Research Article

Genetic cryptic demographic mode shift analysis in buffaloes of Odisha using STR markers shows an absence of recent bottleneck

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ABSTRACT

India is known for its livestock diversity, particularly in the buffalo population, which is highly diversified and the country is placed top among the buffalo rearing across the globe. Odisha state is home to exemplary and varied buffalo populations, featuring prominent breeds and populations like Chilika, Kalahandi, Paralakhemundi, Sambhalpuri, and the latest registered breed, Manda. It is intriguing to know if some rare alleles are contributing to the survival and adaptability of Odisha buffalo breeds in the niche, which is generally hostile to other Indian buffalo breeds. The data already generated using a set of 20 highly polymorphic heterologous markers on 192 animals of Chilika, Kalahandi, Paralakhemundi and Sambhalpuri buffalo populations was analyzed for the current study. The mutation drift equilibrium analysis for buffalo populations revealed no significant heterozygosity excess, when evaluated using three different models of evolution viz. infinite alleles model (IAM), step-wise mutation model (SMM) and two-phase model (TPM). The buffalo populations, few of them being small in size, still did not show significant heterozygosity excess under any of the three models of microsatellite evolution, thus indicating the absence of any recent genetic bottleneck. Mode shift analysis displayed a normal L-shaped curve which is indicative of the absence of any recent past bottleneck. The results of the present study will help formulate rational breeding strategies as well as the conservation of unique germplasm of Odisha buffaloes.

Key words: Bottleneck, Buffaloes, Mutation drift equilibrium, Odisha

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INTRODUCTION

India is one of the 17 countries harboring highly diverse species, which is reflected in its highly diversified buffalo population. India has seen the highest buffalo population, with a percentage increase of 13-20% during the decades of 1991-2002 and 2003-2017 (Hegde, 2019). Buffalo belongs to the Bovidae family, further classified into two main species: the Asiatic buffalo (*Bubalus bubalis*) and the African buffalo (*Syncerus caffer*). The domestication of Asiatic buffaloes, also known as water buffaloes and located in India, started there 5,000 years ago (Kierstein *et al*., 2004). The water buffaloes are classified into two classes based on their habitat, riverine and swamp, which diversified approximately 10,000 to 1.7 million years ago, long before domestication of the buffaloes (Michelizzi *et al*., 2010). Except for Chilika buffaloes, which feature a small minority of hybrid riverine swamp animals, all of the Odisha buffalo breeds are predominantly riverine (Singh *et al*., 2017; Kumar *et al*., 2019a; Singh *et al*., 2020).

The population bottleneck is a central paradigm of population genetics and conservation biology (Nei *et al*., 1975). A sharp reduction in the size of a population leading to a decrease in variability and distorting the population structure is defined as a 'genetic bottleneck'. Various approaches have been implemented for bottleneck detection. At the demographic level, the regular census of livestock populations can record the population bottleneck, but in India, a periodic census is not available at the breed level for several reasons (Kataria *et al*., 2010). Alternately, genetic bottleneck analysis is preferred for such endemic or remote livestock populations' bottleneck monitoring. The genetic bottleneck analysis based on the SSR markers primarily investigates the allele deficiency based on two main approaches: the first involves testing for heterozygosity excess under three different mutation models: IAM (Kimura and Crow, 1964), SMM (Kimura and Ohta, 1978), and TPM (Di Rienzo *et al*., 1994), and the second involves plotting the frequency of allele distribution, also known as a 'site-frequency spectrum graph' or 'mode-shift analysis'. The cryptic bottleneck occurring due to poor census, high variance in family size, artificial insemination, or polygamous or polyandrous breeding systems needs to be monitored by genetic bottleneck methods, in terms of estimated effective population size (Ne) (Kataria *et al*., 2010).

Odisha being a coastal area, the environmental conditions are severe and ever-changing; therefore, the buffaloes of Odisha province (Chilika, Kalahandi, Paralakhemundi, and Sambhalpuri) are highly diversified to adapt to the highly adverse conditions of their tropical habitat (Singh *et al*., 2018; Kumar *et al*., 2019b; Mishra *et al*., 2020; Singh *et al*., 2021; Kumar *et al*., 2022). The underlying cause for this adaptation can be assumed to be at the genetic level, specifically that the presence of rare alleles is responsible for resistance to fluctuating conditions. The introduction of milch breeds, especially Murrah, in these areas, must have created selective pressure on particular alleles, which resulted in the loss of those beneficial rare alleles (Kathiravan *et al*., 2009). The microsatellite markers are useful in revealing if the given population has undergone a bottleneck. The microsatellite marker has been utilized for mutation drift analysis in several Indian buffalo breeds (Kataria *et al*., 2009a; Kataria *et al*., 2009b; Mishra *et al*., 2009; Mishra *et al*., 2010; Joshi *et al*., 2015). Therefore, the present study was proposed to see whether the populations of Odisha state, mainly Chilika, Kalahandi, Paralakhemundi and Sambhalpuri, have undergone any bottleneck in the recent period. This has been achieved by using 20 highly polymorphic microsatellite markers.

MATERIALS AND METHODS

Population selection and microsatellite genotyping

The already generated data on 20 highly polymorphic microsatellite markers on Chilika, Kalahandi, Paralakhemundi, and Sambhalpuri (n=48, each) that have been submitted in the Dyrad, open data publishing platform under DOI: 10.5061/dryad.s4mw6m97q (Kumar *et al*., 2022) was utilized in the present study. The breeding tracts of the buffalo populations are depicted in Fig. 1. Briefly, the STR data generation involved isolating DNA from whole blood samples obtained from unrelated the buffaloes and amplifying the aforementioned microsatellite markers (ILSTS025, ILSTS056, ILSTS019, ILSTS033, CSSM33, ILSTS061, ILSTS028, HEL13, ILSTS058, ILSTS052, CSSM19, CSSM57, ILSTS030, CSSM47, ILSTS060, ILSTS089, CSSM66, ILSTS026, CSSM45, and ILSTS029), using primer sets previously published (Kataria *et al*., 2009a). Following the verification of the amplified products on agarose gel electrophoresis, the four PCR products were multiplexed, primers end-labeled with different dyes in a single well, with GS500LIZ (Applied Biosystems, USA) serving as an internal lane control. The PCR products were then genotyped using an automated DNA sequencer (ABI 300). Following post-fragment analysis on an automated DNA sequencer, allele size information was extracted from the raw data using GENEMAPPER software (Imle, 2005), and further GenAlEx 6.5 software (Peakall and Smouse, 2006) was used to export the data in the GENEPOP format.

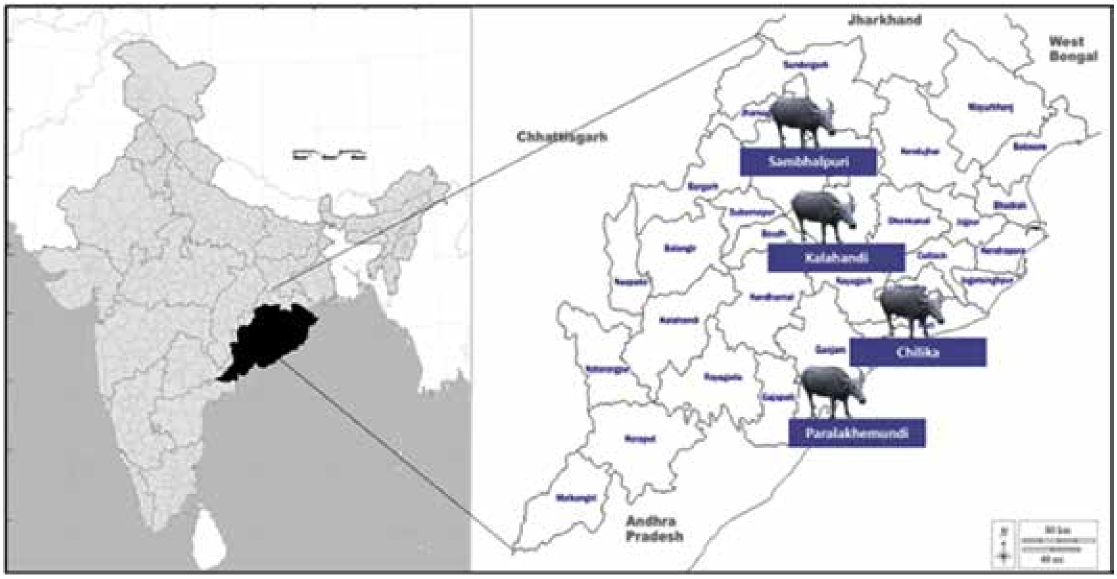

Fig. 1: *Geographical distribution of Odisha buffalo populations considered in the present study.*

Statistical Analysis

“BOTTLENECK” (Piry *et al.*, 1999) software was used to analyze mutational drift equilibrium. In an Excel file, a chart was created using the allele frequency data. To determine if the population was in mutational drift equilibrium, four distinct tests were run using allele frequency data: the sign test, the standardized differences test, the Wilcoxon sign rank test, and a qualitative test of mode shift.

RESULTS AND DISCUSSION

Bottleneck analysis in Odisha buffaloes (Chilika, Kalahandi, Paralakhemundi, and Sambhalpuri) was performed using different methods in this study by using 20 microsatellite markers to understand the change in effective population size (Ne). The absence of bottleneck in Manda, another registered buffalo breed of Odisha has been reported earlier (Kumar *et al.*, 2022). The reduction in the effective population size leads to the progressive reduction of allele numbers and heterozygosity, resulting in the bottleneck in a breed/population (Cornuet and Luikart, 1996). Both the number of alleles (k) and gene diversity will decline in a population that has experienced a bottleneck due to a reduction in effective population size, although the rate of gene diversity reduction will be slower than that of allele number reduction (k). This leads to an increase in the observed gene diversity than the observed one (Kathiravan *et al.*, 2010).

Sign rank, standardized differences, and Wilcoxon tests were used to assess if these populations had recently undergone any bottlenecks under three mutation models—the two-phase model (TPM), which is better suited to account for the evolution of microsatellites, the infinite allele model (IAM), and the stepwise mutation model (SMM). The ‘BOTTLENECK’ software used for the aforesaid analysis assumes the population to be under mutation drift equilibrium. The variance for TPM was set at 30 and the proportion of SMM in TPM (%) was set to 70 with 1000 iterations under the 3 mutation models with 3 statistical tests during analysis. The sign test within the IAM mutational model established significant deviations from mutation drift equilibrium. The heterozygous deficit was observed to be highest in Paralakhemundi (10 loci). The heterozygous excess (He excess expected/ observed< 0) was found in Kalahandi under IAM model under the sign test (Table 1).

Table 1: Mutation drift equilibrium was tested under different mutational models with different statistical tests for four buffalo populations.

Breeds	Mutation Models	Sign Test		Standardized Differences Test		Wilcoxon Signed-Rank Test
		H_{ex}/ H_{d}	Level of significance	T2	Level of significance	Level of significance (one-tail probability test for H_{ex})
Sambhalpuri	IAM	12/8	Ns	1.561	ns	*
	SMM	4/16	***	-9.098	ns	ns
	TPM	9/11	Ns	-2.297	*	ns
Kalahandi	IAM	13/7	Ns	0.485	ns	ns
	SMM	4/16	***	-12.869	ns	ns
	TPM	8/12	Ns	-3.993	***	ns
Paralakhemundi	IAM	10/10	Ns	-1.975	*	ns
	SMM	2/18	***	-20.054	ns	ns
	TPM	3/17	***	-8.692	ns	ns
Chilika	IAM	12/8	Ns	-0.286	ns	ns
	SMM	2/18	***	-14.51	ns	ns
	TPM	6/14	**	-5.094	ns	ns

***$P<0.001$; **$0.001<P<0.01$; *$0.01<P<0.05$; ns $P>0.05$; H_{ex}/H_{d}= Obs heterozygous excess/ heterozygous deficit

In the IAM model any mutation in the previous allele leads to the formation of a new allele irrespective of the existing one (Kimura and Crow, 1964). Under the IAM model, the standardized differences test and Wilcoxon signed-rank test revealed no significant heterozygosity excess in all the buffalo breeds. In the standardized differences test, the T2 values were found to be positive in all the buffalo breeds except for Paralakhemundi (P<0.05) and Chilika breeds (P>0.05). The one-tail Wilcoxon signed-rank test for heterozygosity excess only for Sambhalpuri (P>0.05) (Table 1). The observed heterozygous excess loci were found to be higher in Nagpuri (Kataria *et al.*, 2009a), UP buffaloes (Joshi *et al.*, 2015) than all Odisha buffaloes. In the IAM mutation model under various tests, only Sambhalpuri was found to have a significant heterozygous excess when compared with previous reports (Kathiravan *et al.*, 2009; Mishra *et al.*, 2009; Kataria *et al.*, 2010, Kathiravan *et al.*, 2010), except Paralakhemundi which showed a higher value, equal to that of South Kanara buffalo.

The SMM mutational model assumes the allele maneuver as a unit with bidirectional sense, positive or negative (Kimura and Ohta, 1978). The heterozygous deficient was found to be significantly (P < 0.05) higher than the heterozygous excess under SMM ranging from 16 (Sambhalpuri, and Kalahandi) to 18 (Paralakhemundi and Chilika) loci out of 20 loci included for analysis (Table 1). The T2 values are found to be negative in all the breeds with a high negative in Paralakhemundi and Chilika breeds (Table 1). The negative T2 values indicate tilting towards heterozygosity deficiency (Kataria *et al.*, 2010), and the high negative values in all Odisha populations indicate tilting towards heterozygosity deficiency. In the standardized difference test, the T2 values were found to be negative (P>0.05) in all the buffalo breeds analyzed. Wilcoxon signed-rank test (one-tail probability test for H_{ex}) was non-significant for all the buffalo populations under the stepwise mutation model. The observed heterozygous excess loci were found to be lower in all Odisha breeds when compared with other Indian breeds (Kathiravan *et al.*, 2009; Kataria *et al.*, 2009a; Kataria *et al.*, 2009b; Mishra *et al.*, 2009; Joshi *et al.*, 2015). In the TPM mutation model, all the buffalo populations had heterozygous deficit higher than heterozygous excess (p<0.05), except for Sambhalpuri the high value of heterozygous deficit was non-significant (p>0.05). The expansion of all Odisha populations is reflected in significant negative T2 values under TPM and (standardized differences test) which are consistent with results from previous reports in Banni (Mishra *et al.*, 2009), SouthKanara (Kathiravan *et al.*, 2010) and UP buffaloes (Joshi *et al.*, 2015). But it was found only in the SMM model (Kathiravan *et al.*, 2009; Kataria *et al.*, 2009a).

The TPM is better suited for microsatellite data than SMM or IAM (Kathiravan *et al.*, 2010). In all the Odisha buffalo populations studied under the TPM model (sign test), the number of loci with observed heterozygosity excess was lower than expected. The statistically significant p value was found in Paralakhemundiand Chilika buffalo populations with sign test suggesting deviation from mutation drift equilibrium. In the standardized differences test, The T2 values were found to be negative in all buffalo breeds with a highly significant value across all the populations except Paralakhemundi and Chilika buffaloes. Wilcoxon signed-rank test revealed one-tail test of heterozygosity excess to be non-significant P values (P>0.05). Thus, rejects the null hypothesis and confirms the absence of any bottleneck in all the Odisha buffaloes. The Odisha buffaloes showed lower heterozygous excess loci under TPM than other Indian buffaloes (Kathiravan *et al.*, 2009; Kataria *et al.*, 2009a). The results are in contrast to the previous reports on UP buffaloes (Joshi *et al.*, 2015). The observed heterozygous excess loci were more than Odisha buffaloes, except Sambhalpuri but Kalahandi showed the same number of loci with observed heterozygous excess with South Kanara under the TPM model (Kathiravan *et al.*, 2010). Toda reported higher heterozygous excess under the TPM model (Kataria *et al.*, 2009b).

The heterozygosity excess was found to be found to be non-significant except for Sambhalpuri (under IAM and TPM), Kalahandi (under TPM), and Paralakhemundi (IAM). The heterozygosity excess was found in the Kalahandi buffalo population under IAM model but the values were statistically non-significant. The Odisha buffalo populations studied have no bottleneck since the populations that recently underwent bottleneck have a significant excess of heterozygosity. However, under both SMM and TPM models, none of the buffalo populations showed heterozygosity excess. The SMM showed the same results as previous reports on buffalo and poultry along with in poultry, were as it was contrasting under TPM model (Kataria *et al.*, 2010; Kathiravan *et al.*, 2009; Tantia *et al.*, 2006).

The Marathwada, Banni, Nagpuri, Jaffarabadi, Toda, Assamese Murrah, Chilika, Pandharpuri, Surti, Jaffarabadi, Nili-Ravi were not found to be undergoing bottleneck in the recent past except Mehsana breed (Kataria *et al.*, 2010; Kathiravan *et al.*, 2009; Mishra *et al.*, 2009; Kataria *et al.*, 2009a; Kataria *et al.*, 2009b; Mishra *et al.*, 2010). In the present report, the Odisha buffalo breeds were also not found to have undergone any bottleneck in the recent past. Apart from the mutational models used to estimate the mutation drift equilibrium, we have also assessed mode-shift distortion to graphically evaluate the allele frequency distribution as an indicator for genetic bottleneck. The qualitative test for mode shift was performed in all the Odisha populations using the allelic frequency. The absence of low-frequency alleles indicates the cause of the bottleneck, due to increasing inbreeding and along with selective breeding that decreases the fitness of a particular breed. Thus, all are reported for mode shift analysis as normal L-shaped curve which are indicative of absence of any recent past bottleneck in all the buffalo populations (Fig. 2).

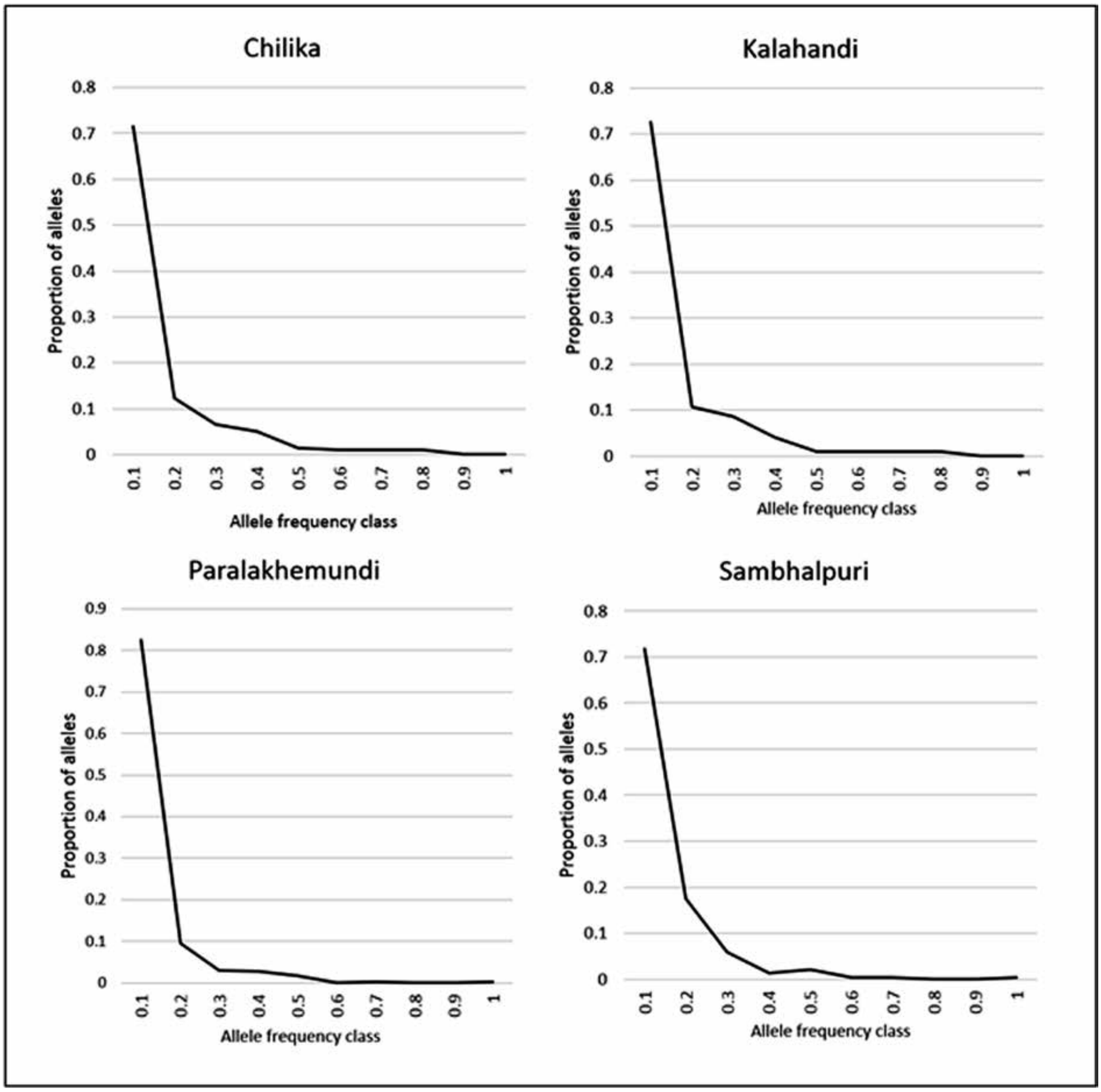

Fig. 2: *Allele frequency distribution in different buffalo populations showing normal L-shaped mode shift graph.*

The normal L-shaped curves are shown in other Indian buffaloes (Kataria *et al*., 2009a, Kataria *et al*., 2009b, Mishra *et al*., 2009, Mishra *et al*., 2010, Joshi *et al*., 2015). These buffaloes did not show significant heterozygosity excess under any of the three models of microsatellite evolution, thus indicating an absence of any recent genetic bottleneck. In conclusion, the populations are deviating from mutation drift equilibrium, there was no significant heterozygosity excess indicating an absence of genetic bottleneck in the recent past. Thus, there is a need for conservation of these highly diversified buffaloes.

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Data availability: Data that support the findings of the study, has been deposited at Dryad data repository (https://datadryad.org/stash), and is available under doi: https://doi.org/10.5061/dryad. s4mw6m97q.

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