GENETIC DIVERSITY AND EFFECTIVE POPULATION SIZE OF EIGHT IRANIAN CATTLE BREEDS

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SUMMARY

Iranian cattle breeds are currently under-represented in studies regarding genetic variability and conservation effort even though they are settled in a region that is believed to be one of two ancient cattle domestication centres. This study provides first population genetic parameters for eight Iranian cattle breeds collected from across the entire country to highlight the necessity for conservation programs. High density genome-wide SNP chips were used. None of the Iranian breeds showed a decreased heterozygosity compared to outgroup breeds (Holstein, Jersey, and Brahman) and inbreeding coefficients were low. Nevertheless, estimated effective population sizes were <10 for the Mazandarani, Sarabi, and Kermani breeds and it is predicted that most of the genetic variability will be lost within 20-30 generations if no intervention measures are taken. Effective population size estimates varied between chromosomes with occasional extremely high values, especially for Najdi, Pars, and Kermani which have high proportions of indicine ancestry as represented by the Brahman outgroup.

INTRODUCTION

Two separate domestication events gave rise to the variety of cattle breeds we see today. India is the origin of humped zebu cattle, and the Fertile Crescent of the Near East is the region of origin for humpless taurine cattle (Loftus *et al.* 1994). Iran covers the Eastern side of the Fertile Crescent and the native cattle breeds represent an extensive biological resource for origin and domestication studies. Iran is home to a large number of cattle breeds, however, the number of indigenous animals is declining with breeds, such as the Golpayegani, becoming extinct and other indigenous breeds endangered. The loss of these breeds or their genetic diversity, which is the ultimate source of adaptive variation to environmental pressures, will significantly limit the genetic resources available to future breeding programs (Herrero-Medrano *et al.* 2013).

Genetic characterization of the Iranian breeds provides the first step towards breed conservation, which largely depends on existing genetic variability and effective population sizes. A few studies attempted to examine the genetic diversity of Iranian native cattle (Mirhoseinie *et al.* 2005; Valizadeh *et al.* 2012). However, these studies concentrated on only a few breeds and were based on small numbers of markers (Mirhoseinie *et al.* 2005; Valizadeh *et al.* 2012). In this study, a dense SNP dataset was used to investigate genetic diversity, inbreeding, and effective population sizes in eight Iranian native cattle breeds.

MATERIALS AND METHODS

Data. Hair samples of individuals from eight Iranian cattle breeds were collected throughout the country. The Iranian breeds could be clustered into four taurine populations (humpless), and four indicine populations expressing a hump and often a pronounced dewlap and pendulous ears. Additionally, randomly selected subsets from larger populations of Holstein, Jersey and Brahman cattle were included to anchor the Iranian breeds towards taurine and indicine origins.

All animals were genotyped with the 700k Bovine BeadChip (Illumina Inc, San Diego, CA, USA). Genotypes were subject to a stringent quality control (Gondro *et al.* 2014) with a GC score

>0.9, call rates per marker >90% and per animal >70% (reduced due to small sample size). Markers were excluded that deviated (1) in their heterozygosity by more than 3 standard deviations from the mean heterozygosity or (2) from Hardy-Weinberg equilibrium at P-value$<10^{-16}$. Only markers that were present in all breeds including outgroups were kept for further analyses which reduced numbers considerably (283,028 SNPs).

Analyses. Population parameters such as average minor allele frequency (MAF) and average heterozygosity (He) were calculated per breed. Inbreeding coefficients (F_{IS}) were estimated according to Weir and Cockerham (1984). Based on an unsupervised analysis in ADMIXTURE 1.23 (Alexander *et al.* 2009) for two ancestral populations we calculated the percentage of indicine origin (as represented by the Brahman population) in contrast to the taurine origin (as represented by the Holstein and Jersey populations) in the Iranian breeds.

Effective population sizes were estimated with the *LDNe* program (Waples and Do 2008). *LDNe* conducts population size estimations based linkage disequilibrium as represented by pairwise correlations (r^2) according to Hill (1981) and Waples (2006). Only markers with allele frequencies >0.01 were used and the mating system was assumed to be random. Estimates were carried out per chromosome. For the purpose of conservation genetics, changes in heterozygosity over time (generations 1-50) were estimated as $H_t = H_0 (1 - 1/(2 N_e))^t$ where H_t and H_0 are heterozygosity at generation t and generation zero, respectively, and N_e is the effective population size as estimated with the *LDNe* program and averaged across all chromosomes.

RESULTS AND DISCUSSION

The Iranian cattle breeds showed similar heterozygosity levels compared to the applied outgroup breeds. Inbreeding coefficients were close to zero indicating no apparent loss of genetic diversity (Table 1). The Pars breed had the highest inbreeding coefficient (0.121; Table 1). Surprisingly, the Holstein cattle showed an increase in heterozygosity as indicated by a negative inbreeding coefficient (-0.109; Table 1). This unexpected result indicates that further interpretation of results concerning the Holstein breed have to be taken in the context of this study, as Holsteins are generally reported to have a decreased heterozygosity due to strong artificial selection (Rodriguez-Ramilo *et al.* 2015).

Table 1. Breed description and population genetics parameters for 11 cattle breeds after quality control

Breed	N	N_e ±se	Appearance	MAF	He	F_{IS}	% indicine
Jersey	15	10.6 ±1.00	taurine	0.22	0.32	-0.041	0.00001
Holstein	15	7.7 ±0.91	taurine	0.24	0.36	-0.109	2.5
Kurdi	7	31.1 ±10.6	taurine	0.26	0.36	0.005	32.3
Sarabi	19	6.1 ±0.84	taurine	0.25	0.34	-0.023	42.1
Pars	7	63.7 ±28.4	taurine	0.21	0.26	0.121	80.9
Kermani	9	8.8 ±2.03	taurine	0.20	0.27	0.083	82.6
Taleshi	7	72.0 ±20.1	indicine	0.23	0.32	0.017	61.5
Mazandarani	10	3.1 ±0.87	indicine	0.23	0.32	0.018	65.6
Najdi	7	165.5 ±56.8	indicine	0.21	0.31	-0.001	75.1
Sistani	9	45.2 ±19.8	indicine	0.16	0.23	0.059	95.3
Brahman	15	35.0 ±5.99	indicine	0.16	0.23	0.004	99.9

Ne: effective population size; MAF: minor allele frequency; He: heterozygosity; F_{IS}: inbreeding coefficient

Notably, the more indicine a breed was (as represented by the percentage of Brahman origin) the lower the heterozygosity (Table 1). However, this trend can be explained by ascertainment bias where indicine loci are often less variable due to mostly taurine breeds used in design of the SNP chip (Lachance and Tishkoff 2013). Interestingly, some of the breeds that were classified into taurine and indicine based on their outer appearance were re-grouped based on their genetic constitution. As such, Taleshi and Mazandarani expressed dewlaps and slight humps but had less indicine ancestry than Pars and Kermani (taurine appearance; Table 1). This discrepancy shows how important genetic analyses are to correctly assign individuals to breeds and ancestries.

Effective population sizes based on LD were on average lowest for Mazanderani, Sarabi, and Kermani (Table 1). For these breeds, a close monitoring of potential inbreeding depressions is warranted; however, the small sampling size might have led to a downward bias of estimates. The Sarabi breed is currently the most used dairy breed in the North-West of Iran and the small effective population size of this breed might also stem from a semi-established breeding program by the Iranian government. Thus, individuals for mating could be artificially restricted. Effective population sizes for Holstein and Jersey were much lower compared to previous reports (Rodriguez-Ramilo *et al.* 2015; Stachowicz *et al.* 2011) and the downward bias of our study is most likely due to the small sample size. Nevertheless, comparisons can be made within the results of this study.

Effective population sizes showed a large variation depending on the chromosome that was used for estimation. Whilst estimates of most chromosomes resulted in N_e <100, chromosomes 3, 6, 10, 11, 13, 20, 23, 25, and 29 showed highly increased effective population sizes (Figure 1). Breeds with chromosome-wise outstanding N_e were Najdi, Pars and Sistani which are all breeds with an increased indicine/Brahman proportion. Taleshi showed highest chromosome-wise N_e for the taurine classified breeds (Figure 1).

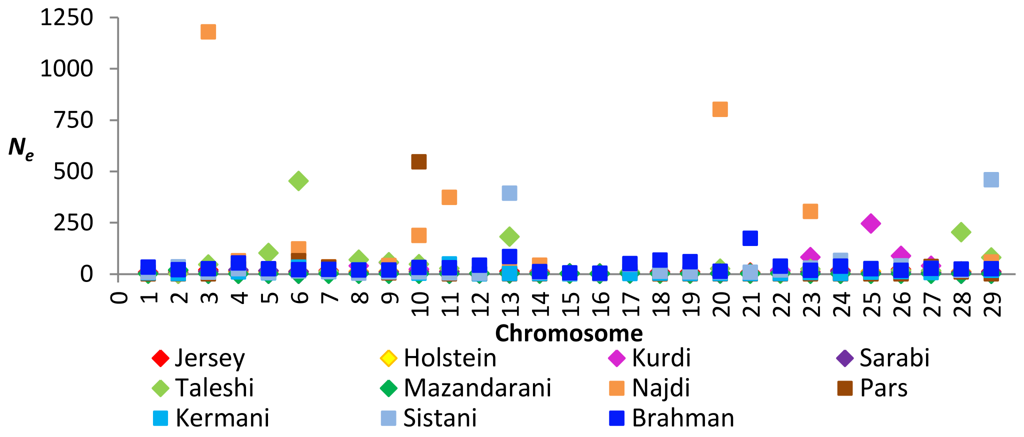

Figure 1. Effective population sizes (N_e) per chromosome for 11 cattle breeds.
diamonds: taurine breeds; squares: indicine breeds

According to Waples (2006; *equation 7*), N_e is dependent on LD (r^2) and sample size. Even though sample sizes in this study varied slightly between populations, we can deduce that chromosomes and breeds with outstandingly high N_e must have a very weak LD (small r^2). Linkage disequilibrium between markers is also an important measure to estimate the phenotypic variance that is explained by the genetic sequence. Esquivelzeta-Rabell *et al.* (2014) demonstrated in sheep that the variance explained differed between chromosomes. Even though not completely transferable between sheep and cattle, chromosomes with the highest explained variance were also the chromosomes in this study that show outstandingly high N_e estimates (except chromosomes

3 and 29).

Based on average heterozygosities and N_e estimates, genetic variability over the subsequent generations can be predicted if no intervention is made via conservation programs. Breeds with the smallest effective population sizes will lose almost all of their genetic variability within the next 20-30 generations (**Figure 2**). As previously stated, conservation programs should aim to maintain the genetic variability of these breeds to preserve this valuable resource for the future.

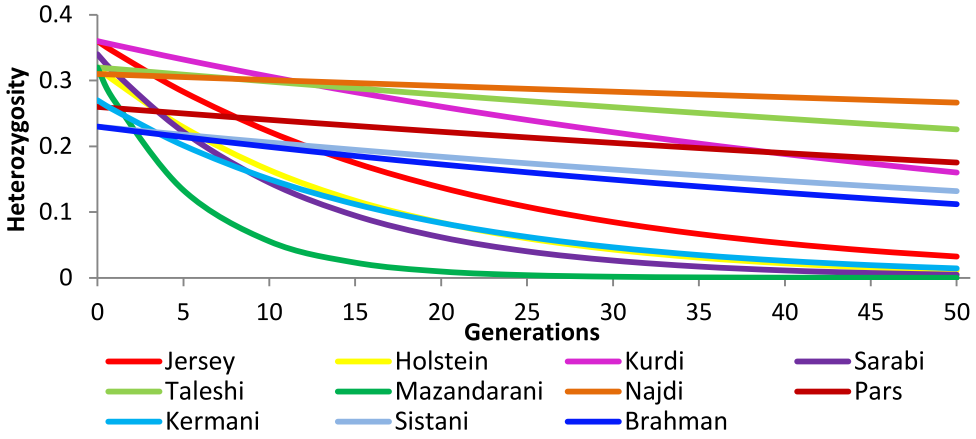

Figure 2. Decay of heterozygosity over 50 generations in 11 cattle breeds.

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