Cattle Genetic Resources of India

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PREFACE

The enormous and diverse cattle genetic resources of India are signified in the form of 30 documented breeds of zebu cattle (Acharya and Bhat 1984) besides numerous populations yet to be characterized and defined. Present day cattle breeds have evolved through mutation and genetic drift, as well as human-guided artificial selection. Majority of Indian cattle breeds belong to draft category, as cattle development in India principally rested on the production of bullock energy required for conventional agricultural operations and load pulling. The Kherigarh breed of Indian zebu cattle (Bos indicus) evolved as a draft breed over centuries and become adapted to harsh native environments, developed resistance to tropical diseases and capable of sustenance on low quality roughage and grasses. The breed is primarily employed for agricultural operations, for carrying loads and transportation. The breeding tract of this breed encompasses Nighasan and Pallia blocks of Lakhimpur Kheri District (27N54 80E48) of Uttar Pradesh State, on the foothills of the Himalayas. The breed is primarily maintained by small, marginal and landless labourers.

The animals of the breed are draft type with 362.5 kg average lactation milk yield and an average lactation length of 10.08 months. Animals of the breed are lighter in appearance than Hariana. Bullocks are fast in speed and good for light draught (carting) and agricultural operations. Animals of this breed are kept by and large on zero input system. The declining population indicated that this precious cattle breed will be lost if proper attention is not paid immediately to conserve it. This calls for urgent conservation and improvement strategies for the breed.

Kherigarh cattle breed has been explored morphometrically by field visits and genetically by exploiting FAO recommended microsatellite markers to present current status of this breed. The study is a contribution to the ongoing global endeavor of phenotypic and genetic characterization of livestock genetic resources.

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Introduction

Livestock of a given species are often derived from a single domestication event, followed by genetic and phenotypic radiation, through natural and artificial selection. Thus, selection has created huge genetic diversity among breeds. This diversity is perceptible in the form of local adaptation to rigorous and unpredictable environments, resistance to many of the leading indigenous diseases, and vastly improved and specialized production ability. Nearly all wild ancestors of livestock are either extinct or exist in small populations with limited genetic diversity. Thus, contrary to many agricultural plant species, most or all genetic diversity in livestock exists within and between the available domesticated farm animals. Extensive, but still inadequate efforts are in progress to characterise livestock at the phenotypic and molecular levels, and to document their production environments, utilization and status.

Measuring diverse attributes of a population is important to its characterization, taking account of phenotypic traits, reproduction, geographic distribution, origin and habitat. The genetic characterization of populations, breeds and species allows the evaluation of genetic variability, a fundamental element in working out breeding strategies and genetic conservation plans. Molecular markers have been comprehensively exploited to access this variability as they contribute information on every region of the genome, regardless of the level of gene expression.

Kherigarh, a draught breed of cattle is native of Lakhimpur Kheri district of Uttar Pradesh and a few animals are also available in adjoining Pilibhit district. The breed derives its name from a village ***"Kherigarh"*** situated in the Shinghai area in the Nighasan Tehsil of Lakhimpur Kheri. The bullocks of this breed are active and have remarkable speed and stamina. Cows are poor milkers. Joshi and Phillips (1953) conducted an FAO study on "Zebu Cattle of India and Pakistan" and classified the cattle breeds into 6 groups.

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The Kherigarh breed of cattle falls in the Group I with other breeds like Kenkatha, Malvi, Tharparker and Kankrej. This group included lyre-horned grey cattle with wide foreheads, prominent orbital arches and thin faces having flat or dished in profiles.

Geographic Distribution and Topography

The native tract of the Kherigarh cattle is Lakhimpur Kheri and Pilibhit districts of Uttar Pradesh. The breed is mainly concentrated along the banks of river Sharda in Nighasan and Pallia Blocks of Lakhimpur Kheri district (27N54 80E48).

The predominant soil of the area consists of deep alluvial with occasional limestone. The entire breeding tract is plain area covered by considerable forests. The climate of Lakhimpur Kheri district may be characterized as sub-tropical. Normal average rainfall has been reported as 1093mm whereas it increases upto 1600mm during flood years. The temperature range of different seasons of Lakhimpur Kheri is as follows:

Winter	October to February	30°C to 4°C
Summer	March to June	43°C to 20°C
Rainy Season	July to September	35°C to 20°C

Rice and sugarcane are the major crops in the area. Other crops viz. wheat, arhar *(Cajanus cajan)*, bengal gram *(Cicer arietinum)*, mustard, maize, jowar *(Sorghum vulgare)* and bajra *(Pennisetum typhoides)* are also cultivated in the area. The vegetable crops of the area include potato and onion, however, farmers also grow common green vegetables. Progressive farmers also cultivate green fodder that includes berseem *(Trifolium alexandrinum)*, lucerne *(Medicago sativa)*, jowar *(Sorghum vulgare)* and bajra *(Pennisetum typhoides)*.

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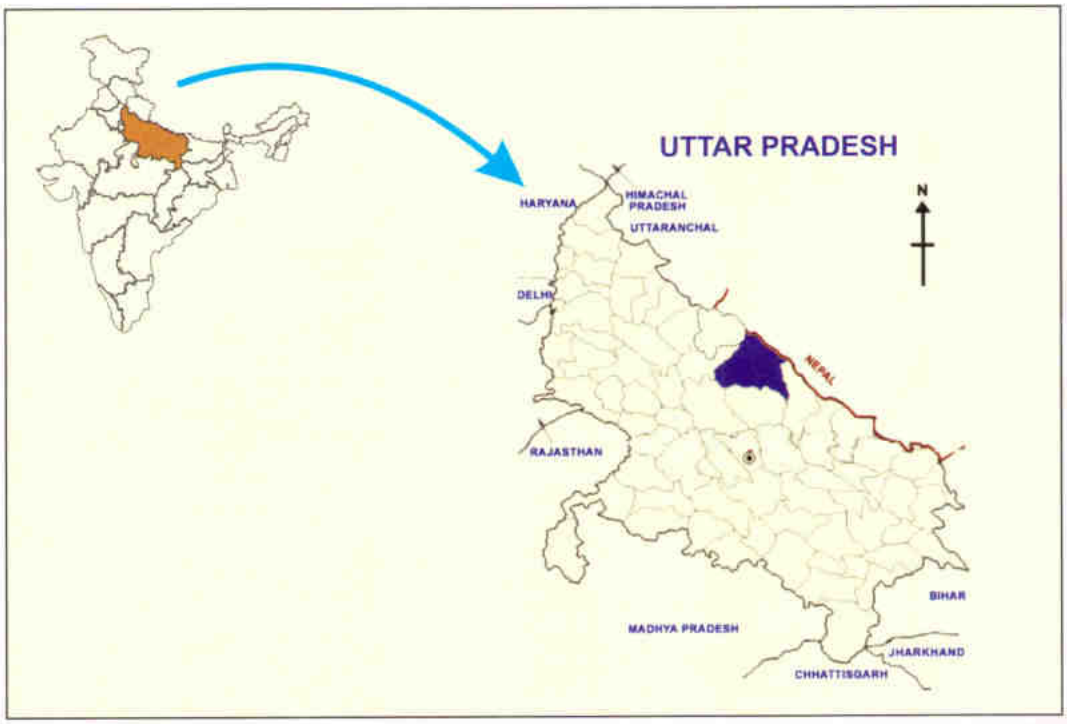

Fig.1: Breeding tract of Kherigarh cattle in India

Socio-economic Profile of the Farmers

The Kherigarh cattle are mainly maintained by small and marginal farmers and landless labours. The land-holding size of the farmers maintaining the breed ranged between 1 to 12 acres. Majority (71 %) of the cattle were with the farmers having <5 acres of land. In majority of the cases, the cattle holding size ranged between 1-6 animals.

The progressive farmers (local, as well as migrated from Punjab) do not maintain animals of this breed. They either maintain crossbred cattle or buffaloes. The real custodians of the typical Kherigarh breed are the traditional cattle owners mainly *Yadav / Ahir* community. *Tharu* tribal community settled around the forest area of Dudhwa National Park and on

Fig.2: A Kherigarh Calf

the Indo-Nepal Border also maintain some of the animals of this breed. In the Pallia Kalan Block, majority of the farmers (92%) were not milking their cows. The milk was left for suckling by calves. The source of income for the farmers was from sale of bullocks and in few cases sale of dung for manure purpose. Majority of the farmers were using dung for manure purpose in their own agricultural fields. The bullocks are sold for Rs 5000-8000 per pair in different livestock trade markets locally known as *Mandies*. A few healthy and stout bullocks pair fetch around Rs 12,000.

A few such Mandies are *Dakrava Choraha, Dubagga, Gola, Bodhia, Moodha Swaran* and *Bujhava Pallia.* True to the breed animals are available in the interior area of the breeding tract mainly around Sharda and Saryu rivers and around Dudhwa National Park. Liking for cattle rearing has reduced considerably due to shrinkage in grazing land, replacement of Kherigarh bullocks by buffalo bullocks/tractors and ban on entry of the livestock in the surrounding forest for grazing. The farmers who used to maintain large herds (around 500) are now maintaining herds of 20-100 Kherigarh animals only.

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Fig.3: A pair of Kherigarh bullocks

Survey

Actual breeding tract of the Kherigarh was delineated by NBAGR scientists with the help of discussions with District/tehsill/Block level officers of Animal Husbandry Department (Uttar Pradesh) and based on surveys in that area. The villages were divided into 3 strata on the basis of concentration/density of animals viz. Central (30-45% Kherigarh population), Adjacent (20-30% Kherigarh population) and Peripheral (<20% Kherigarh population). The distribution of villages on the basis of these strata was as follows:

Sr.No.	Category	Villages
1.	Central (Most populous villages)	Jaganpurva, Bhura, Masan Khambh, Gulara
2.	Adjacent (Medium populous area)	Baghaia, Dhayanpur, Kripakund,Gabrola
3.	Peripheral (Least populous area)	Bela Khurd, Bela Tapra, Bodhia Kalan, Kherigarh

Two blocks each in the above strata were selected randomly (a total of six blocks). In each of the selected blocks, two villages were taken randomly. Thus a total of 12 villages were taken up for study. The information on production and reproduction traits was collected by interviewing the farmers and morphometric measurements were physically taken. The farmers were interviewed for the choice of the breed, management practices, technical/ financial inputs, socio-economic utility of the breed, feeding and disease prophylactic practices and level of their technical knowledge for the animal husbandry and other related problems. The cattle population of the villages in general and Kherigarh population in particular were determined.

Measurements were taken on 501 Kherigarh animals of different age-group categories. A total of 50 animals of Kherigarh breed from each village were randomely choosen for morphometric measurements. The information on production and reproduction characteristics of 126 Kherigarh cows was gathered from discussions with the farmers. Reproduction and production traits recorded were: age at first calving, daily milk yield, lactation yield, inter-calving period, lactation period, service period and dry period.

Population Statistics

On the basis of sample survey (Table 1) of the breeding tract, population of the breed was extrapolated in the entire breeding tract. The Cattle Census of the different blocks was obtained from the District Animal Husbandry Department. The cattle population of these villages was 4,024 out of which 998 cattle (24.8%) belonged to Kherigarh breed. Based on total cattle population of Pallia Kalan and Nighasan Blocks (63,346 head) approximate Kherigarh populations in the entire breeding tract was estimated as 15,709 heads.

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Table1: Population statistics in surveyed villages

Village	Total cattle	Kherigarh cattle	Kherigarh cattle measured	Categories of Kherigarh cattle measured			
				Bullocks	Cows	Calves (1yr)	Calves (2yr)
Bela Tapra	123	18	18	4	8	3	3
Bodhia Kalan	737	72	50	8	20	13	9
Bela Khurd	535	58	50	10	20	14	6
Kherigarh	637	123	50	10	30	5	5
Kripakund	128	32	32	4	18	5	5
Jaeanpurva	322	106	50	8	32	5	5
Masan Khambh	205	92	50	20	20	5	5
Bhura	317	142	50	20	20	5	5
Gulara	526	212	50	20	20	5	5
Baehaia	125	25	25	6	8	5	6
Ohayanpur	286	92	50	16	15	10	9
Gabrola	83	26	26	8	8	5	5
Total	4024	998	501	134	219	80	68

Physical Characteristics of the Breed

The information available on this breed in literature is very limited and only a few reports are available. Acharya and Bhat (1984) reported average adult body weight of Kherigarh cattle as 476 kg in males and 340 kg in female animals. The height, length and heart girth were 1.3, 1.2 and 1.8 meters in males and 1.3, 1.3 and 1.5 meters in females, respectively. However, the number of observations was not mentioned. Nivsarkar *et al.* (2000) described various characteristics of Kherigarh cattle based on earlier reports. Thus recording was done in the field for physical parameters.

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The characteristics recorded were coat color, skin, muzzle, eye lids, hoofs, tail switch; color, shape, size and orientation of horns; length and orientation of ears; description of head and body including hump (large/medium/small) and dewlap (large/medium/small); basic temperament (docile/moderate/aggressive); size of udder and teats (large/medium/small); chest girth; body length; height at withers (cm) and tail length (above hock, at hock, below hock, touching the ground) while surveying in the breeding tract.

Fig.4: Calf of Kherigarh breed

Colour: White and grey coat colour. Kherigarh cattle are lighter in appearance than Hariana.

Horns: Lyre shaped, thin, curving outward, upward, tapering and pointed at the tip. In certain animals, modified shapes are also found. Horns are thick at the base.

Face: Small, narrow, flat or dished face. Forehead broad.

Ears: Small and horizontal.

Neck: Short but strong.

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Hump: Well developed in males as compared to females.

Dewlap: Thin, pendulous, starts below the chin and continues down to the brisket.

Muzzle: Black.

Sheath: Short and moderately tight.

Legs: Thin and straight.

Hooves: Small and Black.

Tail: Long, nearly touching the ground and ending in black switch, white switch in few animals.

Udder: Small-sized and tightly attached to the body.

Teats: Small and cylindrical.

Fig.5: A Kherigarh cow

Morphometric Measurements

Morphometric measurements for body length, height at withers and heart girth are presented in Table 2.

Table 2: The morphometric measurements for body length, height at withers and heart girth

Category	No. of Obs.	Body length (cm)		Height at withers (cm)		Heart girth (cm)	
		Mean	SD	Mean	SD	Mean	SD
Nearly One year	80	71	9.81	87	10.12	104	12.02
Nearly Two year	68	87	6.90	107	9.40	124	8.67
Adult cows	219	112	5.48	124	6.65	152	8.17
Bullocks	134	116	5.66	133	6.60	163	4.75

Management Practices

Kherigarh cattle are maintained almost on zero input system. The animals are taken for grazing in the surrounding forest area in the morning between 8-10AM. The animals cover about 5 to 15 kms in a day for grazing. The animals return to their respective owners in the evening around 5:00PM. No additional fodder or concentrate is provided. Few farmers feed paddy and wheat straw and sugarcane tops to their animals. In Pallia block, very few farmers fed self-grown Berseem to their animals, Animals are either tied in the open or are kept together under the thatched housing (*Chhappar*) with wooden-log fencing.

Fig.6: A healthy pair of Kherigarh bullocks

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The Kherigarh cattle are seldom brought to Veterinary Hospital for treatment due to breed being highly disease resistant (as informed by Veterinary Officers of Palia and Nighasan).

Indigenous Technical Knowledge (ITK)

The owners of the breed are following age-old traditional home-remedies for treatment of their animals. For example, *Neem* leaves and mustard oil are used for treatment of wound. For cure of foot wound, the animal is made to stand in mud. In case of flatulence, mustard oil, *Jeera*, *Ajvayan* etc. are used. In winter months, a few farmers feed Jaggery to their animals to counter cold effect.

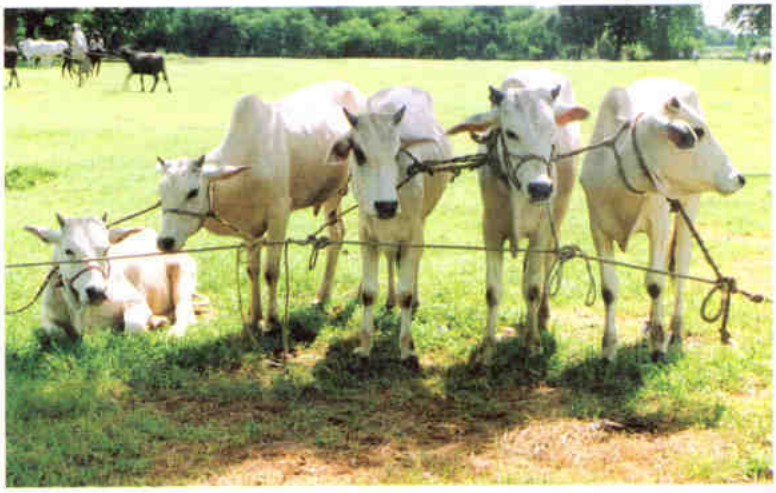


Fig.7: A group of young Kharigarh male

Uncastrated males are used for breeding and work. No separate breeding bulls are maintained. The males are castrated between 3-5 years mostly using the crude method of crushing the testicles. Very rarely the castrator is used.

Performance Parameters

The bullocks are very agile and good for agricultural operations and quick light transport. The animals are aggressive in temperament. A pair of bullocks can haul more than 10 quintals of load in a cart to a distance of 10-15 km without showing signs of much fatigue. The average speed is 4-5 km per hour. A pair of bullocks can plough one acre of land in a day working for 6 to 8 hours.

Cows are poor milkers producing only 0.5-2 kg of milk for 9-12 (10.08±0.09) months. Lactation milk yield averaged 362.50±11.19 kg. Several farmers do not milk the cows and milk is suckled by the calves. Age at first calving ranged between 36-54 (49.3+0.89) months. Service period ranged between 90-150 (129±2.5 days). Calving interval ranged between 13-15 (13.9±0.11) months. The dry period ranged between 60 - 120 days (105±2.93). Bulls attain maturity between 3-3.5 years and start servicing.

Fig.8: Young calf suckling mother's milk

Molecular Genetic Characterization

Microsatellites (highly polymorphic simple sequence repeats) are presently the most favorable molecular markers, essentially owing to the

option of blending their analysis with the polymerase chain reaction (PCR). Microsatellites have been effectively exploited to enlighten bovine domestication and migration pattern (Edwards *et al.* 2000) and to evaluate genetic diversity and relationships among cattle populations (Dorji *et al.* 2003; Jordana *et al.* 2003; Metta *et al.* 2004; Mukesh *et al.* 2004).

Sample collection

Blood samples were acquired from 50 random and unrelated Kherigarh animals from the breeding region following the guidelines of MoDAD (Measurement of Domestic Animal Diversity) (FAO, 1995) programme. To ensure unrelatedness of animals in the absence of reliable pedigreed accounts, animals were selected from distinct villages after interviewing the owners in detail. Blood samples (5-6 ml) were collected from jugular vein of the animal in vacutainers containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant.

Molecular techniques

DNA was extracted from whole blood using standard protocol (Sambrook *et al.* 1989). The DNA isolation procedure encompassed lysis of RBC's, digestion of protein using Proteinase K and precipitation of protein using phenol: chloroform: isoamylalchol. A set of 21 microsatellite markers (Table 3) recommended for cattle in FAO's DADIS MoDAD programme were utilized for generating microsatellite genotyping data in a panel of 47 animals. Since microsatellite markers are co-dominant, 47 samples correspond to 94 alleles for each microsatellite locus. An amalgamation of 21 co-dominant loci and 47 samples were projected to create 1,974 allelic data for the Kherigarh breed.

Polymerase Chain Reaction (PCR) was performed utilizing 50-100 ng of genomic DNA in a 25 µl reaction volume using a PTC-200 Thermal cycler (M J Research Inc., MA, USA). The PCR procedure involved initial

Table 3. Microsatellite markers, sequences, location and annealing temperature

Marker	Primer Sequences	Chromosome Number	Annealing Temp. (°C)
MM8	cccaaggacagaaaagact, ctcaagataagaccacacc	2	55
INRA063	atttgcacaagctaaatctaacc, aaaccacagaaatgcttggaag	18	55
ILSTS030	ctgcagttctgcatatgtgg, cttagacaacaggggtttgg	2	55
BM1818	agctgggaatataaccaaagg, agtgctttcaaggtccatgc	23	58
CSRM60	aagatgtgatccaagagagaggca, aggaccagatcgtgaaaggcatag	10	55
ILSTS054	gaggatcttgattttgatgtcc, agggccactatggtacttcc	21	55
ILSTS005	ggaagcaatgaaatctatagcc, tgttctgtgagtttgtaagc	10	55
HEL5	gcaggatcacttgttaggga, agacgttagtgtacattaac	21	55
ILSTS006	tgtctgtatttctgctgtgg, acacggaagcgatctaaacg	7	56
ILSTS011	gcttgctacatggaaagtgc, ctaaaatgcagagccctacc	14	58
INRA035	atcctttgcagcctccacattg, ttgtgctttatgacactatccg	16	55
ILSTS033	tattagagtggctcagtgcc, atgcagacagttttagaggg	12	55
HEL9	cccattcagtcttcagaggt, cacatccatgttctcaccac	8	59
BM1824	gagcaaggtgtttttccaatc, cattctccaactgcttccttg	1	55
ETH225	gatcaccttgccactatttcct, acatgacagccagctgctact	9	57
HAUT24	ctctctgcctttgtccctgt, aatacactttaggagaaaaata	22	52
ILSTS034	aagggtctaagtccactggc, gacctggtttagcagagagc	5	57
HAUT27	ttttatgttcatttttgactgg, aactgctgaaatctccatctta	26	55
HEL1	caacagctatttaacaagga, aggctacagtccatgggatt	15	55
MM12	caagacaggtgtttcaatct, atcgactctggggatgatgt	9	55
INRA005	caatctgcatgaagtataaatat, cttcaggcataccctacacc	12	55

denaturation at 95° C for 1 min, 30 cycles of '95 °C for 1 min, precise annealing temperature of primer for 1 min, 72° C for 1 min' and finally extension at 72° C for 5 min. The PCR products were resolved on 6% denaturing polyacrylamide gels (Sequi GT System, Bio-Rad) and sized using a 10 bp

ladder (Invitrogen, Life Technologies, CA, USA) as standard for sizing. Gels were stained using silver staining (Bassam *et al.*, 1991) and genotypes scored manually.

Statistical analysis

Observed and expected heterozygosity estimates were computed after Nei (1973) as executed in POPGENE software (Yeh *et al.* 1999). The observed number of alleles and effective number of alleles were also evaluated applying POPGENE software. Allelic frequencies were utilized for assessing Polymorphic Information Content (PIC), a measure of informativeness of a marker and it was calculated as per Botstein *et al.* (1980) using the formula:

$$PIC = 1 - \sum_{i=1}^{k} x_i^2 - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2x_i^2 x_j^2$$

Where k is the number of alleles and x_i and x_j are the frequencies of the i^{th} and j^{th} alleles, respectively.

Departure from Hardy-Weinberg equilibrium was derived using the exact test of POPGENE. Heterogeneity of deviations from Hardy-Weinberg equilibrium among the microsatellite loci was investigated by considering the deviations as correlation coefficients and tested accordingly (Barker *et al.* 2001). Heterozygote deficiencies were articulated as $F_{IS} = (H_o - H_e) / H_e$ where, H_o and H_e are the observed and expected frequency of heterozygotes, respectively. Linkage (Genotypic) disequilibrium among the microsatellite loci was analyzed employing F-STAT version 2.9.3, an update version 1.2 (Goudet 1995) for 21 microsatellite loci. To test the recent genetic bottleneck, the program BOTTLENECK (Piry *et al.* 1999) was used.

Genetic Variability in Kherigarh Cattle

All of the 21 microsatellite loci, which have been identified to be polymorphic in a variety of *Bos taurus* and *Bos indicus* breeds (Dorji *et al.*

2003; Jordana *et al.* 2003; Metta *et al.* 2004; Mukesh *et al.* 2004), amplified successfully and produced definite banding patterns from which individual genotypes could be ascertained. Linkage disequilibrium was not detected between the investigated loci. Thus all the loci were retained for the analysis. The genetic variability parameters of Kherigarh cattle measured were, (1)

Table 4. Estimates of various parameters in Kherigarh cattle

Locus	N_o	N_e	PIC	Heterozygosity		Heterozygote deficiency, f (F_{IS})
				Observed	Expected	
MM8	7	4.11	0.726	0.681	0.765	0.099
INRA063	7	1.96	0.458	0.489	0.495	0.000
ILSTS030	5	4.62	0.749	0.809	0.792	-0.0321
BM1818	9	4.07	0.733	0.630	0.762	0.1639
CSRM60	5	3.64	0.688	0.564	0.735	0.2221
ILSTS054	6	4.21	0.730	0.553	0.771	0.2746
ILSTS005	5	3.52	0.666	0.681	0.724	0.0493
HEL5	5	2.93	0.603	0.261	0.673	0.6040
ILSTS006	5	3.81	0.692	0.697	0.749	0.0554
ILSTS011	4	2.46	0.530	0.468	0.601	0.2122
INRA035	7	4.75	0.764	0.717	0.798	0.0913
ILSTS033	4	2.63	0.543	0.511	0.626	0.1751
HEL9	9	6.52	0.828	0.636	0.856	0.2483
BM1824	4	3.17	0.627	0.575	0.692	0.1607
ETH225	8	2.60	0.596	0.565	0.623	0.0821
HAUT24	5	3.01	0.610	0.432	0.677	0.3527
ILSTS034	10	6.30	0.824	0.711	0.851	0.1547
HAUT27	6	2.81	0.589	0.386	0.652	0.4006
HEL1	6	2.80	0.604	0.583	0.652	0.0930
MM12	8	5.59	0.799	0.435	0.830	0.4705
INRA005	6	3.75	0.689	0.674	0.742	0.0800
Mean	6.24	3.77	0.669	0.574	0.717	0.188
S. D.	1.7	1.24	0.097	0.131	0.091	

N_o Observed number of alleles
N_e Effective number of alleles

observed and effective number of alleles, (2) observed and expected heterozygosity, (3) heterozygote deficiency at each of the 21 microsatellite loci are included in Table 4.

Across the 21 microsatellites scrutinized, a total of 131 distinct alleles were identified in Kherigarh cattle. The allele frequency data revealed a reasonable amount of polymorphism in Kherigarh cattle. The number of observed alleles oscillated between 4 (ILSTS011, ILSTS033, BM1824) and 10 (ILSTS034) with an overall mean number of alleles (MNA) per locus of 6.24±1.7. The FAO has specified a minimum of four distinct alleles per locus for proficient judgment of genetic differences between breeds. By this criterion, all 21 microsatellites employed in this study displayed ample polymorphism for evaluating genetic variation within breed and exploring genetic differences between breeds. The observed number of alleles for all the 21 loci exceeded the effective number of alleles which varied from 1.96 (INRA063) to 6.52 (HEL09) with a mean of 3.77±1.24 (Table 4).

Genetic markers demonstrating PIC values higher than 0.5 are normally considered as informative in population genetic analyses (Botstein *et al.* 1980). The average PIC was 0.67±0.10. Consequently, with the exception of INRA063 all loci were informative as seen in the taurine and indicus breeds investigated earlier using microsatellite markers (Kumar *et al.* 2003; Metta *et al.* 2004; Mukesh *et al.* 2004).

The observed heterozygosity averaged over the 21 loci was 0.574±0.131, which was lower than the expected heterozygosity (Table 4). The average expected heterozygosity (Nei 1973) within the Kherigarh population ranged from 0.495 (INRA63) to 0.856 (HEL09) with an overall mean of 0.717±0.091. Kherigarh cattle, thus, encompassed considerable measure of genetic variation. The average observed heterozygosity estimation in this study (0.574 ± 0.131) is marginally lower than that illustrated in seven Italian cattle breeds 0.6 – 0.68 (Del Bo *et al.* 2001) and five Swiss cattle breeds 0.60-0.69

(Schmid *et al.* 1999). Fairly comparable levels of heterozygosity were reported in Deoni cattle breed (0.59) of India (Mukesh *et al.* 2004) and twelve west/central African cattle breeds 0.506-0.697 (Ibeagha-Awemu *et al.* 2004). However, lower heterozygosities (0.42 and 0.53) and lower numbers of alleles than Kherigarh have been reported in two Indian zebu cattle breeds viz Sahiwal and Hariana (Mukesh *et al.* 2004) whose populations are in rapid decline in India. With the fall in observed number of alleles (Hariana 6.5 and Sahiwal 5.2) heterozygote deficiency (F_{IS}) increased from 0.211 in Hariana to 0.326 in Sahiwal cattle (Mukesh *et al.* 2004). Although the observed number of alleles in Kherigarh cattle (6.24) was lower than Hariana cattle (6.5), the F_{IS} (0.188) did not display a proportionate increase. This suggests that this breed retains considerable genetic variability and only moderate levels of heterozygote deficiency despite its declining population in the breeding region.

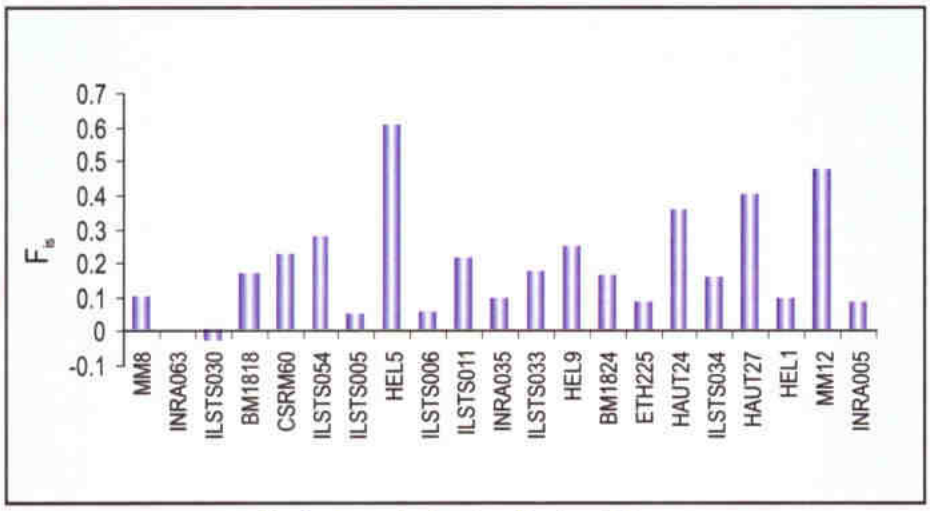
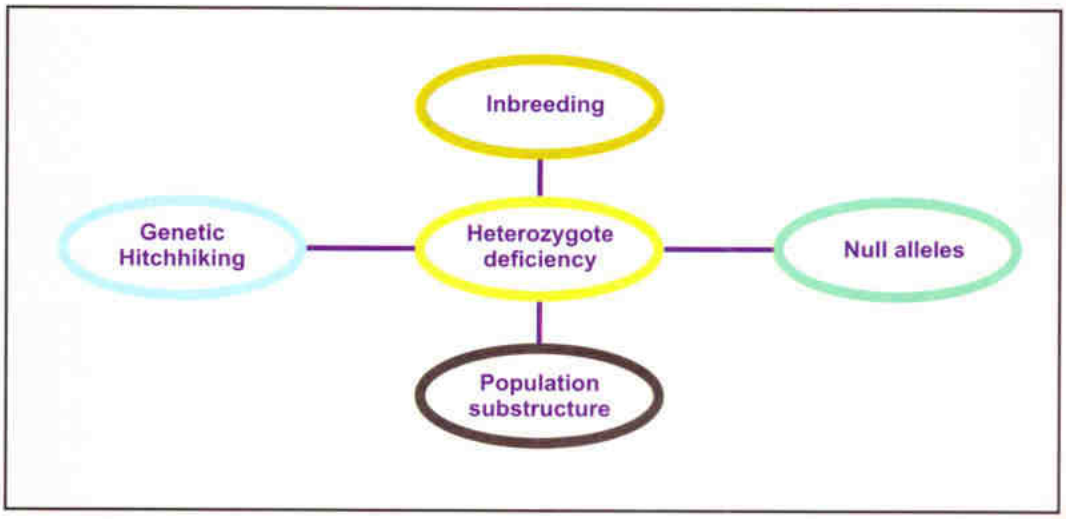


Fig.9: A graph showing F_{IS} at each locus

Within-population heterozygote deficiency estimate f (F_{IS}) was significantly positive, as derived from table-wide randomizations ($P< 0.05$). The f-estimates ranged between -0.032 and 0.604 with an average of 0.188. Thus on an average a substantial shortfall (18.8%) of heterozygotes existed

in the Kherigarh population. All of the 21 microsatellite markers, except INRA063 and ILSTS030, contributed to this observed heterozygote shortage. Reasons for heterozygote deficincies in populations (Nei 1987) are depicted below.



The fundamental cause for low heterozygosity in Kherigarh cattle is likely to be inbreeding prompted by the above expressed issues and demonstrated by the overall positive f-value (Weir 1996). All the scrutinized loci were observed to be neutral (except ILSTS030, HEL9 and MM12) when probed with Ewens-Watterson neutrality test suggesting that homozygosity in Kherigarh cattle is unlikely to be the result of selection. Null alleles are largely unlikely to be segregating at all the loci. Likewise prospective Wahlund effects (localities with subpopulations) may not account significantly for the observed heterozygote deficit.

The heterozygote deficiency detected in this population is likely to be a manifestation of diminished population size and confinement to a small breeding territory, coupled with lack of sufficient number of breeding males in the breeding region. The Kherigarh cattle population has shrunk to just about 15,709 heads in the entire breeding tract. Moreover, male calves of six

to twelve months of age are traded to farmers outside the breeding region to be exploited in agricultural operations and transportation after castration, thus leading to their genetic death. As a result breedable males are significantly reduced in the breeding tract. Altogether the effective population size is curtailed and breeding between relatives stimulates inbreeding and genetic drift.

When a population goes through bottleneck rare alleles tends to be lost and the average number of alleles per locus, or allelic diversity, is reduced. Heterozygosity, however, is not reduced proportionally, because rare alleles contribute little to heterozygosity. The difference between allelic diversity and heterozygosity is used as the basis for statistical tests to detect the presence of recent genetic bottleneck (Piry *et al*. 1999). The allele frequency spectra was visualized through the qualitative graphical method of Cornuet and Luikart (1996). The microsatellite alleles were organized into 10 frequency classes, which permit checking whether the distribution followed the normal L-shaped form, where alleles with low frequencies (0.01-0.1) are the most numerous. The observed distribution suggests that the population did not encounter a genetic bottleneck in the recent past.

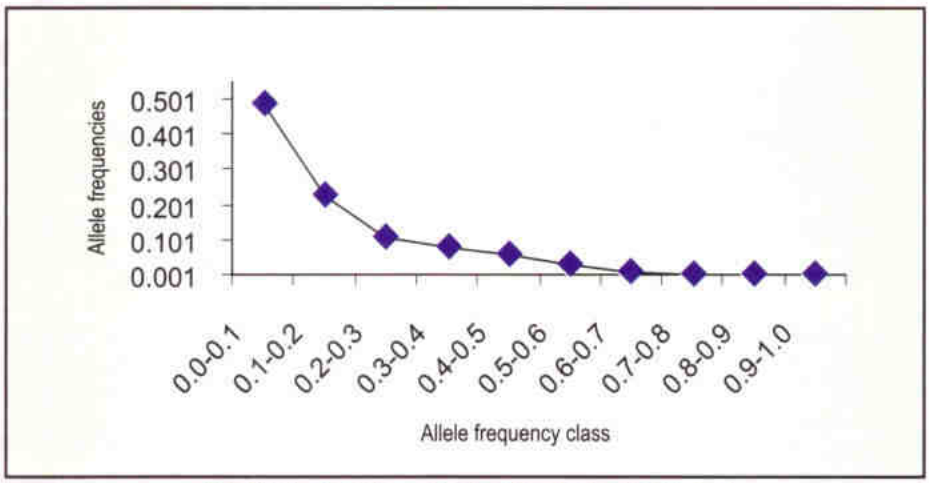


Fig.10: L-shaped graph showing lack of bottleneck

KHERIGARH

The significant level of variability in Kherigarh cattle, notwithstanding its diminished population size, is indicative of a valuable reservoir of genetic diversity in this breed. This fact coupled with the evident environmental adaptation of the Kherigarh emphasises the importance of genetic regulation and conservation of this indigenously evolved draft breed and its sustainable utilization.

Recommendations

The present status of Kherigarh cattle breed revealed that the animals of this breed must be conserved and improved on priority. This precious cattle breed will be lost if proper attention is not paid immediately. Conservation and improvement, both the models need to be implemented effectively to make the program successful. The following issues need inclusion in the policy and immediate implementation in the state for overall development of Kherigarh cattle breed:

- Breed society should be formed which should be responsible for overall maintenance and development of the breed in the breeding tract.
- At least one nucleus herd of Kherigarh must be established in its native tract under *in situ* conservation model. The elite animals should only be procured from the field / native tract for establishing the farm. Sufficient funds should be provided to the cattle farm to maintain this breed on scientific lines.
- Pure breeding and selection at initial level and field progeny scheme thereafter should be followed at the farm. Young bulls should be produced in sufficient number to meet the requirement of the field animals.
- Incentives should be given to the farmers maintaining the Kherigarh cows.
- Exhibitions should be organized in the breeding tract to reward the best Kherigarh animals.
- Norms should be prepared to check the migration of purebred animals from the breeding tract. Information about the new place of the animal must be available with breed society.

- Artificial Insemination (AI) should be initiated in breeding tract so that indiscriminate use of the bulls could be checked. Semen of the breed must be available in the breeding tract at AI centres.
- Use of exotic semen for AI should be banned in this breed.
- Provision should be made to castrate the non-descript males through legislation and it should be strictly implemented to check the indiscriminate breeding.

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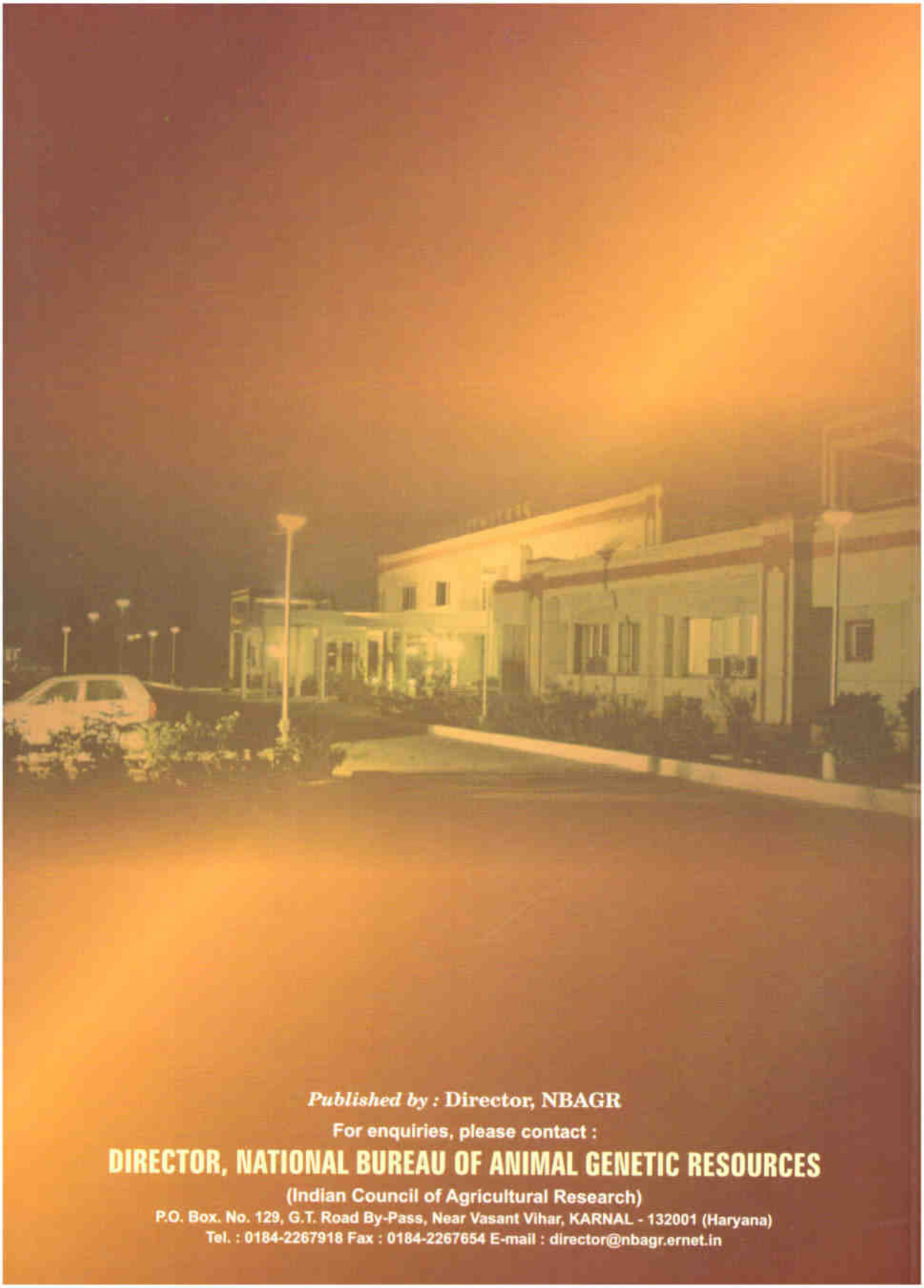

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