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# GENEALOGICAL DECOMPOSITION OF THE EFFECTIVE POPULATION SIZE: A CASE STUDY ON CROATIAN AUTOCHTHONOUS CATTLE BREEDS

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## SUMMARY

**Effective population size ( $N_e$ ) is one of the most important tools used to assess genetic diversity for conservation purposes. Using pedigree data of three Croatian autochthonous cattle breeds (Buša, Istrian and Slavonian Syrmian Podolian) the effective maternal ( $N_{eF}$ ), paternal ( $N_{eM}$ ) and combined maternal-paternal ( $N_{eFM}$ ) population size was estimated. Additionally, we estimated the effective population size based on the census population sex ratio ( $N_{es}$ ), the effective population size from the individual increase in inbreeding ( $N_{eFi}$ ) and the effective population size from individual increase in coancestry ( $N_{eCi}$ ). We compared these sizes with the values obtained for 20 additional cattle populations, as well as with the newly calculated  $N_{eFM}$ . The effective population sizes calculated for three autochthonous breeds were consistently the lowest in amongst all the considered cattle breeds. Utilisation of extremely small numbers of breeding males is the main reason for the observed reduction in the effective population size. The decomposition of effective population size into maternal and paternal components is shown to be an informative parameter in detecting the reduction of the effective population size as a consequence of unequal sex contribution. Still, the impact of the pedigree depth and completeness on the  $N_{eF}$ ,  $N_{eM}$  and  $N_{eFM}$  estimation remain to be analysed. A large deviation between  $N_{es}$  and all other methods of  $N_e$  estimation was observed and it is our recommendation that breeders and stakeholders should consider using alternative methods of  $N_e$  estimation when planning breeding programmes as well as in the determination of the endangered status of animal populations.**

**Key-words: genealogical analysis, effective population size, cattle, sex ratio**

## INTRODUCTION

Assessment of genetic diversity is necessary for autochthonous genetic stock conservation (Alvarez et al., 2012). Genealogical records, or pedigrees, are both historically and presently important sources for the estimation of quantitative genetic and diversity parameters. Leroy et al. (2014) states that the effective population size ( $N_e$ ), as developed by Wright (Wright, 1931), stands out amongst the many tools used to assess genetic diversity for conservation purposes.  $N_e$  is defined as the size of an ideal (Wright-Fisher) population ( $N$ ) where individuals are monoecious, selfing is possible, and the amount of genetic drift will be the same as in the actual population being considered (Allendorf, 2013). The simplest method of calculating  $N_e$  is based on the sex ratio within the population. Since pedigree informa-

tion is unavailable for many animal populations, this is the method routinely used by the Food and Agriculture Organization (FAO) and the European Association for Animal Production (EAAP) when estimating the effective population size of animal populations. However,  $N_e$  can be estimated from different sources of information, including demographic information, pedigrees or molecular data. The choice of the method of estimation becomes very important in order to obtain a result which can be used to effectively manage animal populations.

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Several alternative genetic diversity indicators have been proposed and of those the most widely used ones are: the genetic drift through temporal changes in allele frequencies (variance of effective population size), the increase in homozygosity (inbreeding effective population size), or the rate at which the unique alleles are lost (eigenvalue effective population size) (Leroy et al., 2014). None of these methods separate the effective population sizes of males and females, rather they combine them. On the other hand, the ratio of the effective population sizes of both sexes is one of the critical points for the estimation of the overall effective population size, so our approach was to estimate these parameters separately.

Recently, mitochondrial DNA (mtDNA) was used to compute the maternal effective population size, along with the discrete generations equivalent and inbreeding parameters (Alvarez et al., 2012). In this pilot study we aimed to extend the genealogical approach by Alvarez et al. (2012) and estimate the effective maternal ( $N_{eF}$ ) and paternal ( $N_{eM}$ ), as well as the combined maternal-paternal ( $N_{eFM}$ ) effective population size in three Croatian autochthonous cattle breeds (Buša, Istrian and Slavonian Sarmian Podolian) using their respective pedigrees. We also estimated the effective population size based on the sex ratio ( $N_{es}$ ), the effective population size from the individual increase in inbreeding ( $N_{eFi}$ ) and the effective population size from the individual increase in coancestry ( $N_{eCi}$ ), and compared them with values obtained for 20 additional cattle populations (Leroy et al., 2014), as well as to the newly calculated  $N_{eFM}$ .

## MATERIAL AND METHODS

Pedigree files for three autochthonous cattle populations: Buša (CRB), Istrian (IST) and Slavonian Sarmian Podolian cattle (SSP) in Croatia have been analysed. The largest pedigree was the IST cattle pedigree (4752), followed by the BH cattle (1707) and the SSP cattle (1129) pedigrees.

The reference population was defined as individuals born in the period from 2004 to 2014 and all effective population sizes were calculated only for these populations. The pedigrees were checked for errors using the programs CFC (Sargolzaei, M., 2006) and Endog (Gutiérrez and Goyache, 2005).

The effective population size based on census population sex ratio ( $N_{es}$ )

$$N_{es} = \frac{4MF}{M + F},$$

was calculated for all three pedigrees.

The following genealogical parameters were then computed in Endog:

a) effective population size from individual increase in inbreeding ( $N_{eFi}$ ) according to Gutiérrez et al. (2009):

$$\Delta F_i = 1 - \sqrt[2]{1 - F_i},$$

b) effective population size from individual increase in coancestry ( $N_{eCi}$ ) according to Cervantes et al. (2010):

$$\Delta C_i = 1 - \sqrt[2]{1 - C_i}$$

Maternal ( $N_{eF}$ ) and paternal ( $N_{eM}$ ) effective population sizes were calculated using the version of the in-house program MaGellAn (work in progress) by the method employed by Alvarez et al. (2012):

$$N_{ef} = \frac{1}{\Delta PI},$$

where PI is defined as the probability where individuals share the same dam or haplotypic line by chance (Bowling et al., 2000). The paternal ( $N_{eM}$ ) effective population size was calculated using the same method by simply reversing the sexes within the pedigrees.

The results obtained in this way via MaGellAn were then used to calculate the combined maternal-paternal effective population size  $N_{eFM}$  based on the same formula as for  $N_{es}$ , substituting  $N_{eM}$  instead of M and  $N_{eF}$  instead of F.

## RESULTS AND DISCUSSION

Three Croatian autochthonous cattle breed populations have been analysed. A total of 3350 IST, 1357 CRB and 866 SSP cattle were included in the reference population (*Pref*). The mean inbreeding percentage (*F*) was largest in CRB (6,59), followed by IST (5,1) and SSP (3,81) cattle populations. When compared to *F* values from 20 cattle populations by Leroy et al. (2014) our populations had at the same time the largest inbreeding and the smallest complete generation equivalents (2, 3 and 3,1) of all cattle populations analysed. The estimated effective population sizes of the three Croatian autochthonous cattle populations, calculated by different approaches, are shown in Table 1.

**Table 1. Estimated effective population sizes Croatian autochthonous cattle breeds**

Parameters	CRB	IST	SSP
Reference population ( <i>Pref</i> )	1357	3350	866
Individual increase in inbreeding effective population size ( $N_{eFi}$ )	13	22	29
Individual increase in coancestry effective population size ( $N_{eCi}$ )	13	19	12
Maternal effective population size ( $N_{eF}$ )	147	78	13
Paternal effective population size ( $N_{eM}$ )	18	2	3
Founder sex ratio effective population size ( $N_{eFM}$ )	64	8	8
Census population sex ratio effective population size ( $N_{es}$ )	1244	3316	861

Buša (CRB), Istrian (IST) and Slavonian Sarmian Podolian cattle (SSP)

The effective population size estimates  $N_{eFi}$  and  $N_{eCi}$  showed values in the range 12-29 for all three cattle populations. The combined maternal-paternal effective

population size  $N_{eFM}$  resulted in a larger range from 8 for IST and SSP to 64 for CRB cattle. By comparing the  $N_{eFi}$  and  $N_{eCi}$  estimates with those for the 20 cattle populations from Leroy et al. (2014) our cattle populations showed consistently smaller effective population sizes than other populations, up to twice as small as the smallest  $N_{eFi}$  and  $N_{eCi}$  values in the comparison dataset, 52 and 58, respectively. By estimating  $N_{eFM}$  through decomposition to  $N_{eF}$  and  $N_{eM}$  we noticed large differences between  $N_{eF}$  and  $N_{eM}$ . The  $N_{es}$  values, on the other hand, strongly deviate from all other  $N_e$  estimates, being in the range from 861 to 3316, due to the relatively balanced census population sex ratio in the three populations. This overestimation trend is also clearly seen when looking at the  $N_{es}$  values of the 20 cattle populations from Leroy et al. (2014). Here, it is evident that the census population sex ratio is overestimated. By comparing the census population sex ratios ( $N_M/N_F$ ) with effective population sex ratios ( $N_{eM}/N_{eF}$ ) a two-three fold drop in the values was observed: 0,6→0,3 for CRB, 0,8→0,25 for IST and 1,1→0,4 for SSP. Thus, we consider that the low number of breeding males is the critical factor that caused small effective population size estimates in the analysed breeds.

The low effective population size for IST population is in concordance with Curik et al. (2014) where the current effective population size ( $N_{eLD}=12$ ) was estimated by high-throughput molecular data following the linkage disequilibrium approach described in Flury et al. (2010). At the same time, according to the Croatian Agricultural Agency report (2013) the breed status is highly endangered with  $N_e$  estimated to 152 (721 cows and 40 bulls) when calculated from the census population sex ratio.

Our recommendation is that breeders and stakeholders should not rely only on  $N_{es}$  when planning breeding programmes or estimating the endangered status of animal populations, as other methods provide more concordant  $N_e$  estimates.

## CONCLUSION

The effective population sizes calculated in three autochthonous Croatian cattle breeds were consistently lower in comparison to other cattle breeds from Leroy et al. (2014), even those with smaller reference populations than Croatian breeds, such as Ferrandaisex ( $P_{ref}=587$ ). Utilisation of an extremely small number of breeding males in CRB, IST and SSP breeding is the main reason for the observed reduction in the effective population size. Decomposition of the effective population size to the maternal and paternal components has been shown to be an informative parameter in detecting the reduction of the effective population size due to the unequal sex contribution. Still, the impact of the pedigree depth and completeness on  $N_{eF}$ ,  $N_{eM}$  and  $N_{eFM}$  estimation remains to be analysed.

A large deviation between  $N_{es}$  and all other methods of  $N_e$  estimation was observed and it is our recommenda-

tion that breeders and stakeholders should be considered using alternative methods of  $N_e$  estimation when planning breeding programmes, as well as in the determination of the endangered status of animal populations.

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