 7.7%. The cumulative marginal contribution of the most influential ancestors to the reference population are represented in Fig. 3. According to the Fig. 3, a small number of ancestors contribute heavily to the reference population, but the rest of the genes are derived from a large number of ancestors each with a very small contribution. The effective number of ancestors of the reference population was 40. The founder genome equivalent of the reference population was 21.08 and, as expected, was smaller than both $f_a$ and $f_e$.

**Table 2.** Criteria calculated from the probabilities of gene origin

| | |
|---|---|
| Total number of founders (f) | 355 |
| Effective number of founders (fe) | 95 |
| Number of ancestors to explain: | |
| 50% of gene pool | 16 |
| 75% of gene pool | 44 |
| 100% of gene pool | 191 |
| Effective number of ancestors (fa) | 40 |
| Contribution of the main ancestor, (%) | 7.7 |
| Founder genome equivalent (fg) | 21.08 |

### 3.4 Effective population size

Effective population size of a population is a measure of its genetic behavior, relative to that of an ideal population. The effective population size estimated based on the individual increase in pairwise coancestry using the method of Cervantes et al. (2011), was 96 animals with standard error of 2.05.

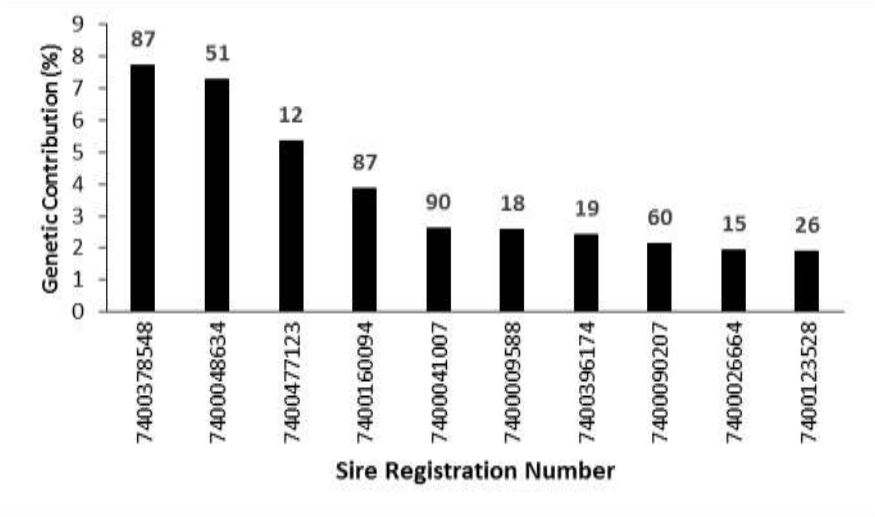



**Figure 2.** Marginal genetic contribution of the 10 most influential sires with respective number of offspring

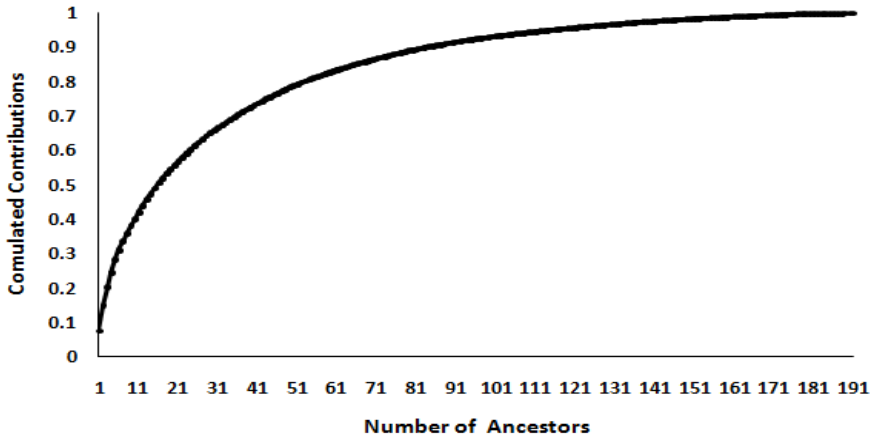



**Figure 3.** Cumulated marginal genetic contributions of ancestors to the reference population

## IV. DISCUSSION

Conservation of genetic diversity is now universally accepted as being vital for sustainable management of these resources. In this regard, effective management of farm animal genetic resources requires comprehensive knowledge of the breed's characteristics, including data on population size and structure and genetic diversity [18,19]. The present study provided useful information regarding the status of genetic diversity in the closed nucleus of Iranian Sangsari sheep.The estimated generation interval of the Sangsari sheep (4.1 yr) in the current study was slightly higher than has been observed for some other Iranian breeds. The average generation interval of 3.33, 3.42, 3.45, 3.35 and 3.39 years has been reported for the Iranian Baluchi, Lori-Bakhtiari, Zandi, Afshari and Iran Black sheep, respectively [20, 21, 22, 23, 24]. This implies the differences in replacement policies between breeds. But estimated generation interval in this study was close to the reports for Charmoise, Romanov, Berrichon du Cher, Blanc du Massif Central, Limousin and Roussin de La Hague breeds of France [25,26].

Completeness and depth of the pedigree are important factors, which affect the estimated inbreeding coefficients and relationship among animals and, to a lesser extent, affect also the estimated generation intervals and effective numbers of founders and ancestors [27]. In the current study, pedigree completeness of animal in the reference population was in acceptable level and was comparable to the values reported in other analysis on sheep populations [23,25,26,28,29].Average inbreeding coefficients of animals in the present study were low. Only 6.2% of animals of the reference population with PCI greater than 0.6, had inbreeding coefficients greater than 6.25%, which was the level reached by cousin mating, while 87% of animals had an inbreeding coefficient of less than 3.125%. In conservation programs, the rate of inbreeding is the most important parameter in monitoring genetic diversity in terms of effective population size [30]. The rate of inbreeding needs to be

limited to maintain diversity at an acceptable level. In this study the rate of inbreeding, was 0.12%s per generation and was less than the critical levels (1% per generation) recommended by FAO (1998) and Bijma(2000). Similarly, an increase in inbreeding per generation of 0.14 to 0.21 in some French meat sheep and of 0.17 and 0.21 in the Iranian Lori-Bakhtiari and Moghani sheep were reported [23,26,33]. Nevertheless, a higher rate of inbreeding per generation has been reported for Danish population of Texel, Shropshire, and Oxford Down (1%), and also for Iranian Baluchi (0.66) and Zandi sheep (0.76) [22,34,35]. Such differences may be partly described by the different mating strategies and depth of the analyzed pedigree. The average coancestry of animals in a population predicts the average inbreeding coefficient in the subsequent generation [6]. The average coancestry of 2.2% between animals of the reference population is equal to an average inbreeding of about 1.1% in their progeny under random mating. Considering the estimated average inbreeding of animals in the reference population (1.33%) we can conclude that mating is carried out without structuring the population.

Other measurements providing information about the genetic diversity of a studied population are the effective number of founders, ancestors, and founder genomes. The parameters derived from the probabilities of gene origin precisely describe genetic diversity of populations after a small number of generations, although inbreeding coefficient and effective population size are important in monitoring diversity over longer periods of time [12,30]. The considerable difference between the effective number of founders and the effective number of ancestors in this study show that narrow bottlenecks have occurred since the foundation of the population. The ratio of fe/fa in this study (2.37) was higher than previously reported for other Iranian sheep breeds, indicating stronger bottleneck impact in Sangsari sheep compared with those breeds [20,22,23]. The ratio found in this study agrees with those reported for French Charmoise, Romanov, Solognote, Berrichon du Cher and Limousin breeds [25,26]. Another important reason for the loss of genetic diversity is random genetic drift. In small populations genetic drift is usually the dominant force causing allele frequency change [14]. The fg/fe ratio measures the impact of genetic drift excluding the effect of founder contributions on genetic diversity, such that lower ratios are associated with higher impact of genetic drift [36]. The fg/fe ratio in this study was lower than those reported for other Iranian Sheep breeds, reflecting a higher impact of random genetic drift on the loss of genetic diversity in Sangsari sheep. Overall, probability of gene origin analysis indicate that genetic diversity has been lost due to the unbalanced contributions of founders, bottlenecks and random genetic drift.

The FAO (1998) set an effective population size of 50 animals as a critical level. In conservation biology, the effective population size should not be lower than 50 to avoid extinction in the short term, and not lower than 500 to avoid extinction in the long term [37]. However, according to Frankham et al. (2014), genetically effective population size (Ne) = 50 is inadequate for preventing inbreeding depression, with Ne $\geqslant$ 100 being required to limit loss in total fitness to $\leqslant$10%. Also they stated that, even Ne = 500 is too low for retaining evolutionary potential for fitness in perpetuity; a better approximation is Ne $\geqslant$ 1000. Leroy et al. (2013), reported that factors such as method used, species and population structure should be considered in the interpretation and comparison between the estimated effective population sizes. Comparing the results of analysis on cattle and sheep populations in the literature, indicate the favorable situation of sheep in terms of the effective population size. This can be partly attributed to the widespread use of artificial insemination in cattle and its effect on making differences in male progeny sizes that is not the case in sheep as in cattle and a ram cannot provide as many doses as a bull [25,29]. The estimated realized effective population size in this study was in the intermediate situation compared to other analysis on sheep populations. Leroy et al. (2013) estimated the average effective population sizes of 189 with the range of 28-449 for 40 French sheep population using the same method as used in this study. The estimated realized effective population size of the Sangsari sheep in this study (96) was close to the estimates for Iranian Kermani (100) and Lori-Bakhtiari sheep (101) [23,39]. Also, it was comparable with the estimates for the Cotentin breed of France and Finnseep of Finland [25,40]. Nevertheless, it was higher than the estimates for Iranian Zandi, Afshari and IranBlack sheep [20,21,24].

## V. CONCLUSION

In general, this study demonstrated the status of genetic diversity in a closed nucleus of Iranian Sangsari sheep by pedigree analysis. The estimated rate of inbreeding and effective population size were in acceptable level when considering that population was closed with no entry of animals from other herds. However, according to the parameters derived from the probability of gene origin, genetic diversity has been lost compared to the founder population, due to the random genetic drift and disequilibrium between contributions of parents. Various other studies also demonstrated genetic drift as the main cause of loss of genetic diversity in small closed population [2,30]. Therefore, there is a need to increase the Ne so as to prevent further effects of random genetic drift. The application of optimum contribution selection in Sangsari sheep nucleus can help in this regard to achieve desired genetic gain while maintaining the genetic diversity at an acceptable level.

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