

## PRELIMINARY ANALYSIS OF GENETIC DIVERSITY IN IMPROVED VALACHIAN AND TSIGAI BREEDS USING GENEALOGICAL INFORMATION

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**Abstract.** The objective of this study was to analyse genetic variability of Improved Valachian and Tsigai sheep breeds using pedigree information. The programme ENDOG was used for analyses. The reference populations were considered live animals born during a period of four consecutive years from 2007 to 2010 and consisted of 14,652 (Improved Valachian) and 10,601 animals (Tsigai). The pedigree populations were considered all animals with genetic ties to the reference populations and consisted of 45,135 (Improved Valachian) and 28,292 animals (Tsigai). The mean values of inbreeding were 0.22% (Improved Valachian) and 0.49% (Tsigai). The mean values of average relatedness were 0.19% (Improved Valachian) and 0.49% (Tsigai). The mean values of individual increase in inbreeding were 0.06% (Improved Valachian) and 0.12% (Tsigai). The realized population size - computed taking into account individual increase in inbreeding was 893 (Improved Valachian) and 403 animals (Tsigai). The equivalent number of founders was 7,143 (Improved Valachian) and 4,187 animals (Tsigai). The effective number of founders was 1,232 (Improved Valachian) and 434 animals (Tsigai). The effective number of ancestors was 608 (Improved Valachian) and 264 (Tsigai). The number of ancestors explaining 50% of genetic variability was 246 (Improved Valachian) and 107 (Tsigai).

**Keywords:** sheep, pedigree, identity by descent, gene origin.

**Introduction.** Genetic variability of a population can be described using the pedigree information. The coefficient of inbreeding is defined as the probability that two genes in an individual are identical by descent (Wright, 1922). The coefficient of average relatedness of each individual is defined as the probability that an allele randomly chosen from a population belongs to a given animal (Gutiérrez and Goyache, 2005). The realized effective population size (Cervantes et al., 2003) is defined as the global effective size over time which has led to the present inbreeding level. Parameters derived from probabilities of gene origin (effective number of founders and effective number of ancestors, number of ancestors explaining 50% of genetic variability) are additional indicators of genetic variability that are less sensitive to length and completeness of the pedigree (Lacy, 1989; Boichard et al., 1997). Genetic variability is crucial for population survival (Lacy, 1995) and provides future opportunities for selection, improvement and adaptation (Golden et al., 2000; Márquez et al., 2010). Its loss can negatively affect the economically and biologically important traits.

The most important sheep breeds in Slovakia are Improved Valachian and Tsigai. These traditional breeds have selection programmes aimed at improvement of milk and meat production (including on-farm performance testing), and are of great importance for land management. Their genetic variability from the perspective of the available pedigree information has not been studied until now.

The objective of this study was to perform pedigree analysis in the Improved Valachian and Tsigai breeds in order to assess inbreeding, average relatedness, individual increase in inbreeding and realized effective population size for each population. The parameters derived from probabilities of gene origin, effective number of founders,

effective number of ancestors and number of ancestors explaining 50% of genetic variability, were computed.

**Material and methods. Data.** Data of the Improved Valachian and Tsigai breeds were provided by the Slovak Breeding Services of the Slovak Republic, s.e. An individual pedigree record included animal, sire and dam unique identification numbers, date of birth, animal sex and flock. The whole pedigree files consisted of 45,135 (Improved Valachian) and 28,292 animals (Tsigai). The individual coefficients of inbreeding and of average relatedness were computed for all animals. Their evolution over time was assessed by grouping animals across the years of birth. The analyses based on probabilities of gene origin were done for a group of youngest animals (live animals born in the most recent years i.e. 2007-2010). Also, the mean values of inbreeding, of average relatedness as well as of individual increase in inbreeding and the realized effective population size were calculated for these youngest animals (reference population). The reference population consisted of 14,652 animals (Improved Valachian) and 10,601 animals (Tsigai), respectively. The ENDOG programme v.4.8 (Gutiérrez and Goyache, 2005) was used for all analyses. Additional statistical analyses were done using the SAS 9.2 software (2009).

**Pedigree completeness.** The degree of pedigree completeness (proportion of known ancestors in each generation) was assessed by the index as proposed by MacCluer et al. (1983).

The number of equivalent complete generations traced for each animal, which is defined as the sum over all generations of the proportion of known ancestors in each generation, was computed considering Maignel et al. (1996) as  $(1/2)^n$ , where  $n$  is the number of generations separating the animal from each known ancestor (parent=1, grandparent=2 etc.).

**Probabilities of gene origin.** Founders are animals with unknown parents. If animal has only one unknown parent, the unknown parent is considered as a founder (Lacy, 1989). The effective number of founders ( $f_e$ ) is defined as the number of equally contributed founders that would be expected to produce the same genetic diversity as in the population under study. It was computed as  $f_e = 1 / \sum_{k=1}^f q_k^2$ , where  $q_k$  is the expected

proportional genetic contribution of founder  $k$ . For a given total number of founders, the more balanced their expected genetic contributions, the higher effective number of founders (Huby et al., 2003). The difference between the number of founders and the effective number of founders indicates non-proportional contributions of founder animals.

The ancestors (founders or not) were identified using the method proposed by Boichard *et al.* (1997). The effective number of ancestors ( $f_a$ ) is the minimum number of ancestors explaining the complete genetic diversity of a population under study. This parameter accounts for the losses of genetic variability produced by the unbalanced use of animals as parents of the next generation and was computed as  $f_a = 1 / \sum_{j=1}^a q_j^2$ , where  $q_j$  is the marginal

contribution of founder  $j$ , which is not explained by any other ancestor chosen before (contribution independent of the contributions of the other ancestors).

The effective number of ancestors ( $f_a$ ) is by nature lower than the effective number of founders ( $f_e$ ) and the difference is due to bottlenecks (Boichard et al., 1997).

**Inbreeding coefficients, coefficients of average relatedness and the realized effective population size.** Inbreeding is defined as the probability that an individual has two identical alleles by descent and was computed following Meuwissen and Luo (1992).

Average relatedness for each individual is defined as the probability that an allele randomly chosen from the whole population in the pedigree belongs to a given animal and was computed as the average of the coefficients in the row corresponding to the individual in the numerator relationship matrix (Gutiérrez and Goyache, 2005).

The realized effective population size is defined as the global effective size over time which has led to the present inbreeding level in a population and was estimated from individual increase in inbreeding (Gutiérrez et al., 2008; 2009). This was computed as:

$\Delta F_i = 1 - \sqrt[t]{1 - F_i}$ , where  $F_i$  is the individual coefficient of inbreeding and  $t$  is the number of equivalent generations. Averaging of individual increases in inbreeding enables to compute the realized effective population size:

$$\bar{N}_e = 1 / (2\Delta F), \text{ as proposed by Cervantes et al. (2008)}$$

to overcome such limitations as generation overlapping, selection and non-random mating.

**Results. Pedigree completeness.** Table 1 shows two indicators of pedigree completeness for the reference

population: the number of equivalent complete generations and a proportion of known ancestors in individual generations. The average number of equivalent complete generations was 5 (both breeds). A proportion of 100% to 95% of known ancestors was found in first to third generation. Of the whole population, the proportion of known ancestors was lower (85% to 60%) in first to third generation (results not published). In fourth to sixth generation, the proportion of known ancestors decreased from 85% to 30% (reference population) vs. 45% to 13% (whole population). The phenomenon behind this fact is lack of historical data that went 25 years back (a maximum).

**Probabilities of gene origin.** The parameters derived from probabilities of gene origin are given in Table 1. The equivalent number of founders was computed considering animals with unknown parents and additional animals with only one parent known (weighed as half a founder). Considering the similar pedigree completeness in both breeds and only natural mating applied; larger values of the equivalent number of founders and of the remaining parameters derived from probabilities of gene origin in the Improved Valachian breed are probably due to a larger population size. The effective number of founders was several times smaller as small as the equivalent number of founders. It indicates the unbalanced contributions of founders and was found higher in the Improved Valachian breed. The ratio of about 2 was found between the effective number of founders and the effective number of ancestors. This value indicates that no narrow bottleneck in both populations occurred. Ranking the ancestors by decreasing expected marginal contribution, the number of ancestors needed to explain 50% of genetic variability of the reference population was 246 (Improved Valachian) and 107 (Tsigai).

**Coefficients of inbreeding, coefficients of average relatedness and the realized effective population size.** Table 1 shows further indicators of the genetic variability estimated for the reference populations of the two breeds. Mean values of inbreeding, of average relatedness, of individual increase in inbreeding and the realized effective population size were two times greater for Tsigai than for Improved Valachian. The realized effective population size was estimated taking into account the individual increase in inbreeding as this approach was found more suitable under limited pedigree knowledge available and generation overlapping. In accordance with Gutiérrez et al. (2009), animals with at least two equivalent complete generations were considered when estimating the realized effective population size. The estimated realized effective population size was 893 and 403 (Improved Valachian and Tsigai, respectively).

Across the years of birth of all animals in pedigrees, a detailed insight into evolution of two of these parameters: inbreeding and average relatedness is given in Fig.1. The evolution of equivalent complete generations is also given. Even though the coefficients of inbreeding remained low over the analysed period (both breeds), some differences within and between breeds were found. Due to missing pedigree information, zero and almost

zero values were found till 1998 (Improved Valachian) and 1999 (Tsigai). Since then, the inbreeding level increased in the Tsigai breed to a greater extent. The coefficients of average relatedness showed a regular

increase from 1990 and were of similar values as coefficients of inbreeding, both indicating low genetic relationships among animals. The equivalent complete generations also increased from the early years.

Table 1. Results of pedigree analysis: reference populations of Improved Valachian (IV) and Tsigai (TS)

	IV	TS
Pedigree completeness	No. of EquGen	5
	% of both parents known in generation 2	99.2
	% of both parents known in generation 4	87.7
	% of both parents known in generation 6	33.4
	% of both parents known in generation 8	<2
Parameters derived from probabilities of gene origin	Equivalent No. of founders	7,143
	Effective No. of founders	1,232
	Effective No. of ancestors	608
	NA <sub>50</sub>	246
Inbreeding, mean value (%)	0.22	0.49
% of inbred animals	35	60
Average relatedness, mean value (%)	0.19	0.49
Individual increase in inbreeding, mean value (%)	0.056	0.124
Realized effective population size $N_e$	893	403

EquGen – equivalent generations; NA<sub>50</sub> - No. of ancestors explaining 50% of genetic variability

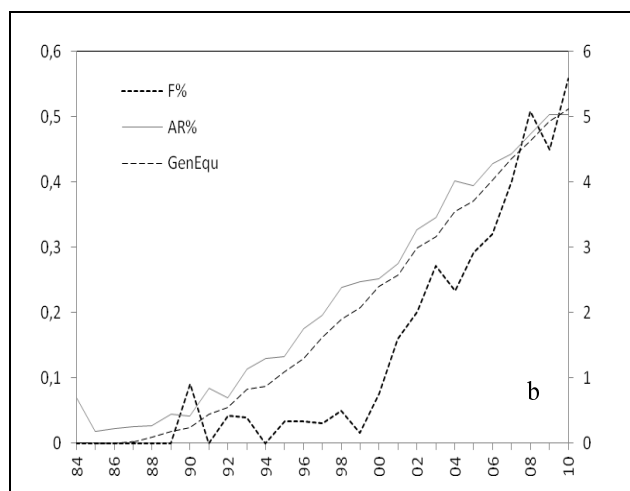
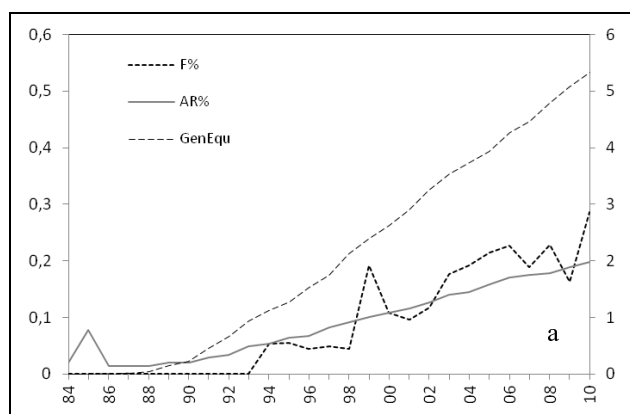


Fig 1. Evolution of inbreeding  $F\%$ , average relatedness  $AR\%$  (left y-axis) and number of equivalent generations (GenEqu) across the years of birth (right y-axis): (a) Improved Valachian, (b) Tsigai

**Discussion.** According to the results presented, the analysed populations of the Improved Valachian and Tsigai breeds do not have the genetic variability issues and their within-population variability is good. However, the limited information was available for accurate assessment of genetic variability. When comparing pedigrees of sheep breeds, the pedigree completeness measured by the number of equivalent complete generations estimated for Improved Valachian and Tsigai was similar to Roussin de la Hague and Solognote (Danchin-Burge et al., 2010), or to Limousine (Huby et al., 2003). It was worse than in Bleu du Maine and in Charmoise (Danchin-Burge et al., 2010), or in White Alpine and in Black-Brown Mountain (Hagger, 2002). On the contrary, it was better than in Xalda (Goyache et al., 2003; Álvarez et al., 2008), in Zandi (Ghafouri-Kesbi, 2010) and in Valachian (Oravcová and Krupa, 2011).

When comparing the parameters derived from probabilities of gene origin obtained in this study to those reported by Huby et al. (2003) or Danchin-Burge et al. (2010) for French sheep breeds of similar pedigree depth, the effective number of founders and of ancestors were found greater in the Slovak breeds. Hagger (2002), who analysed a population of the White Alpine sheep (18,214 animals in the reference population), also found smaller effective number of founders and effective number of ancestors. Azor et al. (2008), who analysed a population of the Spanish Merino breed (43,976 animals in the reference population), found the effective number of founders and of ancestors similar to those estimated for the Tsigai breed.

It was generally accepted that the pedigree knowledge affects the estimates of inbreeding coefficients. When a large proportion of animals has unknown parents, the inbreeding level within the population can be

underestimated (Boichard et al., 1997). The pedigree completeness of the Slovak sheep showed good quality for animals born in the most recent years (more than 60% in five generations) as a result of regularly decreasing numbers of founders over time. On the contrary, the older animals were of lower pedigree completeness. The increased pedigree completeness was due to improved animal recording system and selection practice that reflected a need to provide good pedigree knowledge for candidate breeding animals. The average inbreeding coefficients were found low. Individual inbreeding coefficients were found between 0% and 25% for animals born in the most recent years. The proportion of inbred animals (of all born over the period 2007-2010) was 35% and 60% (Improved Valachian and Tsigai). Of these, only 1% and 3% (Improved Valachian and Tsigai) had individual inbreeding coefficients greater than 6.25%. These findings are probably a result of good mating management, large population sizes and partially limited knowledge of pedigrees. The trends of inbreeding (0% to 0.3% for Improved Valachian and 0% to 0.6% for Tsigai) were lower than those reported by Maiwashe and Blackburn (2009) and Li et al. (2009) for the Navajo-Churro sheep breed (0% to 1.2%) and Finnsheep (1% to 2.95%).

In addition to previous indicators, the average relatedness and the realized effective population size, which was estimated from the individual increase in inbreeding, confirmed good genetic variability in both breeds. The ram to ewe ratio was 1 to 10 (up to 50 a maximum) as only natural mating is applied. Flock reproduction management depended mainly on own sires that were replaced early to avoid inbreeding. As a consequence, sire-progeny generation intervals were shorter than dam-progeny generation intervals (results not published).

**Conclusion.** Based on results of pedigree analysis, the within-population variability in populations under the study is good. Because of both the routine genetic evaluation system and the national programme to increase resistance to scrapie being in place, an increasing impact of selection on the within-population variability is expected. The large genetic basis and good mating programme can be useful when maintaining low genetic relationships between candidate breeding animals.

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