



Estimation of inbreeding and effective population size in Istrian cattle using molecular information

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ABSTRACT

To provide preliminary insight in the conservation risk status in Istrian cattle we analysed ROH inbreeding and effective population size in 15 individuals, mostly bulls, using BovineSNP50K BeadChip. We obtained very high inbreeding level, although with broad confidence interval, and very low effective population size. While the results obtained are preliminary (small sample size) and should be treated with caution, the high recent inbreeding and small effective population size suggest additional monitoring of the conservation risk status of the Istrian cattle.

(Keywords: Istrian cattle, Inbreeding, Runs of homozygosity, effective population size, single nucleotide polymorphism)

INTRODUCTION

Istrian cattle, colloquially called Boškarin, is the autochthonous breed spread mainly over the Istrian peninsula. The breed belongs to the group of grey cattle breeds that are scattered over the Balkan and neighbouring countries (Croatia, Bulgaria, Greece, Hungary, Italy, Romania, Serbia, Turkey and Ukraine) and that are considered as direct descendants from the Auroch (*Bos primigenius*). In the last 50 years the number of Istrian cattle individuals has been reduced dramatically. Inbreeding level and effective population size (N_e) are among the most important conservation genetic parameters. Classical inbreeding and N_e estimates rarely work well in real populations as they are mostly based on inaccurate pedigree records or, in case of N_e estimation, on robust demographic parameters that do not completely recognise the history of the population (bottlenecks, preferential mating or population subdivision). The rapid development of new molecular technologies enabled high-throughput genotyping of individual animals at available prices. Consequently, those technological achievements provide new views on old problems and reinforce estimation of inbreeding and N_e from molecular markers. Runs of homozygosity (ROH) were recently proposed as a useful concept in quantifying individual inbreeding in humans (McQuillan *et al.*, 2008), cattle (Ferenčaković *et al.*, 2011; Purfield *et al.*, 2012) and pigs (Bosse *et al.*, 2012), performing even better than traditional estimates calculated from the pedigree. Sved (1971) and Hill (1981) showed that linkage disequilibrium (LD) could be used to estimate N_e . While theoretical basis has been established before, the practical use of LD in estimating N_e started by Hayes *et al.*, (2003) and, further, continued by Tenesa *et al.*, (2007); Qanbari *et al.*, (2009).

The aim of this study was, based on high-throughput genotypes (BovineSNP50K BeadChip), to estimate inbreeding level and effective population size in Istrian cattle. The results obtained will contribute to the conservation management strategy of the Istrian cattle.

MATERIAL AND METHODS

Samples (15) representing Istrian cattle population, mostly bulls, were either taken from the blood (randomly chosen from several private farms in Istria, or were obtained as semen straws (three bulls) from CRSH d.o.o. in Krizevci (www.crsh.hr). As the number of Istran bulls is extremely small we have considered our sample as representative, although, we are aware that larger sample would be more adequate.

After ROH calculation quality control that was performed according to *Ferenčaković et al.* (2013b) we proceed with analyses including information from 42265 SNPs (%), placed on 29 autosomes and with average distance of 59 kb between adjacent SNPs. ROH segments were identified as a part of the genome in which 15 or more consecutive homozygous SNPs at a density of one SNP on every 100 kb are not more than one Mb apart. ROH calculations were done by SNP & Variation Suite (v7.6.8 Win 64; Golden Helix, Bozeman, MT, USA www.goldenhelix.com). The general formula for calculating F_{ROH} from chip data is $F_{ROH} = L_{ROH}/L_{AUTOSOME}$, where L_{ROH} is the total length of all ROH in the genome of an individual while $L_{AUTOSOME}$ refers to the specified length of the autosomal genome covered by SNPs on the chip (here 2,543,177 kb). For each bull, we calculated three inbreeding coefficients ($F_{ROH>4Mb}$, $F_{ROH>8Mb}$ and $F_{ROH>16Mb}$) based on ROH of different minimum lengths (>4, >8 or >16). Different ROH inbreeding coefficients are expected to have differently remote common ancestors (for details see *Curik et al.*, 2014).

Effective population size (N_e) was estimated following the approach described in *Flury et al.* (2010) respecting functional relationship of N_e with correlation r^2 and recombination rate (c), here inter-marker genetic distance between two considered loci with assumption that 1 Mb = 1 cM. Two slightly different formulas were used, one described in Sved (1971) where $r^2 = 1/(1+4 \cdot c \cdot N_{e1})$ and the other described in Weir and Hill (1980) where $r^2 = 1/(1+4 \cdot c \cdot N_{e2}) + (1/n)$ with $n = 2 \cdot$ number of animals (bulls) used in the calculation as a correction factor for a sample size induced LD. Only SNPs with adjacent r^2 values from 0.01 to 0.99 were used in the calculation by *Uimari and Tapio* (2011). Finally, time defined effective population size N_{eT} was derived from 40 marker distance derived categories as described in *Flury et al.* (2010). Current effective population size was predicted based on the regression analysis of estimated values in previous 150 generations. LD (r^2) was estimated using SNP & Variation Suite (v7.6.8 Win 64, Golden Helix, Bozeman, MT, USA www.goldenhelix.com). Data manipulations, numerical calculations and graphical visualisations were done by procedures included in SAS 9.3 (*SAS Institute*, 2011).

RESULTS AND DISCUSSION

Summary statistics of the ROH estimated inbreeding level ($F_{ROH>4Mb}$, $F_{ROH>8Mb}$ and $F_{ROH>16Mb}$) in 15 Istrian cattle bulls are presented in *Table 1*. The estimates obtained (mean and standard deviations) were much higher than those obtained in Brown Swiss, Fleckvieh, Norwegian Red and Tyrol Grey by *Ferenčaković et al.* (2013a) or in Pinzgauer by *Ferenčaković et al.* (2013b). However, one should be aware that the

confidence limits are very broad with values comparable to any population studied so far. One individual had extremely high close inbreeding ($F_{ROH>8Mb}=0.351$ and $F_{ROH>16Mb}=0.287$) indicating the absence of mating strategy respecting avoidance of close inbreeding.

Table 1

Summary statistics of inbreeding calculated from ROH with different lengths (>4 Mb, >8 MB and >16 MB) based on Illumina BovineSNP50K BeadChip in 15 Istrian cattle bulls

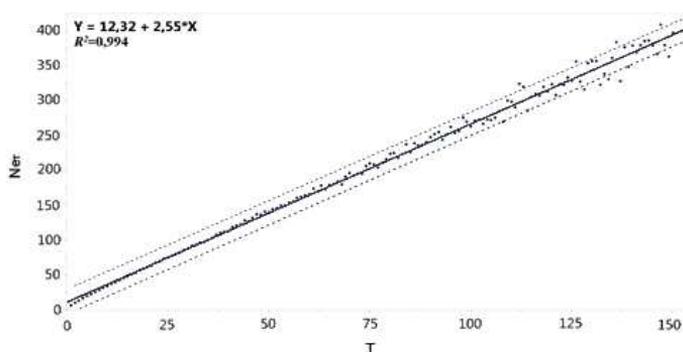
Inbreeding coefficient	Mean	Lower 95% CI	Upper 95% CI	Standard deviation	Range
$F_{ROH>4Mb}$	0.093	0.039	0.147	0.092	0.002-0.368
$F_{ROH>8Mb}$	0.081	0.029	0.133	0.091	0.000-0.351
$F_{ROH>16Mb}$	0.075	0.014	0.096	0.078	0.000-0.287

CI = Confidence interval

Historical estimates of the effective population size (Ne_T) during last 150 generations showed rather linear decrease of 2.55 individuals per generation while predicted current generation effective population size (Ne_0) was equal to 12.32 with 95% confidence interval ranging from 9.58 to 15.06 individuals (*Figure 1*).

Figure 1

Linear regression with 95% confidence interval presenting relationship between historical effective population size (Ne_T) and number of generations in the past (T) while Ne_T values were previously estimated from genomic data of 15 Istrian cattle individuals



Thus, the linear regression function was $Ne_T=12.32+2.55 \cdot T$ with extremely high coefficient of determination ($R^2=0.994$). The obtained prediction for the current effective population size of Istrian cattle was surprisingly small. According to the Croatian Agricultural Agency report (2013) the breed status is highly endangered with Ne estimated to 151.59 (721 cows and 40 bulls) when calculated from the sex ratio

[$N_e = (4 \cdot N_m \cdot N_f) / (N_m + N_f)$, where N_m and N_f represent the number of breeding males and females, respectively].

Although, the sample size was very small, historical estimates of effective population size do represent large number of chromosomal segments originating from much larger number of individuals and, thus, should be less sensitive to the sample size. Still, the interpretation of the results should be considered with caution as we are not fully aware of the magnitude of potential bias resulting from one individual being highly inbred.

CONCLUSIONS

Although, the results obtained are preliminary (small sample size) and should be treated with caution, the appearance of high recent inbreeding in some individuals and small effective population size require additional monitoring of the conservation risk of Istrian cattle population.

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