Genetic diversity among four short stature cattle populations of India

M.S. Tantia, R.K. Vijh, B. Mishra & S.T. Bharani Kumar

*National Bureau of Animal Genetic Resources, P.O. Box 129, **Karnal** 132001, India*

Summary

The genetic diversity, genetic differentiation and relationship between four short stature cattle populations of south India - Punganur, Iduki, Kasargod and Vatakara - was studied, using 24 microsatellite loci. A total of 164 alleles were observed. The mean number of alleles per locus was 6.58 with mean observed and expected heterozygosity values of 0.70 and 0.75 respectively. The relative magnitude of gene differentiation (F_{ST}) was 6% and was significant except between the Iduki and Kasargod populations. The negative F_{IS} values obtained for the majority of loci indicated a lack of population structure in the four populations. Both phylogenetic and correspondence analysis exhibited a closeness between Iduki and Kasargod animals. The results indicated that all four populations were outbred and Kasargod and Iduki animals should be considered as one even though these are reared for different purposes.

Résumé

La diversité génétique, la différence génétique et les relations entre 4 populations bovines de petite taille du Sud des Indes, Punganur, Iduki, Kasargod et Vatakara, ont été étudiés en utilisant 24 loci microsatellites. Un total de 164 allèles ont été observés. Le nombre moyen d'allèles par locus était de 6,58 avec une valeur d'hétérozigosité moyenne observée et espérée de 0,70 et 0,75, respectivement. La grandeur relative du gène de différentiation (FST) était de 6% et était significative sauf entre la population Iduki et la Kasargod. Les valeurs négatives de FIS obtenues pour la majorité des loci indiquent un manque de structure dans les 4 populations. Les analyses de correspondances et phylogénétiques montrent un lien plus étroit entre les animaux Iduki et Kasargod. Les résultats indiquent que les 4 populations étaient hors croisement et les animaux Kasargod et Iduki devraient être considérés comme une seule population, même s'ils sont élevés pour des buts différents.

Resumen

La diversidad genética, la diferencia genética y las relaciones entre 4 poblaciones bovinas de pequeño tamaño des sur de la India, Punganur, Iduki, Kasargod y Vatakara, han sido estudiadas utilizando 24 loci de microsatélites. Un total de 164 alelos han sido observados. El número medio de alelos por locus era de 6,58 con un valor de heterocigosidad media observada y esperada de 0,70 y 0,75, respectivamente. El tamaño relativo del gen de diferenciación (FST) era de 6% y resultaba significativo salvo entre la población Iduki y Kasargod. Los valores negativos de FIS obtenidos para la mayoría de los loci indican una falta de relación estrecha entre los animales Iduki y Kasargod. Los resultados indican que las 4 poblaciones estaban fuera de cruce y que los animales Kasargod y Iduki deberían considerarse como una sola población, a pesar de ser criados con objetivos distintos.

Key words: *Microsatellite genotyping, Statistical analysis, Genetic diversity, Populations, Dendrogram.*

Introduction

India has a large population of humped cattle (*Bos indicus*) estimated to be 186 million (Livestock Census, 2003). Most of the animals are best described as 'local cattle' and are poor yielders of milk. These animals have been mostly kept for draught purposes and have not been subjected to any kind of selection for a specific trait. Of late, cross breeding of indigenous cattle with exotics (*Bos taurus*) has become common especially in the states of India which have natural resources to feed

the cattle. The local cattle of Kerala and Andhra Pradesh have been described as small animals with the height of the animals varying from 90 to 105 cm. There are four populations of these small sized cattle (*Bos indicus*) which inhabit the hilly terrain with an altitude of 1500 meters above mean sea level (Figure 1). These are locally named as Punganur, Iduki, Kasargod and Vatakara. The high range small cattle called Iduki have been reported to number less than 2 000 (AnilKumar and Raghunandanan, 2003). These animals have been bred for meat as sacrificial animals during festival seasons. Kasargod cattle have their utility only as producers of organic manure for areca nut plantations and are very poor yielders of milk. Vatakara and Punganur cattle produce 3-5 kg of milk per day and 500-550 kg per lactation (Rao *et al.*, 2000). All the populations are facing extinction primarily due to economic considerations and are being replaced with crossbreds. The head counts of these cattle have been reduced to a few hundreds (AnilKumar and Raghunandanan, 2003; Rao *et al.*, 2000).

Figure 1. Distribution of four cattle populations.

To date no information is available on the status of these animals at a molecular level. The present study was therefore undertaken to study the genetic diversity, gene flow, genetic structure and relationship among these breeds using 24 microsatellite loci in order to have some genetic basis for conservation or undertaking other management decisions regarding these genetic resources.

Materials and Methods

Microsatellite genotyping

Blood samples were collected from 102 genetically unrelated animals. The DNA was isolated using the standard protocol of Phenol Chloroform extraction (Sambrook *et al*., 1999). Twenty four microsatellite loci were selected for the study, including 13 markers (INRA063, ETH225, ILSTS005, INRA035, ETH152, ETH10, CSSM66, BM1818, ILSTS006, MM12, CSRM60, HAUT24 and HAUT27) that appear in the new FAO MoDAD marker set (Hoffmann *et al*., 2004).

The PCR conditions were standardized for all of the 24 primer pairs selected for the study. The variables, which required standardization, included annealing temperature, $MgCl_2$ concentration, quantity of primers, Taq polymerase and dNTPs. The PCR products were loaded on 6% denatured polyacrylamide gel electrophoresis (PAGE) using urea as denaturing agent. The standard DNA markers were simultaneously run in the gel for sizing of the alleles. The PAGE was run for a sufficiently long period for proper resolution of alleles. The polyacrylamide gel was fixed in acetic acid (10%) and stained with silver nitrate following standard protocol (Bassam *et al.*, 1991). To avoid any mistyping, the various alleles of the different breeds were simultaneously run on the same gel.

Statistical analysis

The deviations from the Hardy-Weinberg Equilibrium (HWE) for all locus-population combinations were determined using GENEPOP ver. 3.3 (Raymond and Rousset, 1995). Hardy-Weinberg proportions were assessed by exact test (Guo and Thompson, 1992), using Markov chain randomization to estimate unbiased p-values for each locus in each population. The allele frequency, mean numbers of alleles per locus,

observed heterozygosity and expected heterozygosity were estimated using POPGENE software (Yeh *et al*., 1999). The PIC value was calculated as per Botstein (1983). The F statistics for each locus (Weir and Cockerham, 1984) between populations (F_{ST}) and the estimate of average inbreeding coefficient (F_{IS}) for each population were calculated and tested using FSTAT version 2.9.3 (Goudet, 2001). The recent bottleneck was inferred for all populations studied using a Wilcoxon signed rank test (Cornuet and Luikart, 1996). The calculations implemented in the program BOTTLENECK version 1.2.02 (Cornuet and Luikart, 1996) were based on 10 000 simulation replicates.

An unweighted pair group method using an arithmetic average (UPGMA) dendrogram was constructed from Nei's standard Genetic Distances (DS, Nei 1972) with DISPAN (Ota, 1993). The robustness of the dendogram topology was evaluated with a bootstrap of 1 000 re-samplings of loci with replacement. The inter-individual distances were estimated using allele sharing distance and neighbor joining algorithms (Saitou and Nei, 1987).

Since the phylogenetic reconstruction may not readily take into account the effects of admixture between the populations, we performed correspondence analysis as an alternative approach to understand the genetic relationship among the populations. The correspondence analysis and number of migrants per generation (Wright, 1969) were calculated using the GENETIX software version 4.05 (Belkhir *et al*., 2004). The analysis of molecular variance (AMOVA) was carried out as implemented in ARLEQUIN Software (Excoffier *et al*., 2005).

Results

Genetic Diversity

A total of 164 microsatellite alleles were amplified in 24 loci and 102 animals belonging to the four breeds . The numbers of alleles observed in the four populations were 113, 131, 134 and 134 in Kasargod, Punganur, Vatakara and Iduki animals, respectively. The number of alleles varied from 4 (ILSTS 97 and ILSTS 101) to 12 (CSSM 66). A total of 21 alleles were found to be private alleles unique to one population (eight in Vatakara, seven in Punganur, five in Iduki and one in Kasargod). The frequencies of 17 private alleles were more than 2.5% in their respective population while four alleles were at low frequency. The maximum frequency of private alleles was 34.21% in Punganur animals (CSSM 66 size 164). The mean numbers of alleles were 4.71±0.32 (Kasargod), 5.58±0.32 (Vatakara), 5.58±0.29 (Iduki) and 5.46±0.34 (Punganur). The mean numbers of alleles were not significantly different among the four cattle populations (Table 1). The effective numbers of alleles were 3.62, 3.53, 2.99 and 3.36 for the four populations respectively as a large proportion of alleles in the populations were at low frequency. The observed heterozygosity ranged from 0.72±0.04 (Kasargod) to 0.78±0.03 (Punganur), while the expected heterozygosity ranged from 0.66±0.02 (Kasargod) to 0.70±0.02 (Iduki). These values were not significantly different among the four cattle populations.

All the loci showed no significant deviation ($P<0.01$) from HWE in the Vatakara, Kasargod, Iduki and Punganur breeds when alternative hypothesis H_1 was heterozygosity deficiency except locus ILSTS 006 in the Iduki and locus MM I2 in the Punganur. When the alternative hypothesis H_1 was heterozygotic excess five loci in Vatakara, three in Iduki and seven in Punganur deviated from HWE. The only locus that was common in the three populations for deviation from HWE was ETH 10. The results of Weir and Cockerham's F-Statistics for each locus across all the populations are given in table 2. The F_{IS} values for most of the loci are negative which shows no inbreeding, rather it indicates the mating of individuals which are less related than the average relationship of the population (outbreeding). The mean F_{IS} value was found to be -0.14 which is significantly different from zero. The relative magnitude of the gene differentiation (F_{ST} estimator) was 6%. The highest numbers of migrants (Nm) were estimated between the Kasargod and Iduki and least between Punganur and Kasargod cattle.

Genetic differentiation among populations

Significant ($P<0.001$) genetic differentiation was detected among all the populations although the differentiation values ranged from 0.032 between the Kasargod and Iduki to 0.70 between the Kasargod and Punganur (Table 3). The genetic relationship between the four populations was determined using Nei's standard genetic distance (D_S). The largest genetic distance was estimated between Punganur and Kasargod cattle, 0.21, while the least distance was between Iduki and Kasargod cattle. The un-rooted UPGMA

Table 1. Number of alleles Observed (No) and Effective (Ne) and Heterozygosities Observed (Ho) and Expected (He).

	Vatakara				Iduki				Kasargod				Punganur			
Locus	No	Ne	Ho	He	No	Ne	Ho	He	No	Ne	Ho	He	No	Ne	Ho	He
ILSTS11	6	3.29	0.89	0.71	4	2.20	0.59	0.56	4	1.96	0.67	0.51	3	2.05	0.85	0.52
ILSTS97	3	2.71	0.59	0.64	4	3.02	0.74	0.68	2	1.86	0.55	0.48	3	2.86	0.92	0.66
HAUT27	7	3.49	0.86	0.73	6	4.52	0.96	0.80	5	3.13	0.80	0.72	3	2.10	0.97	0.53
ILSTS6	3	2.62	0.62	0.63	4	2.22	0.26	0.56	4	3.14	0.36	0.71	5	3.82	0.88	0.75
ILSTS5	5	4.28	0.68	0.78	5	4.19	0.86	0.78	4	3.03	0.83	0.70	5	3.01	0.97	0.68
ILSTS30	5	3.04	0.93	0.68	5	3.05	0.83	0.69	3	2.07	0.83	0.54	4	1.96	0.47	0.50
ILSTS31	3	1.76	0.55	0.44	5	2.65	0.83	0.64	5	2.72	0.83	0.66	4	1.97	0.65	0.50
ILSTS95	6	5.74	0.96	0.84	7	5.90	0.95	0.85	6	4.17	0.80	0.80	7	4.38	0.84	0.78
CSSM66	7	4.23	0.93	0.78	5	3.32	0.74	0.71	6	3.16	0.58	0.71	7	4.11	0.82	0.77
ILSTS26	4	2.00	0.52	0.51	5	2.63	0.73	0.63	3	2.18	0.64	0.57	4	2.15	0.61	0.54
ILSTS101	4	1.52	0.32	0.35	4	2.39	0.78	0.60	4	1.87	0.58	0.49	4	2.58	0.77	0.62
ILSTS92	6	4.50	1.00	0.79	6	3.96	0.83	0.76	4	3.79	1.00	0.77	6	4.63	0.78	0.80
ILSTS36	4	2.98	0.83	0.68	4	3.13	0.62	0.70	5	3.27	0.58	0.72	5	3.25	0.79	0.70
CSRM60	8	6.16	0.97	0.85	9	6.87	0.91	0.87	10	5.88	1.00	0.87	9	5.97	0.97	0.84
ETH225	7	3.40	0.71	0.72	7	3.46	0.78	0.73	3	1.88	0.58	0.49	6	2.76	0.75	0.65
ETH10	7	5.26	1.00	0.82	7	4.34	1.00	0.79	6	3.97	0.91	0.78	5	3.32	0.97	0.71
ILSTS89	6	4.79	0.97	0.81	7	4.18	1.00	0.78	7	4.30	1.00	0.80	8	5.60	0.95	0.83
BM1818	8	4.15	0.72	0.77	7	4.88	0.83	0.81	5	3.32	0.73	0.73	7	4.42	0.87	0.78
ILSTS33	8	3.21	0.86	0.70	7	3.42	0.95	0.72	4	2.59	0.83	0.64	7	2.43	0.61	0.60
HAUT24	6	3.55	0.90	0.73	5	3.69	0.86	0.75	4	2.51	0.43	0.65	4	2.26	0.80	0.57
INRA35	6	4.92	0.72	0.81	7	4.12	0.65	0.77	5	3.10	0.58	0.71	5	2.66	0.63	0.63
INRA63	5	3.96	0.79	0.76	4	1.84	0.35	0.47	4	2.36	0.67	0.60	7	6.13	0.87	0.85
ETH152	4	1.50	0.35	0.34	4	1.78	0.50	0.45	5	2.07	0.58	0.54	6	2.01	0.53	0.51
MM12	6	4.06	0.79	0.77	6	3.08	0.57	0.69	5	3.60	0.92	0.75	7	4.33	0.53	0.78
Mean	5.58	3.63	0.77	0.69	5.58	3.53	0.75	0.70	4.71	3.00	0.72	0.66	5.46	3.36	0.78	0.67
	±0.32	±0.16	±0.03	±0.02	±0.29	±0.18	±0.03	±0.02	±0.32	±0.19	±0.04	±0.02	±0.34	±0.15	±0.03	±0.02

dendrogram reveals that the populations of Kasargod and Iduki are closely related and join first followed by the Vatakara and then the Punganur. The bootstrap values were 61% based on 1 000 replications (Figure 2). The Punganur individuals cluster together (Figure 3) while the rest were admixed in the tree. Figure 4 represents the first three axes which explain 48.67, 34.19 and 17.14% of the total variation respectively. The plot reveals a similar pattern as genetic relationship from phylogenetic analysis. The two populations of Iduki and Kasargod are very similar and form a single cluster and the Punganur formed a distinct cluster. The self assignment gave an accuracy of 95% in the four populations. All the individuals in the Vatakara and Punganur groups were correctly assigned to their respective populations while three individuals of the Iduki and two individuals of the Kasargod were miss-assigned among the two populations owing to low genetic differentiation between the two populations. The Wilcoxon test revealed no evidence for recent genetic bottleneck in any of the four populations. The analysis of Molecular variance revealed significant differentiation among the populations. 12% of the total variation was among populations while 88% variation was among individuals.

Discussion

In this study we investigated the genetic diversity in four south Indian breeds of cattle which are of short stature measuring around one meter in height which have not undergone any selection for milk or draft purposes (AnilKumar and Raghunandanan, 2003). The mean numbers of alleles were not significantly different in the four populations. The results were similar to other *Bos indicus* reports of 5.86 and 5.82 in Red Kandhari and Deoni cattle, respectively (Sodhi *et al.*, 2005). The heterozygosity values are higher than reported in African cattle including *Bos taurus* and *Bos indicus* breeds of cattle, ranging from 0.432 to 0.658 (MacHugh *et al.*, 1997)

Table 2. F statistics and number of migrants (Nm).

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
ILSTS11	-0.34	-0.23	0.08	2.94
ILSTS97	-0.16	-0.07	0.08	2.94
HAUT27	-0.33	-0.23	0.07	3.09
ILSTS6	0.18	0.23	0.07	3.50
ILSTS5	-0.17	-0.13	0.04	6.55
ILSTS30	-0.30	-0.25	0.04	5.46
ILSTS31	-0.31	-0.23	0.06	3.74
ILSTS95	-0.12	-0.06	0.05	5.12
CSSM66	-0.06	0.10	0.15	1.44
ILSTS26	-0.13	-0.07	0.05	4.36
ILSTS101	-0.23	-0.18	0.04	6.40
ILSTS92	-0.18	-0.14	0.03	7.33
ILSTS36	-0.03	-0.01	0.03	9.38
CSRM60	-0.15	-0.12	0.02	9.77
ETH225	-0.12	-0.06	0.05	4.71
ETH10	-0.28	-0.24	0.03	7.33
ILSTS89	-0.25	-0.20	0.04	6.57
BM1818	-0.04	-0.01	0.03	8.98
ILSTS33	-0.25	-0.20	0.04	5.78
HAUT24	-0.15	-0.03	0.10	2.31
INRA35	0.09	0.15	0.06	3.74
INRA63	-0.02	0.10	0.12	1.92
ETH152	-0.09	-0.05	0.04	6.34
MM12	0.04	0.09	0.06	4.24
Mean	-0.14	-0.07	0.06	4.08

Table 3. Between population F_{ST} (Upper triangle) and Nei's standard genetic distance (Lower triangle).

Populations	Vatakara	Iduki	Kasargod	Punganur
Vatakara	-	0.045	0.050	0.066
Iduki	0.151	-	0.032	0.066
Kasargod	0.171	0.134	-	0.070
Punganur	0.188	0.194	0.206	-

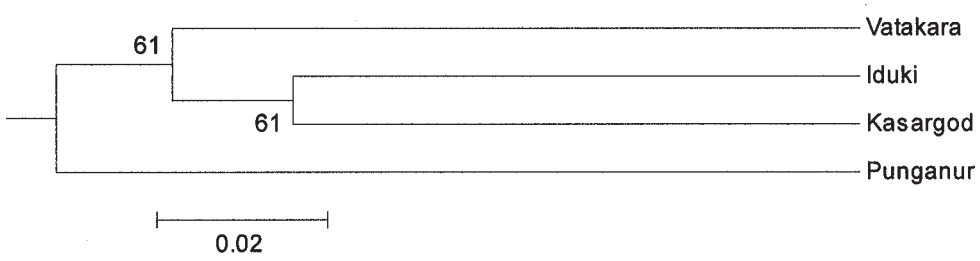

Figure 2. Dendrogram of four cattle populations with bootstrap values.

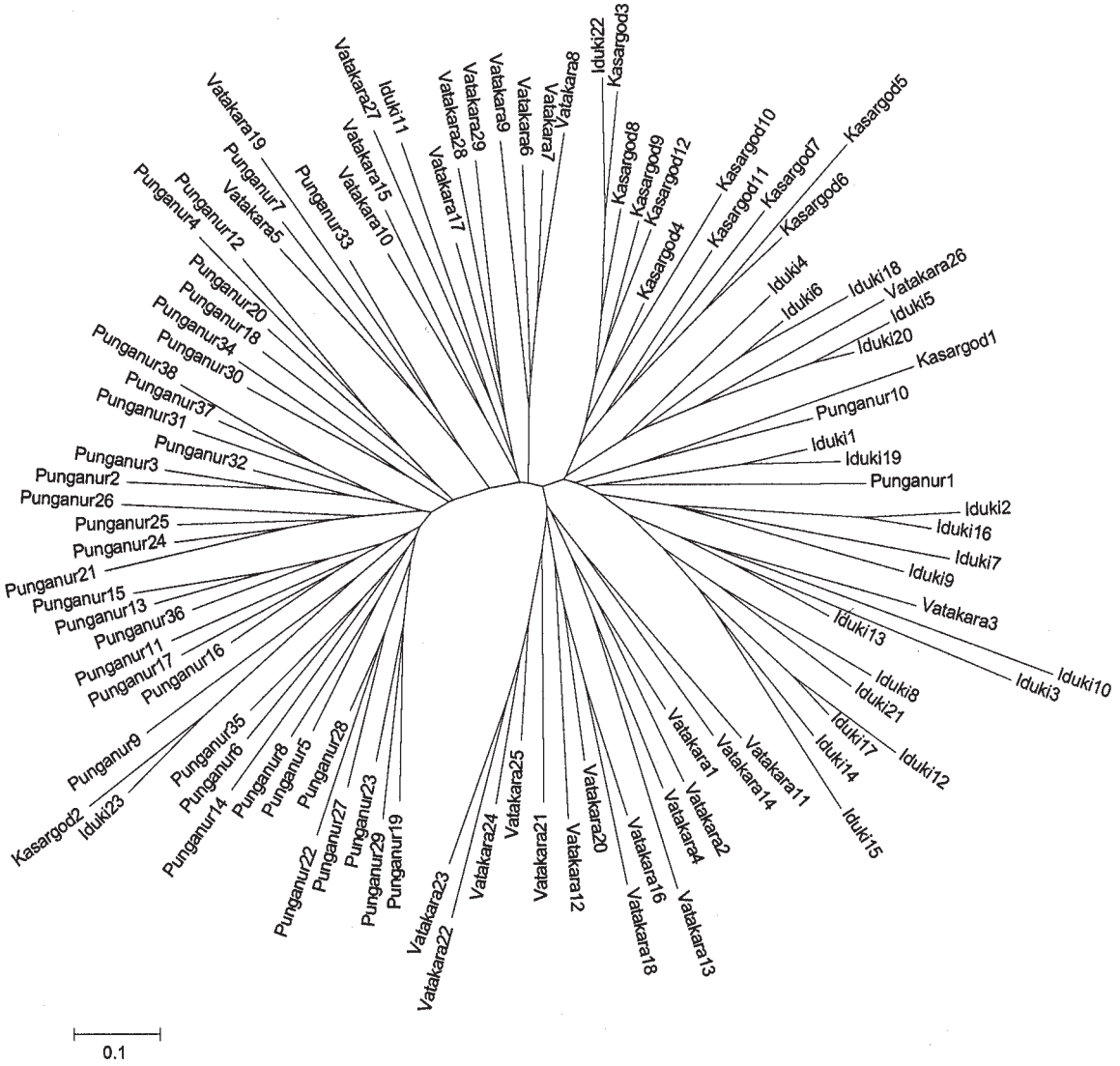

Figure 3. Radiation tree using NJ algorithm and genetic distance by allele sharing.

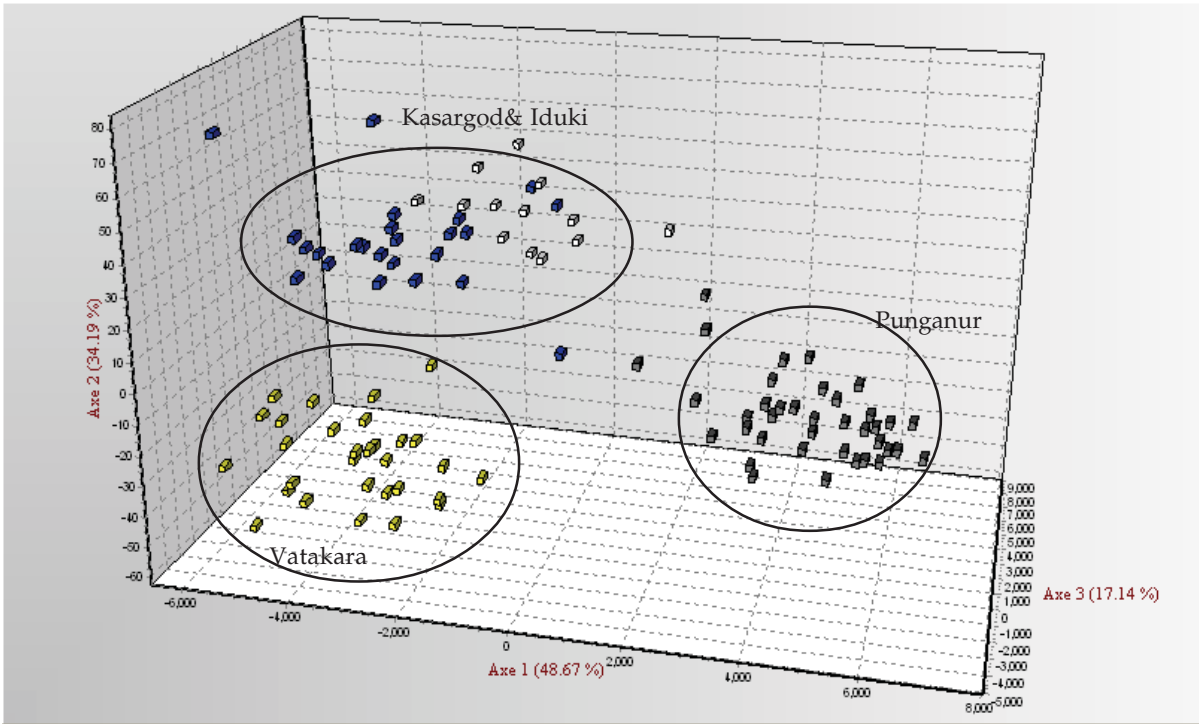

Figure 4. Correspondence analysis of four cattle populations.

or than the one obtained in north European cattle, ranging from 0.45 to 0.67 (Kantanen *et al.*, 2000). This indicates lack of population structure.

The mean F_{IS} values across all the populations was -0.137 which was significantly different from zero and may be due to outbreeding taking place for increase in milk yield. The history reveals large breeds like the Sahiwal and Gir (*Bos indicus*) cattle of India having been used for improving the milk yield of these cattle in the pre-independence era before the introduction of crossbreeding with exotics (*Bos taurus)*. The F_{ST} estimates were significantly different from zero for all the loci with a mean value of 0.058 indicating that the populations are moderately differentiated. The differentiation among the population is low (10.7 - 19.35%) compared to other breeds of cattle (MacHugh *et al.*, 1997; Kantanen *et al.*, 2000) and 11% in *Bos indicus* cattle breeds (Sodhi *et al.*, 2005). This low differentiation may be due to the result of out crossing with other milk breeds for improvement in milk yield. This seems especially the case in Punganur and Vatakara cattle where the size of animals is comparatively larger (more than one meter) and milk yield was also higher. Similarly the genetic distance (0.134) was least between Iduki and Kasargod animals and highest between the Punganur and Kasargod (0.206). The bootstrap values were 61%. The Nm values present an indirect measure of the gene flow and gave values of 7.47 migrants between Kasargod and Iduki animals and least values of 3.31 between Kasargod and Punganur cattle. This represents the effective number of migrants among the populations.

Little or no differentiation between Kasargod and Iduki breeds of cattle is supported by the assignment test. The self assignment gave an accuracy of 95% in the four populations. Similar results of 80-100% correct assignment amongst different breeds have been reported by several authors (MacHugh *et al.*, 1998; Bjørustad and Røed, 2001; Moudet *et al.*, 2002; Achmann *et al.*, 2004). The two populations of Iduki and Kasargod are very similar and have a high gene flow between them and form a single genetic cluster and thus can be considered as one. The stepwise mutation model (SMM) being most conservative did not reveal any evidence for recent genetic bottleneck in any of the four populations. The status report on Punganur cattle (Country Report, 2004) described it as threatened on the basis of phenotypic characteristics and count of heads but that it has been found to be an out-bred population.

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