Genome-wide linkage disequilibrium and past effective population size in three Korean cattle breeds


Summary

The routine collection and use of genomic data are useful for effectively managing breeding programs for endangered populations. Linkage disequilibrium (LD) using high-density DNA markers has been widely used to determine population structures and predict the genomic regions that are associated with economic traits in beef cattle. The extent of LD also provides information about historical events, including past effective population size (Ne), and it allows inferences on the genetic diversity of breeds. The objective of this study was to estimate the LD and Ne in three Korean cattle breeds that are genetically similar but have different coat colors (Brown, Brindle and Jeju Black Hanwoo). Brindle and Jeju Black are endangered breeds with small populations, whereas Brown Hanwoo is the main breeding population in Korea. DNA samples from these cattle breeds were genotyped using the Illumina BovineSNP50 Bead Chip. We examined 13 cattle breeds, including European taurines, African taurines and indicines, and hybrids to compare their LD values. Brown Hanwoo consistently had the lowest mean LD compared to Jeju Black, Brindle and the other 13 cattle breeds (0.13, 0.19, 0.21 and 0.15–0.22 respectively). The high LD values of Brindle and Jeju Black contributed to small Ne values (53 and 60 respectively), which were distinct from that of Brown Hanwoo (531) for 11 generations ago. The differences in LD and Ne for each breed reflect the breeding strategy applied. The Ne for these endangered cattle breeds remain low; thus, effort is needed to bring them back to a sustainable tract.

Keywords genetic diversity, Hanwoo, population parameter

The Food and Agricultural Organization of the United Nations (FAO) documents Korean cattle breeds on its Domestic Animal Diversity Information System. Korean cattle have been classified and named based on their body color and geographical distribution (Dadi et al. 2012; Suh et al. 2014). Brown Hanwoo is widely used for breeding in Korea, Brindle Hanwoo is from the Korean Peninsula and Jeju Black is from Jeju Island (Fig. S1). The Korean government has been trying to improve the genetic performance of these cattle breeds even though the conditions of the breeding populations are not the same. Brown Hanwoo has the largest population (3 million animals) with a well-established breeding program and progeny testing to select bulls (Park et al. 2013), whereas the others (Brindle and Jeju Black) are endangered (Suh et al. 2014), although these breeds have the potential to be developed as beef cattle with quality comparable to that of Brown Hanwoo (Lee et al. 2013c; Han et al. 2015).

Current genetic improvement programs for Korean cattle rely on the framework of genomic selection, population structure, and genetic predictions – which have become routine genomic tools (Lee et al. 2014). Analysis of genomic data is becoming part of the beef cattle industry and useful for the improvement of the effectiveness of genetic programs for endangered breeds such as Brindle and Jeju Black. Linkage disequilibrium (LD) and effective population size (Ne) analyses are important, as these parameters are strongly influenced by historical events, including genetic
Recent population sizes of Jeju Black and Brindle cattle were 500 and 2885 breeding animals respectively (Chikso Management System 2015). These two cattle breeds are managed by artificial insemination (AI) by sires at livestock research centers that belong to the local government. We genotyped all available AI sires for Jeju Black (n = 20) and Brown Hanwoo (n = 667), but we had access to only 10 sire and 10 female Brindle cattle. All animals were genotyped using the BovineSNP50 BeadChip (Illumina). SNP data for 304 animals, obtained from Gautier et al. (2010), were used as representative of European taurine (EUT; Angus, Brown Swiss, Jersey, Hereford and Guernsey), African taurine (AFT; N'Dama, Oulmes Zaer and Sheko), indicine (ZEB; Brahman, Nelore and Madagascar Zebu) and hybrid (H; Beef Master and Santa Gertrudis).

The quality of the SNP data was maintained using the following criteria: SNPs that were out of Hardy-Weinberg equilibrium (P < 1 x 10^{-5}) or had a low call rate (<90%) were removed, and the minimum minor allele frequency of SNPs was limited to 1%. Missing genotypes were imputed within each breed using 30 iterations with BEAGLE 4.0 (Browning & Browning 2007). LD was measured as r^2 among all pairs of syntenic SNPs. SNP cleaning and LD estimation were finished using PLINK v1.07 (Purcell et al. 2007) for each breed. The historical LD phase was calculated using the linear regression of the square root of LD (r) between breeds (Sargolzaei et al. 2008). We used both PLINK and ADMIXTURE v1.23 (Alexander et al. 2009) to determine population structure analyses, i.e. by calculating the multidimensional scaling algorithm and estimating the unsupervised model for ancestors admixture analysis respectively. We also calculated the heterozygosity and runs of homozygosity to examine the genetic diversity of the Korean cattle. N_e was estimated from the LD value following Sved’s (1971) equation as explained by Sargolzaei et al. (2008) and fitted to a non-linear least square regression in R (R Core Team 2015). Then, the equation was plotted following estimated times in the horizontal ordinate (Hayes et al. 2003).

Korean cattle breeds were observed to have high proportions of cleaned SNPs (>71%) from a panel of 54 609 SNPs (Table S1). Despite the small sample size of Brindle and Jeju Black, we were still confident with our results. These sample sizes were adequate if following the recommendation of Khatkar et al. (2008) regarding the minimum number of samples for r^2 analysis, as both Brindle and Jeju Black were classified as endangered (FAO 2015). The expected heterozygosity results proved that our samples were randomly selected (Fig. S2). The run of homozygosity plot (Fig. S3) supports the result that no homozygous region was observed. The other 13 breeds were used for comparison, and the LD results for these breeds should be considered only a rough approximation.

Based on the multidimensional scaling plot (Fig. S4), we suggest that Korean cattle are more liable to be inclined toward EUT because the point distance from Korean cattle to EUT is closer than that to AFT and about half that to ZEB. We performed admixture analysis with K = 2 to K = 20 and found that the minimum error after cross validation was at K = 15, i.e. 0.48702 (Figs. S5 & S6). EUT was seen as dominantly structured (60%) in the Brown Hanwoo population at K = 3. We observed that Korean cattle still have their independent structure in the next K (Fig. S5; K = 7, K = 11, K = 15), which means they may have a common ancestor. The proportions of the genetic mixtures among Korean cattle breeds were different, proving their genetic diversity. Lee et al. (2013a) and Porto-Neto et al. (2014) also performed similar studies, in which similar primary results were observed.

Breed, chromosome and genomic distance are known to have significant effects on LD value (Lu et al. 2012). Brown Hanwoo had a lower LD value, and in all autosomal chromosomes and all distance ranges, the LD value decayed more rapidly compared to those of the other Korean cattle breeds (Tables S2–S4) and other 13 breeds (Fig. 1). EUT breeds tended to have the highest LD values. Brindle and Jeju Black had lower LD values compared to EUT and seemed to overlap with ZEB, AFT and H in the extended distance ranges. The Korean cattle breeds were found to have different LD values, i.e. 0.13, 0.21 and 0.19 for Brown Hanwoo, Brindle, and Jeju Black respectively (Table S1). This demonstrates that the LD pattern is a breed-specific feature that depends on genetic events passed on by individuals in the population (Smith & Kuhnner 2009). The value of adjacent LDs for Brown Hanwoo in the present study was lower compared to the value reported by Edea et al. (2015). The difference could be because of the distinctive types of Hanwoo populations that were analyzed, i.e. steers (Edea et al. 2015) vs. proven bulls in the present study.

The levels of LD seemed to be inversely related to the number of marker pairs (Table S5). Higher LD values were obtained in cases of a smaller number of SNP pairs, which were usually found in the short distance range (<20 kb). This result agrees with similar studies in Hanwoo (Edea et al. 2015) and other cattle breeds (Gautier et al. 2007; de Roos et al. 2008; Sargolzaei et al. 2008; Lu et al. 2012). Unfortunately, estimation bias appeared when LD was analyzed for the short distance range for the other 13 cattle breeds because of the small sample size, as predicted by Khatkar et al. (2008). That is why Angus, N’Dama, Brahman and Beef Master, which are representative of EUT, AFT, ZEB and H respectively, all had very low LD values at a short distance range <20 kb.

Linkage disequilibrium (LD) was persistent between the Korean cattle breeds and extended up to 20 kb (r-values of 0.89 and 0.91 for Brown Hanwoo vs. Brindle and Brown Hanwoo vs. Jeju Black respectively; Table S6). The correlation coefficient is a result of the genetic relationship
between populations (de Roos et al. 2008; Sargolzaei et al. 2008), and a strong relationship is usually found with a close genetic distance (Gautier et al. 2007; de Roos et al. 2008), as shown in our study. Furthermore, the correlation coefficient can be used to estimate population history if genomic distance is assigned as historical time (Hayes et al. 2003) and the correlation coefficient is considered as the population relatedness level. We propose that these Korean cattle breeds came from a common ancestral population.

We identified that chromosomes 21 and 26 had higher LD for all three Korean cattle breeds (Table S7). Given that Lee et al. (2013a) and Li & Kim (2015b) identified significant associations on chromosomes 21 and 26 with growth-related traits in Brown Hanwoo and Porto-Neto et al. (2014) identified significant associations on chromosome 21 with immune system traits, we suggest that those associations would also affect Brindle and Jeju Black cattle. Moreover, Lee et al. (2013b) reported that one major region on chromosome 14 was related to carcass weight in Brown Hanwoo. In the present study, chromosome 14 was also identified as one of the three chromosomes with the highest LD in Brown Hanwoo but not in the other breeds. This might be because of the strict genetic selection through performance test that has been applied only for Brown Hanwoo for the past 30 years (Park et al. 2013; Suh et al. 2014).

**Figure 1** Linkage disequilibrium plot for Brown Hanwoo, Brindle Hanwoo, Jeju Black, European taurines, African taurines, indicines, and hybrids.

**Figure 2** Effective population size ($N_e$) plot for Brown Hanwoo, Brindle Hanwoo, and Jeju Black.

The estimated $N_e$ tended to decrease over time in all three breeds (Fig. 2; Table S8). The high LD of Brindle and Jeju Black contributed to a small $N_e$ (53 and 60 respectively), which is considerably lower than that of the Brown Hanwoo (531) for the past 11 generations. The $N_e$ for Brown Hanwoo in the recent study was lower compared to that in the study performed by Li & Kim (2015a), who reported an $N_e$ value of 630 for Hanwoo 11 generations past. However, our result was higher compared to that in a previous study performed by Lee et al. (2011), where they reported an $N_e$ value of 352 for the 10 past generations. These differences might be due to the sample type and size variances used in these studies, because estimates of $N_e$ depend on the number of cattle alive at any time in the population, generation length and variance in family size (Lu et al. 2012). Furthermore, SNP densities also have influence on the $N_e$ because they depend on LD, which is biased for low marker density (Edea et al. 2015). We used about 43K cleaned SNPs from 667 proven bulls (sires), a higher density than did Li & Kim (2015a), who used about 36K cleaned SNPs from 61 sires and 486 steers, and Lee et al. (2011), who used about 4.5K cleaned SNPs from 232 Hanwoo steers. Moreover, both paternally and maternally inherited genotypes were used in our study, whereas Li & Kim (2015a) used only maternally inherited genotypes.

Low $N_e$ values that have been estimated for Brindle and Jeju Black corroborated the demographic history explained by Dadi et al. (2012), by which the population reduction experienced by both Brindle and Jeju Black cattle accounts for the loss of genetic variation and increase in their inbreeding rate. However, the $N_e$ for both Brindle and Jeju Black populations were still higher than the limit assigned by the FAO, i.e. 50 per generation to maintain breed fitness (Lu et al. 2012).

The $N_e$ of Brown Hanwoo has decreased dramatically in the past 60–70 years, possibly due to population loss during the Korean War during which the number of Brown Hanwoo decreased to 390 000 animals (Song 1994), which is 10 times smaller than the current population. The endangered Brindle and Jeju Black animals have low $N_e$ values because $N_e$ is intensely influenced by the applied selection and inbreeding levels (Hayes et al. 2003; de Roos et al. 2008). Inbreeding most likely occurs because of a limited number of bulls in an isolated natural population (Sargolzaei et al. 2008). The other reason is that Brown Hanwoo was selected as the main breed early in the genetic improvement program, whereas Brindle and Jeju Black were excluded (Suh et al. 2014).

In conclusion, distinct LD and $N_e$ values were identified in three Korean cattle breeds, and the three breeds probably came from a common ancestral population. The differences in LD and $N_e$ for each breed reflect historical events and recent selection through the breeding program. The $N_e$ values for endangered cattle breeds remain low and efforts are needed to maintain its sustainability.

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Supporting information

Additional supporting information may be found online in the supporting information tab for this article:

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