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Genome-wide analysis of runs of homozygosity in six Indian buffalo breeds

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Introduction

Water buffalo (Bubalus bubalis) are central to India's livestock economy, with the country maintaining over half of the global buffalo population (≈109.85 million) and contributing substantially to national milk production (DAHD, 2023; FAO, 2023). Buffaloes also provide draught power, manure, and livelihood security in mixed farming systems (Birthal *et al*., 2015). Buffalo breeds vary widely in population size, breeding structure, and geographic isolation, leading to differences in genetic diversity and vulnerability to inbreeding (FAO, 2015; Groeneveld *et al*., 2010). Buffalo breeding programmes are often constrained by incomplete pedigree recording and unstructured mating, limiting the reliability of pedigree-based inbreeding estimates (Kumar *et al*., 2018). Genome-wide SNP data offer robust alternatives by capturing realised autozygosity and genetic relationships (VanRaden, 2008; Purfield *et al*., 2012). Runs of homozygosity (ROH) enable partitioning of recent and ancient inbreeding, with long ROH reflecting recent inbreeding and short ROH indicating historical events (Ferenčaković *et al*., 2013; Ceballos *et al*., 2018). ROH-based inbreeding coefficients (F_{ROH}) are therefore considered reliable measures across livestock species (Mastrangelo *et al*., 2017).

Despite their importance, genome-wide ROH-based studies across indigenous Indian buffalo breeds remain limited. This study aimed to (i) characterise ROH distribution and length classes in six Indian buffalo breeds, (ii) estimate ROH-based genomic inbreeding coefficients (F_{ROH}), and (iii) assess genomic relationships within and between breeds.

Materials & Methods

A total of 3,429 buffaloes from six breeds i.e. Jafarabadi (JAF, n = 1512), Banni (BN, n = 432), Nili-Ravi (NR, n = 408), Pandharpuri (PD, n = 527), Surti (SR, n = 362), and Toda (TD, n = 188)—were analysed. Samples from JAF, BN, NR, and PD were obtained from pedigree selection programmes under the Rashtriya Gokul Mission (DAHD, Government of India), while SR and TD samples were collected from field populations. All these buffaloes were genotyped with medium density MAHISHCHIP, customized Affymetrix Bovine SNP chip developed by National Dairy Development Board (NDDB), Anand, Gujarat, India consisted 61353 SNP. Genotypic quality control was performed using PLINK v1.9 (Purcell *et al*., 2007) with thresholds of --geno 0.1 and --mind 0.1. After removal of low-quality and non-autosomal markers, 54,909 autosomal SNPs were retained. ROH were identified using a sliding-window approach, with parameters validated based on genomic coverage. Final ROH detection required a minimum length of 1 Mb and ≥35 consecutive SNPs.

ROH were classified into four length categories: 1–4 Mb, 4–8 Mb, 8–16 Mb, and >16 Mb. Individual-level metrics included ROH count, total ROH length, mean ROH length, and maximum ROH length. Genomic inbreeding coefficients (F_{ROH}) were calculated following McQuillan *et al*. (2008) as the proportion of the autosomal genome covered by ROH. F_{ROH} > 4 Mb was used as an indicator of recent-to-intermediate inbreeding.

Genomic relationships among individuals were estimated using the –make-rel and –make-rel square functions in PLINK, and the correlation between F_{ROH} and genomic relationships was assessed following VanRaden (2008). Breed-wise correlations between F_{ROH} and GRM estimates were evaluated to assess the concordance between autozygosity-based and relationship-based measures of realised inbreeding (Bjelland *et al*., 2013; Peripolli *et al*., 2017).

Results

Across the 3,429 buffalo analysed, a total of 39,124 runs of homozygosity (ROH) segments were identified. ROH were unevenly distributed across the genome, with chromosomes 2, 3, 5, 7, and 12 consistently showing higher ROH density across breeds, suggesting the influence of selection, genetic drift, and historical demographic processes.

Breed-wise ROH length-class distributions revealed contrasting demographic patterns (Figure 1).

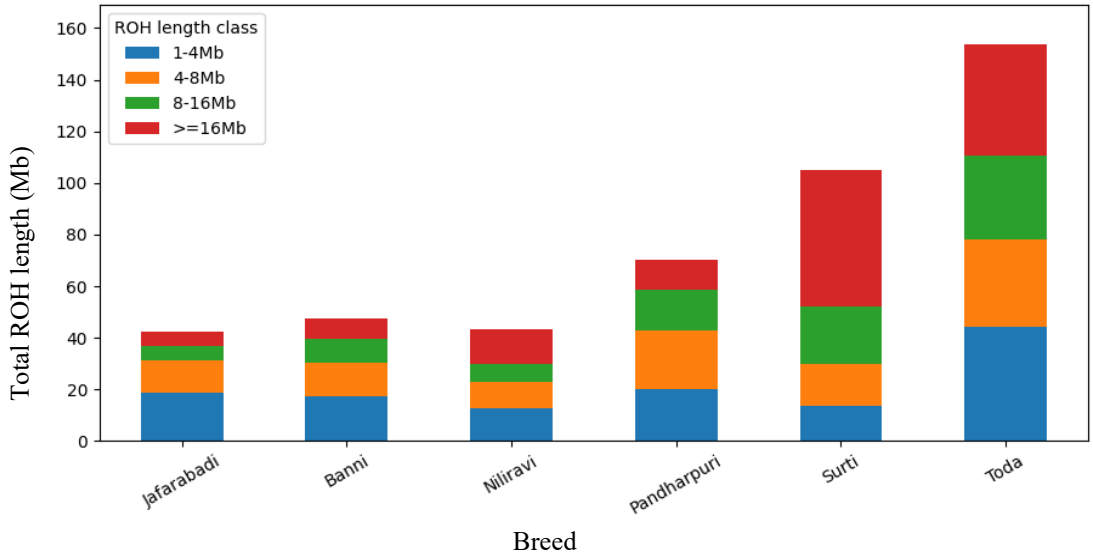

Figure 1. Genome-wide distribution of ROH length classes across Indian buffalo breeds. Stacked bar plot showing the contribution of ROH length classes (1–4 Mb, 4–8 Mb, 8–16 Mb, ≥16 Mb) across breeds.

The longest ROH (130.95 Mb) was detected on chromosome 2 in the Surti breed, indicative of recent inbreeding or a reduced effective population size. The mean genomic inbreeding coefficient across all animals was F_{ROH} = 0.0243, indicating moderate population-level inbreeding. Partitioning inbreeding by ROH length revealed a mean F_{ROH} > 4 Mb of 0.0166, indicating that approximately two-thirds of total autozygosity originated from recent to intermediate inbreeding events. Substantial breed-wise variation was observed (Table 1). Toda showed the highest ROH burden (153.51 Mb; F_{ROH} ≈ 0.062), followed by Surti (130.95 Mb; F_{ROH} ≈ 0.057), whereas Jafarabadi exhibited the lowest ROH coverage (42.27 Mb; F_{ROH} ≈ 0.017).

Mean ROH count varied markedly among breeds, ranging from 26.79 in Toda to 7.72 in Nili-Ravi. Mean ROH length was greatest in Surti (6.63 Mb) and smallest in Jafarabadi (3.90 Mb), reflecting differences in breeding structure, effective population size, and demographic history.

Breed-wise ROH length-class distributions revealed contrasting demographic patterns (Figure 1). Surti and Toda showed an excess of long ROH (>16 Mb), consistent with recent inbreeding and reduced effective population size. Pandharpuri was dominated by intermediate ROH (4–16 Mb), whereas Jafarabadi and Nili-Ravi were enriched for short ROH (1–4 Mb), indicative of ancient inbreeding, admixture, or historically larger population sizes. ROH-based genomic inbreeding coefficients (F_{ROH}) were positively associated with genomic relationship matrix (GRM) estimates across all breeds (Table 2), with correlation coefficients ranging from r = 0.39 in Nili-Ravi to r =

0.69 in Toda. Breeds characterised by higher inbreeding levels and smaller effective population size (Surti and Toda) showed stronger F_{ROH}–GRM associations, whereas larger and more widely distributed breeds (Jafarabadi and Nili-Ravi) exhibited weaker correlations, reflecting greater genetic diversity and a higher contribution of ancient ancestry.

Table 1. Summary of ROH-based genomic inbreeding (F_{ROH}) statistics by breed.

Breed	No. of samples	Mean ROH count	Total ROH (Mb)	F_{ROH}	Genome coverage (%)
Jaffarabadi	1512	7.8	42.27	0.017	1.69
Banni	432	12.56	59.29	0.024	2.37
Nili-Ravi	408	7.72	54	0.015	2.16
Pandharpuri	527	18	84.14	0.034	3.36
Surti	362	14.56	130.95	0.057	5.2
Toda	188	26.79	153.51	0.062	6.1

F_{ROH} was calculated based on runs of homozygosity (ROH) with a minimum length threshold of > 4 Mb

Table 2. Breed-wise correlation between ROH-based inbreeding (F_{ROH}) and genomic relationships (GRM)

Breed	Mean F_{ROH}	Mean within-breed GRM	Correlation
Jafarabadi	0.017	0.024	0.42
Banni	0.024	0.031	0.48
Nili-Ravi	0.015	0.021	0.39
Pandharpuri	0.034	0.045	0.55
Surti	0.057	0.072	0.63
Toda	0.062	0.081	0.69

F_{ROH} was calculated based on runs of homozygosity (ROH) with a minimum length threshold of > 4 Mb

Discussion

This study provides the first comprehensive genome-wide assessment of ROH patterns and length-specific genomic inbreeding across multiple Indian buffalo breeds. The moderate overall F_{ROH} values observed indicate that, at the population level, inbreeding is not excessive, consistent with findings in buffalo and cattle populations maintained under semi-structured breeding systems (Purfield *et al*., 2012; Kim *et al*., 2013; Meyerhoff *et al*., 2019; Mokhber *et al*., 2019). Similar levels of genomic inbreeding have been reported in livestock populations where unstructured or partially controlled mating systems allow maintenance of genetic diversity despite limited pedigree recording. Marked breed-specific differences in ROH profiles reflect contrasting demographic histories and breeding practices. The predominance of long ROH in Surti and Toda indicates recent inbreeding arising from recent common ancestry. This pattern may reflect the combined effects of small effective population size, geographic isolation, and/or intentional mating of related individuals (Ferenčaković et al., 2013; Ceballos *et al*., 2018; Peripolli *et al*., 2017, 2018; Meyerhoff *et al*., 2019). Such patterns have been widely documented in small or isolated livestock populations and highlight the cumulative impact of limited sire usage and restricted gene flow. In contrast, enrichment of short ROH in Jafarabadi and Nili-Ravi suggests ancient inbreeding, historical bottlenecks, or long-term genetic drift rather than recent consanguinity (Mastrangelo *et al*., 2017; Meyerhoff *et al*., 2019; Mokhber *et al*., 2019). The positive correlation between F_{ROH} and GRM estimates confirms that both metrics capture realised identity-by-descent, with stronger correspondence observed in breeds experiencing recent inbreeding. Similar relationships between ROH-based inbreeding and genomic relationships have been reported in buffalo, cattle, and other livestock species, supporting the use of ROH-based

metrics as robust tools for monitoring inbreeding in populations lacking reliable pedigree information (VanRaden, 2008; Bjelland *et al*., 2013; Peripolli *et al*., 2017; Meyerhoff *et al*., 2019).

Conclusions

This study establishes a genomic baseline of autozygosity, inbreeding, and population structure across six Indian buffalo breeds. Substantial breed-specific variation was observed, with evidence of recent inbreeding in Surti and Toda and predominance of ancient autozygosity in Jafarabadi and Nili-Ravi. ROH-based genomic metrics provide a practical and robust framework for monitoring inbreeding and guiding sustainable breeding and conservation strategies in buffalo populations critical for farmer livelihoods in India and globally.

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